Research Article *Journal of Plantation Crops, 2010, 38 (2): 97-104*

Plant growth promoting potential of *Bacillus* spp. isolated from rhizosphere of cocoa (*Theobroma cacao* L.)

Litty Thomas, Alka Gupta*, Murali Gopal, Priya George, George V. Thomas†

Microbiology Section, Central Plantation Crops Research Institute,
Kudlu P.O., Kasaragod - 671 124, Kerala

(Manuscript Received: 04-01-10, Revised: 03-01-10, Accepted: 17-05-10)

Abstract

A total of 185 bacilli were isolated from rhizosphere soils of cocoa from various locations in southern states of India and they were subsequently screened *in vitro* for plant growth promoting (PGP) traits such as production of IAA, ACC deaminase, HCN, siderophore, chitinases and antibiotics, ammonification, ability to grow on N-free media and solubilization of phosphates. It was found that 91 % of the *Bacillus* spp. isolates produced ammonia; less than 20 % isolates produced HCN and siderophore; IAA production was noticed in 22 % isolates and 19 % isolates were found to produce antibiotics. About 49 % of 185 *Bacillus* isolates solubilized phosphates; 50 % of isolates could grow on N-free medium and 71 % of isolates showed ACC deaminase production. The isolates could exhibit upto six PGP traits, which may promote plant growth directly or indirectly or synergistically. Thirty six isolates were selected based on the *in vitro* plant growth promotion potential and were further screened for plant growth promotion under Environmental Plant Growth Chamber (EPGC) conditions. Thirty isolates which showed increase in seedling length in EPGC study were further screened for *in vivo* growth promotion in cowpea seedlings under greenhouse conditions. Out of 30 isolates tested, 11 were found to increase seedling length and fresh and dry weight of cowpea seedlings when compared to the uninoculated control. The results of this study points to the potential of rhizospheric soil bacilli for plant growth promotion. Further studies are necessary to confirm their effectiveness and potential in the field.

Keywords: Bacillus spp., plant growth promoting (PGP) traits, *Theobroma cacao*

Introduction

Cocoa is a commodity produced in the developing countries of the tropics and consumed mostly in the middle-and high-income countries of the world's temperate zones. Cocoa is now grown in some 50 tropical countries, with smallholder farmers growing most of the world's 3 million tons of annual cocoa production (Lass, 2004). Continuous and excess use of chemical fertilizers and other agrochemicals to increase yield may lead to many ill effects on soil and environment, eventually resulting in reduction of crop yield. Biofertilizers from microorganisms can partially replace chemical fertilizers to increase crop production. In principle, biofertilizers are less expensive and are more environmentally friendly than chemical fertilizers.

It has been long recognized that many naturally occurring rhizospheric bacteria and fungi are plant growth promoting and may offer a viable substitute for chemicals. Plants play an important role in selecting and enriching the type of bacteria by the constituents of their root exudates. Most popular bacteria studied and exploited as plant growth promoting rhizobacteria (PGPR) which include the species of fluorescent *Pseudomonas* and *Bacillus*. Numerous *Bacillus* strains display plant growth-promoting activities and have been investigated due to their widespread distribution in soil, ability to colonize the rhizospheres of host plants, and ability to produce a range of plant growth promoting traits. Application of *Bacillus* species to seeds or roots has been shown to cause alteration in the composition of rhizosphere leading to increase in growth and yield of different crops.

Screening for the selection of effective PGPR strains is very critical to achieve improved growth and

†*Director, *E mail: agcpcri@yahoo.co.in*

yield of agricultural crops using microbial inoculant technology. Recent research has identified isolates of many endophytic fungi especially *Trichoderma* species (Bae *et al*., 2009) and *Bacillus* spp. (Rachel *et al*., 2008) that are endophytic on cocoa including above ground tissues. PGPR based products with strains of *Bacillus* are more successful in the field due to the resistant spore forming characteristics. Therefore, *Bacillus* spp. can be applied as booster inoculants to increase the efficacy of plant growth promotion and elicitation of systemic disease protection in the field (Kloepper *et al.,* 2004). The present studies were undertaken to characterize *Bacillus* species from the rhizosphere of healthy cocoa plants and to study their plant-growth promotion effects by inoculating the rhizobacteria on test crop of cowpea.

