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ORIGINAL ARTICLE

Plant Growth Promoting Bacteria from Cow Dung Based Biodynamic Preparations

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Abstract Indigenous formulations based on cow dung fermentation are commonly used in organic farming. Three biodynamic preparations viz., Panchagavya (PG), BD500 and 'Cow pat pit' (CPP) showed high counts of lactobacilli (10^9 ml^{-1}) and yeasts (10^4 ml^{-1}). Actinomycetes were present only in CPP (10^4 ml^{-1}) and absent in the other two. Seven bacterial isolates from these ferments were identified by a polyphasic approach: *Bacillus safensis* (PG1), *Bacillus cereus* (PG2, PG4 PG5), *Bacillus subtilis* (BD2) *Lysinibacillus xylanilyticus* (BD3) and *Bacillus licheniformis* (CPP1). This is the first report of *L. xylanilyticus* and *B. licheniformis* in biodynamic preparations. Only three carbon sources—dextrose, sucrose and trehalose out of 21 tested were utilized by all the bacteria. None could utilize arabinose, dulcitol, galactose, inositol, inulin, melibiose, raffinose, rhamnose and sorbitol. All the strains produced indole acetic acid (1.8–3.7 μg ml^{-1} culture filtrate) and ammonia. None could fix nitrogen; but all except *B. safensis* and *B. licheniformis* could solubilize phosphorous from insoluble tri-calcium phosphate. All the strains except *L. xylaniliticus* exhibited antagonism to the plant pathogen *Rhizoctonia bataticola* whereas none could inhibit *Sclerotium rolfsi*. In green house experiment in soil microcosms, bacterial inoculation significantly promoted growth of maize; plant dry weight increased by ~21 % due to inoculation with *B. cereus* (PG2). Results provide a basis for understanding the beneficial effects of biodynamic preparations and industrial deployment of the strains.

Keywords *Bacillus* spp. · Biofertilizer · BD500 · Cow pat pit · Fermentation · *Lysinibacillus* sp. · Panchagavya

Introduction

Many agricultural products based on indigenous fermentation technologies are used in organic farming like biodynamic preparations and liquid manures. Cow dung is an integral component of all these preparations and serves as a source of inoculum of beneficial microorganisms. '*Panchagavya*' (PG; Sanskrit for a blend of 'five products from cow') is a traditional product prepared in India by fermenting cow dung, cow urine, milk, curd and clarified butter (*ghee)* [1]. BD500, a biodynamic preparation that is also called as horn manure and *'Cow pat pit'* (CPP) are preparations from cow dung that are used in organic farming [2, 3]. Biodynamic products are included in the list of materials and techniques permitted in organic farming by an EC regulation (834/2007). These contain macro- and micro-nutrients, amino acids, growth promoting substances like indole acetic acid, gibberellins and beneficial microorganisms. Beneficial effects of biodynamic preparations have been reported on lentil and wheat [4]. Spraying a 3 % solution of PG along with soil application of biogas slurry improved the yields of maize and sunflower [1]. Biodynamic sprays increased the yields of cereals and vegetables, during the years when yields were low [5].

Presence of naturally occurring beneficial microorganisms, predominantly bacteria, yeast, actinomycetes, and certain fungi have been reported in cow dung [6]. Research related to isolation and characterization of the beneficial

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attributes of the bacteria present in biodynamic preparations are few [7, 8]. Definitive proof is required whether the bacteria in such formulations have any PGPR attributes, and if so whether they can improve plant growth under defined conditions in soil microcosms, that overcome the drawbacks of field experiments by eliminating the errors arising from spatial variability of soil physico-chemical and fertility properties that occur in field gradients. There are no reports on the definitive identification of the beneficial bacteria in such biodynamic preparations using molecular methods. In the present work the microbial composition of three biodynamic preparations *viz*., PG, BD500 and CPP was analyzed. Seven bacterial isolates were identified by morphological, biochemical and molecular features and their plant growth promoting attributes were evaluated in the laboratory and their potential for improving plant growth was tested in vivo on maize crop in soil microcosms.

