

Original Research Article

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Systematic Screening Strategies for Identifying Elite Plant Growth Promoting Rhizobacteria for Coconut (*Cocos nucifera* L.)

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ABSTRACT

Elite strains of plant growth promoting rhizobacteria were proficiently selected based on the strategic screening approaches. These included *in vitro* testing for plant growth promotion attributes, seedling bioassay under controlled and under greenhouse conditions. A total of 483 morphologically distinct bacteria [156 rhizosphere fluorescent *Pseudomonas* spp. (RSF), 206 rhizosphere *Bacillus* spp. (RSB) and 121 endophytic *Bacillus* spp. (EB)] were isolated from the roots and rhizosphere soil of coconut palms growing in different states of India. These were characterized for various plant growth promotion traits that have direct and indirect effects on plant growth. All the isolates were assessed and given scores based on their *in vitro* assay of plant growth promotion traits. A total of 129 isolates, which secured more than 11 points, were further selected for seedling bioassay under controlled conditions. Greenhouse trial was conducted with 72 isolates and results further proved the efficiency of screened isolates for plant growth promotion. This strategic screening helped in shortlisting a total of 20 best PGPR (7 fluorescent *Pseudomonas* spp. and 13 *Bacillus* spp.) out of 483 isolates. The present study clearly showed that the rhizosphere of coconut palms harbour varied populations of fluorescent pseudomonads and *Bacillus* spp. that possess diverse plant growth promotion properties, and some of the efficient ones could be developed in to bioinoculants for use in coconut nurseries and plantations.

Keywords

Screening PGPR, Fluorescent pseudomonads, *Bacillus* spp., Coconut, Plant growth promoting traits

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Introduction

The coconut is a tropical tree species, mainly grown and harvested by small-scale farmers. Production of coconuts is concentrated on the island and coastal areas, with Indonesia, Philippines, India and Brazil being the major producers. India is the third largest coconut producing country in the world, its cultivation

spread in 1.89 million hectares of land (FAOSTAT data, 2016). Coconut is classified as a "functional food" because it provides many health benefits beyond its nutritional content. As every bit of the coconut is used for human intake, its cultivation practices should be in more healthy, organic and eco-friendly manner. Hence, it is desirable that the natural wonder 'coconut' is not exposed to harmful

and hazardous synthetic chemicals as fertilizers and pesticides. Research on the microorganisms in the root region has proven beneficial to the eco-friendly farming practices. Rhizosphere and endorhizosphere microorganisms have a great impact on root biology, influence nutrition, plant growth, uptake and development (Mantelin and Touraine, 2004). Rhizosphere organisms that have been well studied for their beneficial effects on plant growth and health are the nitrogen-fixing bacteria, mycorrhizal fungi, plant growth-promoting rhizobacteria (PGPR), biocontrol microorganisms, mycoparasitic fungi, and protozoa (Mendes *et al.*, 2013). PGPR were first used for agricultural purposes in the former Soviet Union and India in the early 20th century and are now being tested for plant growth promotion worldwide (Lucy *et al.*, 2004) in many plants including many crop plants (Dutta and Thakur, 2017; Vargas *et al.*, 2017). The most common non-symbiotic genera with plant growth promotion activity included *Pseudomonas*, *Bacillus*, *Azospirillum*, *Azotobacter*, *Enterobacter* and *Serratia* (George *et al.*, 2013; Vargas *et al.*, 2017; Dutta and Thakur, 2017). As a perennial crop, coconut palm is likely to recruit highly potent 'candidates' from the pool of soil microorganisms to maintain a 'rhizo-microbiome' for enduring mutual interaction between them. The occurrence of higher population of beneficial plant-associated microbes was reported in the rhizosphere of coconut palms (Gopal *et al.*, 2005; Rajendran *et al.*, 2007; George *et al.*, 2013). Enhanced plant growth and nutrient uptake has been reported in seedlings of perennial crops such as cocoa (Thomas *et al.*, 2011), oil palm (Amir *et al.*, 2005) and oak (Domenech *et al.*, 2004) upon inoculation with PGPR. Among rhizosphere microorganisms, the fluorescent pseudomonads have emerged as the largest and potentially most promising group of cultivable PGPR with the production of versatile metabolites (Santoro *et al.*, 2016).

Likewise, *Bacillus* spp. attracted considerable attention because of their advantages over other PGPR strains in inoculant formulations, stable maintenance in rhizosphere soil, and greater potential in sustainable agriculture (Govindasamy *et al.*, 2010).

An earlier report on the ability of PGPR such as *Bacillus coagulans* and *Brevibacillus brevis* improving the seedling growth of coconut (Gupta *et al.*, 2006) forms the basis for this initiative on a very detailed and meticulous study of PGPR in coconut. Towards this aim, a study was taken up involving collection of soil and root samples from the rhizosphere of coconut palms growing in different locations in five southern states of India. A total of 483 morphologically distinct fluorescent *Pseudomonas* spp. and *Bacillus* spp. were isolated and purified from these samples. We affirmed the antagonistic activity and phosphate solubilization associated with some of these isolates in our previous reports (George *et al.*, 2011, 2012a, b). Here we propose a screening strategy to select potent plant growth promoting rhizobacteria (PGPR) with multiple PGP traits from this microbial collection that could be developed into bioinoculants to enhance the health, vigour and growth of coconut seedlings.

Materials and Methods

Isolation of fluorescent *Pseudomonas* spp. and *Bacillus* spp.

The root and rhizospheric soil samples collected from yielding, healthy coconut palms growing in Kerala, Karnataka, Tamil Nadu, Andhra Pradesh and Maharashtra states of India (Fig. 1a) were used for isolation of fluorescent *Pseudomonas* spp. and *Bacillus* spp. The enumeration studies in coconut palms clearly indicated that the fluorescent pseudomonads and *Bacillus* spp. occurred in good numbers in the rhizosphere of this

perennial plantation crop (George *et al.*, 2011, 2012b; Fig. 1b, c). A total of 156 rhizospheric fluorescent *Pseudomonas* spp. (RSF), 206 rhizospheric *Bacillus* spp. (RSB) and 121 root endophytic *Bacillus* spp. (EB) isolates, representing each location were purified on King's B agar (KBA), Gould's S2 agar and Nutrient agar (NA). They were identified as fluorescent *Pseudomonas* spp. and *Bacillus* spp. based on the preliminary identification methods. The methods for collection, population, isolation and preliminary identification of the isolates were done as reported elsewhere (George *et al.*, 2011, 2012b).

Screening of isolates *in vitro* for plant growth promotion traits

All the isolates were characterized for their direct and indirect plant growth promoting traits such as growth on N-free medium (Jensen's medium, Himedia), phosphate solubilization (Pikovskaya's agar, Himedia) (George *et al.*, 2012b), production of IAA (Brick *et al.*, 1991), 1-aminocyclopropane carboxylic acid (ACC)-deaminase activity (Klee *et al.*, 1991), production of siderophores (Schwyn and Neilands, 1987), ammonification (Cappuccino and Sherman, 1992), production of HCN (Bakker and Schippers, 1987) and chitinase activity (Renwick *et al.*, 1991). The ability of isolates to produce antibiotics was detected by agar well technique (Fuhrmann, 1994). Antifungal activity was detected against two different coconut pathogens (*Thielaviopsis paradoxa* and *Ganoderma applanatum*) (George *et al.*, 2012b). For studying these properties, bacterial suspension having 10^8 c.f.u. ml^{-1} was spot inoculated or streaked on different media as per the requirement of the procedure. All the isolates were assessed and scored in a scale of 1 to 3 based on their performance in above plant growth promotion traits (Table 1). Maximum 3 points were given to HCN production,

growth on N-free medium, high ammonifiers and good growth on minimal media containing ACC. Antibiotic producers were assessed based on the inhibition zone size, siderophore producers according to the diameter of orange halo and phosphate solubilizers and chitin degradation based on the size of clearing zones on a scale of 1 to 3. Antifungal activity was evaluated according to their percentage of inhibition.

Screening of isolates by seedling bioassay in plant growth chamber

Selected isolates were grown in King's B (KB)/Nutrient Broth (NB) for 24 h at 30 °C in a refrigerated incubator shaker (Innova Model 4335, USA). Paddy seeds (*Oryza sativa* cv. Aiswarya) were surface sterilized with 0.1% HgCl_2 for 4 minutes and washed repeatedly with sterile distilled water. Seeds were immersed in bacterial culture broth (10^8 cells ml^{-1}) for 10 minutes and then transferred to the petriplates (5 seeds per plate) containing soft water agar (0.7%) and incubated in an Environmental Growth Chamber (Sanyo, Japan) at 25°C, 85% RH, 10 h light and 14 h dark cycle for 10 days. Three replications were maintained. Control was maintained by placing surface-sterilized seeds (without culture) for germination and growth under same conditions. Shoot and root lengths of the seedlings were measured. Data were statistically analysed using Duncan's multiple range test.

