Int.J.Curr.Microbiol.App.Sci (2018) *7*(5): 1051-1074

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 05 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.705.130

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

Priya George[1,2*], Alka Gupta[1], Murali Gopal[1], Litty Thomas[1] and George V. Thomas[1,3]

[1]Microbiology Section, ICAR-Central Plantation Crops Research Institute, Kudlu P.O., Kasaragod – 671 124, India
[2]ICAR-National Institute of Abiotic stress Management, Baramati, Pune-413115, India
[3]Council for Food Research and Development, Konni, Kerala, India

**Corresponding author*

Keywords

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

Article Info

Accepted:
10 April 2018
Available Online:
10 May 2018

A B S T R A C T

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.

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* (Geroge *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 µS/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 weigh. 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 $\log_{10}$ cfu g^{-1} fresh weight of soil. Low population levels of fluorescent pseudomonads were detected in the non-treated control plants, up to 2 $\log_{10}$ 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 $\log_{10}$ cfu g^{-1} of soil, when compared to control (average of 5.44 $\log_{10}$ 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	Ammonification*	Siderophore (zone in mm)	IAA*	ACC*	Chitinase (zone in mm)	Antibiotics (zone in mm)*	Antagonism**		Assessment 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. ǂ 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
***Bacillus* 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**
***Pseudomonas* 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	% increase in SL	FW	DW
Bacillus sp. ESB15	+	-	-	M	G	-	L	-	13	59	80	5	36	-	6
Bacillus sp. KnSB6	+	-	-	H	-	-	L	-	10	74	62	14	13	9	-
Bacillus sp. TSB16	+	-	-	H	-	M	L	-	-	57	56	11	43	65	163
Bacillus sp. KiSB10	-	10	+	M	-	H	L	-	-	71	-	7	29	50	-
Bacillus sp. RSB14	-	16	+	M	12	-	L	-	12	57	80	10	10	35	20
Bacillus sp. TEB2	-	-	+	M	-	L	M	-	-	57	64	20	21	-	-
Bacillus sp. TEB4	-	-	+	L	-	M	L	-	-	57	64	17	35	-	25
Bacillus sp. HEB8	-	-	-	M	-	H	M	-	11	74	56	30	30	21	25
Bacillus sp. HEB10	-	-	-	H	-	M	M	-	14	74	56	24	11	-	-
Bacillus sp. KiEB23	-	10	+	H	G	-	-	-	-	71	44	34	14	8	100
Bacillus sp. KiEB25	-	6	+	H	-	-	L	-	9	80	80	34	13	41	20
Bacillus sp. KiEB31	-	10	+	H	-	-	L	-	-	71	44	47	11	-	7
Bacillus sp. PoEB5	+	14	+	M	26	-	L	-	-	60	76	14	16	6	-
Pseudomonas sp. ChSF180	-	16	-	H	14	H	L	-	-	-	44	48	28	45	140
Pseudomonas sp. KnSF208	-	42	-	H	12	H	L	-	-	-	-	10	12	57	100
Pseudomonas sp. ASF285	-	16	-	L	12	L	L	L	-	43	49	12	29	33	13
Pseudomonas sp. PoSF314	-	20	-	M	20	H	L	H	-	40	47	17	14	23	50
Pseudomonas sp. TSF7	-	16	-	H	16	H	L	H	-	43	56	21	33	14	50
Pseudomonas sp. RSF266	-	16	-	M	10	H	L	L	-	-	20	25	10	62	150
Pseudomonas 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	*Pseudomonas putida*	99	KF381346
2	KnSF208	*P. putida*	99	KF364491
3	ASF285	*P. monteilii*	99	KF381348
4	PoSF314	*P. plecoglossicida*	99	KF381347
5	TSF7	*P. monteilii*	99	KF381345
6	RSF266	*P. plecoglossicida*	99	KF381349
7	KiSF27	*P. plecoglossicida*	99	KF381344
8	ESB15	*Bacillus cereus*	100	KF381350
9	KnSB6	*B. cereus*	99	KF381351
10	TSB16	*B. megaterium*	99	KF364492
11	KiSB10	*B. megaterium*	99	KF381356
12	RSB14	*B. licheniformis*	99	KF381343
13	TEB2	*B. megaterium*	100	KF381342
14	TEB4	*B. megaterium*	100	KF381354
15	HEB8	*B. cereus*	99	KF381353
16	HEB10	*B. cereus*	99	KF381352
17	KiEB23	*Bacillus* sp.	98	KF381357
18	KiEB25	*B. cereus*	100	KF381358
19	KiEB31	*B. megaterium*	99	KF381355
20	PoEB5	*B. subtilis*	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[#]			
Pseudomonas sp. HSF132	8.22 ab	4.94 a	13.16 a
Pseudomonas sp. ESF153	8.40 ab	5.86 a	14.26 a
