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Efficacy of rhizospheric *Bacillus* spp. for growth promotion in *Theobroma cacao* L. seedlings

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Abstract

Cocoa (*Theobroma cacao* L.) trees harbour a diverse microbial communities of epiphytic, rhizospheric and endophytic organisms, many possessing plant growth promoting abilities. Since plant growth promoting rhizobacteria (PGPR) can significantly promote plant health and sustain agriculture by a variety of mechanisms, their potential for use in cocoa crop can be exploited. PGPR based products with strains of *Bacillus* are more successful in the field due to the fact that they produce spores which offer them ability to tolerate wide range of biotic and abiotic stress. This paper highlights the screening of selected *Bacillus* spp., isolated from the rhizosphere of cocoa growing in different agro-climatic and soil types in Kerala, Karnataka, Tamil Nadu and Andhra Pradesh, on the growth parameters of cocoa seedlings when grown in polybags. Statistically significant increase (P = 0.05) over the control was observed in the tested parameters such as total seedling length (up to 37 %), total fresh weight (up to 73 %) and total dry weight (up to 56 %) of cocoa seedlings when they were inoculated with *Bacillus* spp. The overall improvement in seedling vigour through a significant increase in various growth parameters indicated that the *Bacillus* strains (*Bacillus* sp. ASB3, ASB12, CSB8, CSB16 and CSB17) had positive and effective plant growth promoting ability on cocoa seedlings. In addition to its plant growth promotion abilities the potential PGPR *Bacillus* spp. (isolates CSB8, CSB16, CSB17 and ASB12) also showed high abiotic stress tolerance, growing at higher temperatures (> 50 ^{0}C) and salt concentrations (10 % NaCl). These PGPRs were identified according to Bergey's Manual of Determinative Bacteriology and confirmed by Biolog® GEN III microplate identification system. The results of this study points to the potential of rhizospheric *Bacillus* spp. to enhance growth and vigour of cocoa seedlings when grown in polybags.

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

Introduction

Theobroma cacao is a small tree native to forest of north-central South America. In India, this crop is commonly grown as inter crop in coconut and arecanut cropping system in an area of 34049 thousand hectares in Kerala, Karnataka, Tamil Nadu and Andhra Pradesh (DCCD web portal). The cocoa beans fetch good remuneration to farmers as it is used for producing high-end confectionary and beverage items. The production capacity in India is unable to cater to the local market and every year there is an increased import of cocoa beans and its by-products. This situation offers a good scope for improving the productivity of this crop through various means. One of the less studied aspect in cocoa, in India, is the use of microorganisms as plant growth promoting agents. Elsewhere, it has been reported that inoculation with the VAM fungi *Scutellospora calospora* to the cocoa seedlings along with palm oil mill effluent has significantly increased nutrient uptake and plant growth in unsterilized Oxisol and Ultisol soil types (Azizah Chulan, 1991).

Cocoa trees support a diverse microbial communities (Bopaiah and Shetty, 1991) that includes nitrogen fixing bacteria, plant growth promoting actinomycetes (Barreto *et al*., 2008) in their rhizosphere. Certain strains are referred to as plant growth-promoting

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rhizobacteria (PGPR), which can be used as inoculant biofertilizers (Kennedy *et al*., 2004). In addition to improvement of plant growth, PGPR are directly involved in increased uptake of nitrogen, synthesis of phytohormones, solubilization of minerals such as phosphorus, and production of siderophores that chelate iron and make it available to the plant root (Glick, 1995; Bowen and Rovira, 1999). PGPR based products with strains of *Bacillus* are more successful in the field due to the resistant spore forming characteristics. Diverse populations of *Bacillus* spp. are denizen of agricultural fields and may directly and indirectly contribute to crop productivity. *Bacillus* strains increased total bacteria and PSB population, root and shoot dry weight, as well as total N and P uptake by plants (Canbolat *et al*., 2006).