Screening of isolates by seedling bioassay in greenhouse

Surface sterilized paddy seeds were sown in 11x9 cm sized plastic cups (four seeds/cup) filled with unsterile soil: sand mixture (pH 5.3, EC 90 $\mu\text{S}/\text{cm}$) in 3:1 ratio. One ml of 24 h old bacterial culture (10^8 cells ml^{-1}) was applied on each seed. The cups were placed in greenhouse (Average Temp: 27 °C, Average

Humidity: 91 %). After germination, seedlings were thinned to 2 numbers per cup. Ten replications were maintained for each microorganism. Control treatment received surface-sterilized seeds without application of bacterial culture. Seedlings were uprooted after 20 days of sowing and their shoot and root lengths and fresh and dry weights were recorded. Data were statistically analysed using Duncan's multiple range test. Percentage of increase in total seedling length, fresh weight, and dry weight were calculated. The populations of rhizospheric fluorescent *Pseudomonas* spp./*Bacillus* spp. were enumerated in the rhizosphere soil of paddy seedlings by serial dilution and pour plating method on S2/NA media, respectively.

Identification of screened isolates

The screened plant growth promoting rhizobacteria (PGPR) were identified based on 16S rRNA gene sequence analysis. Genomic DNA of the PGPR was isolated by GenElute Bacterial Genomic DNA Kit (Sigma, USA) and fragment of 16S rDNA gene was amplified by PCR from the isolated genomic DNA using the universal primers F27/R1492 as described earlier (George *et al.*, 2013).

Statistical analysis

The data were analyzed using analysis of variance (ANOVA) and the means were compared using Duncan's Multiple Range Test (DMRT) at P=0.05 level using version 15 of SPSS.

Results and Discussion

***In vitro* characterization for plant growth promotion traits**

The overall results showed that all the isolates had strong tendencies for one or more of the plant growth promoting (PGP) traits tested and

none were negative for all traits. The percent of RSF and bacilli showing plant growth promoting traits is depicted in Figure 2a. Results indicated that ammonifying bacteria were preponderant PGPR in the rhizosphere and roots of coconut. Other traits like HCN production and antibiotic production were found in a few isolates. Nitrogen fixing ability, chitinase activity and antagonism were found more in rhizospheric bacilli, whereas IAA production, phosphate solubilization and siderophore were found more in RSF.

A total of 217 isolates (48 RSB, 37 EB, 132 RSF), whose colonies were immobilized on Whatman filter paper and then treated with Salkowski reagent, produced pink colour on the filter paper (Fig. 2b). Thirty two fluorescent *Pseudomonas* spp. and 17 *Bacillus* spp. exhibited high colour intensity indicative of higher levels of IAA production. Eighty percent of all isolates showed growth on minimal medium containing ACC as the sole source of nitrogen as it would seem to possess low to high activity of ACC deaminase enzyme. A total of 84 isolates showed luxuriant growth on N-free medium. This included 21 bacilli from the rhizosphere, 60 bacilli from endorhizosphere and 3 fluorescent pseudomonads from the rhizosphere. Almost half of the endophytic bacilli showed growth on N-free medium and were presumptive nitrogen fixers. In the present study, most of the fluorescent pseudomonads (147 nos.) were found to be phosphate solubilizers. Two *Pseudomonas* spp. KnSF208 and KnSF227 produced maximum clearing zone of 42 mm diameter (Fig. 2c). The siderophore positive isolates produced orange halo surrounding their colonies on CAS agar medium (Fig. 2d). Again, fluorescent *Pseudomonas* spp. KiSF17 and KiSF33 showed highest affinity for Fe sequestration as indicated by orange halo (30 mm dia.). The production of HCN could be seen only in 4% of the isolates which included 11 *Bacillus* spp. and 8 fluorescent

Pseudomonas spp. and was indicated by change in colour of the filter paper from yellow to orange/brown (Fig. 2e). This study also revealed that chitinase activity was mostly limited to *Bacillus* spp. (131 isolates). Only one isolate of *Pseudomonas* sp. KiSF 13 produced clearing zone of 12 mm diameter on chitin medium (Fig. 2f). Antibiotic production was exhibited by 16 (10% of the total) isolates (Fig. 2g). Growth of *Ganoderma applanatum* and *Thielaviopsis paradoxa* were inhibited by more than 90% isolates with percentage inhibition ranging from 44 to 91% on KBA (Fig. 3 a, b, c, d) / NA (Fig. 3 e, f, g, h) medium. Maximum antagonism that could be achieved by fluorescent *Pseudomonas* spp. and *Bacillus* spp. were 76% and 93%, respectively, in terms of mycelial growth reduction.

All the isolates were assigned scores based on their performance in plant growth promoting traits. As equal importance were given to all the plant growth promoting traits tested, maximum three points were possible for each of the 11 parameters totaling 33 points (Table 1). A total of 129 isolates that scored ≥ 11 , were selected for further studies (Table 2). Among RSF, *Pseudomonas* sp. KiSF16 got maximum score of 21 points followed by *Pseudomonas* spp. PoSF 314 and KiSF17 with 19 points. Among *Bacillus* spp., *Bacillus* sp. PoEB5 isolated from the roots of coconut from Pollachi District of Tamil Nadu scored maximum (17) followed by *Bacillus* spp. RSB14 and KiEB25 (score of 16), isolated from coconut growing in Ratnagiri (Maharashtra) and Kidu (Karnataka), respectively.

Effect of bacterial inoculation on paddy seedlings under controlled conditions

Out of 21 RSF isolates, 13 isolates increased the length of paddy seedlings (ranging from 5 to 48%) as compared to uninoculated control

(Fig. 4a) when tested under controlled temperature, humidity and light conditions. Maximum increase in total length was shown by paddy seedlings inoculated with *Pseudomonas* sp. ChSF180 from Chengannur, Kerala (48%), followed by *Pseudomonas* sp. KiSF27 from Kidu, Karnataka (33%) after 10 days growth period and were significantly different from uninoculated control in DMRT analysis. Nineteen out of 64 RSB positively influenced the growth of paddy seedlings under environmental growth chamber conditions. They showed 3-29% increase in total length of paddy seedlings over respective uninoculated controls with maximum showed by the seedlings inoculated with *Bacillus* sp. ChSB2. Inoculation of paddy seeds with 22 EB isolates increased the total seedling length (upto 47% over control); many isolates increased either root length or shoot length. *Bacillus* sp. KiEB31 (47% increase) performed better than other isolates in stimulating plant growth, which was also statistically significant over the other treatments. The bacterial isolates which showed plant growth promotion either in shoot or root were also selected for further studies. Hence, the initial collection of 483 isolates was shortlisted to 72 PGPR (12 RSF, 35 RSB and 25 EB) after two screening approaches. The individual results of bacterial inoculation studies under controlled conditions are given in supplementary data (Table S1, S2 and S3) and summary of the results is depicted in Table 3.

Effect of bacterial inoculation on paddy seedlings under greenhouse conditions

In the 21-days-duration study with 72 isolates (12 RSF, 35 RSB and 25 EB) conducted under greenhouse conditions, 10 RSF isolates (out of 12 tested) increased all the growth parameters of paddy seedlings (Fig. 4b) with maximum increase recorded in *Pseudomonas* sp. KiSF27 (36%) and two isolates increased at least one

growth parameter. More than 100% increase in dry weight was observed amongst 8 RSF with *Pseudomonas* sp. KiSF24 (isolated from Kidu) recording 200% increase in dry weight. Most of the growth parameters were found to be significantly different from the control.

Among 35 RSB tested, 10 isolates increased all parameters of paddy seedlings while others increased any one of the growth parameters. *Bacillus* sp. TSB16 isolated from Tumkur, Karnataka showed maximum increase in dry weight (163%) and maximum increase in seedling length (48%) was showed by *Bacillus* sp. ESB13 isolated from the rhizosphere of coconut growing in Ernakulam, Kerala. Six of the 25 EB increased all the growth parameters of paddy seedlings, while the remaining ones increased any one of the growth parameters. The maximum increase in total seedling length was shown by *Bacillus* sp. TEB4 (35%), isolated from the roots of coconut palm growing in Tumkur, Karnataka.

Analysis of the rhizosphere soil of paddy seedlings showed the population of fluorescent pseudomonads to be higher when inoculated with RSF, compared to uninoculated control, after 20 days of planting.

It ranged from 3 to 5.92 log₁₀ cfu g⁻¹ fresh weight of soil. Low population levels of fluorescent pseudomonads were detected in the non-treated control plants, up to 2 log₁₀ cfu ml⁻¹ fresh weight of soil. Population of *Bacillus* spp. in the rhizosphere region of paddy seedlings inoculated with RSB and EB ranged from 5.84 – 6.95 log₁₀ cfu g⁻¹ of soil, when compared to control (average of 5.44 log₁₀ g⁻¹ of soil). The individual results of bacterial inoculation studies under greenhouse conditions are given in supplementary data (Table S4, S5 and S6).

Selection and identification of PGPRs

A total of 20 potential PGPR (7 RSF, 5 RSB and 8 EB) were selected based on their overall performance in the above screening strategies. All of the selected PGPR had an assessment score ≥ 11 points in functional traits and increased at least one of the growth parameter in plant assays.