Pseudomonas sp. ESF168	7.00 ab	4.10 a	11.10 a
Pseudomonas sp. KnSF208	8.08 ab	4.86 a	12.94 a
Pseudomonas sp. RSF266	8.86 a	5.20 a	14.60 a
Pseudomonas sp. ASF285	8.54 ab	4.54 a	13.08 a
Pseudomonas sp. PoSF315	4.50 b	5.50 a	10.00 a
Pseudomonas sp. TSF7	8.86 a	5.36 a	14.22 a
Control	6.90 ab	4.82 a	11.72 a
Experiment – II			
Pseudomonas sp. ChSF180	8.58 a	7.96 a	16.54 a
Pseudomonas sp. KiSF27	8.65 a	6.20 ab	14.85 a
Control	6.675 b	4.525 b	11.20 b
Experiment –III			
Pseudomonas sp. RSF259	3.88 b	5.82 bc	9.70 b
Pseudomonas sp. KiSF13	9.70 a	5.70 bc	15.40 a
Pseudomonas sp. KiSF3	8.14 a	6.80 ab	14.90 a
Pseudomonas sp. KiSF16	9.22 a	7.36 ab	16.58 a
Pseudomonas sp. KiSF17	2.40 b	6.32 abc	8.72 ab
Pseudomonas sp. KiSF21	3.24 b	5.20 c	8.44 b
Pseudomonas sp. KiSF24	9.60 a	6.70 abc	16.30 a
Pseudomonas sp. KiSF28	3.54 b	6.00 abc	9.54 b
Control	8.10 a	7.48 a	15.58 a
Experiment – IV			
Pseudomonas sp. RSF257	9.50 a	5.67 a	15.17 a
Pseudomonas sp. PoSF313	7.00 b	4.68 a	11.68 b
Pseudomonas 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#			
Bacillus sp. KnSB22	NG	NG	NG
Bacillus sp. KnSB6	9.63 abc	6.26abcde	15.89abcd
Bacillus sp. KnSB17	6.2fg	6.4abcd	12.6efg
Bacillus sp. KnSB10	7.6 def	5.75cdefg	13.35efg
Bacillus sp. KnSB19	6fg	6.05bcdef	12.05fg
Bacillus sp. KnSB7	NG	NG	NG
Bacillus sp. KnSB3	9.9ab	7.46a	17.36ab
Bacillus sp. KnSB2	9.66bcd	6.3abcde	15.96abc
Bacillus sp. HSB17	9.15 abc	5.3defg	14.45cdef
Bacillus sp. ChSB2	11.2a	6.73abc	17.93a
Bacillus sp. ChSB4	NG	NG	NG
Bacillus sp. ChSB8	9.83ab	6.8abc	16.63abc
Bacillus sp. ChSB9	8cde	4.46g	12.46efg
Bacillus sp. PSB13	6.6efg	6.5abcd	13.1efg
Bacillus sp. ESB2	10ab	7abc	17ab
Bacillus sp. ESB4	7.8def	4.9fg	12.7efg
Bacillus sp. ESB8	5.6g	7.26ab	12.86efg
Bacillus sp. ESB13	7.63 def	7.46ab	15.09bcde
Bacillus sp. ESB14	5g	6.1abcdef	11.1g
Bacillus sp. ESB15	7.5def	7.1abc	14.6cdef
Control	8.86 bcd	5.02efg	13.88def
Experiment II			
Bacillus sp. KnSB13	5.30g	2.41e	7.71bc
Bacillus sp. KnSB15	5.05g	4.08bc	9.13abc
Bacillus sp. KnSB20	6.85bcd	4.00bcd	10.85a
Bacillus sp. TSB13	6.16e	3.73bcd	9.89ab
Bacillus sp. TSB16	6.49cde	4.09bc	10.58a
Bacillus sp. CoSB1	5.53fg	3.53bcd	9.06abc
Bacillus sp. CoSB5	6.32de	3.75bcd	10.07a
Bacillus sp. PoSB7	5.0g	3.57bcd	8.57abc
Bacillus sp. RSB8	5.29g	3.30cd	8.59abc
Bacillus sp. RSB14	7.32b	3.17d	10.49a
Bacillus sp. ESB3	6.96bc	3.67bcd	10.63a
Control	5.97ef	3.58bcd	9.55ab
Experiment III			
Bacillus sp. CoSB3	11.86b	7.86a	19.72a
Bacillus sp. CoSB2	8.5b	6.8abc	15.3b
Bacillus sp. CoSB4	10.14b	6.42abc	16.56b
Bacillus sp. CoSB6	8.72b	6.76abc	15.48b
Bacillus sp. CoSB7	6.16b	5.24c	11.4b
Bacillus sp. CoSB8	9.5b	5.22c	14.72b
Bacillus sp. CoSB10	9.94b	7.02ab	16.96b
Bacillus sp. PoSB2	7.04b	5.65bc	12.69b
Bacillus sp. PoSB8	8.26b	6.18abc	14.44b
Bacillus sp. PoSB10	8.6b	6.5abc	15.1b
Control	12.42b	6.76abc	19.18b
Experiment IV			
Bacillus sp. ASB1	8.9ab	5.68 abc	14.58ab
Bacillus sp. ASB2	5.0bc	5.26 abc	10.26b
Bacillus sp. ASB3	5.74abc	5.84 abc	11.58ab
Bacillus sp. ASB13	8.76ab	5.96 abc	14.72 ab
Bacillus sp. KiSB5	7.1 abc	6.06 abc	13.16 ab
Bacillus sp. KiSB9	9.15ab	4.81c	13.96 ab
Bacillus sp. KiSB10	8.6 abc	6.4a	15.0 ab
Bacillus sp. KiSB21	5.12bc	6.28 abc	11.4 ab
Bacillus sp. KiSB22	6.54 abc	5.348 abc	11.888 ab
Bacillus sp. KiSB24	7.34 abc	6.44a	13.78 ab
Bacillus sp. KiSB27	6.46 abc	5.74 abc	12.2 ab
Bacillus sp. KiSB34	5.628 abc	5.656 abc	11.284 ab
Bacillus sp. TSB2	8.57ab	6.012 abc	14.582 ab
Bacillus sp. TSB3	5.44 abc	5.436 abc	10.876b
Bacillus sp. TSB8	6.04 abc	6.04 abc	12.08 ab
Bacillus sp. TSB21	5.2bc	6.02 abc	11.22 ab
Bacillus sp. RSB3	4.064c	6.32ab	10.384b
Bacillus sp. RSB4	5.5 abc	5.86 abc	11.36 ab
Bacillus sp. RSB15	8.64ab	4.884bc	13.524 ab
Bacillus sp. RSB20	6.84abc	5.4 abc	12.24 ab
Bacillus sp. RSB21	5.4bc	5.8 abc	11.2 ab
Bacillus 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[#]			