Materials and Methods

Biodynamic Preparations

'Panchagavya' was prepared by modification of the method described by Suresh Kumar et al. [9]. A mixture of 5 kg fresh cow dung and 500 g clarified butter (*ghee)* made from cow milk was incubated in a 20 l plastic container for 4 days at room temperature. Twice a day, the mixture is stirred for 20 min with a wooden stick, 10 min in clockwise and 10 min in the opposite direction. On the 5th day, 3 l of cow urine, 2 l of cow milk, 2 l of curd made from cow milk, 500 g sugarcane jaggery, 3 l sugarcane juice, 12 nos. of ripened banana, 3 l of tender coconut water and 100 g brewer's yeast (*Saccharomyces cerevisae*) were added. The contents were incubated for 15 days along with two stirrings daily as described above for 20 min to facilitate aeration. BD500 is a humus mixture prepared by filling the horn of a cow with cow manure and burying it in the ground (40–60 cm below the surface) in the autumn. It is left to decompose during the winter and recovered for use the following spring. For preparation of CPP fresh cow dung obtained from indigenous, pasture grazing and lactating cows is fermented along with crushed eggshell (source of calcium) and basalt dust (source of silica) mixed and placed in a 12 inch deep pit along with biodynamic preparations (BD502–BD507) for catalysing the composting process. In the present study, the commercial formulations (dried powder) of BD500 and CPP were diluted 1:10 with sterile water and used for analysis of microbiological populations and isolation of bacteria.

The biodynamic preparations were serially diluted tenfold and plated on Luria–Bertani (LB) agar, de Man Rogosa Sharpe (MRS) agar, malt agar (with chloramphenicol added after sterilization at 0.5 %) and Actinomycetes isolation agar for the enumeration of bacteria, lactobacilli, yeast and actinomycetes respectively. Based on morphological variations viz., shape, elevation, texture and margin, seven isolates—four from PG, two from BD500 and one from CPP were short listed for evaluation. They were characterised for gram reaction, endospore formation and selected biochemical attributes viz, catalase, nitrate reduction, casein hydrolysis, starch hydrolysis, H_2S production, urease activity, oxidase, citrate utilization, Voges–Proskauer test and gelatin liquefaction [10].

Strain Identity

The isolates were tested for their ability to utilize 21 carbon compounds as sole sources of energy; monosaccharides (adonitol, arabinose, dextrose, dulcitol, fructose, galactose, inositol, mannitol, mannose, rhamnose, sorbitol, xylose), disaccharides (cellobiose, lactose, maltose, melibiose, salicin, sucrose, trehalose), trisaccharides (raffinose) and polysaccharides (inulin). The isolates were inoculated in test tubes containing basal medium with phenol red indicator (Himedia laboratories, India). Carbohydrate discs containing 25 mg of the carbon source impregnated in them were suspended in the broth. After incubation at 24–48 h the tubes were observed for change in color of the medium from red to yellow which was taken as positive for utilization.

The near full length 16S rRNA gene sequences of the bacterial strains were custom sequenced on ABI 3730×1 Genetic Analyzer at Xcelris Labs Ltd., Ahmedabad, Gujarat, India. The sequences were deposited in the NCBI GenBank data base; accession numbers are given in Table 2. Nearest identities of the strains were obtained by comparing sequences of 16S rRNA gene with database in GenBank (http://www.ncbi.nlm.nih.gov/) using the BLASTn program. The strains were also deposited in the culture collection of National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau. U.P., accession numbers are given in Table 2.

