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Assessment of ecological diversity of rhizobacterial communities in vermicompost and analysis of their potential to improve plant growth

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Abstract: Present study was designed to determine the microbial diversity from three distinctive sites (amended with vermicompost) of Gujarat, India. A set of 76 strains were screened from total of 438 strains that exhibit plant growth-promoting (PGP) and antagonistic potential isolated from sites PS1 (Mehsana district), BS2 (Dantiwada district) and VS3 (Gandhinagar district). Their diversity indices were studied for determining the species richness and evenness of screened isolates. Results revealed that site BS2 showed the most significant diversity indices in terms of Shannon (H' 1.525) and Simpson ($1/D$ 5.120) than other two samples. Principal component analysis showed that bacterial diversity (H') was correlated with the soil characteristics. Chickpea and groundnut plants inoculated with MBCU1 and MBCU3 isolates showed an increase in the vegetative growth parameters that evaluate plant growth when compared to uninoculated controls. Strains MBCU1 and MBCU3 were identified as *Pseudomonas stutzeri* and *Pseudomonas mosselii*, respectively, according to sequence analysis of the 16S rRNA gene. These both isolates belong to site BS2 and they showed specific PGP traits suggesting that these isolates can promote plant growth by more than one mechanism with respect to their higher diversity index.

Key words: bioinoculants; evenness; functional diversity; richness; plant growth promoting rhizobacteria; vermicompost.

Abbreviations: PCA, principal component analysis; PGP, plant growth-promoting; PGPR, plant growth-promoting rhizobacteria; PIE, probability of an interspecific encounter.

Introduction

Sustainable agriculture is the management and utilization of the agricultural ecosystem in such a way that include conservation of its biological diversity, productivity, regeneration capacity, vitality and ability to function. It means that it can fulfil – today and in the future – a significant ecological, economic and social function at the local, national and global levels and that it does not harm other ecosystems (Spiertz 2010). Soil amendments have important role in improving the soil fertility and in the prevention of nutrient losses. Vermicompost is proving to be highly nutritive organic fertilizer and more powerful growth promoter over the conventional composts and a protective farm input (increasing the physical, chemical and biological properties of soil, restoring and improving its natural fertility) against the destructive chemical fertilizers, which have destroyed the soil's natural fertility over the years (Sinha et al. 2009). Vermicomposts can significantly influence the growth and productivity of plants (Edwards 1988; Sinha et al. 2009) due to their micro and macro elements, vitamins, enzymes and hormones (Makulec 2002). Vermicompost has been shown to improve the germination, growth, and yield of plants, due to faster release of nutrients than traditional composts, and the production of plant growth hormones (Doan et al. 2013).

Apart from providing mineralogical nutrients, vermicomposts also enriched the biological fertility by adding beneficial microbes to soil. Mucus, excreted through the earthworm's digestive canal, stimulates antagonism and competition between diverse microbial populations resulting in the production of secondary metabolites, such as antibiotics and hormone-like biochemicals, boosting plant growth (Edwards & Bohlen 1996). Earthworms ingest plant growth-promoting rhizospheric bacteria, such as *Pseudomonas*, *Rhizobium*, *Bacillus*, *Azosprillium*, *Azotobacter*, etc., along with rhizospheric soil, and they might get activated or increased due to the ideal micro-environment of the gut and thereby increasing the population of plant growth-promoting rhizobacteria (PGPR) (Sinha et al. 2010). This specific group of bacteria stimulates plant growth directly by solubilization of nutrients, production of growth hormones like indole acetic acid (IAA), gib-

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Table 1. Physicochemical characteristic of three collected samples.

Sample sites	pH	Salinity (mM)	C (%)	P (kg/ha)	K (kg/ha)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)
Site PS1 (Mehsana district)	7.8	1.91	0.37	42	450	7.84	0.40	1.08	5.46
Site BS2 (Dantiwada district)	7.7	1.26	0.39	53	378	5.80	9.86	0.96	8.34
Site VS3 (Gandhinagar district)	6.5	0.40	3.24	87	618	8.34	2.60	1.32	1.32

berellic acid, etc., nitrogen fixation, ammonia production and indirectly by suppressing fungal pathogens through the production of antibiotics, fluorescent pigments, siderophores and fungal cell-wall degrading enzymes, namely chitinases and β-1,3-glucanase (Saraf et al. 2010). Numerous studies demonstrated both a non-specific increase in total microbial biomass and the induction of species-specific microbial assemblages in the rhizosphere. The effect of microbial communities could be translated into functional changes that could then affect plant growth and yield (Tian et al. 2013).