***Bacillus* sp. KiEB3**	10.6abc	6.44abc	**17.04ab**
***Bacillus* sp. KiEB9**	9.9abcd	7.34a	**17.24ab**
***Bacillus* sp. KiEB13**	9.0bcde	5.42cd	**14.42cd**
***Bacillus* sp. KiEB14**	9.2bcde	6.96ab	**16.16abc**
***Bacillus* sp. KiEB15**	9.94abcd	6.92ab	**16.86ab**
***Bacillus* sp. KiEB16**	8.22cdef	5.62bcd	**13.84bcd**
***Bacillus* sp. KiEB19**	5.12f	5.24cd	**10.36d**
***Bacillus* sp. KiEB20**	7.36cdef	6.26abcd	**13.62bcd**
***Bacillus* sp. KiEB23**	12.94a	5.98bcd	**18.92a**
***Bacillus* sp. KiEB25**	10.5abc	6.3abc	**16.8ab**
***Bacillus* sp. KiEB18**	6.7def	6.06abcd	**12.76bcd**
***Bacillus* sp. KiEB26**	6.1ef	5.94bcd	**12.04cd**
***Bacillus* sp. KiEB27**	7.0def	6.44abc	**13.44bcd**
***Bacillus* sp. KiEB29**	6.98def	6.46abc	**13.44bcd**
***Bacillus* sp. KiEB31**	12.28ab	6.26abcd	**18.54a**
Control	7.64cdef	4.94d	**12.58bcd**
Experiment II			
***Bacillus* sp. HEB7**	6.8bc	5.92a	**12.72abc**
***Bacillus* sp. HEB10**	8.7ab	5.76a	**14.46ab**
***Bacillus* sp. HEB6**	6.68bc	4.5a	**11.18bcd**
***Bacillus* sp. HEB1**	5.3cd	4.6a	**9.9cd**
***Bacillus* sp. HEB2**	5.4cd	4.32a	**9.72cd**
***Bacillus* sp. HEB16**	7.64abc	4.44a	**12.08abc**
***Bacillus* sp. HEB8**	9.86a	5.3a	**15.16a**
***Bacillus* sp. TEB2**	9.04ab	4.98a	**14.02ab**
***Bacillus* sp. TEB4**	8.6ab	5.06a	**13.66ab**
***Bacillus* sp. TEB11**	6.96bc	4.8a	**11.96abc**
***Bacillus* sp. TEB20**	3.2d	4.5a	**7.7d**
***Bacillus* sp. TEB22**	7.14abc	4.52a	**11.66abc**
Control	6.9bc	4.82a	**11.7abc**
Experiment III			
***Bacillus* sp. AEB5**	14.44a	6.68ab	**21.12a**
***Bacillus* sp. AEB10**	11.6abc	6.68ab	**17.84abc**
***Bacillus* sp. AEB11**	10.68abcd	5.16ab	**15.84bcd**
***Bacillus* sp. CoEB1**	10.98abc	6.88a	**17.86abc**
***Bacillus* sp. CoEB2**	9.36abcd	5.9ab	**15.26bcd**
***Bacillus* sp. CoEB3**	12.42ab	6.76ab	**19.18ab**
***Bacillus* sp. CoEB4**	8.86bcd	6.08ab	**14.94bcd**
***Bacillus* sp. CoEB5**	9.52abcd	6.54ab	**16.06abcd**
***Bacillus* sp. CoEB6**	8.48bcd	4.98b	**13.46cd**
***Bacillus* sp. CoEB7**	7.36cd	5.8ab	**13.16cd**
***Bacillus* sp. CoEB8**	6.12d	5.76ab	**11.88d**
***Bacillus* sp. PoEB2**	10.28abcd	6.84a	**17.12abc**
***Bacillus* sp. PoEB3**	12.42ab	6.76	**19.18**
***Bacillus* sp. PoEB4**	12.68ab	6.48ab	**19.16ab**
Control	12.42ab	6.76ab	**19.18ab**
Experiment IV			
***Bacillus* sp. AEB3**	5.71d	3.21e	**8.92e**
***Bacillus* sp. TEB18**	6.53b	4.21b	**10.74b**
***Bacillus* sp. REB1**	5.10e	4.25a	**9.35d**
***Bacillus* 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#							
***Pseudomonas* sp. RSF257**	13.67 b	26.66 a	40.32 b	4	62	113	**5.52a**
***Pseudomonas* sp. KnSF208**	17.22 ab	26.34 a	43.57 b	12	57	100	**5.50a**
***Pseudomonas* sp. RSF266**	17.56 ab	25.24 a	42.80 b	10	62	150	**5.50a**
***Pseudomonas* 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							
***Pseudomonas* sp. ChSF180**	9.76 a	18.82 ab	28.58 b	25	45	140	**5.79a**
***Pseudomonas* sp. PoSF314**	7.00 b	18.60 ab	25.60 bc	12	23	50	**5.65a**
***Pseudomonas* sp. KiSF16**	6.00 b	14.00 c	20.00 d	-	-	100	**4.97a**
***Pseudomonas* sp. KiSF27**	10.45 a	20.83 a	31.28 a	36	14	-	**5.92a**
***Pseudomonas* 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							
***Pseudomonas* sp. KiSF24**	13.34 ab	22.86 b	36.20 bc	4	55	200	**5.47a**
***Pseudomonas* sp. HSF132**	15.22 a	22.46 b	37.680 bc	8	27	100	**5.47a**
***Pseudomonas* 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#							
***Bacillus* sp. CoSB10**	14.46a	21.8ab	36.26a	7	8	50	**6.46ab**
***Bacillus* sp. CoSB5**	14.1a	21.08ab	35.18a	3	4	50	**6.51ab**
***Bacillus* sp. CoSB3**	13.8a	22.14ab	35.94a	6	9	50	**6.20a**
***Bacillus* sp. RSB22**	15.82a	21.34ab	37.16a	9	6	50	**6.67ab**
***Bacillus* sp. RSB15**	13.68a	20.56ab	34.24a	1	2	50	**7.15a**
***Bacillus* sp. RSB3**	14.42a	21.9ab	36.32a	7	5	-	**7.00a**
***Bacillus* sp. RSB14**	14.00a	23.5a	37.50a	10	35	20	**6.73ab**
Control	13.82a	20.22b	34.04a				**5.30b**
Experiment II							