Beneficial effects of *Bacillus* spp. have been reported in many crops, including horticultural, oil seed crops, etc. but in cocoa report are scanty. In cocoa previous research has focused mainly on the biological control of cocoa pathogens and so far very limited attempts have been made to evaluate the promotion of seedling growth upon PGPR inoculation. Bae *et al.* (2009) reported that colonization of cocoa seedlings by *Trichoderma hamatum* isolate DIS 219b enhanced growth and delays the onset of the drought response in *Theobroma cacao*. Therefore, the present study was undertaken to investigate the effect of selected rhizospheric *Bacillus* spp. on the growth parameters of cocoa seedlings when grown in polybags.

Materials and Methods

Selection of potential isolates

All *Bacillus* spp. (*Bacillus* sp. ASB3, ASB12, CSB8, CSB16, CSB17, TSB 15, TSB 17 and KDSB3) used in this present study were selected from a collection of *Bacillus* spp. isolated from the rhizosphere of *Theobroma cacao* L. growing in different agro-climatic zones of Kerala, Karnataka, Tamil Nadu and Andhra Pradesh. These *Bacillus* spp. were reported to possess multiple plant growth promoting traits such as phosphate solubilization, growth on nitrogen free medium, ability to produce siderophores, indole-3-acetic acid (IAA), 1-aminocyclopropane-1-carboxylate (ACC) deaminase, chitinase and antibiotics (Litty *et al*., 2010).

Stress tolerance of the plant growth promoting *Bacillus* spp.

Preliminary experiments were conducted to study stress responses of the *Bacillus* spp. with in respect of growth temperature, growth pH and salt (NaCl) concentration. The ability of the isolates to grow in diverse temperature range was carried out by growing each bacterial isolate on TSA agar plates and incubated separately at different temperatures i.e. 4, 15, 30, 40, 45, 50, 55 and 60 °C. The ability of the isolates to tolerate alkaline or acid pH was studied by growing the bacteria at 28±2 ^{0}C in Trypticase Soy Broth (TSB), prepared in buffers with different pH from 4.2 to 9.0 (4.2, 5.2, 6.2, 7.2, 8.2 and 9.0). The intrinsic resistance of the *Bacillus* spp. against salinity was evaluated by observing the growth on TSA medium amended with various concentrations of NaCl (2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 %).

Evaluation of plant growth promotion ability of the selected *Bacillus* spp.

For evaluation of growth promotion under polybag conditions seedlings were treated with fresh suspension of respective *Bacillus* spp. Experiments were conducted using one month old healthy seedlings of cocoa (Bulk Forastero) obtained from CPCRI Region Station, Vittal, Karnataka. *Bacillus* spp. were inoculated in their respective broths and incubated for 48-60 h so as to reach 10^8 cells ml^{-1}. This culture broth was used as inoculum for cocoa seedlings. 1-2 month old cocoa seedlings were transferred to polybags containing potting mixture (soil, vermicompost and sand in the ratio 3:1:1 at a rate of 10 kg per polybag). Each seedling was inoculated with 500 ml of culture broth. A completely randomized design was employed with 20 replicates per treatment. Cocoa seedlings with uninoculated broth served as control. 500 ml of respective *Bacillus* sp. were applied as booster dose after three months of planting. Moisture was maintained by watering at regular intervals. At the end of the experimental period (six months), the cocoa seedlings were uprooted and growth parameters such as number of leaves, roots, number of branches, collar girth, length of shoot and root, fresh weight of shoot and root were recorded. Cocoa seedlings were dried in an oven at 60 ^{0}C till constant dry weight was obtained and then dry weight of shoot and root was recorded.

Statistical analysis

The data collected in this study was subjected to analysis of variance (ANOVA) and the means of the treatments were compared at CD value at P= 0.05.

Identification of efficient *Bacillus* spp.

Bacillus spp. were identified according to Bergey's Manual of Determinative Bacteriology- 9th edition and Reva *et al*.(2001) and their identity was confirmed by Biolog® GEN III microplate identification system (Biolog, Hayward, CA, USA), which provided 94

phenotypic tests (71 carbon source utilization assays and 23 chemical sensitivity assays).