Growth Promoting Characteristics and Plant Bioassay

The isolates were tested for plant growth promoting attributes like diazotrophy: growth on Jensen's N free medium; P solubilisation on Pikovskaya agar followed by spectrophotometric quantification of P solubilized in broth after 10 days growth at 28 ± 2 °C in shake cultures at 125 rpm; ammonia production; indole acetic acid production [11], siderophore production was ascertained by growth in chrome azurol S (CAS) medium [12] after 48–72 h. growth at 28 °C The isolates screened for their ability to utilize

ACC (1-aminocyclopropane-1-carboxylic acid) as a sole N source by using MDF (modified nitrogen free-Dworkin and Foster) medium [13]. Screening of bacterial culture against plant fungal pathogens *Rhizoctonia bataticola* and *Sclerotium rolfsi* was done using dual culture technique. The bacterial isolates were screened in green house for their ability to promote plant growth in soil microcosms. 330 ml paper cups were filled with 300 g soil (Vertisol). Seeds of maize (var. hybrid Marvel) were surface sterilized by dipping in 95 % ethanol for 5 min and 0.1 % $HgCl_2$ for 3 min and finally washed 5 times in sterile distilled water. The bacterial strains were grown on LB broth for 6 days with shaking. Farm yard manure was used as a carrier material to prepare the inoculants. FYM was air dried for 3–4 days and passed through 0.2 mm sieve and sterilized three times by steam sterilization (121 °C for 20 min) on successive days and then two times dry heat sterilization (160 °C for 3 h each time). Forty millilitre of the culture broth was added to 100 g FYM in a plastic pouch, mixed by hands and sealed. One gram of each inoculant was added to 10 ml of 1 % carboxy-methyl cellulose (CMC). Then 24 seeds of maize were transferred to the CMC-culture suspension and kept overnight. The seeds were removed aseptically and air dried in a laminar air flow work station. The inoculant coated seeds were sown at four seeds per cup in five replications. After germination, the plants were thinned to maintain three plants in each cup. After 15 days urea was applied as solution to the cups at 40 μg N g^{-1} soil. The cups were watered regularly with tap water (boiled for 30 min and cooled) to maintain optimum moisture. The shoot and root length of the plants and their dry mass was recorded at 5 weeks growth stage (37 days after sowing).

Results and Discussion

The micro-flora of the biodynamic preparations consisted pre-dominantly of lactobacilli (10^9 ml^{-1}) and yeasts (10^4 ml^{-1}) (Table 1). The microflora was thus overwhelmingly bacterial whereas yeasts formed only 0.001 %. In PG, plant growth promoting bacteria that are widely used as biofertilizers have been reported to occur in high numbers e.g., *Azospirillum* (10^{10} ml^{-1}), *Azotobacter* (10^9 ml^{-1}) and *Pseudomonas* (10^6 ml^{-1}) [14]. *Lactobacillus* was also detected in PG but not counted [14]. In our study lactobacilli were present in very high numbers in PG (2.0×10^{10} ml^{-1}) due to addition of milk and milk products during the fermentation. Rupela et al. [2] evaluated six other biodynamic preparations and found the population of bacteria to range from 3.24 $\log_{10}$ ml^{-1} (in BD502) to 6.90 $\log_{10}$ ml^{-1} (in BD500). Yeasts counts were ten-fold higher in PG as compared to the more humified preparations like BD500 and CPP because of the low pH as well as the fact that yeast was added as inoculum. In our study, actinomycetes were absent in PG and BD500 (Table 1); in the former it may due to the very low pH (3.7) and also competition for substrate from the fast growing bacilli. Substrate competition might also be responsible for its absence in BD500. But in CPP actinomycetes were detected possibly due to their stimulation by the added calcium source. Stalin et al. [3] enumerated the micro organisms from several organic and biodynamic manures, among them CPP manure contained the highest bacterial load (4.8×10^6 cfu g^{-1}); *Bacillus subtilis* was predominant in CPP manure.

The 16S rRNA gene sequence of the bacteria revealed that all the strains isolated from PG belonged to *Bacillus safensis* or *Bacillus cereus.* Strains from BD500 belonged to *B. subtilis* and *Lysinibacillus xylanilyticus.* The strain from CPP belonged to *Bacillus licheniformis.* In milk and milk products, *B. licheniformis* and *B. cereus* are the most frequently isolated bacilli [15]. Recently Giannattasi et al. [7] characterized the bacterial and fungal communities of BD500 using ARISA (Automated rRNA Intergenic Spacer Analysis) fingerprints. BD500 was found to harbor a bacterial community of 2.38×10^8 cfu g^{-1} dry weight dominated by Gram positives with minor instances of Actinobacteria and Gamma proteobacteria. The culturable fraction was dominated by *Bacillus* species.