Chickpea and groundnut are two of the most important crops cultivated in India. Gujarat as the largest producer in India accounts to 36% of total production of groundnut in India (Pandya & Saraf 2013). Chickpea is also important pulse crop of India in terms of both area and production. India is the largest producer of chickpea in the world sharing 65.25% and 65.49% (FAOSTAT 2010; http://faostat.fao.org/site/567) of the total area (11.97×10^6 ha) and production (10.89×10^6 tons), respectively. Chickpea is a resource poor farmers' crop and receives very less inputs in terms of biofertilizers and biopesticides, which is reflected in poor crop health succumbing to heavy pest infestations. To make its cultivation sustainable, it is necessary to create awareness among the farmers about the application of PGPR, which would enhance the vegetative yield of chickpea and groundnut.

In this context, the present study was conducted to study the bacterial diversity persisting in the ecological niche of vermicompost and their behaviour in accordance to their plant growth-promoting (PGP) and antifungal attributes. A single index of diversity limits the amount of information that can be conveyed. An alternative is to examine the structure of a community in terms of its species abundance distribution or spatial patterning in more details (Magurran & McGill 2011). The objectives of present study were: (i) to isolate the predominant bacterial species from three different sites; (ii) to estimate the species richness and evenness diversity index; (iii) to do the principal component analysis between diversity and soil factors; (iv) to evaluate some of their PGP and antifungal attributes under *in vitro* experiments; and (v) to assess application of potential PGPR strains as bioinoculants in order to enhance growth of chickpea and groundnut seedlings under green house experiments.

Material and methods

Vermicompost sampling and rhizobacterial isolation

Based on the structure and functionality of soil and the culture conditions, three distinctive soil sites were selected all over the state of Gujarat (India). They were labelled as PS1, BS2 and VS3. Site PS1 was sodic soil highly deficient in Zn content amended with vermicompost from Mehsana district. Site BS2 was fertile land located in Agriculture University at Dantiwada amended with vermicompost. Soil of site VS3 was also amended with vermicompost from Gandhinagar district. All three samples were taken for the determination of pH, salinity, total organic C, P, K, Fe, Zn, Cu and Mn (Table 1). Ten g samples of each were suspended in sterile saline solution (0.85% NaCl) and were incubated at 28 °C on shakers with 150 rpm for 16 h. Aliquots (0.1 mL) from serially diluted samples were inoculated on differential media like YEM agar, NFB agar, Pikovskaya agar, MRBA agar, King's agar, and Ashby's mannitol agar in triplicates according to Jha et al. (2010). Number of colonies obtained on all these agar plates was counted and their colony characteristics were recorded. Fast growing prominent colonies were selected for further studies. The isolates were studied for their morphological characteristics after performing Gram staining. They were purified by sub-culturing and preserved on their respective basal medium containing 40% glycerol at −4 °C.

Functional diversity and data analysis

After isolation of the maximum number of bacteria to avoid the loss of bacterial variability, different tests were performed to select the bacteria chosen, so that only putative beneficial ones remain. The tests were performed *in vitro* to check biochemical activities that correspond with potential PGPR traits. Based on the Bergey's Manual of Systematic Bacteriology, biochemical tests were carried out for their preliminary identification. Isolates were screened for their known traits, associated with the ability to function as PGPR. Each *in vitro* test was replicated three times. These isolates were checked for their PGP and antagonistic potentials, like phosphate solubilization (Gaur 1990), siderophore production (Schwyn & Neilands 1987), IAA production (Sarwar & Kremer 1995), HCN production (Castric 1975; Morrison & Askeland 1983), dual culture technique (Skidmore & Dickinson 1976) and lytic enzymes production (Cattelan et al. 1990) as per standard methods reported. The results obtained from PGPR diversity analysis were used to calculate richness metrics (Margalef 1972; Menhinick 1964), evenness metrics (Simpson 1949; Hurlbert 1971; Pielou 1975; Smith & Wilson 1996), and diversity metrics (Shannon & Weaver 1949; Simpson 1949; Hurlbert 1971) and Berger-Parker index (Berger & Parker 1970) as reported. Principal component analysis (PCA) was used to determine the statistical correlation between soil properties and population diversity (Rico et al. 2004).