Materials and Methods

Isolation of rhizobacteria

Rhizosphere soil samples were collected from cocoa plants (Forastero variety) of various locations in Southern states of India. From each field site, five rhizospheric cocoa-growing soils were randomly selected and a composite sample of rhizosphere soil (roots + adhering soil) was collected. From each sample, 10g of soil was aseptically weighed and transferred to 90 ml of sterile distilled water, which was shaken for 30 min at 180 rpm and then heated on water bath at 80 ^{0}C for 20 min for isolating endospore forming *Bacillus* spp. A series of 10-fold dilutions were made for each sample. Later they were spread and pour plate were made on nutrient agar and incubated at 30 ^{0}C for 48-96 h. Colony counts were taken and morphologically different colonies were selected, picked for purification and purified colonies were stored at 4 ^{0}C.

In vitro assessment of *Bacillus* spp. for their plant growth promoting potential

The ability to produce siderophore, solubilize phosphate and production of chitinase were determined in terms of change in colour of the specific dye and clearance zone around the colony, respectively. Growth of bacteria in DF salts minimal medium supplemented with 3 mM ACC and Jensen's agar medium were taken as the presence of aminocyclopropane-1-carboxylate (ACC) deaminase activity and nitrogen fixation ability. The HCN and IAA production were determined by change in colour of the indicator.

i) Siderophore production ability

Ability of the rhizobacterial isolates to synthesize siderophore under iron limiting conditions was assessed by universal chemical assay (Schwyn and Neilands, 1987). Rhizobacteria plated on blue agar CAS medium were incubated at 30 ^{0}C. Siderophore activity was detected after 24 h in terms of the intensity of colour change (blue to orange-yellow).

ii) Phosphate solubilization ability

This was detected by means of a plate assay using Pikovskaya (PVK) agar, which results in formation of a clear halo. Bacterial growth and clearing of turbidity were taken as measures of phosphate solubilization and diameter of the activity zone was recorded.

iii) 1-Aminocyclopropane-1-carboxylate (ACC) deaminase production

ACC-deaminase production was determined by assessing the ability of rhizobacterial isolates to utilize ACC as sole source of N. The isolates were spot inoculated in DF salts minimal medium supplemented with 3 mM ACC (Harry *et al*., 1991). Growth of bacteria in media was taken as the presence of ACC- deaminase activity.

iv) Production of ammonia

Bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 ml peptone water in each tube and incubated for 48–72 h at 30 ^{0}C. Development of brown to yellow colour in the presence of Nessler's reagent was a positive test for ammonia production.

v) HCN production

All the isolates were screened for the production of hydrogen cyanide by adapting the method of Bakker and Schippers (1987). Briefly, nutrient agar amended with 4.4 g glycine/l and bacteria were streaked. A Whatman No.1 filter paper soaked in 1 % ammonium carbonate in 0.5 % picric acid solution was placed on the top of the plate. Plates were sealed with parafilm and incubated at 30 ^{0}C for 7 days. Development of orange to red colour indicated HCN production.

vi) Assay for indole-acetic acid (IAA) production

For detection of IAA, the method of Brick *et al*. (1991) was slightly modified and standardized. Isolates were streaked on LB medium containing 5mM L-Tryptophan. Sterile Whatman No.1 filter paper was placed on inoculated plate. After 3 days of incubation, the filter paper was transferred to another petriplate containing Salkowsky-reagent saturated filter paper. Development of a pink colour within 30 min on filter paper was considered as positive result. The intensity of colour was noted.

vii) Ability to grow on nitrogen-free medium

Growth on N-free medium was determined by using Jensen's medium (HiMedia).

viii) Chitinase production

Production of chitinase enzyme was determined as described by Renwick *et al*. (1991) on a defined medium by using colloidal chitin as substrate. Presence of clearing zone was recorded as positive result.

ix) Antibiotic production

The capability of isolates to produce antibiotic was studied using agar well method. Supernatant of the culture was added to the wells made on TSA plates, which already had been spread plated with appropriate soil dilution. Presence of inhibition zone around the well was recorded after 48 h of incubation.

Environmental Plant Growth Chamber (EPGC) Assay

Cocoa isolates were tested on short duration test plant cowpea for determining their plant growth promoting potential. Cowpea seeds were surface sterilized by 0.1 % $HgCl_2$ for four min and washed with sterile distilled water five times. Surface sterilized seeds were allowed to dry in airflow for a few minutes. Bacterization of the seeds was achieved by soaking seeds in broth for 10 min. Seeds treated with sterile broth served as untreated control. Seeds were aseptically transferred to petriplates with soft agar. Five seeds were sown in each Petri plate with two repeats. Observations were taken on the 7^{th} day. Seedling bioassays were done in a growth chamber, which was maintained at 25 ^{0}C, 85 % humidity with 10 h light and 14 h dark cycles. Increases in seedling length and fresh weight compared to control were measured.