Among the 13 *Bacillus* spp., 8 were root endophytes and rest were from the rhizosphere of coconut. The details of the isolates are given in Table 4 and their plant growth promoting traits in Table 5.

Table.1 Scheme for the assessment/ evaluation of PGPR

HCN	PS (Zone in mm)	N	Ammoni fication*	Sideroph ore (zone in mm)	IAA*	ACC*	Chitinase (zone in mm)	Antibiotics (zone in mm)*	Antago nism**		Assesse nt score‡
									GA	TP	
+	1-14	+	L	1-9	L	L	1-8	L	L	L	1
	15-28		M	10-19	M	M	9-15	M	M	M	2
	≥ 29		H	≥20	H	H	≥16	H	H	H	3

HCN-Hydrogen cyanide production, PS- phosphate solubilization, N-growth on N-free medium, IAA- IAA production, ACC- ACC deaminase production, mm= diameter in mm, GA- antagonism against *Ganoderma applanatum*, TP- antagonism against *Thielaviopsis paradoxa*., '+' = positive, '-' = negative, *The activity/production was qualitatively assessed in three broad categories: H (high), M- (medium), L (low), **Low (L), medium (M) and high (H) represented inhibition ranges of 1-20, 21-40 and ≥41% and 40-59, 60-79 and 80-100% for *Pseudomonas* spp. and *Bacillus* spp., respectively. ‡ For negative reaction, no score was assigned.

Table.2 Assessment of coconut RSF, RSB and EB based on plant growth promotion traits

Group	Number of Isolates	Assessment points in range				
		1-5	6-10	11-15	16-20	21-25
RSF	156	14	121	15	5	1
RSB	206	18	125	62	1	-
EB	121	8	68	42	3	-
Total	483	40	314	119	9	1
Total number of isolates selected for seedling bioassay under controlled conditions			129 (RSF-21, RSB-63, EB-45)			

RSF – rhizospheric fluorescent *Pseudomonas* spp., RSB- rhizospheric *Bacillus* spp., EB- endophytic *Bacillus* spp.

Table.3 Over view of the results of bacterial inoculation on paddy growth in seedling bioassay

Isolates	Total no. of isolates tested	Increase in total seedling length (%)				
		1-10	11-20	21-30	31-40	41-50
Number of isolates						
RSF	21	5	1	4	1	1
RSB	63	9	7	3	0	0
EB	45	8	5	3	5	1
Total	129	22	13	10	6	2

RSF – rhizospheric fluorescent *Pseudomonas* spp., RSB- rhizospheric *Bacillus* spp., EB- endophytic *Bacillus* spp.

Table.4 Details of selected PGPR

PGPR	Coconut variety/ Part of isolation	Place/State	Medium used for isolation	Assessment score
<i>Bacillus</i> spp.				
ESB15	WCT/Rhizosphere	Ernakulam, Kerala	NA	13
KnSB6	WCT/Rhizosphere	Kunnamkai, Kasaragod, Kerala	NA	12
TSB16	TT/Rhizosphere	Tumkur, Karnataka	NA	11
KiSB10	WCT/Rhizosphere	Kidu, Karnataka	NA	12
RSB14	WCTxMYD/Rhizosphere	Ratnagiri, Maharashtra	NA	16
TEB2	TT/Root	Tumkur, Karnataka	NA	11
TEB4	TT/Root	Tumkur, Karnataka	NA	11
HEB8	WCT/Root	HDMSCS, CPCRI, Kasaragod, Kerala	NA	13
HEB10	WCT/Root	HDMSCS, CPCRI, Kasaragod, Kerala	NA	14
KiEB23	WCT/Root	Kidu, Karnataka	NA	11
KiEB25	WCT/Root	Kidu, Karnataka	NA	16
KiEB31	WCT/Root	Kidu, Karnataka	NA	11
PoEB5	ECTxMYD/Root	Pollachi, Tamil Nadu	NA	17
<i>Pseudomonas</i> spp.				
ChSF180	WCT/Rhizosphere	Chengannur, Alapuzha, Kerala	KBA	12
KnSF208	WCT/Rhizosphere	Kunnamkai, Kasaragod, Kerala	S2	12
ASF285	ECT/Rhizosphere	Ambajipetta, Andhra Pradesh	S2	14
PoSF314	ECTxMYD/Rhizosphere	Pollachi, Tamil Nadu	KBA	19
TSF7	TT/Rhizosphere	Tumkur, Karnataka	KBA	17
RSF266	WCTxMYD/Rhizosphere	Ratnagiri, Maharashtra	KBA	12
KiSF27	WCT/Rhizosphere	Kidu, Karnataka	S2	15

Table.5 Plant growth promoting traits possessed by the selected PGPR

PGPR	HCN	PS (mm)	N-fixation	Ammonia	Siderophore	IAA	ACC d	Antibiotic	Chitinase	Antagonism *		Seedling bioassay	Green house studies		
										GA	TP		% increase in SL	SL	FW
<i>Bacillus</i> sp. ESB15	+	-	-	M	G	-	L	-	13	59	80	5	36	-	6
<i>Bacillus</i> sp. KnSB6	+	-	-	H	-	-	L	-	10	74	62	14	13	9	-
<i>Bacillus</i> sp. TSB16	+	-	-	H	-	M	L	-	-	57	56	11	43	65	163
<i>Bacillus</i> sp. KiSB10	-	10	+	M	-	H	L	-	-	71	-	7	29	50	-
<i>Bacillus</i> sp. RSB14	-	16	+	M	12	-	L	-	12	57	80	10	10	35	20
<i>Bacillus</i> sp. TEB2	-	-	+	M	-	L	M	-	-	57	64	20	21	-	-
<i>Bacillus</i> sp. TEB4	-	-	+	L	-	M	L	-	-	57	64	17	35	-	25
<i>Bacillus</i> sp. HEB8	-	-	-	M	-	H	M	-	11	74	56	30	30	21	25
<i>Bacillus</i> sp. HEB10	-	-	-	H	-	M	M	-	14	74	56	24	11	-	-
<i>Bacillus</i> sp. KiEB23	-	10	+	H	G	-	-	-	-	71	44	34	14	8	100
<i>Bacillus</i> sp. KiEB25	-	6	+	H	-	-	L	-	9	80	80	34	13	41	20
<i>Bacillus</i> sp. KiEB31	-	10	+	H	-	-	L	-	-	71	44	47	11	-	7
<i>Bacillus</i> sp. PoEB5	+	14	+	M	26	-	L	-	-	60	76	14	16	6	-
<i>Pseudomonas</i> sp. ChSF180	-	16	-	H	14	H	L	-	-	-	44	48	28	45	140
<i>Pseudomonas</i> sp. KnSF208	-	42	-	H	12	H	L	-	-	-	-	10	12	57	100
<i>Pseudomonas</i> sp. ASF285	-	16	-	L	12	L	L	L	-	43	49	12	29	33	13
<i>Pseudomonas</i> sp. PoSF314	-	20	-	M	20	H	L	H	-	40	47	17	14	23	50
<i>Pseudomonas</i> sp. TSF7	-	16	-	H	16	H	L	H	-	43	56	21	33	14	50
<i>Pseudomonas</i> sp. RSF266	-	16	-	M	10	H	L	L	-	-	20	25	10	62	150
<i>Pseudomonas</i> sp. KiSF27	-	14	+	H	24	M	H	-	-	-	-	33	40	14	-

HCN-Hydrogen cyanide production, PS- phosphate solubilization, N-fixation- capability to fix nitrogen, Ammonia-ammonification, Siderophore- siderophore production, IAA- IAA production, ACCd- ACC deaminase production, Antibiotic- antibiotic production, Chitinase- chitinase production, antagonism- % inhibition showed towards GA and TP on NA/KB, GA- antagonism against *Ganoderma applanatum*, TP- antagonism against *Thielaviopsis paradoxa*, +positive, - negative/no increase, H- high, M- medium, L-low, SL-seedling length, FW- fresh weight, DW- dry weight.