Genomic DNA sequencing and phylogenetic analysis

Genomic DNA isolation was performed (Sambrook et al. 1989) and a complete 1.6-kb 16S rDNA region was amplified using the universal primer 1F-AGCGGCGGACGGGTGA GTAATG and 1509R-AAGGAGGGGATCCAGCCGCA. The DNA sequencing was performed using an ABI Prism Sequence Detection System. The BLASTn search program (http://www.ncbi.nlm.nih.gov/) was used to look

for nucleotide sequence homology. The sequences obtained were then aligned by ClustalW using MEGA 4.0 software (Tamura et al. 2007) and a neighbour-joining tree was generated using the software.

In vivo experiment on plant growth promotion by native PGPR isolates

Only two strains were selected on the basis of their highest PGP and biocontrol potential under the *in vivo* experiments. Chickpea and groundnut seeds were sterilized with 70% ethanol for 2 min and in 2% sodium hypochlorite for 2 min, followed by washing ten times in sterile tap water. For this experiment, pure cultures were grown in nutrient broth at 28 °C and diluted to a final concentration of 10^8 colony forming units per mL in sterile saline water (0.85%). The surface-sterilized seeds were immersed in appropriate PGPR suspension for 1 h, air-dried, and sown immediately. The following treatments with three replicates were investigated with two individual experiments: (i) control (without bacterial inoculation); (ii) *Pseudomonas stutzeri* (MBCU1); and (iii) *Pseudomoas mosselli* (MBCU3). Pots were sterilized with 20% sodium hypochlorite solution and filled with sterile soil. The chickpea and groundnut seeds (10 seeds in each pot) were sown in plastic pots filled with 1 kg sterile field soil. The pots were arranged in a completely randomized factorial design. The seedlings were grown at a temperature of 28–32 °C and 85% relative humidity, on a day-night cycle of 13–14 h natural light. The pots were watered to 50% water-holding capacity and were maintained at this moisture content by watering to weight every day. For each species and treatment, the plants of three pots were harvested at 3 weeks after the emergence of seedlings, washed and various vegetative parameters like root length, shoot length, number of leaves, chlorophyll content, fresh weight and dry weight were studied (Pesqueira et al. 2006).

Statistical analysis

To evaluate the efficiency of rhizobacteria in pot experiments, a completely randomized block design was used. To identify significant treatment, analysis of variance (ANOVA) was carried out. Mean values were compared at significance levels of 1% and 5%. The ANOVA indicated significances of treatment and effects.

Results

Isolation and characterization of rhizobacteria

Isolates showing variation in their morphological characteristics, such as size, shape, texture, pigmentation, etc., were selected and purified by sub-culturing. Only those isolates which gave maximum growth after 24 h of incubation, termed as fast growers, were selected for further studies. They were purified on their respective medium. Each isolate was ranked and assigned on the basis of its ability to produce the both PGP and antagonistic characteristics. Based on fast growing characteristics, total 132 isolates were screened from site PS1, 101 isolates from BS2 and 205 isolates were screened from VS3 sample. All the 438 isolates were further screened for both the PGP and antagonistic characteristics. Individually, 28 isolates from site PS1, 21 isolates from site BS2 and 27 isolates from site VS3 were screened on the basis of their above-mentioned attributes. Their diversity indices were studied for determining the species

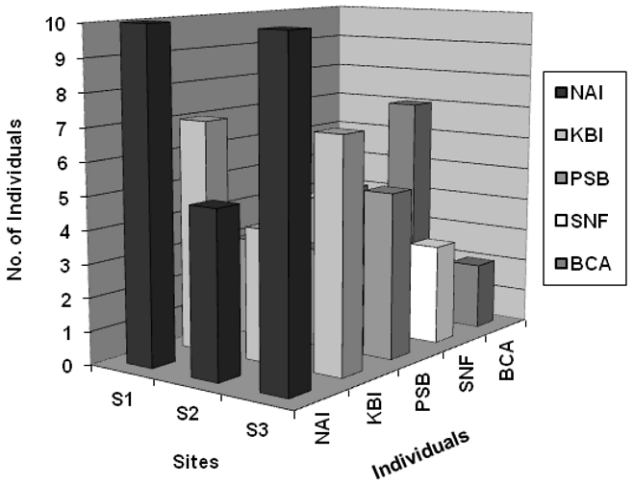

Fig. 1. Comparison of number of individuals in all three samples selected for diversity study. Abbreviations: NAI, nutrient agar isolates; KBI, King's agar isolates; PSB, phosphate solubilizing bacteria; SNF, symbiotic nitrogen fixers; BCA, biological control agents (antagonistic bacteria).

