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RESEARCH ARTICLE

Isolation and Characterization of *Bacillus* Consortia for Plant Growth Promotion in Rice (*Oryza sativa L.*)

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ABSTRACT

In this study, soil samples from the rhizosphere of various uncultivated weeds were collected from fifteen different locations of Gujarat. Heat treatment was given at 65°C for 20 minutes prior to initial screening for spore-forming *Bacillus* spp. Among them, 20 nitrogen-fixing (NFB), 27 phosphate solubilising (PSB) and 15 potassium mobilizing (KMB) isolates were screened primarily. After molecular identification only *Bacillus* isolates were further selected and characterized. Three superior *Bacillus* isolates were selected from each category by secondary screening. All isolates belonging to different category were compatible in nature and showed significant ammonia production, ARA, phosphate solubilisation, potassium mobilization, siderophore production, IAA production and organic acid producers. The relative expression analysis of three genes *NRT2.1* (Nitrate transporter), *PT6* (Phosphorus transporter), and *AKT1* (Potassium transporter) at transcriptional level were performed in the juvenile root tissues of Rice using qRT-PCR technique at 15th, 30th and 45th days after transplanting with *Actin* as a internal control. Rice transplants were inoculated with different NPK fixing *Bacillus* consortia, which significantly improved growth parameters as compared to control in field study. Various growth and yield attributing characters of rice *viz*, plant height (at 60 DAT, 90 DAT, and at harvesting), number of productive tillers, days to 50 % flowering, 100 seed weight, grain yield, straw yield, as well as dry biomass per hectare were significantly influenced by different treatments. An application of 100 % RDF with NPK consortia (T_7) treatment of rice crop resulted in significantly higher plant height (49.25 cm at 60 DAT, 107.00 cm at 90 DAT, 129.50 cm at harvesting), days to 50% flowering (75.63), 100 seed weight (3.53 g), number of productive tillers per plant (12.13), grain yield (6889.00 kg ha^{-1}), straw yield (8754.25 kg ha^{-1}) and dry biomass (7889.25 kg ha^{-1}) followed by the treatments T_4 (T_2 + N consortia), T_5 (T_2 + P consortia), T_6 (T_2 + K consortia), and T_2 (RDF (NP) 100%). Nutrient content in grain and straw was differing significantly due to different treatments. However, significantly higher N, P, K, content in straw (1.28 %, 0.15 %, and 1.52 %) and grain (2.06 %, 0.23 %, and 0.25 %) respectively, were recorded under the treatment T_7. The spore forming *Bacillus* consortia was able to survive at a wide range of temperature and pH fluctuations and found to be effective as N-fixers, P-solubilizers, K-mobilizers, siderophore producers, IAA producers with having antagonistic activity against rice pathogen *Magnaportha oryzae*.

Keywords: *Bacillus*, Plant Growth Promotion, Nitrogen Fixation, Phosphate Solubilization

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important amongst all staple food crops that have a role in providing the food to more than half of the world's population. Among the cereals, rice is of importance which is taken as regular meal over 40 % of the world's community, especially, in Asia which has made rice as the most important agricultural produced in most of the developing countries. Rice grow in complex environmental conditions having plenty of microorganisms. Soil microorganisms show effects on rice ranging from favourable effects caused by many soil microorganisms to hazardous effects caused by plant pathogens. The exact mechanisms by which PGPR enhances the plant growth promotion is (i) Increasing solubilization of nutrients thereby providing bioavailable forms of potassium, phosphorus, and other nutrients and trace elements (Ortiz *et al*., 2020). (ii) Aiding or enhancing a symbiotic nitrogen fixation (Yaashikaa *et al*., 2020) or indirectly affecting symbiotic nitrogen fixation, nodulation or nodule occupancy (Bessadok *et al*., 2020). (iii) Affecting the concentration of plant growth promoters and production of phytohormones like auxins, cytokinins and gibberellins (vi) Synthesis of antibiotic, fungicidal compounds and other pathogen-depressing substances such as siderophores, cyanide and chelating agents that protect plants from diseases (Solmaz *et al*., 2020). (v) These organisms can also increase plant tolerance to various adverse environmental conditions and stress like high temperature, flooding (Gupta and Pandey, 2019), salt stress (Grossi *et al*., 2020), etc.

Historically, several approaches for restoring the soil health and maximizing plant growth have been used in agriculture. Such approaches include amending soil with organic materials, using crop rotations, and using cover crops in between growing seasons. A more practical and cost-effective alternative approach is to increase the soil microbial populations by providing the treatment to plants and soils with cultured microbial communities. Jain and Saxena, 2019 showed different applications of plant growth promoting rhizobacteria against rice but one restraint of the biofertilizer is that there is large variation in soil salinity, pH, and an atmospheric temperature where most of the biofertilizers could not tolerate harsh environmental conditions which further leads to the gradual loss and washout of microbes from the soil. Treatment of rice with individual microbe for different plant growth promotion activities is comparatively costlier, also. Thus, for maximizing and sustaining agricultural production, integrated nutrient management practices including the use of *Bacillus* consortia along with inorganic fertilizer could be a preferred alternative.