Green house studies

For the evaluation of growth promotion under green house conditions, seeds were sown in plastic cups filled with unsterile soil: sand mixture (3:1 ratio). PGPR treated and an untreated control in each treatment was maintained. The inoculation treatments were set up in a completely randomized design with 20 replicates/culture. Four surface sterilized seeds of cowpea were sown per cup. The rhizobacteria were grown in NB medium and inoculation was carried out by soil drench with 1 ml of a bacterial suspension, which resulted in inoculam density of 10^6 cfu /ml. After germination, plants were thinned to two per pot. Plants were kept under natural photoperiod (16 h light/8 h dark) in the greenhouse. Twenty days after inoculation, seedlings were harvested and seedling length, fresh weight and dry weight were measured.

Result and Discussion

***In vitro* assessment of *Bacillus* spp. for their plant growth promoting potential**

A total of 59 samples were collected from different locations in south India and 185 isolates of endospore forming bacteria (54 isolates from Kerala, 80 from Karnataka, 33 from Tamil Nadu and 18 from Andhra Pradesh) were obtained (Table 1).

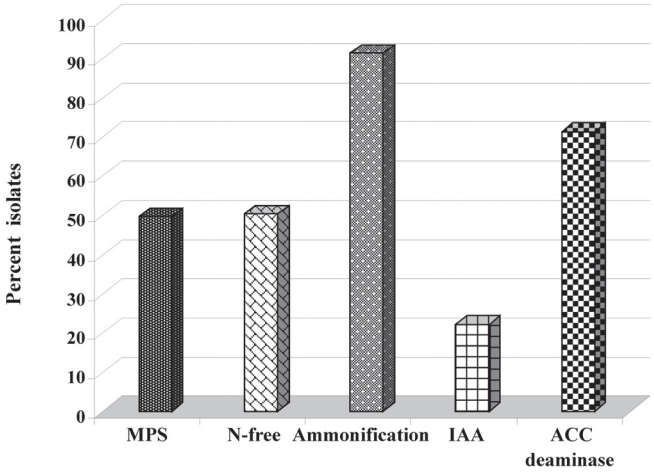
Table 1. Details of *Bacillus* spp. isolated from cocoa rhizosphere

State	Name of place	Total no. of *Bacillus* spp. obtained
Kerala	Kasaragod	29
	Wayanad	12
	Kozhikode	13
	Sub-total	54
Karnataka	Tumkur	46
	Kidu	17
	Vittal	17
	Sub-total	80
Tamil Nadu	Coimbatore	21
	Pollachi	12
	Sub-total	33
Andhra Pradesh	Ambajipetta	18
Total		185

Qualitative phosphate solubilization activity was verified for all 185 isolates and 49 % strains studied were able to solubilize phosphate in the Pikovskaya's agar. Phosphorus, one of the main nutrients limiting plant growth, is rapidly immobilized after addition to soil as a soluble fertilizer, becoming unavailable to the plant. Therefore, bacterial activity is highly important with respect to supplying plants with phosphorus. *Bacillus spp*. is known to promote plant growth by phosphate solubilization (Hariprasad *et al*., 2009). The results in the solubilization of inorganic phosphates by *Bacillus* spp. in the present study corroborate the earlier reports (Hariprasad *et al*., 2009; Ahmad *et al*., 2008). Test for nitrogen fixing bacteria to improve the plants nitrogen nutrition showed that 50 % of the *Bacillus* spp. could grow on N-free medium. Ammonification, an important step in the transformation of organic nitrogen to ammoniacal form, would enhance soil nitrogen content by the ammonifying character of the PGPR isolates. Production of ammonia was common among the tested isolates (91 % of *Bacillus* spp.). ACC-deaminase activity of PGPR strains is known to enhance the root length and growth by sequestering and hydrolyzing ACC from germinating seeds and thereby increasing the active

rhizosphere zone. Test for their ability to produce ACC deaminase revealed that almost 71 % of the *Bacillus* spp. were able to synthesize ACC deaminase. Similar findings were reported by Raddadi *et al.* (2008). Qualitative phenotypic test for ACC deaminase activity in *Bacillus thuringiensis* showed that seven strains are able to grow on salt minimal medium containing ACC as sole nitrogen source, indicating the expression of the *accd* genes. *Bacillus* spp. can also promote plant growth by producing the phytohormone IAA. IAA increases root size and distribution, resulting in greater nutrient absorption from the soil. When screened for auxin production, 22 % of *Bacillus* spp. produced IAA in the presence of tryptophan. The root exudates of various plants contain rich supplies of tryptophan which can be utilized by microorganisms for synthesis and release of auxins as secondary metabolites in the rhizosphere. Auxin production potential of plant associated *Bacillus* spp. was reported (Ahmad *et al*., 2008).