Table.6 Identity of selected PGPR by 16S rDNA sequencing and BLAST comparison

Sl. No.	PGPR	Best classified BLAST hit	Similarity (%)	NCBI GenBank accession numbers
1	ChSF180	<i>Pseudomonas putida</i>	99	KF381346
2	KnSF208	<i>P. putida</i>	99	KF364491
3	ASF285	<i>P. monteilii</i>	99	KF381348
4	PoSF314	<i>P. plecoglossicida</i>	99	KF381347
5	TSF7	<i>P. monteilii</i>	99	KF381345
6	RSF266	<i>P. plecoglossicida</i>	99	KF381349
7	KiSF27	<i>P. plecoglossicida</i>	99	KF381344
8	ESB15	<i>Bacillus cereus</i>	100	KF381350
9	KnSB6	<i>B. cereus</i>	99	KF381351
10	TSB16	<i>B. megaterium</i>	99	KF364492
11	KiSB10	<i>B. megaterium</i>	99	KF381356
12	RSB14	<i>B. licheniformis</i>	99	KF381343
13	TEB2	<i>B. megaterium</i>	100	KF381342
14	TEB4	<i>B. megaterium</i>	100	KF381354
15	HEB8	<i>B. cereus</i>	99	KF381353
16	HEB10	<i>B. cereus</i>	99	KF381352
17	KiEB23	<i>Bacillus</i> sp.	98	KF381357
18	KiEB25	<i>B. cereus</i>	100	KF381358
19	KiEB31	<i>B. megaterium</i>	99	KF381355
20	PoEB5	<i>B. subtilis</i>	99	KF364490

Table.S1 Effect of inoculation of rhizospheric fluorescent pseudomonads on paddy growth in seedling bioassay

Treatment	Root length* (cm)	Shoot length* (cm)	Total seedling length* (cm)
Experiment – I [#]			
<i>Pseudomonas</i> sp. HSF132	8.22 ab	4.94 a	13.16 a
<i>Pseudomonas</i> sp. ESF153	8.40 ab	5.86 a	14.26 a
<i>Pseudomonas</i> sp. ESF168	7.00 ab	4.10 a	11.10 a
<i>Pseudomonas</i> sp. KnSF208	8.08 ab	4.86 a	12.94 a
<i>Pseudomonas</i> sp. RSF266	8.86 a	5.20 a	14.60 a
<i>Pseudomonas</i> sp. ASF285	8.54 ab	4.54 a	13.08 a
<i>Pseudomonas</i> sp. PoSF315	4.50 b	5.50 a	10.00 a
<i>Pseudomonas</i> sp. TSF7	8.86 a	5.36 a	14.22 a
Control	6.90 ab	4.82 a	11.72 a
Experiment – II			
<i>Pseudomonas</i> sp. ChSF180	8.58 a	7.96 a	16.54 a
<i>Pseudomonas</i> sp. KiSF27	8.65 a	6.20 ab	14.85 a
Control	6.675 b	4.525 b	11.20 b
Experiment –III			
<i>Pseudomonas</i> sp. RSF259	3.88 b	5.82 bc	9.70 b
<i>Pseudomonas</i> sp. KiSF13	9.70 a	5.70 bc	15.40 a
<i>Pseudomonas</i> sp. KiSF3	8.14 a	6.80 ab	14.90 a
<i>Pseudomonas</i> sp. KiSF16	9.22 a	7.36 ab	16.58 a
<i>Pseudomonas</i> sp. KiSF17	2.40 b	6.32 abc	8.72 ab
<i>Pseudomonas</i> sp. KiSF21	3.24 b	5.20 c	8.44 b
<i>Pseudomonas</i> sp. KiSF24	9.60 a	6.70 abc	16.30 a
<i>Pseudomonas</i> sp. KiSF28	3.54 b	6.00 abc	9.54 b
Control	8.10 a	7.48 a	15.58 a
Experiment – IV			
<i>Pseudomonas</i> sp. RSF257	9.50 a	5.67 a	15.17 a
<i>Pseudomonas</i> sp. PoSF313	7.00 b	4.68 a	11.68 b
<i>Pseudomonas</i> sp. PoSF314	9.75 a	4.85 a	14.60 ab
Control	7.33 b	4.83 a	12.17 ab

*Values are mean of 5 replications. Mean followed by the same letters in a column in an experiment are not significantly different from each other according to DMRT analysis at P= 0.05.

[#]Experiment were conducted in different batches with separate uninoculated control in each batch

Table.S2 Effect of inoculation of rhizospheric *Bacillus* spp. on paddy growth in seedling bioassay

Treatment	Root length* (cm)	Shoot length* (cm)	Total seedling length* (cm)
Experiment I			
<i>Bacillus</i> sp. KnSB22	NG	NG	NG
<i>Bacillus</i> sp. KnSB6	9.63 abc	6.26abcde	15.89abcd
<i>Bacillus</i> sp. KnSB17	6.2fg	6.4abcd	12.6efg
<i>Bacillus</i> sp. KnSB10	7.6 def	5.75cdefg	13.35efg
<i>Bacillus</i> sp. KnSB19	6fg	6.05bcdef	12.05fg
<i>Bacillus</i> sp. KnSB7	NG	NG	NG
<i>Bacillus</i> sp. KnSB3	9.9ab	7.46a	17.36ab
<i>Bacillus</i> sp. KnSB2	9.66bcd	6.3abcde	15.96abc
<i>Bacillus</i> sp. HSB17	9.15 abc	5.3defg	14.45cdef
<i>Bacillus</i> sp. ChSB2	11.2a	6.73abc	17.93a
<i>Bacillus</i> sp. ChSB4	NG	NG	NG
<i>Bacillus</i> sp. ChSB8	9.83ab	6.8abc	16.63abc
<i>Bacillus</i> sp. ChSB9	8cde	4.46g	12.46efg
<i>Bacillus</i> sp. PSB13	6.6efg	6.5abcd	13.1efg
<i>Bacillus</i> sp. ESB2	10ab	7abc	17ab
<i>Bacillus</i> sp. ESB4	7.8def	4.9fg	12.7efg
<i>Bacillus</i> sp. ESB8	5.6g	7.26ab	12.86efg
<i>Bacillus</i> sp. ESB13	7.63 def	7.46ab	15.09bcde
<i>Bacillus</i> sp. ESB14	5g	6.1abcdef	11.1g
<i>Bacillus</i> sp. ESB15	7.5def	7.1abc	14.6cdef
Control	8.86 bcd	5.02efg	13.88def
Experiment II			
<i>Bacillus</i> sp. KnSB13	5.30g	2.41e	7.71bc
<i>Bacillus</i> sp. KnSB15	5.05g	4.08bc	9.13abc
<i>Bacillus</i> sp. KnSB20	6.85bcd	4.00bcd	10.85a
<i>Bacillus</i> sp. TSB13	6.16e	3.73bcd	9.89ab
<i>Bacillus</i> sp. TSB16	6.49cde	4.09bc	10.58a
<i>Bacillus</i> sp. CoSB1	5.53fg	3.53bcd	9.06abc
<i>Bacillus</i> sp. CoSB5	6.32de	3.75bcd	10.07a
<i>Bacillus</i> sp. PoSB7	5.0g	3.57bcd	8.57abc
<i>Bacillus</i> sp. RSB8	5.29g	3.30cd	8.59abc
<i>Bacillus</i> sp. RSB14	7.32b	3.17d	10.49a
<i>Bacillus</i> sp. ESB3	6.96bc	3.67bcd	10.63a
Control	5.97ef	3.58bcd	9.55ab
Experiment III			
<i>Bacillus</i> sp. CoSB3	11.86b	7.86a	19.72a
<i>Bacillus</i> sp. CoSB2	8.5b	6.8abc	15.3b
<i>Bacillus</i> sp. CoSB4	10.14b	6.42abc	16.56b
<i>Bacillus</i> sp. CoSB6	8.72b	6.76abc	15.48b
<i>Bacillus</i> sp. CoSB7	6.16b	5.24c	11.4b
<i>Bacillus</i> sp. CoSB8	9.5b	5.22c	14.72b
<i>Bacillus</i> sp. CoSB10	9.94b	7.02ab	16.96b
<i>Bacillus</i> sp. PoSB2	7.04b	5.65bc	12.69b
<i>Bacillus</i> sp. PoSB8	8.26b	6.18abc	14.44b
<i>Bacillus</i> sp. PoSB10	8.6b	6.5abc	15.1b
Control	12.42b	6.76abc	19.18b
Experiment IV			
<i>Bacillus</i> sp. ASB1	8.9ab	5.68 abc	14.58ab
<i>Bacillus</i> sp. ASB2	5.0bc	5.26 abc	10.26b
<i>Bacillus</i> sp. ASB3	5.74abc	5.84 abc	11.58ab
<i>Bacillus</i> sp. ASB13	8.76ab	5.96 abc	14.72 ab
<i>Bacillus</i> sp. KISB5	7.1 abc	6.06 abc	13.16 ab
<i>Bacillus</i> sp. KISB9	9.15ab	4.81c	13.96 ab
<i>Bacillus</i> sp. KISB10	8.6 abc	6.4a	15.0 ab
<i>Bacillus</i> sp. KISB21	5.12bc	6.28 abc	11.4 ab
<i>Bacillus</i> sp. KISB22	6.54 abc	5.348 abc	11.888 ab
<i>Bacillus</i> sp. KISB24	7.34 abc	6.44a	13.78 ab
<i>Bacillus</i> sp. KISB27	6.46 abc	5.74 abc	12.2 ab
<i>Bacillus</i> sp. KISB34	5.628 abc	5.656 abc	11.284 ab
<i>Bacillus</i> sp. TSB2	8.57ab	6.012 abc	14.582 ab
<i>Bacillus</i> sp. TSB3	5.44 abc	5.436 abc	10.876b
<i>Bacillus</i> sp. TSB8	6.04 abc	6.04 abc	12.08 ab
<i>Bacillus</i> sp. TSB21	5.2bc	6.02 abc	11.22 ab
<i>Bacillus</i> sp. RSB3	4.064c	6.32ab	10.384b
<i>Bacillus</i> sp. RSB4	5.5 abc	5.86 abc	11.36 ab
<i>Bacillus</i> sp. RSB15	8.64ab	4.884bc	13.524 ab
<i>Bacillus</i> sp. RSB20	6.84abc	5.4 abc	12.24 ab
<i>Bacillus</i> sp. RSB21	5.4bc	5.8 abc	11.2 ab
<i>Bacillus</i> sp. RSB22	9.74a	6.0 abc	15.74a
Control	8.2abc	5.802 abc	14.002 ab

*Values are mean of 5 replications. Mean followed by the same letters in a column in an experiment are not significantly different from each other according to DMRT analysis at P= 0.05.