The composition of these ferments reflects the composition of cowdung used in fermentation. Swain and Ray [6] identified *Bacillus, Corynebacterium, Lactobacillus, Leuconostoc, Bifidobacterium*, *Enterococcus* and *Streptococcus* in cowdung. The predominant fungal genera were *Aspergillus, Rhizopus, Trichoderma* with the remaining

Table 1 Microbial Populations of biodynamic preparations in specific media (cfu ml^{-1})

Sample	pH	*Lactobacillus* spp. ($\times 10^9$) (MRS agar)	Bacteria ($\times 10^6$) (LB agar)	Yeast ($\times 10^4$) (Malt agar)	Actinomycetes ($\times 10^3$) (Actinomycetes isolation agar)
PG	3.7	20.0 ± 2.4	3.8 ± 0.7	15.0 ± 1.2	Nil
BD500[a]	6.9	3.0 ± 0.4	0.4 ± 0.04	5.0 ± 1.0	Nil
CPP[a]	6.6	2.0 ± 4.0	8.2 ± 1.2	1.0 ± 0.35	10.0 ± 1.2

[a] Samples were one-tenth dilution of the powder formulations; ±SEM

Table 2 Genomic identity of the bacterial strains isolated from biodynamic preparations and their morphological and biochemical characteristics

Code	Isolates	Colony characteristics	16S rRNA homology (%)	NCBI acc. no.	NBAIM acc. no.	NR	CH	O	CU	VP
PG1	*Bacillus safensis*	White, spreading, feathery	100.0	KF 804070	B-01476	−	+	+	+	+
PG2	*B. cereus*	White, raised, smooth margin	99.1	KF 804071	B-01477	+	+	−	−	+
PG4	*B. cereus*	Off white, round, smooth margin	99.1	KF 804072	B-01478	+	+	−	−	+
PG5	*B. cereus*	Off white, flat, round, smooth margin	99.5	KF 804073	B-01479	+	+	−	−	+
BD2	*B. subtilis*	Off white, spreading, irregular margin	99.2	KF 804074	B-01480	+	+	−	+	+
BD3	*Lysinibacillus xylanilyticus*	Off white, round, smooth margin, shiny, raised, translucent	95.3	KF 804075	B-01481	−	−	+	−	−
CPP1	*B.licheniformis*	White, spreading, irregular margin	98.6	KF 804076	B-01482	+	−	+	+	+

All the strains were gram positive, endospore formers, motile, catalase positive, positive for starch hydrolysis and gelatin liquefaction. All were negative for H_2S production and urease

NR nitrate reduction, *CH* casein Hydrolysis, *O* oxidase, *CU* citrate utilization, *VP* voges–Proskauer test

belonging to unidentified yeasts and other species. Recently bacteria were identified in cow dung through metagenomic approach [16] and found to belong mainly to the phyla Bacteroidetes (38.3 %), Firmicutes (29.8 %), Proteobacteria (21.3 %) and Verrucomicrobia (2 %). *L. xylanilyticus*, a novel species has been reported from forest humus [17] but ours is the first report on its isolation from biodynamic preparations. Also this is the first report of *B. licheniformis* in biodynamic preparations.

All the seven bacterial strains isolated in this study were gram positive, endospore forming rods that exhibited white to off white colony colour in agar plates. All were catalase positive and also positive for starch hydrolysis and gelatin liquefaction. All were negative for H_2S production and urease activity. Results of other biochemical tests are given in Table 2. Only three carbon sources out of 21 tested were utilized by all the bacterial strains: dextrose, sucrose and trehalose. None of the strains could utilize arabinose, dulcitol, galactose, inositol, inulin, melibiose, raffinose, rhamnose, and sorbitol. Cellobiose was utilized only by *B. cereus.* Maltose was utilized by *B. cereus* and *L. xylanilyticus*. Mannose was utilized by both *B. cereus* and *B.safensis* but mannitol was utilized only by *B. licheniformis*. The results on the utilization of carbon sources were in conformity with the earlier reports on the catabolic ability of *B. subtilis* and *B.cereus* [18]; *B.licheniformis* [19] and *B.safensis* [20]. The sole exception was the inability of *B.cereus* in the present study to utilize citrate which did not agree with Collins and Lyne [18] but agreed with another recent report [21].