Table 2. Richness metrics.

Value index	Sample 1	Sample 2	Sample 3	Relationship
Margalef's index	1.200	1.314	1.214	S1<S3<S2
Menhinick's index	0.945	1.091	0.962	S1<S3<S2

richness and evenness of PGPR in these samples. Phosphate solubilizing bacteria were observed to be maximum in VS3 in comparison with BS2 and PS1. The BS2 showed the maximum 33% biocontrol agents as compared to other two sites PS1 and VS3. The PS1 and VS3 exhibited a higher percentage of *Pseudomonas*, probably due to abundance of this bacteria naturally present inside the microflora. The number of PGPR strains isolated from three different samples showed maximum bacterial population on nutrient agar plates followed by King's agar medium and then phosphate solubilizers (Fig. 1).

Functional and informative diversity

Species richness includes Margalef's equation and Menhinick's equations based on the assumption that a relationship between S (number of species) and N (total number of individuals) exists. Sample BS2 showed the highest Margalef' index $D_{\mathrm{Mg}} = 1.314$ and Menhinick's index $D_{\mathrm{Mn}} = 1.091$ as compared to other two samples (Table 2). A high Shannon diversity index calculated from the number of groups and number of individuals per group was found at all three samples. The diversity indices were as follows: sample PS1 ($H' = 1.509$, $n = 28$), sample BS1 ($H' = 1.525$, $n = 21$) and sample VS3 ($H' = 1.466$, $n = 27$). Sample BS1 showed the highest Simpson diversity ($1/D = 5.120$), Hurlbert diversity (PIE = 0.805), Berger-Parker index ($1/d = 3.003$) as shown in Table 3. Evenness metrics all attempt to

Table 3. Diversity metrics.

Study metrics	Sample 1	Sample 2	Sample 3	Relationship
Shannon diversity (H')	1.509	1.525	1.466	S3<S1<S2
$e^{H'}$	4.520	4.595	4.332	S3<S1<S2
Simpson diversity ($1/D$)	4.673	5.120	4.389	S3<S1<S2
Hurlbert diversity (PIE)	0.785	0.805	0.772	S3<S1<S2
Berger-Parker Index ($1/d$)	2.801	3.003	2.703	S3<S1<S2
Mean	2.86	3.01	2.73	
SD	1.74	1.87	1.64	

Table 4. Evenness metrics.

Value metrics	Sample 1	Sample 2	Sample 3	Relationship
Shannon evenness (J')	0.938	0.945	0.911	S3<S1<S2
Simpson evenness ($1/D/S$)	0.935	1.024	0.888	S3<S1<S2
Smith Wilson evenness	0.880	0.884	0.795	S3<S1<S2
Sheldon $e^{H'}/S$	0.904	0.919	0.866	S3<S1<S2
Mean	0.914	0.943	0.863	
SD	0.027	0.059	0.050	

Table 5. Plant growth promoting and antifungal properties of bacterial isolates.

Strains	Phosphate[a]	Sid[b]	IAA[c]	HCN[d]	Lytic enzyme production[e]			Antagonism[f]		
					Chi	Glu	Prot	MP	RS	FO
MBCU11	15	19	11	+	+++	+	++	+++	+	++
MBCU13	23	26	45	+	++	+	+++	+++	+++	+++
MBCU15	19	24	30	+	+	+	–	++	++	++
MBCU19	19	19	92	+	+	++	++	+	++	+++
MBCU1	24	24	28	+	+++	++	++	+++	+++	++
MBCU2	21	23	19	+	+++	–	–	+++	+++	+++
MBCU25	21	19	49	+	++	–	++	+	–	++
MBCU27	19	24	55	+	+	–	+++	+	+	+
MBCU31	16	16	–	–	+	–	++	+++	–	–
MBCU33	15	19	56	+	++	–	–	+	–	–
MBCU35	15	22	51	–	+	–	+	+	–	+++
MBCU3	17	16	70	–	+++	++	+++	+++	+	+
MBCU39	16	17	61	–	+	+++	++	+	++	++
MBCU4	17	15	52	–	+++	–	++	+++	+	+++
MBCU43	13	16	46	–	++	–	–	+++	–	+
MBCU45	20	16	49	+	+++	+++	+	+++	+	+
MBCU49	15	18	44	+	+++	++	+	+	–	+
MBCU5	14	22	–	+	++	++	+	+++	++	+++
MBCU53	18	24	68	+	++	+	+	+++	+	+
MBCU55	12	19	51	–	+	+	–	+++	–	+
MBCU57	20	16	40	+	+	+	++	+	–	+
MBCU59	10	16	–	–	+	++	+++	++	–	+
MBCU6	16	19	–	+	+++	+	+	+++	–	+
MBCU63	14	21	59	–	+	+	–	++	+++	+
MBCU65	14	15	–	+	+	+	+	+	+	+
MBCU67	17	17	21	+	+	+	–	+	++	++
MBCU69	13	12	47	–	+	+	++	+++	+	+
MBCU7	15	28	–	+	+++	–	–	+++	+++	+
MBCU73	25	22	–	–	+	–	+++	++	–	+
MBCU75	22	13	–	–	+	–	–	+	–	++
MBCU79	23	19	–	–	+	+	–	+++	++	+++