Bacillus consortia could be used as a biofertilizer input to increase crop productivity and sustainability as it is spore forming (could sustain harsh conditions) and are economical. *Bacillus* spp. play a significant role in improving soil fertility by fixing atmospheric nitrogen, solubilizing insoluble phosphates, mobilizing potassium, and producing plant growth promoting substances in the soil. Hence, plants treated with beneficial *Bacillus* typically exhibits enhanced "nutrient utilization efficiency". This response means that at a given level of soil fertility, plants treated with *Bacillus* acquire more nutrients from the soil and that result in higher levels of key nutrients in plant tissues and reduced levels of chemical fertilizers' use. Applied efficient consortia can reduce the use of chemical fertilizer by 25 % (Mano *et al*., 2007) and the quality of plant growth and yields can be maintained at levels equivalent to those that result with full fertility rates. Therefore, to maintain the balance between chemical fertilizers and natural *Bacillus* consortia potentially for improving yield of rice, the objective of this research were as follows.

MATERIALS AND METHODS

Isolation, purification, and identification of NFB, PSB, and KSB

1g sample was suspended in 5 ml autoclaved NaCl saline. After sedimentation of solid particles, clear supernatant was collected in microfuge tubes. Dilutions were made up to 10^{-8} and heat treatment was given at 65°C for 20 minutes for the selection of spore-forming *Bacilli* (Hernandez *et al*., 1996). After the heat treatment, 100µL of the diluted suspension was spread over in selective agar media plates.

Media

For the isolation of NFB, PSB, and KMB, Jensen's nitrogen-free (JNF) agar media, Pikovaskya (PVK) medium agar, and Aleksandrov agar media with mica powder (MP) as a sole source of potassium were used, respectively (Dutta *et al*., 2017; Akintokun *et al*., 2019).

Isolation of NFB, PSB, and KMB

For the isolation of NFB, PSB and KMB, heat-treated diluted samples were spreaded over JNF, PVK, and Aleksandrov with MP agar media plates, respectively and growth characteristicsin the case of PSB and KMB developed transparent zones against the opaque background. Colonies were selected and isolated based on zone formed around the colonies (Suleman *et al*., 2018; Jha *et al*., 2017); only those isolates were selected whose Solubilizing Index (SI) was higher comparatively.

Solubilising index (SI) of the isolates was determined (Wang *et al*., 2020). Further isolates were inoculated into respective media and a decrease in the pH was again monitored.

Identification of the bacterial isolates

Identification of the isolates using the Biolog system

Biolog carbon substrates utilization patterns Biolog GP2 MicroPlates (Biolog, Inc., Hayward, CA, USA) were inoculated in duplicate using the standard procedures and were incubated at 30-35^{oC} for 24-36 hrs (Atefeh *et al*., 2018).

Molecular characterization of the rhizospheric isolates

The selected rhizospheric isolates were identified through 16S rDNA gene sequencing (Alsohim *et al*., 2020).

Quantitative estimation of NFBs

Ammonia production

All the *Bacilli* isolates were tested for ammonia production on peptone water broth using the method described by Cappuccino and Sherman (Dutta *et al*., 2017).

Secondary screening of PSBs

Quantitative estimation of solubilized P by *Bacilli* isolates were done by the vanadomolybdophosphoric yellow color method in pikovskaya's broth media containing 5000 µg/ml tricalcium phosphate (Dutta *et al*., 2017).

Secondary screening of KMBs

The *Bacilli* isolates showing a better zone of solubilization on Aleksandrov agar plates were further checked for their capability to release K from broth media (with 1% muscovite mica) using method described by Sugumaran and Janarthanam (Akintokun *et al*., 2019).

Biochemical characterization of NFB, PSB, and KMB

Selected isolates were biochemically characterized by different biochemical test which includes

Siderophore production and quantification assay

All the *Bacilli* isolates competent in nitrogen fixation, phosphate solubilization, and potassium mobilization were checked for siderophore production using method given by Schwyn and Neilands on the Chrome-Azurol S (CAS) agar medium while the quantification of siderophore production was done by the method described by Meyer and Abdallah Meyer using succinate broth media (Pahari *et al*., 2017).

IAA production

The selected *Bacilli* isolates were inoculated in LB medium supplemented with 1 mgml^{-1} of tryptophan to determine IAA production by the method described by Brick *et al*.,2018.

A field study was conducted in kharif 2017 and 2018 at Main Rice Research Station, NAU, Navsari. To evaluate the effect of NPK fixing *Bacillus* consortium on Rice (GNR-3), soil of pH 8.39 having organic carbon 0.66% and available N, P & K 292, 35 and 385 kg/ha, respectively, was used.

P solubilizing and K mobilizing *Bacillus* isolates were grown in tris minimal medium supplemented with glucose as sole carbon sources. The detection and quantification of organic acids was carried out on Thermo Scientific Fisher High-Performance Liquid Chromatogram (HPLC) equipped with PDA detector, Thermo Scientific Fisher plus autosampler, Thermo Scientific Fisher Smartline pump, Thermo Scientific Fisher inline degasser, and HyperSil Gold C-18 column 250 mm × 4.6 mm with 5 µm particle size (Thermo Fisher, Austria).