Bacterial isolates were examined for colony morphology (on Nutrient Agar), cell shape, Gram's reaction (Gram staining kit, HIMEDIA, India) and ability to form endospores as per the standard procedures. Oxidase activity was determined using commercially available discs (HIMEDIA, India). Catalase activity, utilization of citrate, Voges – Proskauer test, gelatin liquefaction test, nitrate reduction, starch and casein hydrolysis and arginine dihydrolase activity were performed according to standard microbiological procedures. The ability of the isolates to grow in the absence of oxygen was tested using the anaerobic growth chamber (HIMEDIA, India). Growth of *Bacillus* spp. at different temperatures (4 to 60 ^{0}C) and various NaCl concentrations (0 to 12 %) on TSA medium were determined.

Phenotypic fingerprinting using BIOLOG microbial ID system

The test organism was inoculated on Biolog Universal Growth Agar (BUGA) and incubated for 24 h at 33 ^{0}C. Cultures were transferred to Inoculating fluid A (IF A) using Biolog inoculatorz and the inoculum density was adjusted to 98 % T using Biolog turbidimeter. Cell suspension was then aseptically transferred in to a multi channel pipette reservoir. Using multi channel pipette, cell suspension was inoculated into Biolog Gen III Microplates and incubated at 33 ^{0}C. The optical density at 590 nm produced from the reduction of tetrazolium violet in each well was read after 24 h using a Biolog Microplate reader (version 5.1.1) in conjunction with the Microlog software. An identification was attained when it compared the pattern formed in the well with possible patterns in the database (Microstation/ Microlog Version 5.1.1). A species ID was called if the SIM and DIST values were >0.500 and <5.00, respectively.

Result and Discussion

In the present investigation, eight *Bacillus* spp. isolates were evaluated for their effect on seedling growth of cocoa in polybag conditions to ascertain the possible role this *Bacillus* spp. for enhancing cocoa growth. Previous study shows that many of the selected *Bacillus* spp. shared common plant growth promoting characteristics. Seven *Bacillus* spp. were positive for P-solubilization and ACC deaminase production, six were positive for the production of antibiotics, five isolates were able to grow on N-free media and four isolates produced siderophore and inhibited *P. palmivora* under *in vitro* conditions (Table 1). Earlier reports suggests that bacteria possessing these traits can increase plant growth. 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, wheat (Cakmakci *et al*., 2007), apples (Aslantas *et al*., 2007) and tomato (Hariprasad *et al*., 2009).

Stress tolerance of the plant growth promoting *Bacillus* spp.

The introduction and persistence ability of a strain are affected by a number of biotic factors like high salt,

Table 1. Phenotypic characteristics of *Bacillus* spp. selected for growth promotion studies in cocoa seedlings

PGPRs	Isolates obtained from	Characterization for PGP traits										
		HCN	P-solubilization (zone in mm)	Growth on N-free medium	Ammonification*	Siderophore (zone in mm)	IAA*	ACC deaminase activity*	Antibiotics (zone in mm)	Chitinase (mm)	Antagonism against *P. palmivora*	
Bacillus sp. ASB 3	Ambajipetta, Andhra Pradesh	-	6	-	H	-	-	L	5	8	-	
Bacillus sp. ASB 12	Ambajipetta, Andhra Pradesh	-	6	+	H	8	-	L	7	-	39	
Bacillus sp. KDSB 3	Kidu, Karnataka	-	9	+	H	14	-	L	-	-	-	
Bacillus sp. TSB 15	Tumkur, Karnataka	+	6	-	M	-	-	L	3	6	52	
Bacillus sp. TSB 17	Tumkur, Karnataka	+	6	-	L	-	M	L	-	-	-	
Bacillus sp. CSB 8	Coimbatore, Tamil Nadu	-	6	+	M	6	-	L	6	-	30	
Bacillus sp. CSB 16	Coimbatore, Tamil Nadu	+	-	+	M	-	-	L	6	-	36	
Bacillus sp. CSB 17	Coimbatore, Tamil Nadu	-	6	+	H	11	-	-	5	-	-	