[a] Phosphate solubilization (mm); [b] siderophore production (mm); [c] IAA production (µg/mL), [d] HCN production; [e] chitinase (Chi), β-1,3–glucanase (Glu) and protease (Prot) production, [f] MP, RSA and FO – *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium oxysporum* and, respectively; + small halos <0.5 cm wide surrounding colonies, ++ medium halos > 0.5 cm, +++ large halos > 1.0 cm wide surrounding halos.

examine how abundance is apportioned among species within a community. Table 4 showed that sample BS2 had highest Shannon ($J' = 0.945$), Simpson ($1/D/S = 1.024$), Smith and Wilson ($E_{\text{SmithWilson}} = 0.884$) and Sheldon ($e^{H'}/S = 0.919$) was calculated by mathematical calculation.

PCA was studied to investigate the relationships between bacterial diversity (H') and soil factors. The

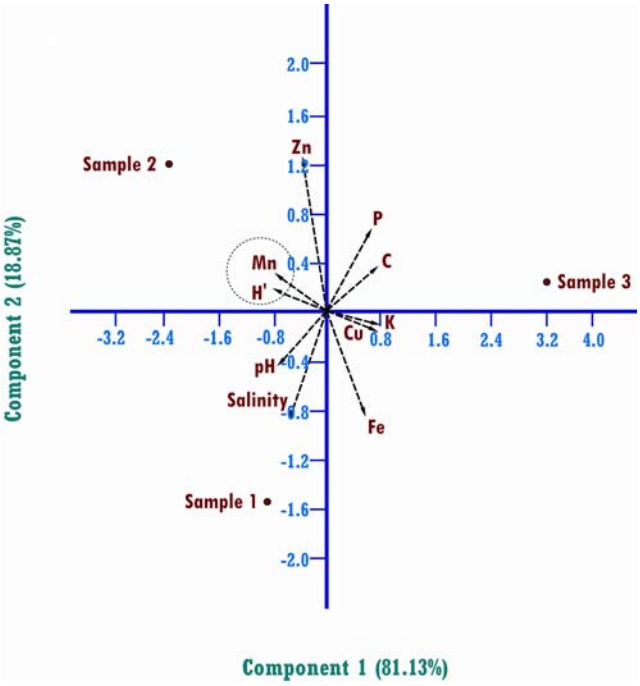

Fig. 2. Principal component analysis of sample sites related to soil composition (pH, salinity, C, P, K, Fe, Zn, Cu, Mn) that have shown some statistical correlation with diversity index (H').

principal components of PCA (PCA1 and PCA2) explained with component 1 accounting for 81.13% and component 2 for 18.87% of the total variation. Mn content had a direct correlation with bacterial diversity as well, where as K and Cu contents had an inverse correlation (Fig. 2).