Inoculation

For present study, consortium of nine *Bacillus* was used such as *Bacillus zhangzhouensis* (BLAST Sequence ID: MN647597), *Bacillus megaterium* (MN647598), *Lysinibacillus macroides* (MN647599), *Bacillus aryabhattai* (MN647600), *Bacillus megaterium* (MN647601), *Brevibacillus agri* (MN647602), *Lysinibacillus macroides* (MN647594), *Lysinibacillus macroides* (MN647595), and *Bacillus megaterium* (MN647596) were used.

Inoculation before sowing: Rice (GNR-3) transplants were treated *Bacillus* consortium. The experiment conducted with eleven treatments of a different combination of NPK fixing *Bacillus* consortium, T1: Absolute control, T2: RDF (NP) 100% (100 kg N, 30 kg P), T3: Consortia NPK, T4: T2 + N consortia, T5: T2 + P consortia, T6: T2 + K consortia, T7: T2 + T3, T8: 75% RDF (NP), T9: T8 + T3, T1075% RDN + 100% RDP + N consortia, T11: 75% RDP + 100% RDN + P consortia..The experiment was replicated four times in RBD manner with plot size of 3 m X 5 m. Remaining practices were followed as per package of practices described.

Molecular study of NPK transporters by qRT-PCR analysis

Fresh root samples were collected and quickly frozen in liquid nitrogen for total RNA extraction. Samples from all the treatments were analyzed in triplicate for following molecular parameters analysis.

Statistical Analysis

A randomized block design (RBD) was used for all experiments in this paper, with four replications for each treatment. Here the data represented are pooled data from the year 2016-17 and 2017-18. Statistical analysis of the various observations and measurements was carried out using analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Rice crop, the backbone of food self-sufficiency, is facing a sustainability problem due to modern agricultural practices with indiscriminate use of chemical fertilizers and pesticides. The concern like declining crop productivity, depletion of soil organic carbon and mineral nutrients content, etc. are the consequents of unbalanced and injudicious use of chemical fertilizers and pesticides, which adversely effects the soil structure, microflora, and soil health. Also, unbalanced and injudicious use of chemical fertilizers and pesticides are environmental burden. *Bacillus* spp. were a dominant bacterial group in the leaves and roots of rice plants; *B. megaterium*, *B. luciferensis*, *Brevibacilllus agri* were isolated from the roots and *B. pumilus*, *B. subtilis*, *Paenibacillus amylolyticus* from the seeds and leaves (Mano *et al*., 2007). Thus, the application of *Bacillus* consortia along with conventional fertilizers improves rice productivity and soil health.

Plant growth is a multi-complex phenomenon and *Bacillus* rhizobacteria acts as bio-stimulant, bio-fertilizer and plant-protective agent or sometimes have role for bioremediation of pollutants (Agarwal *et al*., 2017). *Bacillus* spp. are responsible for multifarious metabolic functions, and interfere in soil fertility, plant health, growth, nutrient cycling, organic matter formation, decomposition and soil structure maintenance (Chauhan, 2017).

Fifteen rhizospheric samples were collected from different weed plants from uncultivated lands across the Gujarat (Surat, Idar, Palanpur, Jamnagar, and Junagadh) for the isolation of NFB's, PSB's, and KMB's. As there was no added application of fertilizers in uncultivated lands, the plants growing in that environment might be dependent on natural microflora for the availability of nutrients for the growth of weed plants.

The results obtained for biolog and molecular sequencing were mentioned in Table 1. The results for ammonia production, ARA, Phosphate solubilization, potassium mobilization, IAA and siderophore production were mentioned in Table 2.

Ammonia production has an important role to play in the accumulation of nitrogen and helps in promoting root, shoot growth along with biomass production which ultimately accelerates plant growth (Dutta and Thakur, 2017). *Bacillus* varied in acetylene reduction, which is an indirect measure of N-fixing potential while also being a specific means for monitoring of functional nitrogenase activity. Hala (2020) revealed that inoculation of nitrogen-fixing bacteria to upland rice can increase tiller formation, shoot and root dry weight and plant Total N production.

Microbes enhance the P availability to plants by mineralizing and solubilizing P in the soil (Liu *et al*., 2019). The microbial biomass in soil contains a significant amount of P (typically 10-15 kg ha^{-1}, but as high as 100 kg ha^{-1}), accounts for 2-5 % of total P and around 10-15 % of the soil organic P (Bargaz *et al*., 2018). Plant growth substances produced by PSBs improve plant growth by their direct effects on plant metabolic processes. PSBs also induce the proliferation of lateral roots and root hairs that results in increased nutrient absorbing surfaces. Initially, the phosphate solubilization was less but as soon as the incubation period increased, all three *Bacillus* isolates exhibited the ability to solubilize calcium phosphate and helps in making phosphorus available for plants to absorb. KMBs mobilize potassium to help plant in its growth promotion and also protect the plants from salinity injury by enhancing its growth-related characters such as stomatal conductance, electrolyte leakage and lipid peroxidation. Plant inoculated with KMBs also accumulates more type and number of soluble carbohydrates in leaves under salinity, which helps the plant to overcome osmotic stress (Gore *et al*., 2019).