- = no activity, + = positive result, mm = radius in mm, * The activity/ production was qualitatively assessed in three broad categories: H (high), M (medium) and L (low)

high water potential, high pH, and high temperature (Johri *et al.*, 1999). Test for the responses of the selected bacteria to different temperatures revealed that four *Bacillus* spp. (isolates CSB8, CSB 16, CSB17 and ASB 12) have good growth at higher temperatures (> 50 ^{0}C). *Bacillus* sp. CSB8, isolated from cocoa growing in Coimbatore, Tamil Nadu showed the potential to tolerate a maximum temperature of 60 ^{0}C and was able to grow on TSA medium amended with 12 % NaCl. *Bacillus* sp. CSB16 could able to grow at 55 ^{0}C and also showed intrinsic resistance to 12 % of NaCl in TSA, where as none of the isolates could tolerate 14 - 20 % NaCl. The ability to adapt to temperature stress may be important in the survival of the microorganisms during drought. Two *Bacillus* spp. (*Bacillus sp.* CSB 17 and *Bacillus* sp. ASB12) could tolerate temperature of 55 ^{0}C and bear NaCl concentraction of 10 %. *Bacillus* sp. KDSB3 and *Bacillus* sp. TSB 15 were the most sensitive strains to temperature (40 ^{0}C). All the selected *Bacillus* spp. showed tolerance to 6 % NaCl incorporated in TSA medium except one *Bacillus* sp. KDSB 3. The growth of test isolates under different pH condition was found to increase linearly from pH 5 to 9. The tolerance of the *Bacillus* spp. recorded at different incubation temperatures, pH and NaCl gradient is showed in Table 2. Identification of stress tolerant microbes is certainly useful in order to formulate effective cultures which can survive and persist for longer period and work more efficiently under such climatic conditions (Gupta *et al*., 2002).

Evaluation of plant growth promotion ability of the selected *Bacillus* spp.

All *Bacillus* spp. selected for plant growth experiments, significantly increased atleast one aspects of cocoa seedling growth. Statistically significant increase (P = 0.05) over the control was observed in the tested parameters such as total seedling length, fresh weight and dry weight of shoot and root and collar girth of cocoa seedlings when they were inoculated with PGPR cultures (Table 3 and Fig. 1). *Bacillus* sp. ASB3, obtained from the rhizosphere of cocoa growing in Ambajipetta, Andhra Pradesh, are found to be the best plant growth promoter for cocoa seedlings. This PGPR showed significant increase in all the tested growth parameters except in case of increasing the number of branches. Maximum increase in the total seedling length and dry mass of cocoa seedlings was shown by the same isolate by 36 and 56 %, respectively, over control. Previous reports revealed the strain had an intrinsic ability for the solubilization of phosphate, production of ACC deaminase, antibiotics and chitinase. Behind all these beneficial effects the

Table 2. Tolerance to temperature, pH and growth on gradient of NaCl concentration showed by *Bacillus* spp.

PGPRs	Growth at different temperature (^{0}C)								pH tolerated	NaCl concentration (%)					
	4	15	30	40	45	50	55	60		2	4	6	8	10	12
Bacillus sp. KDSB 3	-	-	+	+	-	-	-	-	6.2-9.0	+	-	-	-	-	-
Bacillus sp. ASB 3	-	+	+	+	+	+	-	-	6.2-9.0	+	+	+	-	-	-
Bacillus sp. ASB 12	-	-	+	+	+	+	+	-	6.2-8.2	+	+	+	+	+	-
Bacillus sp. CSB 8	-	-	+	+	+	+	+	+	5.2-9.0	+	+	+	+	+	+
Bacillus sp. CSB 16	-	-	+	+	+	+	+	-	6.2-9.0	+	+	+	+	+	+
Bacillus sp. CSB 17	-	-	+	+	+	+	+	-	6.2-8.2	+	+	+	+	+	-
Bacillus sp. TSB 15	-	+	+	+	-	-	-	-	6.2-9.0	+	+	+	-	-	-
Bacillus sp. TSB 17	-	-	+	+	+	+	-	-	6.2-9.0	+	+	+	+	-	-

- = no growth, + = growth present

Table 3. Effect of inoculation of *Bacillus* spp. isolates on leaf length (LL), number of leaves (NL), number of branches (NB), number of roots (NR), root width (RW), collar girth (CG), shoot length(SL), root length (RL), total seedling length(TSL), fresh weight of shoot (FWS), fresh weight of root (FWR), total fresh weight (TFW), dry weight of shoot (DWS), dry weight of root (DWR) and total dry weight (TDW) of cocoa seedlings under polybag conditions