PGP and antifungal attributes of bacterial isolates

All selected 76 isolates were characterized on the basis of their PGP and antagonistic traits. A total of 31 isolates demonstrated all the characteristics, like phosphate solubilization, siderophores, IAA, HCN, lytic enzymes (chitinase, β-1,3-glucanase and protease) and antagonism against fungal phytopathogens under *in vitro* conditions (Table 5). When the phosphate solubilization potential of the 76 isolates was studied, 31 isolates showed a zone of phosphate solubilization on Pikovskaya's agar medium using TCP. The zone of phosphate solubilization on agar plates ranged from 12 to 25 mm within 4 days of incubation. Siderophore production of the 76 isolates was determined by change in colour of the CAS agar medium from dark blue to yellow or purplish pink around the colony. Of the 76 isolates, 31 isolates demonstrated siderophore production. The zone of siderophore production on the CAS agar plate ranged from 12 to 26 mm after 72 h of incubation. A total of 22 isolates (28.95%) produced IAA in the presence of tryptophan. The IAA production ranged from 11 to 70 µg/mL after 72 h of incubation. Among the 76 isolates, 31 isolates tested positive for chitinase, 20 isolates produced β-1,3-glucanase and 21 isolates produced protease on respective medium after 5 days of incubation. All the isolates were screened for antagonistic activity against *M. phaseolina*, *F. oxysporum* and *R. solani*, respectively. The maximum inhibition in radial growth of both fungal pathogens viz. *M. phaseolina* and *R. solani* was caused by 31 isolates from total 76 isolates in dual culture method after 7 days of incubation. Notably, isolate MBCU1 and MBCU3 showed maximum PGP attributes as well as the highest efficiency to inhibit all three fungal pathogens. Hence MBCU1 and MBCU3 were selected for further study for their identification and pot trials.

Phylogenetic analysis

Phylogenetic analysis of 16S rDNA sequence of MBCU1 showed that the strain had the highest sequence similarity with *Pseudomonas stutzeri* (Fig. 3) and the MBCU3 exhibited the highest sequence similarity with *Pseudomonas mosselii* (Fig. 4). Sequence data reported in the present study have been deposited in the GenBank nucleotide sequence database under the accession numbers KC669688 (MBCU1) and KC669689 (MBCU3).

Growth promoting effect of selected bacterial treatment on chickpea and groundnut seedlings

A green house experiment was conducted to assess the efficiency of the two isolates viz. MBCU1 and MBCU3 as bioinoculant in improving the chickpea and groundnut seedling at 21 days after sowing (Table 6). The groundnut seedlings inoculated with MBCU1 showed

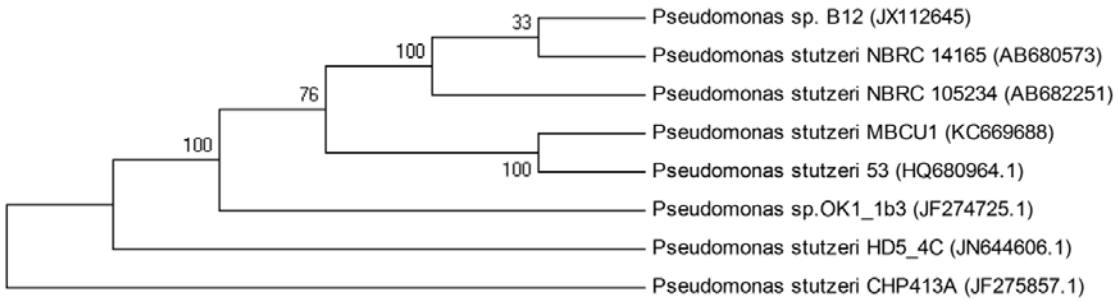

Fig. 3. Phylogenetic analysis of MBCU1 based on 16S rRNA gene sequences available from the European Molecular Biology Laboratory data library constructed after multiple alignments of data by ClustalX. Distances and clustering with the neighbour-joining method were calculated by using MEGA 4.0.

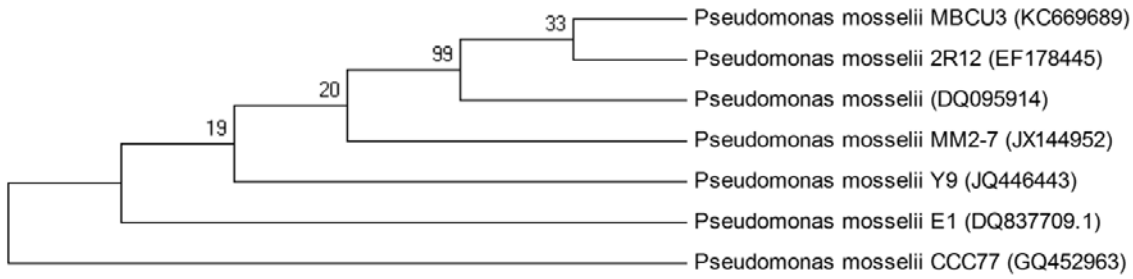

Fig. 4. Phylogenetic analysis of MBCU3 based on 16S rRNA gene sequences available from the European Molecular Biology Laboratory data library constructed after multiple alignments of data by ClustalX. Distances and clustering with the neighbour-joining method were calculated by using MEGA 4.0.