Other important traits of PGPR that may indirectly influence the plant growth, tested in this study were the production of siderophore, HCN, chitinase and antibiotics. Less than 20 % of *Bacillus* spp. produced HCN, siderophore and antibiotics. Siderophores bind to the available form of iron (Fe^{3+}) in the rhizosphere, thus, making it unavailable to the phytopathogens and protecting the plant health. Production of fungal cell wall degrading enzyme was analyzed because this is an important mechanism of fungal inhibition. Forty three isolates (23 % of *Bacillus* spp.) were found to produce chitinase, a fungal cell wall degrading enzyme. Screening results of PGP traits are depicted in Fig. 1 and 2. It was found that higher percentage of *Bacillus* spp. from cocoa rhizosphere exhibited direct PGP traits like phosphate



MPS - mineral phosphate solubilization, N-free- growth on N-free agar medium, IAA - indole acetic acid, ACC- amino cyclopropane -1- carboxylate (ACC) deaminase

Fig. 1. Direct plant growth promoting activities of test isolates

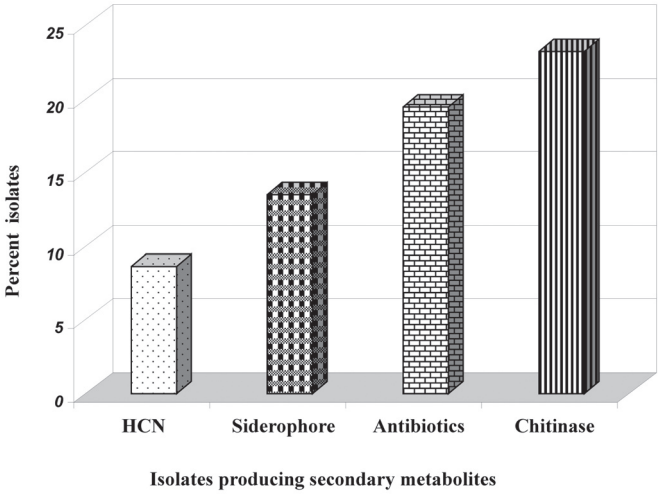


Fig. 2. Indirect plant growth promoting activities of test isolates

solubilization, ammonification, ability to grow on N-free media and ACC deaminase (≥ 50 %), whereas less than 25 % of *Bacillus* spp. showed indirect PGP traits such as production of HCN, siderophore, chitinases and antibiotics. Among the direct PGP traits less number of isolates 41 out of 185 (22 % of *Bacillus* spp.) showed IAA production.

In the present study, it was found that the bacilli isolated from the cocoa rhizosphere were functionally diverse and possessed more than one plant growth promoting traits. Difference in the production of PGPR traits were observed among the isolates and it may be attributed to the various biosynthetic pathways or mechanisms, location of the genes involved, regulatory sequences and also dependent on environmental conditions. All the 185 *Bacillus* spp. were assessed and scored based on *in vitro* characterization and 36 *Bacillus* spp., having combination of PGP activities (score of 9 and above) were selected (Table 2). The isolates could exhibit upto six PGP traits, which may promote plant growth directly or indirectly or synergistically. The maximum score was acquired by two isolates, *Bacillus* sp. ASB 12 (from Andhra Pradesh) and *Bacillus* sp. CSB 17 (from Tamil Nadu). Both the isolates could solubilize phosphate, exhibit growth on N- free media and were capable of producing ACC deaminase, ammonia, siderophore and antibiotics.