Siderophores chelate iron from mineral phases by the formation of soluble Fe^{3+} complexes that can be absorbed by energy-dependent membrane transport system and finally make soluble Fe^{3+} available to plants. It also binds with the Fe^{3+} in the rhizosphere and efficiently prevents the propagation of fungal pathogens by depriving them of available iron (Chandra *et al*., 2018, Wang *et al*., 2020). IAA is the core member of auxins' family produced by plants as it has an important role to play in numerous plant activities such as embryo development, leaf formation, root initiation and development, phototropism, geotropism, fruit development, abscission. etc. IAA helps in the enhancement of root length with an increase in the number of root branches, root hairs and root laterals that aid in the

uptake of nutrients from surrounding (Sheela *et al.*, 2018).

When grown on 100mM of Glucose, all the P solubilizing and K mobilizing *Bacillus* isolates were attributed to produce different concentrations of gluconic acid, oxalic acid, citric acid, and pyruvic acid (Table 3). Banik and Dey, (1982) reported that the isolates produce organic acids such as acetate, lactate, oxalate, tartrate, succinate, citrate, gluconate, ketogluconate, glycolate, etc. Further, secreted organic acid reduce the pH of the medium and solubilize P from rock phosphate (Nahas *et al.*, 1996). The P releasing ability of several PSMs such as *Rahnella aquatilis*, *Erwinia herbicola*, *Pseudomonas cepacia* and *E. asburiae* PSI3 have also been reported to be a result of their ability to secrete gluconic acid (Gyaneshwar *et al.*, 1999; Rodrıguez and Fraga, 1999; Sharma *et al.*, 2005).

The relative expression analysis of 3 transportation related genes *NRT2.1* (Nitrate transporter-2.1), *PT6* (Phosphorus transporter-6), and *AKT1* (Potassium transporter-1) at transcriptional level were performed in the juvenile root tissues of rice using qRT-PCR technique at 15th, 30th and 45th DAT with *Actin* as a indigenous absolute control. (Table No. 4). The expression of *NRT2.1* (Accession No. AB008519) gene upregulated in treatment T_7 showed 2.60 folds at 15 DAT compared to absolute control, and then gradually the expression of *NRT2.1* downregulated with the reduced availability of nitrate at 30 and 45 DAT. The status of N in rice plant as well as local signals in roots trigger morphological changes in the whole system of root where NO_3 acts locally to induce different morphological responses. The *NRT2* family plays an important role in NO_3 acquisition from the soil environment (Morère-Le Paven *et al.*, 2011). The expression of *PT6* gene downregulated in treatment T_7 showed 0.48 folds at 15 DAT compared to absolute control, and then gradually the expression of *PT6* upregulated with the reduced availability of phosphate at 30 and 45 DAT. The uptake and transportation of phosphorus in plants are mediated by phosphate transporters. They play pivotal roles in regulation of absorption and utilization of phosphorus for plants under diversified phosphate-supply conditions. As one member of high affinity phosphate transporter gene family, *OSPT6* was reported to play an important role in enhancing plant resistance to low phosphorus stress through enhanced expression (Tatry *et al.*, 2009). The expression of *AKT1* gene upregulated in treatment T_7 showed 1.95 folds at 15 DAT compared to absolute control, and then gradually the expression of *AKT1* downregulated with the reduced availability of potassium at 30 and 45 DAT. Soils that are K^+ deficient are becoming increasingly common and K^+ fertilization is a considerable cost to farming. K^+ is essential for a number of biochemical and biophysical functions in plant cells (Zörb *et al.*, 2014) and its cytoplasmic concentration is believed to be tightly controlled (Kronzucker *et al.*, 1998). To maintain adequate K^+ levels, plants have a number of K^+ uptake systems of which *AKT1* is one of the major transporters.