***Bacillus* sp. KiSB5**	11b	23.15cd	34.15bc	14	50	-	**6.83a**
***Bacillus* sp. KiSB9**	12.5a	26ab	38.5a	28	50	-	**6.65a**
***Bacillus* sp. KiSB10**	13.25a	25.5b	38.75a	29	50	-	**6.54a**
***Bacillus* sp. KiSB21**	10.75b	24.85bc	35.6b	18	50	-	**6.44a**
***Bacillus* sp. KiSB24**	7.6d	27.4a	35b	16	50	-	**6.43a**
***Bacillus* sp. ASB1**	10.25bc	23.25cd	33.5bc	11	50	-	**6.51a**
***Bacillus* sp. ASB13**	10.75b	21.5de	32.25c	7	100	-	**6.68a**
Control	9.25c	20.8e	30.05d	12			**5.47b**
Experiment III							
***Bacillus* sp. KnSB2**	18.14a	25.72a	43.86ab	3	-	-	**6.46a**
***Bacillus* sp. KnSB3**	16.76a	23.62ab	40.38ab	6	9	-	**6.50a**
***Bacillus* sp. KnSB6**	16.76a	24.74ab	41.5ab	13	9	-	**6.68a**
***Bacillus* sp. KnSB10**	19.4a	24.96a	44.36a	12	9	-	**6.30a**
***Bacillus* sp. KnSB15**	17.88a	26.14a	44.02ab	1	27	-	**6.32a**
***Bacillus* sp. KnSB17**	16.4a	23.3ab	39.7ab	13	-	-	**6.41a**
***Bacillus* sp. KnSB19**	18.66a	25.74ab	44.4ab	9	45	-	**6.76a**
***Bacillus* sp. KnSB20**	18.74a	24ab	42.74ab	12	18	-	**6.72a**
Control	18.14a	21.02b	39.16b				**5.84b**
Experiment IV							
***Bacillus* sp. PSB13**	18.0cd	27.0a	45.0ab	17	-	-	**6.95a**
***Bacillus* sp. HSB17**	19.9ab	26.18a	46.18a	20	5	-	**6.91a**
***Bacillus* sp. ESB2**	19.24bc	25.54a	44.78ab	17	-	-	**6.67a**
***Bacillus* sp. ESB3**	18.3bcd	27.48a	45.78a	19	11	-	**6.32a**
***Bacillus* sp. ESB8**	16.7d	25.12a	41.82b	9	-	-	**6.71a**
***Bacillus* sp. ChSB8**	16.74d	25.4a	42.5b	11	-	-	**6.86a**
Control	21.2a	17..18b	38.38c				**5b**
Experiment V							
***Bacillus* sp. ESB13**	20.42a	25.7a	46.14a	48	12	40	**6.75a**
***Bacillus* sp. ESB14**	16.48bc	24.76ab	41.24ab	33	-	23	**6.55a**
***Bacillus* sp. ESB15**	17.4abc	24.94ab	42.34ab	36	-	6	**5.95a**
***Bacillus* sp. TSB2**	17.64abc	24.2ab	41.84ab	35	18	40	**6.61a**
***Bacillus* sp. TSB8**	20.6a	23.96ab	44.56ab	43	35	75	**6.77a**
***Bacillus* sp. TSB13**	17.9abc	22.22b	40.12b	29	-	6	**5.84ab**
***Bacillus* 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#							
***Bacillus* sp. PoEB3**	15.5bc	19.63a	35.13ab	7	47	20	**6.41a**
***Bacillus* sp. PoEB5**	19.02a	19.28a	38.3a	16	6	-	**7.00a**
***Bacillus* sp. TEB18**	13.72c	20.66a	34.38ab	4	47	20	**6.53a**
***Bacillus* sp. KiEB25**	18.02ab	19.34a	37.36a	13	41	20	**6.79a**
Control	15.38bc	17.6a	32.98b				**5.30b**
Experiment II							
***Bacillus* sp. HEB8**	14.82ab	22.03a	36.85a	30	21	25	**6.98a**
***Bacillus* sp. HEB10**	13.68bc	17.58b	31.26bc	11	-	-	**6.98a**
***Bacillus* sp. TEB2**	14.36abc	19.85ab	34.21ab	21	-	-	**7.04a**
***Bacillus* sp. TEB4**	17.32a	20.7a	38.02a	35	-	25	**7.17a**
***Bacillus* sp. TEB22**	14.4abc	20.54a	34.94ab	24	-	-	**7.72a**
Control	10.85c	17.39b	28.24c				**5.30b**
Experiment III							
***Bacillus* sp. KiEB3**	12.26a	21.52a	33.78a	8	42	75	**6.79a**
***Bacillus* sp. KiEB9**	10.75a	20.56a	31.31a	-	-	50	**6.83a**
***Bacillus* sp. KiEB13**	12.1a	19.1a	31.2a	-	-	75	**2.311754c**
***Bacillus* sp. KiEB14**	12.14a	21.73a	33.87a	8	-	50	**7.029384a**
***Bacillus* sp. KiEB15**	13.72a	19.36a	33.08a	6	-	100	**6.908485a**
***Bacillus* sp. KiEB16**	14.73a	20.87a	35.6a	14	-	100	**6.672098a**
Control	11.2a	20.08a	31.28a				**5.30103b**
Experiment IV							
***Bacillus* sp. KiEB23**	16.6b	24.75a	41.35a	14	8	100	**7.133539a**
***Bacillus* sp. KiEB31**	18.66a	21.76bc	40.42a	11	-	7	**6.908485a**
***Bacillus* sp. AEB5**	12.27d	20.37c	32.64d	-	23	33	**6.869232a**
***Bacillus* sp. REB1**	17.98ab	20.02c	38b	5	-	7	**6.755875a**
***Bacillus* sp. CoEB1**	14.12c	21.58bc	35.7bc	-	54	87	**1.869232c**
***Bacillus* sp. CoEB3**	11.86d	21.72bc	33.58cd	-	15	33	**6.851258a**
***Bacillus* sp. PoEB2**	17.54ab	23.3ab	40.84a	13	8	60	**6.963788a**
***Bacillus* sp. PoEB4**	11.68d	21.84bc	33.52cd	-	61	140	**7.012837a**
Control	14.87c	21.27bc	36.14b				**5.380211b**
Experiment V							
***Bacillus* sp. HEB7**	13.58a	21.2a	34.78a	2	5	-	**6.70757a**
***Bacillus* 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.