Environmental Plant Growth Chamber (EPGC) Assay

Thirty six *Bacillus* spp. from the rhizosphere of cocoa, selected based on their performance in plant growth promoting traits, were further tested on cowpea seeds for their effect on seedling growth by measuring shoot and root length *in vitro* in growth chamber (Table 3;

Table 2. Scoring of *Bacillus* spp. based on their plant growth promoting traits

State	Name of place	Total no. of *Bacillus* spp. tested	Number of *Bacillus* spp. falling in different assessment range in *in vitro* characterization			
			1-4*	5-8	9-12	13-16
Kerala	Kasaragod	29	11	11	7	0
	Wayanad	12	8	3	1	0
	Kozhikode	13	7	5	1	0
	Sub-total	54	26	19	9	0
Karnataka	Tumkur	46	25	19	2	0
	Kidu	17	9	6	1	1
	Vittal	17	7	10	0	0
	Sub-total	80	41	35	1	1
Tamil Nadu	Coimbatore	21	2	7	10	2
	Pollachi	12	3	6	3	0
	Sub-total	33	5	14	13	2
Andhra Pradesh	Ambajipetta	18	2	8	7	1
Total		185	74	75	32	4
No. of *Bacillus* spp. selected for EPGC screening				36		

*Assessment score given to each PGPR isolate based on the results of *in vitro* characterization for PGP traits

EPGC- Environmental Plant Growth Chamber

Fig. 3). Thirty *Bacillus* spp. induced significant effects on any one of the tested parameters i.e., shoot length or root length as compared with non-treated control. Eighteen isolates showed increase in total seedling length, whereas 12 isolates showed increases in either shoot length or root length. *Bacillus* sp. TSB 15, an isolate from Karnataka, showed 57 % increase in seedling length. Based on the selection criterion of concomitant increase in the tested parameters, 30 *Bacillus* spp. were considered promising. The good results obtained *in vitro* could not be dependably reproduced under *in vivo* conditions. The variability in the performance of PGPR may be due to various environmental factors that may affect the growth

Table 3. Screening of *Bacillus* spp. for growth promotion under EPGC conditions

State	*Bacillus* spp. tested	No. of *Bacillus* spp. showing increase in		
		Shoot length	Root length	Total seedling length
Kerala	9	1	2	1
Karnataka	4	0	1	3
Tamil Nadu	15	4	3	8
Andhra Pradesh	8	1	0	6
Total	36	6	6	18
No. of isolates selected for green house studies			30	

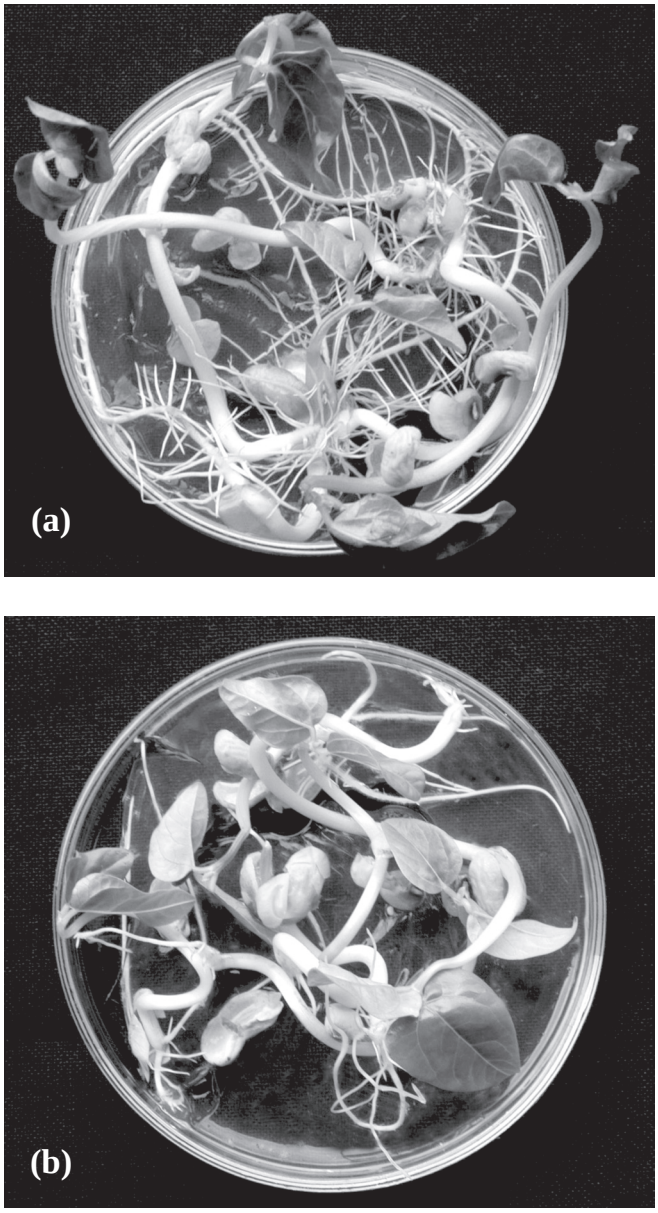


Fig. 3. Cocoa isolate (*Bacillus* sp. CSB 17) showing enhanced root development of cowpea seedlings (a) as compared to uninoculated control (b) under Environmental Plant Growth Chamber conditions

and exert their effect on the plant. The selected *Bacillus* spp. were further screened under greenhouse conditions.