Table 6. Effect of two PGPR strains on the growth of chickpea and groundnut seedlings.[a]

Parameters	Groundnut seedlings			Chickpea seedlings		
	Control	MBCU1	MBCU3	Control	MBCU1	MBCU3
Root length (cm)	10	20*	13^{ns}	12	13.2^{ns}	12.8**
Shoot length (cm)	12	25^{ns}	18*	19.5	24.2*	23.3
No. of leaves	20	32^{ns}	26**	74	136*	110^{ns}
No. of lateral roots	46.5	55.5**	51.5^{ns}	25	45^{ns}	35^{ns}
Fresh weight (g)	1.34	2.10*	1.53^{ns}	0.87	1.20*	1.15^{ns}
Dry weight (g)	0.30	0.41^{ns}	0.37^{ns}	0.30	0.38*	0.33*
Chlorophyll (mg/g)	1.76	3.50**	2.42^{ns}	1.76	2.82**	2.31^{ns}

[a] Values are the mean of triplicates. * Significant at 5% LSD; ** significant at 1% level of LSD as compared to control.

an increase in all the vegetative growth parameters, like seedling root length (100%), shoot length (108.33%) and dry weight (36.67%) over uninoculated control. On the other hand, chickpea seedling inoculated with MBCU3 showed an increase in all the vegetative growth parameters, like seedling root length (55.29%), shoot length (96.75%) and dry weight (35.71%) over uninoculated control.

Discussion

In our study, finally 76 various types of rhizobacteria were found to be more potential from three sites treated with vermicompost from Gujarat, India. Their diversity indices were further studied for determining the species richness and evenness of PGPR from three samples. Usually Shannon index value was found between 1.5–3.5, rarely surpassing 4, high values being produced only when there are huge number of species in the sample (Margalef 1972). Our results also showed Shannon index between 1.5–3.5 and the site BS2 demonstrated the maximum high value. Sometimes Shannon index is so narrowly constrained that it can make interpretation difficult. Similarly we found values of PS1 and BS2 as $H' = 1.509$ and $H' = 1.525$, respectively, that may have little inventiveness whether the two sites in question have similar diversities or are substantially different. Some investigators sidestep the problem by using $e^{H'}$ instead of H'. The $e^{H'}$ is an intuitively meaningful measure as it gives the number of species that would have been found in the sample (Whittaker 1972). Thus $H' = 1.509$ becomes $e^{H'} = 4.520$, and $H' = 1.525$ becomes $e^{H'} = 4.595$. Kaiser et al. (2000) used this approach when examining the effects of disturbance on marine benthic communities. In Shannon index and species richness, higher the value means larger diversity. In the present study, BS2 is richer followed by PS1, while VS3 showed the least diversity of PGPR. Simpson index is one of the most meaningful and robust diversity measures available. In the present case, the site BS2 shows high microbial diversity followed by PS1 according to the Simpson index, the sample VS3 being least diverse.

Hurlbert (1971) suggested to use the probability of an interspecific encounter (PIE), which gives the probability that two randomly sampled individuals from assemblage represent two different species. This index is closely related to Simpson's (1949) "measure of concentration". PIE assumes that each individual within a community can encounter or interact with every other individual. The BS2 showed both the highest value of PIE diversity and Simpson diversity as compared to other samples diversity metrics (Table 3). The Berger-Parker index (Berger & Parker 1970) provides a simple and easily interpretable measure of dominance. As with the Simpson index, the reciprocal form of Berger-Parker index may be adopted so that an increase in the value of the index accompanies an increase in diversity and a reduction in dominance. Thus while the Shannon index results (which emphasizes the richness component of diversity) showed the BS2 as the most diverse, the Simpson and Berger-Parker measures (which place more weight on evenness), conclude that the PGPR strain from BS2 has the highest diversity. Simpson's diversity measure emphasizes the dominance, as opposed to the richness, component of diversity; it is not, in fact,

a pure evenness measure. As Simpson's index increases, diversity increases (Simpson 1949).