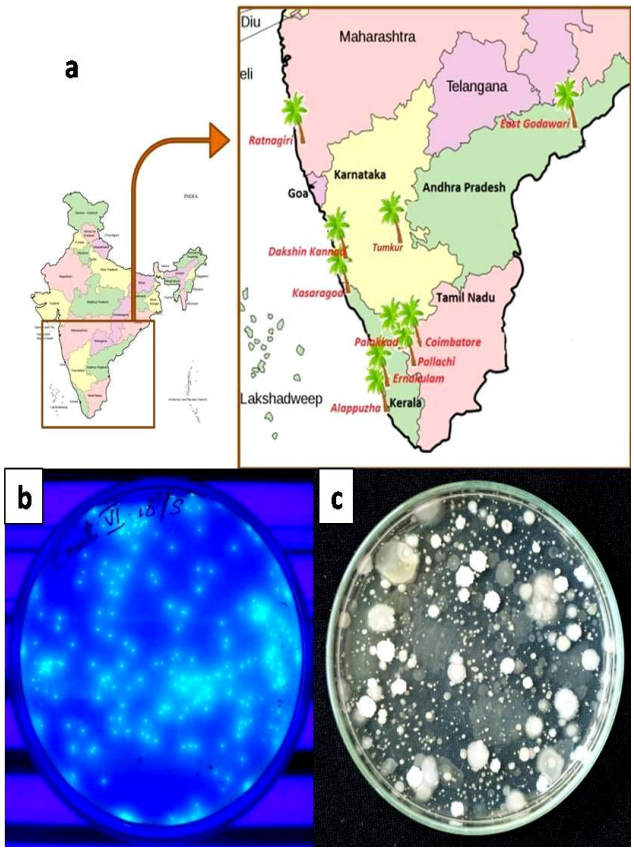


Fig.2 *In vitro* characterization of fluorescent pseudomonad isolates a) percentage of isolates showing plant growth promoting traits (HCN- hydrogen cyanide production, MPS- mineral phosphate solubilization, N- nitrogen fixation (presumptive), AMM- ammonia production, SID- siderophore production, IAA- 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