Breidenbach *et al.*, (2016) showed increase in plant height of rice plants inoculated with beneficial microorganisms. This is because of increase in absorption of nutrients by plant, improvement of soil characteristics such as contents of organic materials and increase in accessible NPK. All applied biofertilizer treatments resulted in significant increase in productivity of straw and grain/plant and in the total weight of thousand grains (Obid *et al.*, 2016). The highest number of productive tillers due to application of RDF with *Bacillus* consortia can be attributed to adequate supply of nutrients due to inoculation of *Bacillus* consortia. The results were in accordance to Fakir *et al.*, (2007). The ultimate objective of all the agronomic studies is to optimize the yield of any crop. The grain yield of a crop is the net resultant of various factors and is valid criterion for comparing the efficiency of different inputs in a given situation. Grain yield in rice is primarily a function of effective tillers, number of grains per spike and thousand grain weight. The grain yield of a crop depends on the manner in which the dry matter produced during the vegetative phase of the crop is distributed among various sinks *i.e.* the vegetative and reproductive parts. Nitrogen is known to promote tillering, improve length and width of leaves, which in turn increase the dry matter and are responsible for increase in straw yield. Gopalswamy and Vidhyasekaran (1988), and Hartmann *et al.*, (2009) reported that the application of biofertilizers had increased the formation and development of numerous root branching, root hairs, and primary and secondary lateral roots which increases the nutrient uptake capacity of roots. This effect on the root system as well as more root colonization and root proliferation are probably due to the growth hormones secreted by the bacteria and also NPK fixation by it. The increased NPK uptake from the soil might have correspondingly increased the biomass to some extent. Significantly higher straw N, P and K content were observed in treatment having combined application of biofertilizers with inorganic fertilizers as compared to control treatment. The organic and inorganic acids convert tricalcium phosphate to di and monobasic phosphates, resulting in an enhanced availability of the phosphorous to the plant. Sheng and He (2006) reported that organic acids, e.g. citric, oxalic, tartaric, succinic acids etc., produced by rhizobacteria are able to chelate metals and mobilize K from K-containing minerals. The N content in grains had increased in treatments inoculated with *Bacillus* consortia

over uninoculated treatments. The grain P content had increased due to inoculation of PSB with P fertilizers in soil. Sarkar (2007), and Alam (2007) reported that the inoculation of seedlings with PSB strains significantly increased the content of P in both straw and grain of rice. The K content in grains had increased in treatments inoculated with *Bacillus* consortia compared to uninoculated treatments. Sarkar (2007) reported that the inoculation of seedlings with PSB strains significantly increased potassium uptake by plant and the K content in grains. The results revealed that available soil phosphorus (Olsen-P) significantly increased with combined incorporation of biofertilizer and inorganic fertilizers. Therefore, use of biofertilizer with chemical fertilizer can play an important role in improving P bioavailability. The increase in soil P content might be due to the P-solubilizing potential of the isolates used in biofertilizer. This may be attributed to the production of organic acids, chelating oxo-acids and solubilization of inorganic insoluble phosphates by microorganisms. The present study indicated that application of biofertilizers along with inorganic fertilizers increase the available potassium content in soil. This may be due to variety of soil microbes which can release soluble potassium from potassium-bearing minerals. These microbes release organic acid, which quickly dissolves rock and chelate silicon ions, releasing K ions into the soil (Bennett *et al*., 1998 and Friedrich *et al*., 2004). It has been shown that *Bacillus mucilaginosus* and *Bacillus edaphicus* can generate polysaccharide and carboxylic acids, such as tartaric acid and citric acid, to solubilize K compounds (Richard *et al*., 1989 and Lin *et al*., 2002). The presence of indigenous potassium mobilizing microbes might have cause to increase the concentration of available soil potassium.

Conclusion

The present investigation concluded that integrated application of NPK fixing consortium *Bacillus* biofertilizer along with inorganic fertilizers significantly improved the available NPK in soil as compared to inorganic fertilizers alone. The treatment having 100 % RDF + NPK fixing *Bacillus* consortia was found to be most effective in all the parameters in treatment having recommended dose of inorganic nutrients. T_{10} and T_{11} were also found effective which states that our *Bacillus* consortium is significant in decreasing the amount of inorganic fertilizers used in field. The enhanced *Bacillus* dynamics in turn increased plant growth parameter, dry biomass and rice yield as well as soil health due to more availability of nutrients and plant growth promoting substances, thus satisfying all the criteria for sustainability.

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Table 1: Identification of bacteria by Biolog and 16S rDNA sequencing

Seq. Name	Biolog Name Sequence	Blast Sequence ID	Accession No.	BLAST Sequence Name	% Identity	Query Cover
NAUN1	*Bacillus zhangzhouensis*	MN647597	MN006154	*Bacillus zhangzhouensis* strain BF-4D-10	97.66	99
NAUN2	*Bacillus megaterium*	MN647598	KF836360	*Bacillus megaterium* strain CMB-08	96.33	95
NAUN3	*Lysinibacillus macroides*	MN647599	MN538917	*Lysinibacillus macroides* strain SMV311	96.53	94
NAUP1	*Bacillus aryabhattai*	MN647600	MH010075	*Bacillus aryabhattai* strain RW024	96.53	99
NAUP2	*Bacillus megaterium*	MN647601	MH260977	*Bacillus megaterium* strain MGB2003	97.48	92
NAUP3	*Brevibacillus agri*	MN647602	MK281590	*Brevibacillus agri* strain ChemUPES_4	95.88	97
NAUK1	*Lysinibacillus macroides*	MN647594	MN538923	*Lysinibacillus macroides* strain SMV329	100	98
NAUK2	*Lysinibacillus macroides*	MN647595	MG892813	*Lysinibacillus macroides* strain SWTPB26	96.76	97
NAUK3	*Bacillus megaterium*	MN647596	KP997271	*Bacillus megaterium* strain FB133M	98.84	100