PGPRs	LL (cm)	NL	NB	NR	RW (cm)	CG (cm)	SL (cm)	RL (cm)	TSL (cm)	FWS (g)	FWR (g)	TFW (g)	DWS (g)	DWR (g)	TDW (g)
Expt. I															
ASB12	27.93	51.35*	1.95	79.20	4.20	5.35*	82.62	64.50	147.12	162.90	58.30*	221.20	59.60*	20.48*	80.08*
CSB17	28.99	47.10	1.60	94.95*	4.35	5.62*	89.30*	58.77	148.07	162.42	52.82	215.25	55.82*	20.06*	75.88*
TSB15	27.76	35.10	0.60	58.20	3.68	4.98*	82.30	65.01	147.31	134.90	37.20	172.10	53.40	15.91	69.315
TSB17	26.97	45.89	1.05	78.00	4.32	5.15*	81.97	60.77	142.747	146.26	47.39	193.65	52.84	18.08*	70.92*
KDSB3	27.10	25.90	0.25	53.60	3.86	4.74*	82.95	66.36	149.31*	140.52	40.50	181.02	50.84	15.67	66.52
Expt. II															
CSB 8	29.36*	35.00*	1.10*	44.05	3.445	5.53*	77.38*	51.85*	129.15*	138.85*	40.15*	179*	61.47*	19.76	81.235*
CSB16	29.88*	27.10*	0.15	54.00*	3.81*	5.17*	82.28*	60.2*	142.4*	129.67*	44.59*	174.27*	56.55*	20.8	77.35*
ASB3	33.31*	31.85*	0.33	52.36*	4.25*	5.93*	92.82*	58.19*	151.02*	139.45*	46.4*	185.85*	61.2*	28.02*	89.22*

*Significant at P= 0.05 as compared to uninoculated control

Fig.1. PGPR-induced enhanced shoot development of cocoa seedlings as compared to uninoculated control (on extreme left)

mechanism possibly involved is the presence of these PGP traits. The total dry mass across treatments ranged from 8 to 56 %. Of the *Bacillus* spp. inoculants *Bacillus* sp. CSB 16 produced the highest root length (37 %), while *Bacillus* spp. CSB17 and TSB 17 were found to be incapable to enhance root length. A maximum increase of 40 % collar girth was recorded in cocoa seedlings inoculated with *Bacillus* sp. CSB 17. The seedlings inoculated with *Bacillus* spp. CSB 16 had the greatest difference in root width (41 %) and number of roots (29 %) as compared to control seedlings. An incremental effect on growth was observed in cocoa seedlings on inoculation with *Bacillus* spp. reveals, broad spectrum plant growth promoting activity by the isolates. In general, across treatments, it could be observed that *Bacillus* spp. inoculation enhanced collar girth and dry weight, as compared to other growth parameters. *Bacillus* spp. were known to have beneficial effects on the growth of different crop plants. Treating Jetropha seeds with *Bacillus pumilus* (IM-3) resulted in enhanced dry shoot mass (473 %), dry total plant mass (407 %), and chlorophyll content (82 %) over control (Desai *et al.*, 2007). *B. licheniformis* and *B. pumulis* enhanced the growth of *Pinus pinea* (Probanza *et al.*, 2002). Formulations of *B. subtilis* AF 1 promoted the growth of pigeon pea both in greenhouse (Manjula and Podile, 2001) and field (Manjula and Podile, 2005) with consistency. Coinoculation of *Bacillus* NEB strains with *Bradyrhizobium japonicum* generally promoted soybean plant growth and nodulation under either optimal or suboptimal root zone temperatures.

Identification of efficient *Bacillus* spp.

Effcient PGPRs were tendatively identified based on morphological and biochemical characteristics (Bergey's Manual of Determinative Bacteriology- 9th edition and Reva *et al*.(2001) as *Bacillus licheniformis* CSB 17, *Bacillus subtilis* ASB 12, *Bacillus subtilis* CSB16, *Bacillus subtilis* CSB8, *Bacillus cereus* and related species ASB3, *Bacillus cereus* and related species TSB 15, *Bacillus cereus* and related species KDSB 3, *Bacillus kobensis* TSB 17 (Table 4). The Biolog microbial ID system rapidly provides the metabolic profile of the isolates on the basis of the differential utilization of carbon sources or resistance to inhibitory chemicals. The 'phenotypic fingerprint' thus generated is used to identify the organism at the species level. In an early study evaluating the Biolog system (Miller and Rhoden, 1991), the acceptable instrument readings at the end of a 24 h incubation time for identification of clinical isolates was as follows: similarity index <0.5 (no identification), similarity index 0.50–0.75 (good identification), and similarity index >0.75 (excellent identification) with indexes >0.50 acceptable for genus and species

Table 4. Biochemical tests for the identification of *Bacillus* spp.