NG- No germination of paddy seeds

#Experiment were conducted in different batches with separate uninoculated control in each batch

Table.S3 Effect of inoculation of endophytic *Bacillus* spp. on paddy growth in seedling bioassay

Treatment	Root length* (cm)	Shoot length* (cm)	Seedling length* (cm)
Experiment I[#]			
<i>Bacillus</i> sp. KiEB3	10.6abc	6.44abc	17.04ab
<i>Bacillus</i> sp. KiEB9	9.9abcd	7.34a	17.24ab
<i>Bacillus</i> sp. KiEB13	9.0bcde	5.42cd	14.42cd
<i>Bacillus</i> sp. KiEB14	9.2bcde	6.96ab	16.16abc
<i>Bacillus</i> sp. KiEB15	9.94abcd	6.92ab	16.86ab
<i>Bacillus</i> sp. KiEB16	8.22cdef	5.62bcd	13.84bcd
<i>Bacillus</i> sp. KiEB19	5.12f	5.24cd	10.36d
<i>Bacillus</i> sp. KiEB20	7.36cdef	6.26abcd	13.62bcd
<i>Bacillus</i> sp. KiEB23	12.94a	5.98bcd	18.92a
<i>Bacillus</i> sp. KiEB25	10.5abc	6.3abc	16.8ab
<i>Bacillus</i> sp. KiEB18	6.7def	6.06abcd	12.76bcd
<i>Bacillus</i> sp. KiEB26	6.1ef	5.94bcd	12.04cd
<i>Bacillus</i> sp. KiEB27	7.0def	6.44abc	13.44bcd
<i>Bacillus</i> sp. KiEB29	6.98def	6.46abc	13.44bcd
<i>Bacillus</i> sp. KiEB31	12.28ab	6.26abcd	18.54a
Control	7.64cdef	4.94d	12.58bcd
Experiment II			
<i>Bacillus</i> sp. HEB7	6.8bc	5.92a	12.72abc
<i>Bacillus</i> sp. HEB10	8.7ab	5.76a	14.46ab
<i>Bacillus</i> sp. HEB6	6.68bc	4.5a	11.18bcd
<i>Bacillus</i> sp. HEB1	5.3cd	4.6a	9.9cd
<i>Bacillus</i> sp. HEB2	5.4cd	4.32a	9.72cd
<i>Bacillus</i> sp. HEB16	7.64abc	4.44a	12.08abc
<i>Bacillus</i> sp. HEB8	9.86a	5.3a	15.16a
<i>Bacillus</i> sp. TEB2	9.04ab	4.98a	14.02ab
<i>Bacillus</i> sp. TEB4	8.6ab	5.06a	13.66ab
<i>Bacillus</i> sp. TEB11	6.96bc	4.8a	11.96abc
<i>Bacillus</i> sp. TEB20	3.2d	4.5a	7.7d
<i>Bacillus</i> sp. TEB22	7.14abc	4.52a	11.66abc
Control	6.9bc	4.82a	11.7abc
Experiment III			
<i>Bacillus</i> sp. AEB5	14.44a	6.68ab	21.12a
<i>Bacillus</i> sp. AEB10	11.6abc	6.68ab	17.84abc
<i>Bacillus</i> sp. AEB11	10.68abcd	5.16ab	15.84bcd
<i>Bacillus</i> sp. CoEB1	10.98abc	6.88a	17.86abc
<i>Bacillus</i> sp. CoEB2	9.36abcd	5.9ab	15.26bcd
<i>Bacillus</i> sp. CoEB3	12.42ab	6.76ab	19.18ab
<i>Bacillus</i> sp. CoEB4	8.86bcd	6.08ab	14.94bcd
<i>Bacillus</i> sp. CoEB5	9.52abcd	6.54ab	16.06abcd
<i>Bacillus</i> sp. CoEB6	8.48bcd	4.98b	13.46cd
<i>Bacillus</i> sp. CoEB7	7.36cd	5.8ab	13.16cd
<i>Bacillus</i> sp. CoEB8	6.12d	5.76ab	11.88d
<i>Bacillus</i> sp. PoEB2	10.28abcd	6.84a	17.12abc
<i>Bacillus</i> sp. PoEB3	12.42ab	6.76	19.18
<i>Bacillus</i> sp. PoEB4	12.68ab	6.48ab	19.16ab
Control	12.42ab	6.76ab	19.18ab
Experiment IV			
<i>Bacillus</i> sp. AEB3	5.71d	3.21e	8.92e
<i>Bacillus</i> sp. TEB18	6.53b	4.21b	10.74b
<i>Bacillus</i> sp. REB1	5.10e	4.25a	9.35d
<i>Bacillus</i> sp. PoEB5	7.14a	3.70c	10.84a
Control	5.97c	3.58d	9.55c

*Values are mean of 5 replications. Mean followed by the same letters in a column in an experiment are not significantly different from each other according to DMRT analysis at P= 0.05.

[#]Experiment were conducted in different batches with separate uninoculated control in each batch

Table.S4 Effect of inoculation of rhizospheric fluorescent pseudomonads on growth parameters of paddy seedlings in green house assay

Treatment	Root length* (cm)	Shoot length* (cm)	Total seedling length* (cm)	% increase in			† log cfu/g fresh soil*
				TSL	FW	DW	
Experiment - I[#]							
<i>Pseudomonas</i> sp. RSF257	13.67 b	26.66 a	40.32 b	4	62	113	5.52a
<i>Pseudomonas</i> sp. KnSF208	17.22 ab	26.34 a	43.57 b	12	57	100	5.50a
<i>Pseudomonas</i> sp. RSF266	17.56 ab	25.24 a	42.80 b	10	62	150	5.50a
<i>Pseudomonas</i> sp. ASF285	21.14 a	29.09 a	50.23 a	29	33	13	5.50a
Control	15.58 b	23.27 a	38.85 b				2b
Experiment – II							
<i>Pseudomonas</i> sp. ChSF180	9.76 a	18.82 ab	28.58 b	25	45	140	5.79a
<i>Pseudomonas</i> sp. PoSF314	7.00 b	18.60 ab	25.60 bc	12	23	50	5.65a
<i>Pseudomonas</i> sp. KiSF16	6.00 b	14.00 c	20.00 d	-	-	100	4.97a
<i>Pseudomonas</i> sp. KiSF27	10.45 a	20.83 a	31.28 a	36	14	-	5.92a
<i>Pseudomonas</i> sp. TSF7	8.43 ab	18.50 ab	26.93 bc	17	14	50	5.79a
Control	7.05 b	15.88 c	22.93 d				2b
Experiment – III							
<i>Pseudomonas</i> sp. KiSF24	13.34 ab	22.86 b	36.20 bc	4	55	200	5.47a
<i>Pseudomonas</i> sp. HSF132	15.22 a	22.46 b	37.680 bc	8	27	100	5.47a
<i>Pseudomonas</i> sp. ESF153	15.30 a	24.04 b	39.34 ab	13	27	100	4.47a
Control	12.20 b	22.70 b	34.90 c				2b

*Values are mean of 10 replications. Mean followed by the same letters in a column in an experiment are not significantly different from each other according to Duncan's multiple range test at P= 0.05.

TSL- total seedling length, FW- fresh weight, DW- dry weight, cfu- colony forming unit.

- No increase

[#]Experiment were conducted in different batches with separate uninoculated control in each batch

[†] Population of fluorescent *Pseudomonas* spp. on s2 medium

Table.S5 Effect of inoculation of rhizospheric *Bacillus* spp. on growth parameters of paddy seedlings in green house assay