Table 2: PGP potential of *Bacillus* rhizobacteria

Sr. No	Name of the isolates	Quantification
Nitrogenase activity (n moles C2H4/h-1 culture-1)	NAUN1	678.57
	NAUN2	735.11
	NAUN3	699.75
Ammonia Production (µmol mL-1)	NAUN1	0.64
	NAUN2	2.04
	NAUN3	0.94
Phosphate Solubilization (µg ml-1)	NAUP1	401.94
	NAUP2	376.74
	NAUP3	308.16
Potassium Mobilization (µg ml-1)	NAUK1	194.24
	NAUK2	228.14
	NAUK3	162.28
	NAUP1	71.32
IAA Production (µg ml-1)	NAUN1	84.74
	NAUN3	86.12
	NAUP1	118.85
	NAUP2	68.92
	NAUP3	146.68
	NAUK2	87.34
Siderophore Production (%)	NAUN1	37.46
	NAUP3	17.56
	NAUK1	32.78
	NAUK2	39.18
	NAUK3	48.15

Table 3: HPLC profiling of organic acids secreted by selected rhizobacteria

Rhizobacteria	Gluconic acid (mM)	Oxalic acid (mM)	Citric acid (mM)	Pyruvic acid (mM)
NAUP1	6.200	0.124	0.270	0.960
NAUP2	3.730	0.075	0.150	0.520
NAUP3	2.980	0.060	0.120	0.420
NAUK1	1.500	0.030	0.620	1.860
NAUK2	2.400	0.048	0.105	0.339
NAUK3	1.210	0.024	0.054	0.157

Table 4: Relative gene expression of NPK transporters

Treatment	Fold expression of *NRT2.1* gene LOC_Os02g02170			Fold expression of *PT6* gene LOC_Os08g45000			Fold expression of *AKT1* gene AK120308		
	15D	30D	45D	15D	30D	45D	15D	30D	45D
T_1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
T_2	1.57	1.53	1.28	0.58	0.66	0.86	1.47	1.21	1.19
T_3	1.05	1.02	1.01	0.81	0.92	0.95	1.11	1.04	1.03
T_4	2.15	1.88	1.37	0.55	0.64	0.85	1.78	1.25	1.24
T_5	1.87	1.69	1.29	0.56	0.64	0.83	1.69	1.23	1.22
T_6	1.80	1.64	1.29	0.60	0.66	0.85	1.63	1.22	1.21
T_7	2.60	2.19	1.48	0.48	0.57	0.79	1.95	1.61	1.34
T_8	1.11	1.10	1.03	0.66	0.81	0.92	1.18	1.10	1.07
T_9	1.13	1.11	1.06	0.65	0.78	0.90	1.35	1.13	1.09
T_{10}	1.47	1.39	1.17	0.65	0.76	0.91	1.37	1.14	1.10
T_{11}	1.42	1.47	1.21	0.61	0.71	0.90	1.40	1.15	1.15

Table 5: Effect of different treatments on plant height in rice

NO.	Treatments	Plant height 60 Days (cm)			Plant height 90 Days (cm)			Plant height Harvesting (cm)		
		2017-18	2018-19	Pooled	2017-18	2018-19	Pooled	2017-18	2018-19	Pooled
T_1	Absolute control	27.75	24.75	26.25	79.25	75.25	77.25	109.00	103.50	106.25
T_2	RDF (NP) 100%	43.50	36.75	40.13	99.25	98.75	99.00	123.75	121.00	122.38
T_3	Consortia NPK	30.25	25.50	27.88	83.25	81.50	82.38	114.25	107.50	110.88
T_4	T_2 + N consortia	47.00	46.25	46.63	102.25	102.50	102.38	125.75	125.00	125.38
T_5	T_2 + P consortia	47.00	43.00	45.00	100.25	99.50	99.88	124.75	122.50	123.63
T_6	T_2 + K consortia	45.75	39.25	42.50	97.75	97.50	97.63	124.25	122.25	123.25
T_7	T_2 + T_3	49.00	49.50	49.25	108.50	105.50	107.00	128.75	130.25	129.50
T_8	75% RDF (NP)	39.25	30.00	34.63	86.75	86.50	86.63	119.50	113.00	116.25
T_9	T_8 + T_3	42.25	36.25	39.25	94.50	91.25	92.88	120.25	117.25	118.75
T_{10}	75% RDN + 100% RDP + N consortia	42.75	30.25	36.50	97.25	92.00	94.63	121.25	116.50	118.88
T_{11}	75% RDP + 100% RDN + P consortia	43.00	33.25	38.13	91.75	97.00	94.38	123.00	113.25	118.13
	S. Em. ±	2.67	2.59	1.87	3.49	3.15	2.24	6.12	4.33	3.52
	C. D. (P=0.05)	7.72	7.48	5.29	10.06	9.11	6.32	17.68	12.50	9.95
	C.V. %	12.86	14.44	13.59	7.37	6.76	7.07	10.09	7.37	8.88