Green house studies

Growth promotion efficiency of selected isolates (selection made on the basis of assessment score obtained in *in vitro* characterization for plant growth promoting traits and seedling bioassay) were evaluated under greenhouse condition on test crop, cowpea. The growth promotion by bacilli was assessed by treating the cowpea seeds with each bacterial isolates separately using the suspension. In general, most of the isolates tested (27 out of 30 *Bacillus* spp.), showed positive growth responses for the parameters measured under green house

conditions compared with the non-bacterized controls (Table 4). Eleven *Bacillus* spp. (Isolates CSB 8, CSB 11, CSB 14, CSB 16, CSB 17, CSB 20, PSB 1, ASB 3, KDSB 3, TSB 15, TSB 17) showed increases in all the three parameters i.e., seedling length, fresh weight and dry weight. Seven *Bacillus* spp. increased seedling length and fresh weight, two *Bacillus* spp. showed increase in fresh weight and dry weight. One *Bacillus* sp. promoted only the seedling length and four could enhance only the seedling dry weight. Three isolates did not show any response on the growth parameters measured under green house conditions. *Bacillus* sp. KGSB 1, isolated from Kasaragod, Kerala, showed maximum of 38 % increase in dry weight. Maximum increase in fresh weight (31 %) was showed by *Bacillus* sp. KDSB 3, an isolate from Kidu, Karnataka. The highest increase in shoot length was obtained upon inoculation with *Bacillus* sp. KDSB 14 (20 % increase over control), isolated from the same location (data not shown). Analysis of the data obtained from green house studies showed that all the selected 11 *Bacillus* isolates, which showed significant

Table 4. Plant growth promoting effects of selected *Bacillus* spp. under green house conditions

Total no. of isolates tested	No. of isolates showing increases in							Negative for all parameters
	SL	FW	DW	SL + FW	SL + DW	FW + DW	SL+ FW +DW	
30	1	2	4	7	0	2	11	3

SL - Seedling length, FW - Fresh weight, DW - Dry weight

effect in green house studies, were positive for ammonification and produced ACC deaminase in *in vitro* studies (Table 5). Ten *Bacillus* spp. could solubilize inorganic phosphate, eight *Bacillus* spp. produced antibiotics, five isolates exhibited growth on nitrogen free medium and chitinase production. Four isolates produced siderophores. Three isolates produced HCN and one showed the ability to produce IAA. An incremental effect on growth observed in cowpea on inoculation with rhizobacteria indicated a broad spectrum plant growth promoting activity by the isolates. All the traits of PGPR might not get expressed at a given point of time. Thus, the more the presence of the PGPR traits in an individual organism, the more the chances of the organism being a successful inoculant strain. Similar multiple PGP activities among PGPR have been reported earlier (Ahmad *et al*., 2008; Farah *et al*., 2006).

The results of our study under green house condition showed an increase in seedling length of cowpea (upto 20 %), fresh weight (upto 31 %) and dry weight (upto 38 %) in response to inoculation with selected *Bacillus* spp. The plant growth stimulation effect of the bacilli tested can be explained not only by their ACC-deaminase activity and/or P-solubilization ability, but also by their ability to grow in N-free conditions, ammonification and IAA production. Ghosh *et al*. (2003) reported that soil inoculation with ACC-deaminase producing *Bacillus circulans*, DUC1, *Bacillus firmus* DUC 2 and *Bacillus globisporus* DUC3 increased the root and shoot length and fresh and dry weight of potted canola plants. Trials with rhizosphere associated plant growth promoting N_2 fixing and p-solubilizing *Bacillus* spp. indicated yield increases in rice, barley (Cakmakci *et al*., 2001 and 2007), wheat (Cakmakci *et al*., 2007), canola (De Freitas, 1997), apples (Aslantas *et al*., 2007), tomato (Hariprasad *et al*., 2009). The possible role of phosphate solubilization by PGPR isolates in enhancing the cowpea growth in this study is further substantiated by the fact that all isolates selected from green house assay were positive for P- solubilization under *in vitro* studies. Soil inoculation with phosphate-solubilizing *Bacillus* spp. can solubilize fixed soil P and applied phosphates resulting in a better plant development and higher yields (Canbolat *et al*., 2006). Biological nitrogen fixation may be facilitated further by the enhancement in the active root zone by increase in the root length and growth due to ACC- deaminase activity of the inoculant strains and thus enhanced nutrient mobilization, capture, harvest and uptake by the plant from the limited pool of nutrients in the rhizosphere (Dey *et al*., 2004). N_2- fixing bacteria inoculation significantly increased uptake of N, Fe, Mn and Zn by barley seedling as compaired to the uninoculated control (Cakmakci *et al*., 2007). Root and shoot weights were enhanced by IAA producing *Bacillus* strains isolated from different wild plant species (Ali *et al*., 2009). Application of IAA to P- deficient plants increased root surface area, carbohydrate release and acid phosphatase activity (Aslantas *et al*., 2007).