Treatment	Root length* (cm)	Shoot length* (cm)	Total seedling length* (cm)	% increase in			† log cfu/g fresh soil*
				TSL	FW	DW	
Experiment I[#]							
<i>Bacillus</i> sp. CoSB10	14.46a	21.8ab	36.26a	7	8	50	6.46ab
<i>Bacillus</i> sp. CoSB5	14.1a	21.08ab	35.18a	3	4	50	6.51ab
<i>Bacillus</i> sp. CoSB3	13.8a	22.14ab	35.94a	6	9	50	6.20a
<i>Bacillus</i> sp. RSB22	15.82a	21.34ab	37.16a	9	6	50	6.67ab
<i>Bacillus</i> sp. RSB15	13.68a	20.56ab	34.24a	1	2	50	7.15a
<i>Bacillus</i> sp. RSB3	14.42a	21.9ab	36.32a	7	5	-	7.00a
<i>Bacillus</i> sp. RSB14	14.00a	23.5a	37.50a	10	35	20	6.73ab
Control	13.82a	20.22b	34.04a				5.30b
Experiment II							
<i>Bacillus</i> sp. KiSB5	11b	23.15cd	34.15bc	14	50	-	6.83a
<i>Bacillus</i> sp. KiSB9	12.5a	26ab	38.5a	28	50	-	6.65a
<i>Bacillus</i> sp. KiSB10	13.25a	25.5b	38.75a	29	50	-	6.54a
<i>Bacillus</i> sp. KiSB21	10.75b	24.85bc	35.6b	18	50	-	6.44a
<i>Bacillus</i> sp. KiSB24	7.6d	27.4a	35b	16	50	-	6.43a
<i>Bacillus</i> sp. ASB1	10.25bc	23.25cd	33.5bc	11	50	-	6.51a
<i>Bacillus</i> sp. ASB13	10.75b	21.5de	32.25c	7	100	-	6.68a
Control	9.25c	20.8e	30.05d	12			5.47b
Experiment III							
<i>Bacillus</i> sp. KnSB2	18.14a	25.72a	43.86ab	3	-	-	6.46a
<i>Bacillus</i> sp. KnSB3	16.76a	23.62ab	40.38ab	6	9	-	6.50a
<i>Bacillus</i> sp. KnSB6	16.76a	24.74ab	41.5ab	13	9	-	6.68a
<i>Bacillus</i> sp. KnSB10	19.4a	24.96a	44.36a	12	9	-	6.30a
<i>Bacillus</i> sp. KnSB15	17.88a	26.14a	44.02ab	1	27	-	6.32a
<i>Bacillus</i> sp. KnSB17	16.4a	23.3ab	39.7ab	13	-	-	6.41a
<i>Bacillus</i> sp. KnSB19	18.66a	25.74ab	44.4ab	9	45	-	6.76a
<i>Bacillus</i> sp. KnSB20	18.74a	24ab	42.74ab	12	18	-	6.72a
Control	18.14a	21.02b	39.16b				5.84b
Experiment IV							
<i>Bacillus</i> sp. PSB13	18.0cd	27.0a	45.0ab	17	-	-	6.95a
<i>Bacillus</i> sp. HSB17	19.9ab	26.18a	46.18a	20	5	-	6.91a
<i>Bacillus</i> sp. ESB2	19.24bc	25.54a	44.78ab	17	-	-	6.67a
<i>Bacillus</i> sp. ESB3	18.3bcd	27.48a	45.78a	19	11	-	6.32a
<i>Bacillus</i> sp. ESB8	16.7d	25.12a	41.82b	9	-	-	6.71a
<i>Bacillus</i> sp. ChSB8	16.74d	25.4a	42.5b	11	-	-	6.86a
Control	21.2a	17.18b	38.38c				5b
Experiment V							
<i>Bacillus</i> sp. ESB13	20.42a	25.7a	46.14a	48	12	40	6.75a
<i>Bacillus</i> sp. ESB14	16.48bc	24.76ab	41.24ab	33	-	23	6.55a
<i>Bacillus</i> sp. ESB15	17.4abc	24.94ab	42.34ab	36	-	6	5.95a
<i>Bacillus</i> sp. TSB2	17.64abc	24.2ab	41.84ab	35	18	40	6.61a
<i>Bacillus</i> sp. TSB8	20.6a	23.96ab	44.56ab	43	35	75	6.77a
<i>Bacillus</i> sp. TSB13	17.9abc	22.22b	40.12b	29	-	6	5.84ab
<i>Bacillus</i> sp. TSB16	19.2ab	25.14ab	44.34ab	43	65	163	6.47a
Control	15.79c	15.3c	31.09c				5b

*Values are mean of 10 replications. Mean followed by the same letters in a column in an experiment are not significantly different from each other according to Duncan's multiple range test at P= 0.05.

TSL- total seedling length, FW- fresh weight, DW- dry weight, cfu- colony forming unit.

- No increase

[#]Experiment were conducted in different batches with separate uninoculated control in each batch

[†] Population of *Bacillus* spp. on NA

Table.S6 Effect of inoculation of endophytic *Bacillus* spp. on growth parameters of paddy seedlings in green house assay

Treatment	Root length* (cm)	Shoot length* (cm)	Total seedling length* (cm)	% increase in			† log cfu/g fresh soil*
				TSL	FW	DW	
Experiment I[#]							
<i>Bacillus</i> sp. PoEB3	15.5bc	19.63a	35.13ab	7	47	20	6.41a
<i>Bacillus</i> sp. PoEB5	19.02a	19.28a	38.3a	16	6	-	7.00a
<i>Bacillus</i> sp. TEB18	13.72c	20.66a	34.38ab	4	47	20	6.53a
<i>Bacillus</i> sp. KiEB25	18.02ab	19.34a	37.36a	13	41	20	6.79a
Control	15.38bc	17.6a	32.98b				5.30b
Experiment II							
<i>Bacillus</i> sp. HEB8	14.82ab	22.03a	36.85a	30	21	25	6.98a
<i>Bacillus</i> sp. HEB10	13.68bc	17.58b	31.26bc	11	-	-	6.98a
<i>Bacillus</i> sp. TEB2	14.36abc	19.85ab	34.21ab	21	-	-	7.04a
<i>Bacillus</i> sp. TEB4	17.32a	20.7a	38.02a	35	-	25	7.17a
<i>Bacillus</i> sp. TEB22	14.4abc	20.54a	34.94ab	24	-	-	7.72a
Control	10.85c	17.39b	28.24c				5.30b
Experiment III							
<i>Bacillus</i> sp. KiEB3	12.26a	21.52a	33.78a	8	42	75	6.79a
<i>Bacillus</i> sp. KiEB9	10.75a	20.56a	31.31a	-	-	50	6.83a
<i>Bacillus</i> sp. KiEB13	12.1a	19.1a	31.2a	-	-	75	2.311754c
<i>Bacillus</i> sp. KiEB14	12.14a	21.73a	33.87a	8	-	50	7.029384a
<i>Bacillus</i> sp. KiEB15	13.72a	19.36a	33.08a	6	-	100	6.908485a
<i>Bacillus</i> sp. KiEB16	14.73a	20.87a	35.6a	14	-	100	6.672098a
Control	11.2a	20.08a	31.28a				5.30103b
Experiment IV							
<i>Bacillus</i> sp. KiEB23	16.6b	24.75a	41.35a	14	8	100	7.133539a
<i>Bacillus</i> sp. KiEB31	18.66a	21.76bc	40.42a	11	-	7	6.908485a
<i>Bacillus</i> sp. AEB5	12.27d	20.37c	32.64d	-	23	33	6.869232a
<i>Bacillus</i> sp. REB1	17.98ab	20.02c	38b	5	-	7	6.755875a
<i>Bacillus</i> sp. CoEB1	14.12c	21.58bc	35.7bc	-	54	87	1.869232c
<i>Bacillus</i> sp. CoEB3	11.86d	21.72bc	33.58cd	-	15	33	6.851258a
<i>Bacillus</i> sp. PoEB2	17.54ab	23.3ab	40.84a	13	8	60	6.963788a
<i>Bacillus</i> sp. PoEB4	11.68d	21.84bc	33.52cd	-	61	140	7.012837a
Control	14.87c	21.27bc	36.14b				5.380211b
Experiment V							
<i>Bacillus</i> sp. HEB7	13.58a	21.2a	34.78a	2	5	-	6.70757a
<i>Bacillus</i> sp. HEB16	13.24a	20.08a	33.32a	-	-	100	6.763428a
Control	13.82a	20.22a	34.04a				0.69897b

*Values are mean of 10 replications. Mean followed by the same letters in a column in an experiment are not significantly different from each other according to Duncan's multiple range test at P= 0.05.

TSL- total seedling length, FW- fresh weight, DW- dry weight, cfu- colony forming unit.

- No increase

[#]Experiment were conducted in different batches with separate uninoculated control in each batch

[†] Population of *Bacillus* spp. on NA

Fig.1 Isolation of fluorescent *Pseudomonas* spp. and *Bacillus* spp. a) Map indicating locations from which coconut roots and rhizospheric samples were collected (sample collection sites are marked with coconut palm and respective district names are given in red colour), b) fluorescent *Pseudomonas* spp. isolated on S2 agar, fluoresced on exposure to UV light, c) *Bacillus* spp. isolated on nutrient agar after heat treatment.