Smith & Wilson (1996) proposed a new index that measures the variance in species abundances, and divides this variance over log abundance to give proportional differences, making thus the index independent of the units of measurement. The maximum value of the index is achieved when abundances are equal and the maximum value is 1. Similarly our results also showed the index value near to 1 (Table 4) with the sample BS2 exhibiting the maximum value (0.884) followed by samples PS1 (0.880) and VS3 (0.795). The Smith evenness index (E) was recommended for general use after comparison with 13 other evenness indices (Smith & Wilson 1996). It is considered the best-discriminating index that is suitable for detecting low evenness in random functions, like a lognormal distribution (Mouilliot & Wilson 2002).

In order to determine the correlation between soil composition and bacterial diversity, PCA was studied. This analysis revealed that soil micronutrients like Mn content directly correlated with bacterial diversity (H'). Aruda et al. (2013) studied bacterial communities in maize soils and observed that a higher bacterial diversity may be influenced by fine particles, such as clay content. Some authors also suggested that soil texture, pH and clay are parameters that might affect the richness and diversity of bacterial communities in the soil (Bashan et al. 1995; Sessitsch et al. 2002, Kemmitt et al. 2006; Beneduzi et al. 2013).

In the present study, more than half of the isolates were able to solubilize phosphates. *In vitro* phosphate solubilization activity has been documented for many PGPR strains isolated from rhizospheric soil (Singh et al. 2010; Muleta et al. 2013). Siderophores are iron chelators synthesized by several PGPR and they play a vital role by directly supplying iron for plant utilization and by removing iron from the environment for the growth of phytopathogens, thereby reducing their competitiveness (Tank et al. 2012). We found several siderophore producing strains belonging to pseudomonads and bacilli. IAA production by PGPR strains in significant amount has also been reported (Jha & Saraf 2011; Patel et al. 2011). Mycolytic enzymes, such as chitinase, β-1,3-glucanase, protease, etc., are being exploited widely for crop disease management practices. Our results showed that potential PGPR strains were able to produce all the three cell wall degrading enzymes which were responsible for fungal growth inhibition. *Pseudomoanas* strain MIP3 produced maximum chitinase (340 µM) and β-1,3-glucanase (450 µM) which caused 87% inhibition of *Aspergillus* spp. (Saraf et al. 2008). Our potential PGPR strains showed maximum radial growth inhibition of *M. phaseolina* by dual culture method. Similar findings have also been reported by other researchers (Singh et al. 2010; Gopalakrishnan et al. 2011). Pathma & Sakthivel (2013) have recently reported a total of 193 vermicompost bacteria that exhibited antagonistic and biofertilizing potential from straw and goat manure based vermicompost.

Seed inoculation with both pseudomonads strains exerted a positive effect on the physiological characteristics of the chickpea and groundnut seedlings. Our study revealed a significantly vegetative growth enhancement of both seedlings as compared to uninoculated control plants. Similarly, Pandya & Saraf (2010) reported that application of bioinoculants to chickpea having potential of phosphate solubilization and siderophore production increases in all the vegetative growth parameters under saline conditions. Patel et al. (2011) also reported that strains MSC1 (*Pseudomonas putida*) and MSC4 (*Pseudomonas pseudoalcaligens*) showed increases in all vegetative parameters of chickpea under saline conditions. Similarly, enhancement of growth parameters of groundnut by pseudomomads has also been reported by a number of authors (e.g., Gupta et al. 2002; Kishore et al. 2005; Bhatia et al. 2008). Recently, Stefan et al. (2013) found that runner bean seed inoculation with two rhizobacterial strains resulted in an increase of photosynthetic activities, water-use efficiency and chlorophyll content along with their significant yield.

Conclusion

Vermicompost enhances soil biodiversity by promoting the beneficial microbes which in turn enhances plant growth directly by production of plant growth-regulating hormones and enzymes and indirectly by controlling plant pathogens. The increase of microbial population may be due to the congenial condition for the growth of microbes within the digestive tract of earthworm and by the ingestion of nutrient rich organic wastes which provide energy and also act as a substrate for the growth of microorganisms. When vermicompost applied with soil, earthworms proliferate beneficial microbes as evidenced by our results when paramount diversity (indices) was observed in site BS2. According to the PCA, the BS2 site contained a rich source of Mn that correlated with maximum bacterial diversity. *In vivo* experiments showed a significant enhancement of chickpea and groundnut seedling treated with beneficial microbes from site BS2 identified as MBCU1 (*Pseudomonas stutzeri*) and MBCU3 (*Pseudomons mosselii*) due to their multiple PGP traits under *in vitro* experiments. Therefore, future investigations will include applications of these potent PGPR along with their biocontrol potential in field conditions.

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