Other attributes like HCN, chitinase, antibiotics and siderophore production etc. reduce the plant mortality and disease severity by suppressing root pathogens and thus enhancing the plant growth. Several studies have demonstrated that production of siderophore, other secondary metabolites and lytic enzymes were most effective in controlling plant root pathogens (Ahmad *et al*., 2008). In natural soil environments where microbial species compete through siderophore-mediated iron uptake, an organism able to utilize siderophores produced

Table 5. Functional characteristics of selected *Bacillus* spp. from the cocoa rhizosphere

PGPRs	Characterization for PGP traits									Growth Chamber assay (% increase over untreated control)	Green house assay (% increase over untreated control)		
	Side-rophore (zone in mm)	HCN	P- solubi-lization (zone in mm)	Growth on N-free medium	Ammoni-fication	IAA	Chitinase (zone in mm)	ACC deaminase	Antibiotics (zone in mm)	Seedling length	Seedling length	Fresh weight	Dry weight
Tamil Nadu isolates													
Bacillus sp. CSB 8	6	-	6	+	+	-	-	+	6	0	8	25	8
Bacillus sp. CSB 11	-	-	6	-	+	-	7	+	6	0	11	15	23
Bacillus sp. CSB 14	-	-	6	+	+	-	-	+	5	9	5	7	2
Bacillus sp. CSB 16	-	+	-	+	+	-	-	+	6	47	13	20	25
Bacillus sp. CSB 17	11	-	6	+	+	-	-	+	5	40	2	13	5
Bacillus sp. CSB 20	-	-	6	-	+	-	6	+	7	0	10	14	29
Bacillus sp. PSB 1	4	-	6	-	+	-	6	+	-	13	6	19	5
Andhra Pradesh isolates													
Bacillus sp. ASB 3	-	-	6	-	+	-	8	+	5	0	5	7	2
Karnataka isolates													
Bacillus sp. KDSB 3	14	-	9	+	+	-	-	+		28	17	31	7
Bacillus sp. TSB 15	-	+	6	-	+	-	6	+	3	57	6	13	2
Bacillus sp. TSB 17	-	+	6	-	+	+	-	+	-	38	8	18	5

- = no activity, + = positive result, mm = radius in mm

by other organisms may be more competitive in terms of survivability and functioning. Geetha *et al*. (2008) reported that co- inoculation of siderophore producing *Bacillus* isolates with *Rhizobium* spp. enhanced nodulation of pigeon pea under iron-starved conditions.

Cook (1993) suggested that microorganisms isolated from rhizoplane or rhizosphere of a specific crop may be better adapted to that crop and may have greater impact on the health status of the crop than organisms originally isolated from other crops. The multiple plant growth promoting activities and incremental growth influence of some of the promising *Bacillus* spp. in the test plant appears potentially promising for further testing as a bioinoculant in the cocoa plant, where conditions are much more complex than those prevailing *in vitro.* The chances of successful application of isolates would appear high, as it is to be deployed in the same milieu from where it has been isolated.

Acknowledgements

The authors thank ICAR, New Delhi for funding the Network Project on 'Application of Microorganisms in Agriculture and Allied Sectors' in which this work was carried out. Litty Thomas is grateful for the award of Senior Research Fellowship.

References

Ahmad, F., Ahmad, I. and Khan, M.S. 2008. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol. Res*. **163**: 173-181.

Ali, B., Anjum, N.S., Karin, L. and Shahida, H. 2009. Quantification of indole-3-acetic acid from plant associated *Bacillus* spp. and their phytostimulatory effect on *Vigna radiata* L. *World J. Microbiol. Biotech*. **25**: 519-526.