All the bacterial strains were able to produce indole acetic acid (IAA) under in vitro conditions ranging from 1.8–3.7 μg ml^{-1} (Table 3). All the strains produced ammonia but none of them fixed atmospheric nitrogen. All the species except *B. safensis* and *B. licheniformis* could solubilize P from insoluble tri-calcium phosphate producing clearing zones of 10–14 mm diameter in petri plates. The amount of P solubilized ranged from 24.3 to 45.5 μg ml^{-1} of culture filtrate which amounted to solubilization of 2.4–4.4 % of added insoluble tricalcium phosphate P (Table 3). None of the strains produced siderophores or exhibited ACC deaminase activity. All the species except *L. xylaniliticus* exhibited antagonism to the plant pathogenic fungus *Rhizoctonia bataticola.* None of the strains inhibited the disease causing fungus *Sclerotium rolfsi.* Our results agree in few respects with Sreenivasa et al. [22] who found that bacteria from '*Beejamrutha*' (fermented cow urine) were capable of P solubilization and production of growth promoting substances like IAA and GA. They also found the bacteria to be capable of N_2-fixation and suppressing *Sclerotium* sp.

Among the seven strains tested in the green house, only two strains of *Bacillus cereus* (PG_2 and PG_4) could increase the shoot length of maize significantly (Fig. 1). Improvement in root length was significant with the all the isolates except *B. subtilis* (BD_2). Total dry weight of the plants was significantly increased by all the isolates. There was a consistently significant increase in shoot and root length, and shoot and root dry weight by 23.3, 71.6 and 20.5, 20.7 % respectively over uninoculated control due to inoculation with *B. cereus* (PG2). Increase in plant growth may be due to the production of auxin, P solubilization and biocontrol ability. Our result support the work of Nagaraj Naik and Sreenivasa [8] who found that the wheat seeds treated with the isolates (unidentified) obtained from PG increased the germination, seedling length and seedling vigour index.

Plant growth promoting rhizobacteria are known to influence the growth, development, and yield of crops either directly or indirectly through various mechanisms.

Table 3 Attributes of the bacterial strains for promoting plant growth

Strain code	Isolates	Ammonia production	IAA production (μg/ml)	P solubilization		Antagonism to *Rhizoctonia bataticola*
				% P solubilized in 10 days	pH of broth after 10 days	
PG1	*Bacillus safensis*	+++	3.75	3.26	4.83	+
PG2	*B. cereus*	++	3.25	2.98	4.52	+
PG4	*B. cereus*	+++	3.00	4.44	4.68	+
PG5	*B. cereus*	+++	3.25	4.12	4.52	+
BD2	*B. subtilis*	+++	3.00	3.03	4.89	+
BD3	*Lysinibacillus xylanilyticus*	++	2.75	3.34	4.81	−
CPP1	*B. licheniformis*	++	1.75	2.37	4.96	+

All strains negative for nitrogen fixation, siderophore production, ACC deaminase activity