PGPRs												NaCl (%)			Temperature (^{0}C)				Tentative identification
	Anaerobic growth	Starch	Nitrate	V.P	Citrate	Catalase	Oxidase	Motility	Arginase	Caseinase	Gelatinase	6	8	10	45	50	55	60	
CSB8	-	+	+	+	+	+	+	+	ND	+	ND	+	+	+	+	+	+	+	*Bacillus subtilis*
CSB16	-	+	+	+	+	+	+	+	ND	+	ND	+	+	+	+	+	+	-	*Bacillus subtilis*
CSB 17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	*Bacillus licheniformis*
ASB3	+	+	+	+	+	+	+	+	ND	+	ND	+	-	-	+	+	-	-	*Bacillus cereus* and related species
ASB 12	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	*Bacillus subtilis*
TSB 15	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	*Bacillus cereus* and related species
TSB 17	-	+	-	-	+	-	+	+	+	+	+	+	+	-	+	+	-	-	*Bacillus kobensis*
KDSB 3	+	-	+	+	-	+	-	-	+	+	+	-	-	-	-	-	-	-	*Bacillus cereus* and related species

- = no activity, + = positive result, ND = not detected

Table 5. Comparison of conventional biochemical identification with Biolog® GEN III microbial ID system for *Bacillus* spp.

Isolate No.	Conventional identification	Biolog® GEN III microbial ID system	Similarity index value	Distance value
ASB 3	*B. cereus* and related species	*B. cereus / thuringiensis*	0.353	6.798
ASB 12	*B. subtilis*	*B. subtilis* ss *subtilis*	0.79	3.478
KDSB 3	*B. cereus* and related species	*B. pseudomycoides*	0.433	5.274
TSB 15	*B. cereus* and related species	*B. cereus / thuringiensis*	0.432	4.286
TSB 17	*B. kobensis*	*B. megaterium*	0.62	6.114
CSB 8	*B. subtilis*	*B. subtilis* ss *subtilis*	0.82	2.258
CSB 16	*B. subtilis*	*B. subtilis* ss *subtilis*	0.80	2.842
CSB 17	*B. licheniformis*	*B. subtilis* ss *subtilis*	0.77	2.085

identification. In our study, the acceptance of Biolog system identification was based on similar criteria. In the tests conducted, Biolog provided a genus and species identification for five of eight *Bacillus* spp.. *Bacillus* spp. ASB 12, CSB 8 and CSB 16 were identified as *Bacillus subtilis* ss *subtilis* with similarity index > 0.75 as validating the conventional identification. Where as *Bacillus* spp. TSB 17 and CSB 17 were identified as *B. megaterium* (SIM value, 0.629) and B. *subtilis* ss *subtilis* (0.77) respectively, contrary to the results obtained from the conventional identification (Table 5). As the the similarity index obtained by Biolog identification for *Bacillus* sp. ASB 3, *Bacillus* sp. TSB 15 and *Bacillus* sp. KDSB 3 were below the threshold for correct identification, the identity obtained by conventional identification method as *Bacillus cereus* and related species were retained.

The overall improvement in seedling vigour through a significant increase in various physiological parameters and their ability to tolerate high temperature and NaCl suggests that *Bacillus cereus* and related species ASB3, *Bacillus subtilis* ss *subtilis* ASB12, *Bacillus subtilis* ss *subtilis* CSB8, *Bacillus subtilis* ss *subtilis* CSB16 and *Bacillus subtilis* ss *subtilis* CSB17 have a plant growth promoting ability on cocoa seedlings and hence could be used for bioinoculation for better establishment of seedlings. The plants with enhanced seedling vigour can help in better establishment of plantations.

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