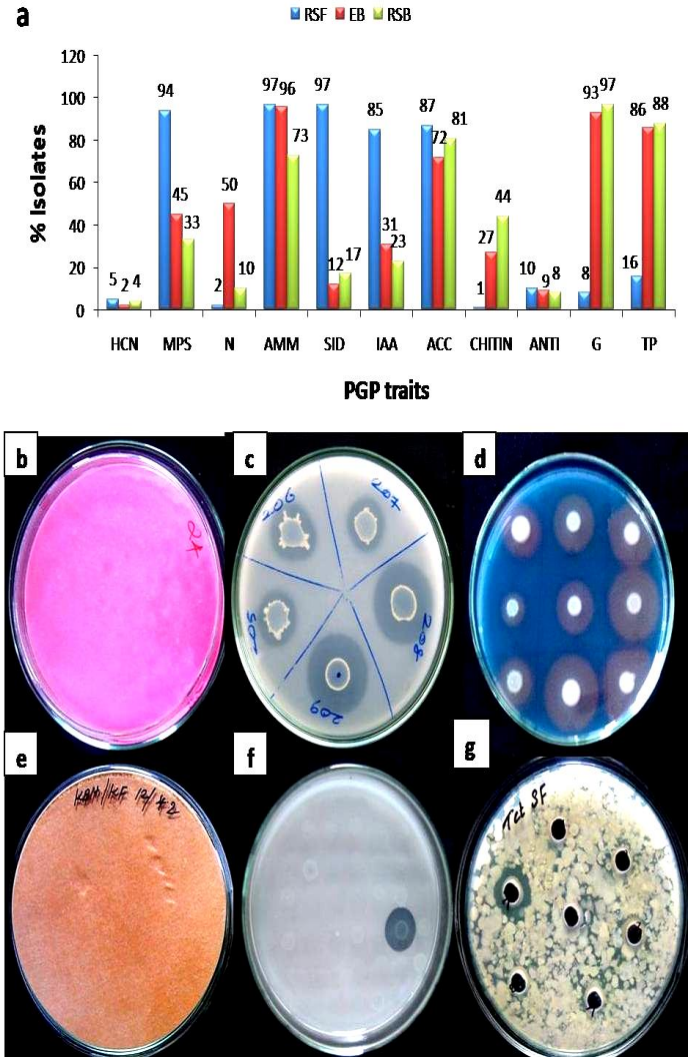


Fig.3 Antagonistic activity showed by isolates. a) Growth of *Ganoderma applanatum* on KBA, b) Growth of *G. applanatum* inhibited by RSF isolates on KBA, c) Growth of *Thielaviopsis paradoxa* on KBA, d) Growth of *T. paradoxa* inhibited by RSF on KBA, e) Growth of *G. applanatum* on NA, f) Growth of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

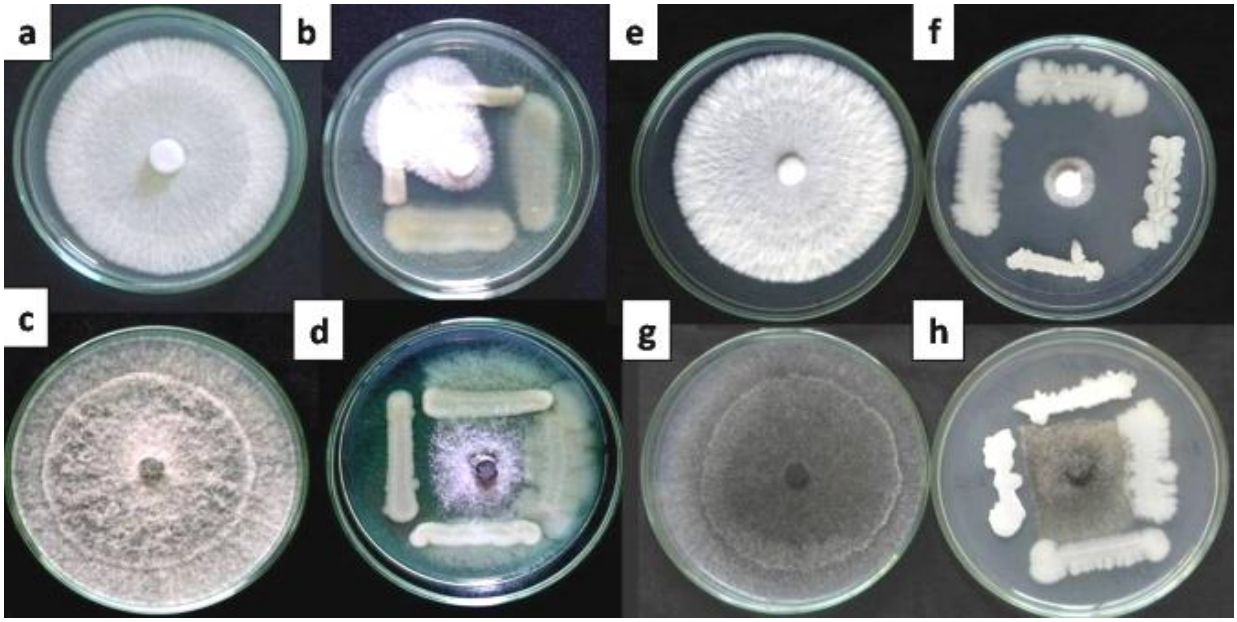
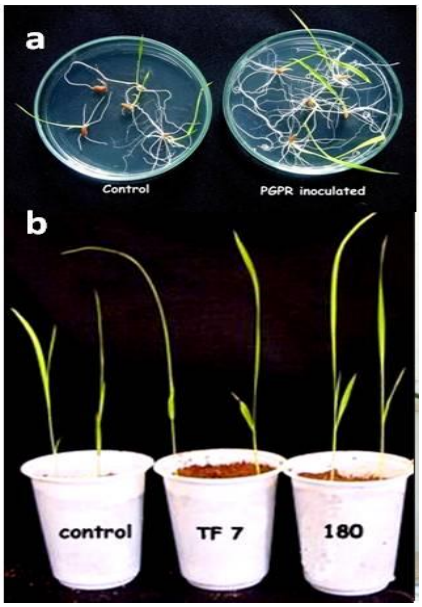


Fig.4 Effect of PGPR inoculations on (a) paddy seedlings under controlled light, temperature and humidity conditions, (b) paddy seedlings under green house conditions



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 (NH_3) 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.

Acknowledgements

We thank Indian Council of Agricultural Research, New Delhi for funding the Network Project on ‘Application of Microorganisms in Agriculture and Allied Sectors’, in which this work was carried out. Priya George is grateful for the award of Senior Research Fellowship.

References

Amir, H.G., Shamsuddin, Z.H., Halimi, M.S., Marziah, M. and Ramlan, M.F. 2005. Enhancement in nutrient accumulation and growth of oil palm seedlings caused by PGPR under field nursery conditions. Communications in Soil Science and Plant Analysis. 36(15-16), 2059-2066.

Bakker, A.W. and Schippers, B. 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp.-mediated plant growth stimulation. Soil Biology and Biochemistry. 19, 451-457.