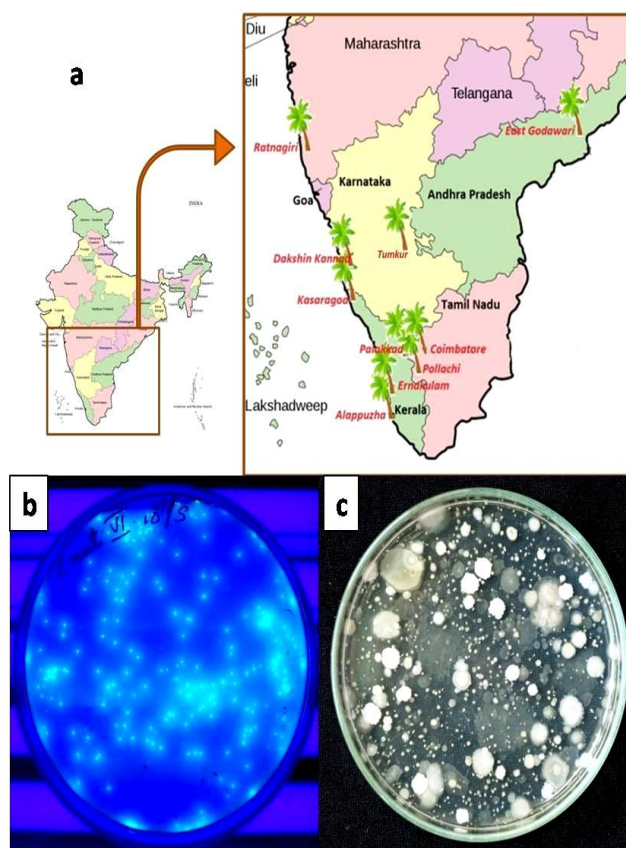


Fig.2 *In vitro* characterization of fluorescent pseudomonad isolates a) percentage of isolates showing plant growth promoting traits (HCN- hydrogen cyanide production, MPS- mineral phosphate solubilization, N- nitrogen fixation (presumptive), AMM- ammonia production, SID- siderophore production, IAA- production of indole acetic acid, ACC- ACC deaminase activity, CHITIN- chitinase activity, ANTI- antibiotic production, G- antagonism against *Ganoderma applanatum*, TP- antagonism against *Thielaviopsis paradoxa*) b) characteristic pink colour developed by IAA producer, c) characteristic p-dissolution halo on Pikovskaya's agar, d) characteristic orange halos formed by siderophore producers on CAS agar, e) characteristic orange colour developed by HCN producer, f) characteristic clearing zone formed by chitinase producer, g) characteristic inhibition zone formed by antibiotic producer

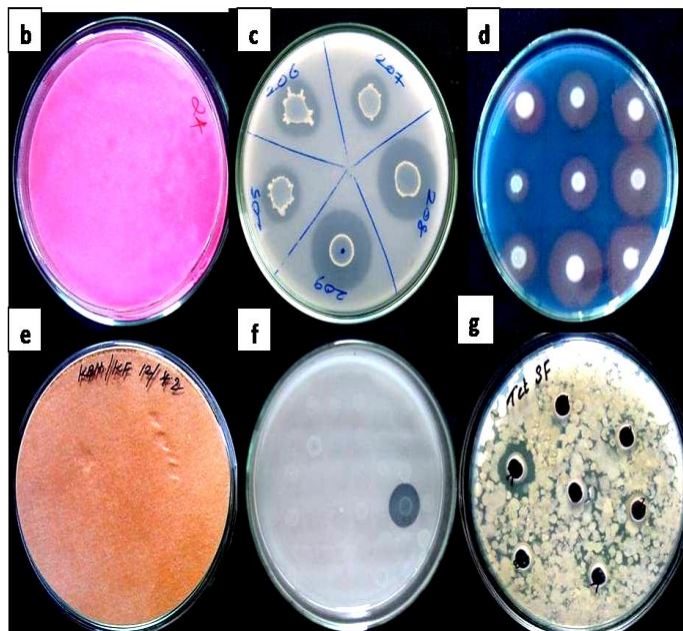
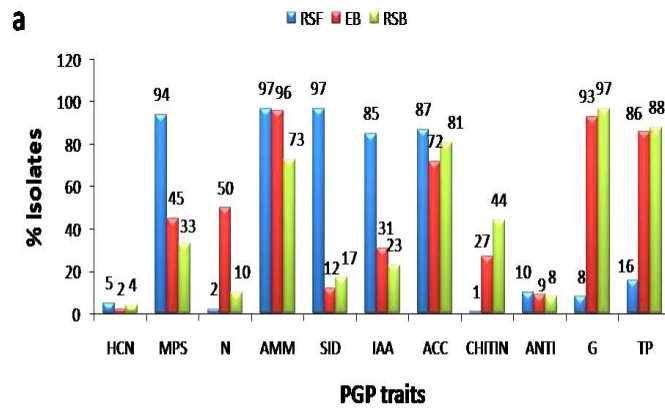


Fig.3 Antagonistic activity showed by isolates. a) Growth of *Ganoderma applanatum* on KBA, b) Growth of *G. applanatum* inhibited by RSF isolates on KBA, c) Growth of *Thielaviopsis paradoxa* on KBA, d) Growth of *T. paradoxa* inhibited by RSF on KBA, e) Growth of *G. applanatum* on NA, f) Growth of *G. applanatum* inhibited by *Bacillus* spp. on NA, g) Growth of *T. paradoxa* on NA, h) Growth of *T. paradoxa* inhibited by *Bacillus* spp. on NA.

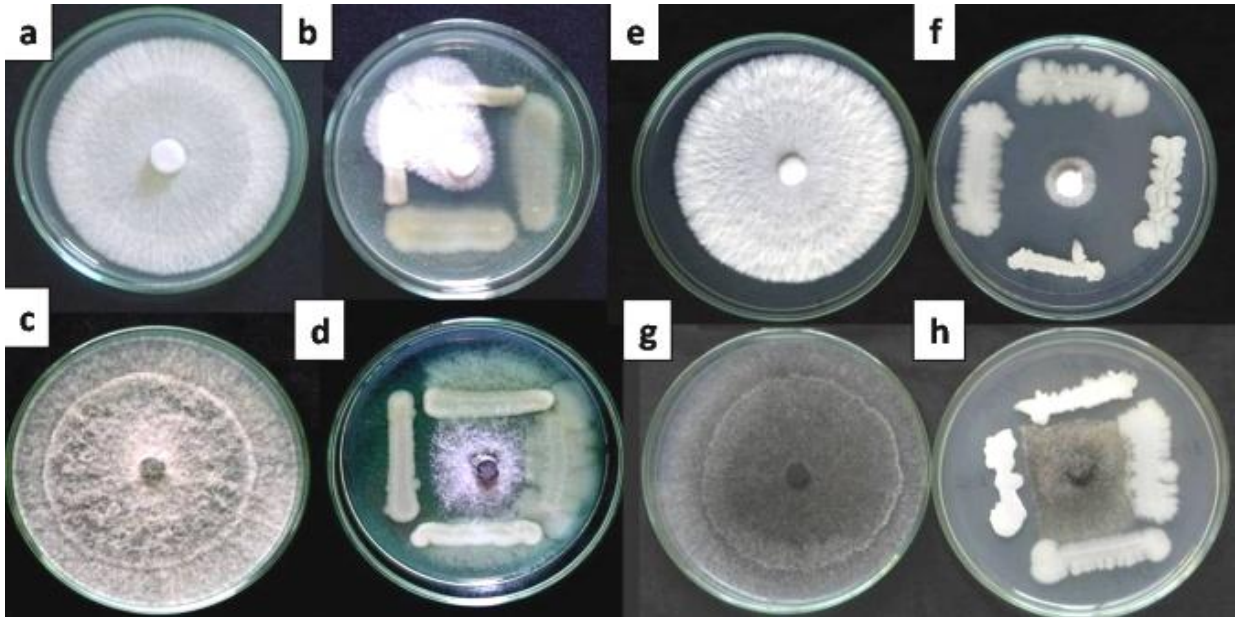
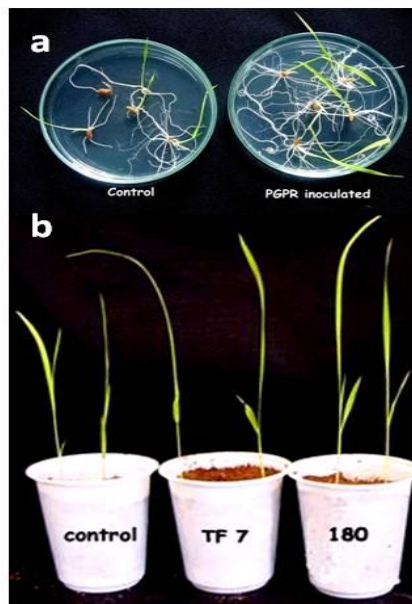


Fig.4 Effect of PGPR inoculations on (a) paddy seedlings under controlled light, temperature and humidity conditions, (b) paddy seedlings under green house conditions



Sequence analysis of the 16S rRNA gene and BLAST sequence comparison confirmed the identity of isolates based on nucleotide homology and phylogenetic analysis as in the preliminary identification. The 16S rDNA sequences were deposited in the NCBI Genbank and accession numbers obtained (Table 6).

Microbial enumeration and isolation studies in coconut palms growing in several parts of Kerala, Karnataka, Tamil Nadu, Andhra Pradesh and Maharashtra under different soil and ecological conditions clearly indicated that the fluorescent pseudomonads and *Bacillus* spp. community occurred in good numbers in the exo- or endo-rhizosphere of this perennial plantation crop thus confirming the presence of many types of plant beneficial microorganisms in coconut (George *et al.*, 2011, 2012a, b). Fluorescent pseudomonads and *Bacillus* spp. are two important groups of PGPR already known for their plant growth promotion properties (Santoro *et al.*, 2016; Govindasamy *et al.*, 2010). We employed different approaches to determine the plant growth promoting potential of these fluorescent pseudomonads and *Bacillus* spp. isolated from coconut rhizosphere as well as the roots. This included *in vitro* testing of plant growth promotion traits, covering both direct and indirect modes of action (Gupta *et al.*, 2000), plant growth promoting ability under controlled and uncontrolled greenhouse conditions.