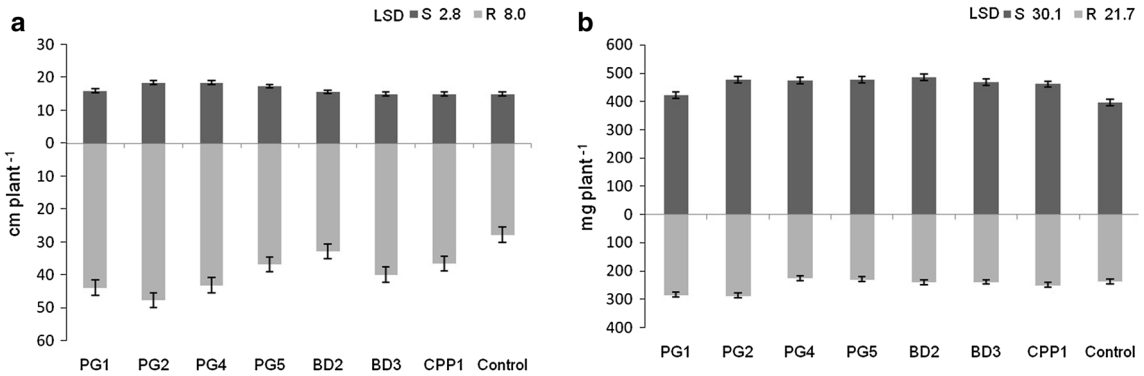

Fig. 1 Effect of the inoculation of bacterial strains isolated from biodynamic preparations on the growth of maize in soil microcosms at 6 weeks. PG1 *Bacillus safensis*; PG2 *B. cereus*; PG4 *B. cereus*; PG5 *B. cereus*; BD2 *B. subtilis*; BD3 *Lysinibacillus xylanilyticus*; CPP1 *B.licheniformis*. LSD values $p = 0.05$ are 2.8 and 8.0 cm plant^{-1} for shoot (S) and root (R) length; 30.1 and 21.7 mg DW plant^{-1} for shoot (S) and (R) root biomass

Direct effects include production of plant hormones such as auxins, gibberellins and cytokinins, supplying biologically fixed nitrogen or solubilizing insoluble phosphates. Indirect mechanisms include suppression of bacterial, fungal and nematode pathogens by production of siderophores, HCN, ammonia, antibiotics, volatile metabolites etc., by induced systemic resistance and by competing with the pathogen for nutrients or for colonization of space [23]. *Bacillus* species are a major component of the heterotrophic soil microflora and are known to influence plant growth through production of auxins [24] and gibberellins [25]. *B. subtilis* isolated from cowdung exhibited biocontrol activity against plant pathogenic fungi *Fusarium oxysporum* and *Botryodiplodia theobromae* and the strain also promoted root elongation in seedlings of *Cicer arietinum* up to 70–74 % as compared to untreated seeds [6]. Further screening of desirable attributes of plant growth promoting organisms from biodynamic preparations would include stability and good survival in soil.

In conclusion, our results on the identification and characterization of culturable bacteria from cow dung based biodynamic preparations show that they are dominated by *Bacillis* spp. This is a new report of the occurrence of *L. xylanilyticus* and *B. licheniformis* in biodynamic preparations. The isolated bacterial strains exhibited plant growth promoting attributes like IAA production, P solubilization, antagonism to *R. bataticola* and improved the growth of maize plants. The results provide a basis for understanding the beneficial effects of biodynamic preparations and for deploying the strains in industrial production of biofertilizer and bio-control agents.

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References

1. Somasundaram E, Amanullah MM, Vaiyapuri K, Thirukkumaran K, Sathyamoorthi K (2007) Influence of organic sources of nutrients on the yield and economics of crops under maize based cropping system. J Appl Sci Res 3:1774–1777