Barriuso, J., Beatriz, R., Solano, J.A., Lucas, Agustín, P.L., Ana, G., Francisco, J. and Gutiérrez, M. 2008. Ecology, Genetic Diversity and Screening Strategies of Plant Growth Promoting Rhizobacteria (PGPR). In: Ahmad, I., Pichtel, J., and Hayat, S. (Eds.), Plant-Bacteria Interactions. Strategies and Techniques to Promote Plant Growth. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, pp.1-17. doi:10.1002/ 9783527621989.ch1

Bashan, Y. and de-Bashan, L.E. 2005. Plant growth promoting bacteria. pp. 103-115. In: Encyclopedia of soils in the environment, Elsevier, Oxford, U.K.Vol.1.

Bonaterra., Ruz, L., Badosa, E., Pinochet, J. and Montesinos, E. 2003. Growth promotion of Prunus rootstocks by root treatment with specific bacterial strains. Plant and Soil. 255, 555-569.

Brick, J.M., Bostock, R.M. and Silverstone, S.E. 1991. Rapid in situ assay for indole acetic acid production by bacteria immobilized on a nitrocellulose membrane. Applied Environment and Microbiology. 21, 613-618.

Cappuccino, J.C. and Sherman, N. 1992. In: Microbiology: A Laboratory Manual, New York, 125–179.

Chakraborty, U., Chakraborty, B. and Basnet, M. 2006. Plant growth promotion and induction of resistance in *Camellia sinensis* by *Bacillus megaterium*. Journal of basic microbiology. 46(3), 186-195.

Das, S.N., Sarma, P.V., Neeraja, C., Malati, N. and Podile, A.R. 2010. Members of *Gamma proteobacteria* and bacilli represent the culturable diversity of chitinolytic bacteria in chitin-enriched soils. World Journal of Microbiology and Biotechnology 26 (10), 1875-1881.

Dey, R., Pal, K.K., Bhatt, D.M. and Chauhan, S.M. 2006. Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. Microbiological Research 159(4), 371-394.

Dharni, S., Srivastava, A.K., Samad, A. and Patra, D.D. 2014. Impact of plant growth promoting *Pseudomonas monteilii* PsF84 and *Pseudomonas plecoglossicida* PsF610 on metal uptake and production of secondary metabolite (monoterpenes) by rose-scented geranium *(Pelargonium graveolenscv*. bourbon) grown on tannery sludge amended soil. Chemosphere. 117, 433-439.

Domenech, J., Ramos-Solano, B., Probanza, A., Lucas-Garcia, J., Colon, J.J. and Gutierrez-Manero, F.J. 2004. *Bacillus* spp. and *Pisolithus tinctorius* effects *on Quercus ilex* ssp. *ballota*: a study on tree growth, rhizosphere community structure and mycorrhizal infection. Forest Ecology Management. 194 (1), 293-303.

Dutta, J. and Thakur, D. 2017. Evaluation of multifarious plant growth promoting traits, antagonistic potential and

phylogenetic affiliation of rhizobacteria associated with commercial tea plants grown in Darjeeling, India. PloS one. 3, 12(8): e0182302.

Fuhrmann, J. J. 1994. Isolation of soil microorganisms producing antibiotic compounds. pp. 379-405. In: Methods of Soil Analysis, Part 2, Microbiological and Biochemical Properties. Weaver, R.W. (Ed.) Soil Science Society of America, Madison, Wisconsin.

George, P., Gupta, A., Gopal, M., Chandra Mohanan, R., Thomas, L. and Thomas, G.V. 2012b. In vitro antagonism of rhizospheric fluorescent pseudomonads of coconut against *Ganoderma applanatum* and *Thielaviopsis paradoxa*, fungal pathogens of coconut. Journal of Plantation Crops. 40(2), 75-81.

George, P., Gupta, A., Gopal, M., ChandraMohanan, R., Litty, T. and Thomas, G.V. 2011. Antagonistic activity of coconut rhizospheric and endophytic *Bacillus* spp. against *Ganoderma applanatum* and *Thielaviopsis paradoxa*. Journal of Plantation Crops. 39 (2), 278-284.

George, P., Gupta, A., Gopal, M., Thomas, L. and Thomas, G.V. 2013. Multifarious beneficial traits and plant growth promoting potential of *Serratia marcescens* KiSII and *Enterobacter s*p. RNF 267 isolated from the rhizosphere of coconut palms (*Cocos nucifera* L.). World Journal of Microbiology and Biotechnology. 29(1), 109-117.

George, P., Gupta, A., Gopal, M., Thomas, L. and Thomas, G.V. 2012a. Screening and in vitro evaluation of phosphate solubilizing bacteria from rhizosphere and roots of coconut palms (*Cocos nucifera* L.) growing in different states of India. Journal of Plantation Crops. 40 (1), 61-64.

Ghosh, S., Penterman, J.N., Little, R.D., Chavez, R. and Glick, B.R. 2003. Three newly isolated plant growth-promoting bacilli facilitate the seedling growth of canola, *Brassica campestris*. Plant Physiology and Biochemistry. 41 (3), 277–281.

Gopal, M., Gupta, A. and Nair, R.V. 2005. Variations in hosting beneficial plant-associated microorganisms by root (wilt)-diseased and field-tolerant coconut palms of West Coast Tall variety. Current Science. 89 (11), 10-15.

Govindasamy, V., Senthilkumar, M., Magheshwaran, V., Kumar, U., Bose, P., Sharma, V. and Annapurna, K. 2010. *Bacillus* and *Paenibacillus* spp.: potential PGPR for sustainable agriculture In: Maheshwari, D.K. (Ed.),. Plant growth and health promoting bacteria, Microbiology Monograph, 18, Springer, Berlin. pp 333–364.

Gupta, A. and Gopal, M. 1999. Plant growth-inhibiting rhizosphere microorganisms: a review. Agricultural Reviews 20(2), 117-122.