All the isolated PGPR (483 numbers) from coconut exo- or endo-rhizosphere possessed at least one plant growth promoting traits *in vitro*. Among coconut endo or exo-rhizosphere bacteria, *Bacillus* spp. were found to be more dynamic in N- fixation ability (40% of *Bacillus* spp.), chitin production (35%) and antagonistic activity (95% and 87% against *G. applanatum* and *T. paradoxa*, respectively), whereas *Pseudomonas* spp.

were good in P-solubilization (94% of RSF), siderophore production (97%) and IAA production (85%). There was a preponderance of ammonifying bacteria and antagonistic bacteria in the rhizosphere and roots of coconut. Ammonifying bacteria help in releasing ammonia (NH₃) from organic matters that are available for uptake by many plants. Seed inoculation of peanut with the ammonifying strain of *Pseudomonas fluorescens* PGPR1 increased the contents of nitrogen in soil, shoot and kernel of peanut (Dey *et al.*, 2006). Rhizobacteria having biocontrol properties are considered as an alternative to chemical pesticides (Zahir *et al.*, 2004). More than 87% of the *Bacillus* spp. were found antagonistic to *G. applanatum* and *T. paradoxa*, fungal pathogens of coconut. The results of our study confirmed the wide distribution of ACC deaminase activity in bacterial genera particularly *Pseudomonas* spp. (Klee *et al.*, 1991) and *Bacillus* spp. (Ghosh *et al.*, 2003) concurrent to previous studies from different crop soils. Although this study identified ACC deaminase activity of the isolates only qualitatively, this is the first study to report the presence of ACC degrading bacteria in the rhizosphere and roots of a perennial plantation crop like coconut. Another interesting result is that more than half of the bacterial isolates (56%) associated with coconut exhibited phosphate solubilization activity as evidenced by the clearing zone around their colonies on Pikovskaya's agar. An increase in P availability to plants through the use of phosphate solubilizing bacteria (PSB) had been reported under field conditions (Otieno *et al.*, 2015). Bacteria with the ability to make atmospheric nitrogen in to available form play a critical role in the plant nutrition as nitrogen is one of the principal plant nutrients. 20% of the isolates showed growth on nitrogen-free medium indicating the ability to fix atmospheric nitrogen with the help of nitrogenase enzyme. Among 84 nitrogen

fixers, 60 isolates were root endophytic *Bacillus* spp. Endophytic nitrogen fixing bacteria were reported from many crop plants like sugarcane, rice etc. (Muangthong *et al.*, 2015; Ji *et al.*, 2014). The production of phytohormones by PGPR is considered to be another important mechanism by which many rhizobacteria promote plant growth (Naveed *et al.*, 2015). In this study, 45% of the total isolates, which included 86% of fluorescent *Pseudomonas* spp., showed the production of indole acetic acid (IAA) under *in vitro* conditions. The results confirmed the findings of Saharan and Nehra (2011) that *Pseudomonas* bacteria, especially *P. fluorescens* and *P. putida*, are the most important kinds of PGPR which produce auxins and promote the yield. The production of siderophores was found to be another most commonly observed plant growth promotion trait possessed by coconut PGPR. Siderophore production had been reported to be commonly associated with fluorescent *Pseudomonas* spp. (Bashan and de-Bashan, 2005). Fifteen percent of the *Bacillus* spp. from the rhizosphere and roots of coconut were also positive for siderophore production. Plants get benefit from this plant growth promotion trait in two ways: from the suppression of pathogens and from enhanced iron nutrition, resulting in increased plant growth (Bashan and de-Bashan, 2005; Gupta and Gopal, 2008). About one-fourth of the isolates exhibited chitinase activity among which *Bacillus* spp. were the predominant; about 35% of the *Bacillus* spp. exhibited chitinase production. Das *et al.*, (2010) reported *Bacillus* sp., *Serratia plymuthica*, and *Enterobacter agglomerans* to be well-known chitinolytic bacteria, which could be used as efficient biological control agents. The production of antibiotics is also considered as one of the most studied and powerful biocontrol mechanisms needed to be possessed by a PGPR for combating phytopathogens (Bashan and de-Bashan,

2005). The overall results of functional traits revealed that all the isolates showed strong tendencies for one or more of the PGPR traits tested and none were negative for all traits. All the isolates were assessed based on the performance in the *in vitro* plant growth promotion tests. An equal importance were given to all the plant growth promoting traits tested and maximum three points were possible for each parameter totaling to 33 points. The best performing isolates (129 nos.) were screened from the collection of 483 by assessing them according to their performance in plant growth promoting traits.

The approach of testing *in vitro* abilities has been proved to be an effective strategy to isolate PGPR; however, there are limitations. Some of the biochemical traits shown *in vitro* are inducible; i.e., they are expressed in certain conditions but not in others. Therefore, after the screening process, the potential PGPR needed to be tested in plants to ensure the same effect occurs in the plant too (Barriuso *et al.*, 2008). Bacterial inoculation had been reported to have a positive influence on various plant-growth parameters, including root and shoot length, and dry biomass (Gupta *et al.*, 1998; Nadeem *et al.*, 2016). The outcome of our seedling bioassay under controlled conditions was that 41% (53 out of 129) isolates increased total seedling length with variable degree of stimulation, 14% showed inhibitory effect (non-significant in DMRT analysis) on plant growth and 44% remained as neutral compared to uninoculated control. The rhizosphere microorganisms, apart from being beneficial, are known to exert neutral and detrimental effects on plant growth and health (Gupta and Gopal, 1999).

Greenhouse studies further proved the efficiency of screened isolates for plant growth promotion in paddy seedlings. All isolates tested were found to be efficient in

increasing either seedling length (up to 48% increase over uninoculated control), or fresh weight (up to 100% increase) or dry weight (up to 200% increase) of paddy seedlings. The strains that exhibited growth promotion of paddy seedlings in green house were very diverse in their traits. Therefore, growth enhancement by these organisms might involve more than one mechanism (Gupta *et al.*, 2000). *Pseudomonas monteilii* PsF84 and *Pseudomonas plecoglossicida* PsF610, isolated from tannery sludge polluted soil, solubilized inorganic phosphorus and were capable of producing indole acetic acid (IAA) and siderophore. They increased the dry biomass of shoot, root, essential oil yield and chlorophyll of rose-scented geranium plants (*Pelargonium graveolens* cv. bourbon) over uninoculated control (Dharni *et al.*, 2014). It had also been reported that the application of bacterial strains with multifaceted traits for plant growth promoting activity is more beneficial than with one plant growth-promoting trait (Indiragandhi *et al.*, 2008). A total of 20 isolates (4% of initial collection) were obtained as efficient PGPR with plant growth promoting abilities through our screening strategies. Some of the isolates selected from greenhouse assay possess direct plant growth promoting activity as well as biocontrol activity.

They inhibited the mycelial growth of *Ganoderma applanatum* and *Thielaviopsis paradoxa* *in vitro*. All the isolates improved paddy seedling growth (11-43% over control) in controlled conditions as well as in uncontrolled greenhouse conditions. Their ability to retain their respective population in the rhizosphere of inoculated seedlings also added to their potency. These results are in agreement with other screening studies e.g., in *Prunus* root stocks, from a starting collection of several hundreds of strains, 20-25% exhibited certain degree of growth promotion activity, but in the final steps of selection,

based on efficacy and consistency only 1-3% were suitable for further use (Bonaterra *et al.*, 2003). Out of 20 potent isolates, 15 exhibited antagonistic activity against both fungal pathogens of coconut, *Ganoderma applanatum*, and *Thielaviopsis paradoxa*. It is expected that plant growth promoting strains with high antagonistic potential will provide additional advantages to the seedlings (coconut) during exposure to pathogens. Identity of the finally selected PGPR was deduced using 16S rDNA sequence analysis with 99-100% similarity. All the identified isolates were reported as plant growth promoting rhizobacteria based on the plant growth promotion assays in greenhouse or field conditions (Dharni *et al.*, 2014; Saharan and Nehra, 2011; Chakraborty *et al.*, 2006; Govindasamy *et al.*, 2010).

The most significant out come in this study is that strategic screening enabled to shortlist a total of 20 best PGPRs (7 *Pseudomonas* spp. and 13 *Bacillus* spp) with multifaceted traits from the preliminary collection of 483 isolates, based on their overall performance in *in vitro* characterization of plant growth promotion traits, seedling bioassay and green house trial. Utilization of these strains will enable to reduce the use of pesticides and fertilizers that are potential pollutants of the environment. The efficacy of the screened exo- or endo-rhizospheric bacterial isolates in improving the growth of coconut needs to be demonstrated and if further developed, could be recommended as efficient bioinoculants for growth and yield improvement in coconut.

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