Table 6: Effect of different treatments on growth parameters in rice

No.	Treatments	Days to 50% flowering			100 seed weight (gm)			No. of productive tillers per plant		
		2017-18	2018-19	Pooled	2017-18	2018-19	Pooled	2017-18	2018-19	Pooled
T_1	Absolute control	84.50	85.00	84.75	3.00	2.75	2.88	5.00	3.75	4.38
T_2	RDF (NP) 100%	85.50	84.00	84.75	3.20	3.08	3.14	8.75	9.00	8.88
T_3	Consortia NPK	83.75	87.75	85.75	3.05	2.84	2.94	5.25	5.75	5.50
T_4	T_2 + N consortia	79.75	74.25	77.00	3.35	3.31	3.33	10.00	11.50	10.75
T_5	T_2 + P consortia	79.00	75.75	77.38	3.37	3.23	3.30	10.00	10.25	10.13
T_6	T_2 + K consortia	80.50	77.50	79.00	3.30	3.18	3.24	9.25	10.25	9.75
T_7	T_2 + T_3	77.50	73.75	75.63	3.37	3.70	3.53	11.50	12.75	12.13
T_8	75% RDF (NP)	82.75	85.00	83.88	3.07	2.97	3.02	7.50	5.50	6.50
T_9	T_8 + T_3	82.50	81.75	82.13	3.20	3.02	3.11	7.75	7.50	7.63
T_{10}	75% RDN + 100% RDP + N consortia	83.75	85.75	84.75	3.15	3.14	3.14	8.50	9.50	9.00
T_{11}	75% RDP + 100% RDN + P consortia	82.75	78.75	80.75	3.16	3.10	3.13	8.25	9.75	9.00
	S. Em. ±	2.75	2.55	1.83	0.10	0.10	0.07	0.39	0.31	0.57
	C. D. (P=0.05)	7.93	7.36	5.16	0.28	0.28	0.20	1.12	0.90	1.78
	C.V. %	6.70	6.30	6.51	6.15	6.32	6.23	9.32	7.19	8.28

Table 7: Effect of different treatments on yield attributing characters in rice

No.	Treatments	Grain Yield (kg/h)			Straw Yield (kg/h)			Dry Biomass (kg/h)		
		2017-18	2018-19	Pooled	2017-18	2018-19	Pooled	2017-18	2018-19	Pooled
T_1	Absolute control	5055.50	4981.50	5018.50	5387.50	5006.25	5196.88	5075.75	5168.25	5122.00
T_2	RDF (NP) 100%	5965.75	6097.25	6031.50	7011.25	6761.25	6886.25	6834.25	7190.75	7012.50
T_3	Consortia NPK	5213.50	5139.00	5176.25	5484.75	5320.75	5402.75	5426.00	5565.25	5495.63
T_4	T_2 + N consortia	6354.00	6580.00	6467.00	7964.50	8244.50	8104.50	7372.50	7658.00	7515.25
T_5	T_2 + P consortia	6232.75	6391.75	6312.25	7620.50	7748.75	7684.63	7048.50	7226.00	7137.25
T_6	T_2 + K consortia	6130.50	6183.50	6157.00	7193.00	7245.75	7219.38	6993.75	6690.75	6842.25
T_7	T_2 + T_3	6759.00	7020.00	6889.00	8662.75	8845.75	8754.25	7760.00	8018.50	7889.25
T_8	75% RDF (NP)	5340.00	5445.75	5392.88	6145.00	5773.25	5959.13	5886.25	5967.75	5927.00
T_9	T_8 + T_3	5544.50	5534.25	5539.38	6423.25	5892.75	6158.00	6399.50	5900.75	6150.13
T_{10}	75% RDN + 100% RDP + N consortia	5691.50	5940.00	5815.75	6391.50	6349.25	6370.38	6619.75	6332.75	6476.25
T_{11}	75% RDP + 100% RDN + P consortia	5726.50	5842.50	5784.50	6596.25	6158.25	6377.25	6751.00	6670.75	6710.88
	S. Em. ±	432.50	226.75	227.19	252.46	234.19	167.76	227.44	270.75	171.84
	C. D. (P=0.05)	1248.99	654.81	641.52	729.06	676.29	473.71	656.80	781.86	485.24
	C.V. %	14.86	7.66	11.76	7.42	7.02	7.23	6.93	8.23	7.61

Table 8: Effect of different treatments on available NPK content in rice straw

NO.	Treatments	Nitrogen content in straw			Phosphorus content in straw			Potassium content in straw		
		2017-18	2018-19	Pooled	2017-18	2018-19	Pooled	2017-18	2018-19	Pooled
T_1	Absolute control	0.63	0.72	0.67	0.07	0.08	0.07	1.28	1.10	1.19
T_2	RDF (NP) 100%	1.16	1.06	1.11	0.13	0.13	0.13	1.38	1.37	1.37
T_3	Consortia NPK	0.72	0.76	0.74	0.08	0.09	0.08	1.31	1.16	1.24
T_4	T_2 + N consortia	1.21	1.18	1.20	0.14	0.14	0.14	1.46	1.49	1.47
T_5	T_2 + P consortia	1.20	1.18	1.19	0.14	0.14	0.14	1.41	1.44	1.43
T_6	T_2 + K consortia	1.16	1.07	1.12	0.13	0.13	0.13	1.40	1.41	1.41
T_7	T_2 + T_3	1.29	1.27	1.28	0.15	0.15	0.15	1.53	1.50	1.52
T_8	75% RDF (NP)	0.84	0.86	0.85	0.09	0.09	0.09	1.33	1.19	1.26
T_9	T_8 + T_3	0.98	1.00	0.99	0.10	0.10	0.10	1.34	1.18	1.26
T_{10}	75% RDN + 100% RDP + N consortia	1.03	1.06	1.05	0.11	0.12	0.11	1.37	1.23	1.30
T_{11}	75% RDP + 100% RDN + P consortia	1.05	0.99	1.02	0.12	0.12	0.12	1.36	1.30	1.33
	S. Em. ±	0.031	0.048	0.028	0.005	0.004	0.003	0.043	0.057	0.037
	C. D. (P=0.05)	0.089	0.137	0.080	0.013	0.013	0.008	0.124	0.165	0.103
	C.V. %	6.02	9.37	7.85	8.13	7.54	7.83	6.23	8.74	7.52