Gupta, A. and Gopal, M. 2008. Siderophore production by plant growth promoting rhizobacteria. Indian Journal of Agricultural Research. 42(2), 153-156.

Gupta, A., Gopal, M. and Tilak K.V.B.R. 2000. Mechanism of plant growth promotion by rhizobacteria. Indian Journal of Experimental Biology. 38(9), 856-862.

Gupta, A., Saxena, A.K., Gopal, M. and Tilak, K.V.B.R. 1998. Bacterization of green gram with rhizosphere bacteria for plant growth. Journal of Scientific and Industrial Research. 57, 726-731.

Gupta, A., Gopal, M. and Thomas, G.V. 2006. Bioaugmentation of *Cocos nucifera* L. seedlings with the plant growth promoting rhizobacteria, *Bacillus coagulans* and *Brevibacillus brevis* for growth

promotion. *In* Proceedings of XXXVI Conference and Annual Meeting of European Society for New Methods in Agricultural Research, Iasi, Romania, Sept. 10-14, 2006.

Indiragandhi, P., Anandham, R., Madhaiyan, M. and Sa, T.M. 2008. Characterization of plant growth-promoting traits of bacteria isolated from larval guts of Diamondback Moth *Plutella xylostella* (Lepidoptera: Plutellidae). Current Microbiology. 56(4), 327-333.

Ji, S.H., Gururani, M.A. and Chun, S.C. 2014. Isolation and characterization of plant growth promoting endophytic diazotrophic bacteria from Korean rice cultivars. Microbiological research. 169(1):83-98.

Klee, H.J., Hayford, M.B., Kretzmer, K.A., Barry, G.F. and Kishore, G.M. 1991. Control of ethylene synthesis by expression of a bacterial enzyme in transgenic tomato plants. Plant cell 3, 1187-1193.

Lucy, M., Reed, E. and Glick, B.R. 2004. Application of free living plant growth-promoting rhizobacteria. Antonie Leeuwenhoek 86 (1), 1–25.

Mantelin, S. and Touraine, B. 2004. Plant growth-promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. Journal of Experimental Botony. 55 (394), 27–34.

Mendes, R., Garbeva, P. and Raaijmakers, J.M. 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS microbiology reviews. 37(5), 634-663.

Muangthong, A., Youpensuk, S. and Rerkasem, B. 2015. Isolation and characterisation of endophytic nitrogen fixing bacteria in sugarcane. Tropical life sciences research. 26(1), 41.

Nadeem, S.M., Ahmad, M., Naveed, M., Imran, M., Zahir, Z.A. and Crowley, D.E. 2016. Relationship between in vitro characterization and comparative efficacy of plant growth-promoting rhizobacteria for improving cucumber salt tolerance. Archives of microbiology. 198(4), 379-387.

Naveed, M., Qureshi, M.A., Zahir, Z.A., Hussain, M.B., Sessitsch, A. and Mitter, B. 2015. L-Tryptophan-dependent biosynthesis of indole-3-acetic acid (IAA) improves plant growth and colonization of maize by *Burkholderia phytofirmans* PsJN. Annals of microbiology. 65(3), 1381-1389.

Otieno, N., Lally, R.D., Kiwanuka, S., Lloyd, A., Ryan, D., Germaine, K.J. and Dowling DN. 2015. Plant growth promotion induced by phosphate solubilizing endophytic Pseudomonas isolates. Frontiers in Microbiology. 6, 745.

Rajendran, L., Karthikeyan, G., Raguchander, T. and Samiyappan, R. 2007. *In vitro* evaluation of bacterial endophytes influence on *Ganoderma lucidum* (leys) karst. mycelial growth. J. Plant Protection Res. 47(4), 426-436.

Renwick, A., Campbell, R. and Coe, S. 1991. Assessment of *in vivo* screening systems for potential biocontrol agents of *Gaeumannomyces graminis.* Plant Pathol. 40, 524-532.

Saharan, B.S. and Nehra, V. 2011. Plant growth promoting rhizobacteria: a critical review. Life Sci. Med. Res. 21, 1-30.

Santoro, M.V., Bogino, P.C., Nocelli, N., Del Rosario Cappellari, L., Giordano, W.F. and Banchio, E. 2016. Analysis of plant growth-promoting effects of fluorescent *Pseudomonas* strains isolated from *Mentha piperita* rhizosphere and effects of their volatile organic compounds on

essential oil composition. Frontiers in microbiology. 7.

Schwyn, B. and Nielands, J.B. 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. 160, 47-56.

Thomas, L., Gupta, A., Gopal, M., George, P and Thomas, G.V. 2011. Efficacy of rhizospheric Bacillus spp. for growth promotion in *Theobroma cacao* L. seedlings. Journal of Plantation Crops. 39(1), 19-25.

Vargas, L.K., Volpiano, C.G., Lisboa, B.B., Giongo, A., Beneduzi, A. and Passaglia, L.M. 2017. Potential of rhizobia as plant growth-promoting rhizobacteria. In Microbes for legume improvement, Springer, Cham. pp. 153-174.

Zahir, Z.A, Arshad M. and Frankenberger, W.T. 2004. Plant growth promoting rhizobacteria: applications and perspectives in agriculture. Adv. Agron. 81, 97-168.

How to cite this article:

Priya George, Alka Gupta, Murali Gopal, Litty Thomas and George V. Thomas. 2018. Systematic Screening Strategies for Identifying Elite Plant Growth Promoting Rhizobacteria for Coconut (*Cocos nucifera* L.). *Int.J.Curr.Microbiol.App.Sci.* 7(05): 1051-1074.
doi: https://doi.org/10.20546/ijcmas.2018.705.130