2. Rupela OP, Gopalakrishnan S, Krajewski M, Sriveni M (2003) A novel method for the identification and enumeration of micro-organisms with potential for suppressing fungal plant pathogens. Biol Fertil Soils 39:131–134
3. Stalin V, Perumal K, Stanley Abraham L, Kalaichelvan PT (2010) Screening and production of subtilin from *Bacillus subtilis* isolated from nutrient-rich organic and biodynamic manures. IUP J Life Sci 4:34–44
4. Carpenter-Boggs L, Reganold JP, Kennedy AC (2000) Biodynamic preparations: short term effect on crops, soils, and weed populations. Am J Altern Agric 15:110–118
5. Raupp J, Koenig UJ (1996) Biodynamic preparations cause opposite yield effects depending upon yield levels. Biol Agric Hortic 13:175–188
6. Swain MR, Ray RC (2009) Biocontrol and other beneficial activities of *Bacillus subtilis* isolated from cowdung microflora. Microbiol Res 164:121–130
7. Giannattasio M, Vendramin E, Fornasier F, Alberghini S, Zanardo M, Stellin F, Concheri G, Stevanato P, Ertani A, Nardi S, Rizzi V, Piffanelli P, Spaccini R, Mazzei P, Piccolo A, Squartini A (2013) Microbiological features and bioactivity of a fermented manure product (preparation 500) used in biodynamic agriculture. J Microbiol Biotech 23:644–651
8. Naik Nagaraj, Sreenivasa MN (2009) Influence of bacteria isolated from Panchagavya on seed germination and seed vigour in wheat. Karnataka J Agric Sci 22:23–231
9. Suresh Kumar R, Ganesh P, Tharmaraj K, Saranraj P (2011) Growth and development of blackgram (*Vigna mungo*) under foliar application of Panchagavya as organic source of nutrient. Curr Bot 2:9–11
10. Cappuccino JG, Sherman N (2004) Microbiology: a laboratory manual, 6th edn. Pearson education, Singapore
11. Bric JM, Bostock RM, Silverstone SE (1991) Rapid in situ assay for indole acetic acid production by bacteria immobilized on a nitrocellulose membrane. Appl Environ Microbiol 57:535–538
12. Schwyn B, Neilands JB (1987) Universal assay for the detection and determination of siderophores. Anal Biochem 160:47–56
13. Jacobson CB, Pasternak JJ, Glick BR (1994) Partial purification and characterization of ACC deaminase from plant growth promoting rhizobacterium *Pseudomonas putida* GR 12-2. Can J Microbiol 40:1019–1025
14. Solaiappan AR (2002) Microbiological Studies in Panchagavya. Bio-control Laboratory, Chengalput, pp 1–2
15. Crielly EM, Logan NA, Anderton A (1994) Studies on the *Bacillus* flora of milk and milk products. J Appl Microbiol 77:256–263
16. Girija D, Deepa K, Xavier F, Antony I, Shidhi PR (2013) Analysis of cow dung microbiota—a metagenomic approach. Indian J Biotech 12:372–378
17. Lee CS, Jung YT, Park S, Oh TK, Yoon JH (2010) *Lysinibacillus xylanilyticus* sp. nov., a xylan degrading bacterium isolated from forest humus. Int J Syst Envol Microbiol 60:281–286
18. Collins CH, Lyne PM (1976) Microbiological methods, 4th edn. Butterworths, Guildford, pp 434–448
19. Vyletelova M, Svec P, Pacova Z, Sedlacek I, Roubal P (2002) Occurence of *Bacillus cereus* and *Bacillus licheniformis* strains in the course of UHT milk production. Czech J Anim Sci 47:200–205
20. Satomi M, La Duc MT, Venkateswaran K (2006) *Bacillus safensis* spp. nov., isolated from spacecraft and assembly-facility surfaces. Int J Syst Evol Microbiol 56:1735–1740
21. Maheswar NU, Sathiyavani G (2012) Solubilization of phosphate by *Bacillus* spp. from groundnut rhizosphere (*Arachis hypogaea* L). J Chem Pharm Res 4:4007–4011
22. Sreenivasa MN, Naik Nagaraj, Bhat SN (2009) Beejamrutha: a source for beneficial bacteria. Karnataka J Agric Sci 22:1038–1040
23. Glick BR (1995) The enhancement of plant growth by free living bacteria. Can J Microbiol 41:109–117
24. Idris EE, Iglesias DJ, Talon M, Borriss R (2007) Tryptophan-dependent production of indole-3- acetic acid (IAA) affects level of plant growth promotion by *Bacillus amyloliquefaciens* FZB42. Mol Plant Microbe Interact 20:619–626
25. Gutierrez-Manero FJ, Ramos B, Probanza A, Mehouachi J, Talon M (2001) The plant growth promoting rhizobacteria *Bacillus pumilus* and *Bacillus licheniformis* produce high amounts of physiologically active gibberelins. Physiol Plant 111:206–211