Table 9: Effect of different treatments on available NPK content in rice grain

NO.	Treatments	Nitrogen content in Grain			Phosphorus content in Grain			Potassium content in Grain		
		2017-18	2018-19	Pooled	2017-18	2018-19	Pooled	2017-18	2018-19	Pooled
T_1	Absolute control	1.41	0.85	1.13	0.17	0.17	0.17	0.20	0.19	0.19
T_2	RDF (NP) 100%	1.92	1.59	1.75	0.20	0.21	0.20	0.23	0.24	0.23
T_3	Consortia NPK	1.49	0.92	1.20	0.18	0.18	0.18	0.21	0.20	0.20
T_4	T_2 + N consortia	1.96	1.87	1.91	0.21	0.22	0.22	0.25	0.25	0.25
T_5	T_2 + P consortia	1.93	1.87	1.90	0.21	0.22	0.21	0.24	0.24	0.24
T_6	T_2 + K consortia	1.91	1.74	1.82	0.20	0.21	0.20	0.24	0.24	0.24
T_7	T_2 + T_3	2.08	2.05	2.06	0.23	0.23	0.23	0.25	0.25	0.25
T_8	75% RDF (NP)	1.62	1.10	1.36	0.18	0.18	0.18	0.21	0.21	0.21
T_9	T_8 + T_3	1.64	1.22	1.43	0.19	0.19	0.19	0.22	0.21	0.22
T_{10}	75% RDN + 100% RDP + N consortia	1.68	1.33	1.50	0.19	0.19	0.19	0.23	0.22	0.23
T_{11}	75% RDP + 100% RDN + P consortia	1.87	1.48	1.67	0.20	0.20	0.20	0.23	0.24	0.23
	S. Em. ±	0.065	0.068	0.102	0.006	0.008	0.005	0.007	0.007	0.005
	C. D. (P=0.05)	0.188	0.195	0.321	0.018	0.022	0.013	0.020	0.021	0.013
	C.V. %	7.33	9.27	8.21	6.36	7.60	7.02	6.04	6.36	6.20

Table 10: Effect of different treatments on available NPK content in soil after harvest

NO.	Treatments	Available Nitrogen in soil (kg/h)			Available Phosphorus in soil (kg/h)			Available Potassium in soil (kg/h)		
		2017-18	2018-19	Pooled	2017-18	2018-19	Pooled	2017-18	2018-19	Pooled
T_1	Absolute control	270.91	265.74	268.33	30.40	31.77	31.08	334.99	305.92	320.45
T_2	RDF (NP) 100%	303.87	303.98	303.92	42.56	41.75	42.15	396.25	387.88	392.06
T_3	Consortia NPK	277.30	275.35	276.32	33.54	34.59	34.06	355.07	358.64	356.86
T_4	T_2 + N consortia	325.11	324.43	324.77	47.36	47.99	47.67	398.95	401.76	400.35
T_5	T_2 + P consortia	319.42	316.94	318.18	44.30	48.30	46.30	404.20	412.52	408.36
T_6	T_2 + K consortia	313.41	312.14	312.77	42.85	44.30	43.57	410.31	414.49	412.40
T_7	T_2 + T_3	342.71	331.64	337.18	49.72	52.17	50.95	423.67	424.49	424.08
T_8	75% RDF (NP)	281.70	280.31	281.00	35.98	32.81	34.39	360.56	369.18	364.80
T_9	T_8 + T_3	283.50	285.07	284.28	36.71	36.30	36.50	363.33	370.71	367.02
T_{10}	75% RDN + 100% RDP + N consortia	287.06	282.82	284.94	38.27	36.81	37.54	370.28	378.37	374.32
T_{11}	75% RDP + 100% RDN + P consortia	293.71	287.37	290.54	40.45	37.04	38.74	375.28	383.63	379.45
	S. Em. ±	9.92	9.00	6.24	1.62	1.39	1.08	11.74	13.15	8.43
	C. D. (P=0.05)	28.64	26.00	17.61	4.69	4.00	3.04	33.89	37.96	23.81
	C.V. %	6.62	6.06	6.35	8.08	6.87	7.50	6.16	6.87	6.53

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