Aslantas, R., Cakmakcy, R. and Sahin, F. 2007. Effect of plant growth promoting rhizobacteria on young apple tree growth and fruit yield under orchard conditions. *Sci. Hort*. **111**: 371-377.

Bae, H., Richard, C.S., Moon, S.K., Soo-Hyung, K., Mary, D.S., Rachel, L.M. and Bailey, B.A. 2009. The beneficial endophyte *Trichoderma hamatum* isolate DIS 219b promotes growth and delays the onset of the drought response in *Theobroma cacao. J. Exp. Bot.* **60**: 3279-3295.

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 reduction. *Soil. Biol. Biochem*. **19**: 452-458.

Brick, J.M., Bostock R.M. and Silverstone S.E. 1991. Rapid *in situ* assay for indoleacetic acid production by bacteria immobilized

on a nitrocellulose membrane. *Appl. Environ. Microbiol*. **57**: 535-538.

Cakmakci, R., Kantar, F. and Sahin, F. 2001. Effect of N_2-fixing bacterial inoculations on yield of sugar beet and barley. *J. Plant Nutr. Soil Sc.* **164**: 527-531.

Cakmakci, R., Mesude ,F. D. and Ummugulsum, E. 2007. The effect of plant growth promoting rhizobacteria on barley seedling growth, nutrient uptake, some soil properties, and bacterial counts. *Turk. J. Agric. For*. **31**: 189-199.

Canbolat, M.Y., Bilen, S., Cakmakci, R., ªahin, F. and Aydin, A. 2006. Effect of plant growth-promoting bacteria and soil compaction on barley seedling growth, nutrient uptake, soil properties and rhizosphere microflora. *Biol. Fertil. Soil*. **42**: 350-357.

Cook, R.J. 1993. Making greater use of introduced microorganisms for biological control of plant pathogens. *Annu. Rev. Phytopathol*. **31**: 53-80.

De Freitas, J.R., Banerjee, M.R. and Germida, J.J. 1997. Phosphate solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola *(Brassica napus* L.). *Biol. Fertil. Soil*. **24**: 358-364.

Dey, R., Pal, K.K., Bhatt, D.M. and Chauhan S.M. 2004. Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. *Microbiol. Res*. **159**: 371-394.

Farah, A., Iqbal, A. and Khan, M.S. 2006. Screening of free-living rhizospheric bacteria for their multiple plant growth-promoting activity. *Microbiol. Res.* **63**: 11-19.

Geetha, R., Sing, F., Desai, A.J. and Archana, G. 2008. Enhanced growth and nodulation of pigeon pea by co-inoculation of *Bacillus* strains with *Rhizobium* spp. *Biores. Tech*. **99**: 4544-4550.

Ghosh, S., Jon, N.P., Rebecca, D.L., Rocky, C. and Bernard, R.G. 2003. Three newly isolated plant growth promoting bacilli facilitate the seedling growth of canola, *Brassica campestris. Plant Physiol. Biochem*. **41**: 277-281.

Hariprasad, P., Navya, H.M., Chandra Nayaka, M. and Niranjana, S.R. 2009. Advantage of using PSIRB over PSRB to improve plant health of tomato. *Biol. Control* **50**: 307-316.

Harry, J.K., Maria, B.H., Keith, A.K., Gerard, F.B. and Ganesh, M.K. 1991. Control of ethylene synthesis by expression of a bacterial enzyme in transgenic tomato plants. *The Plant Cell* **3**: 1187-1193.

Kloepper, J.W., Ryu, C. and Zhang, S. 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathol.* **94**: 1259-1266.

Lass, T. 2004. Balancing cocoa production and consumption. pp. 8–15. In: *Cocoa* futures—a source book on some important issues facing the cocoa industry. (Eds). Flood, J., Murphy, R., CABI-*FEDERACAFE* , USDA, Chinchina´, Colombia.

Rachel, L.M., Nina, K.Z., Bryan, A.B., Siela, N.M., Mark, G. and Paul, A.B. 2008. Bacterial endophytes: *Bacillus* spp. from annual crops as potential biological control agents of black pod rot of cacao. *Biological Control* **46**: 46-56.

Raddadi, N., Cherif, A., Boundabous, A. and Daffonchio, D. 2008. Screening of plant growth promoting traits of *Bacillus thuringiensis. Ann. Microbiol.* **58**: 47-52.

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

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