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SHORT COMMUNICATION

Genetic and functional diversities of bacterial communities in the rhizosphere of *Arachis hypogaea*

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Abstract Bioinoculants are environmentally friendly, energy efficient and economically viable resources in sustainable agriculture. Knowledge of the structure and activities of microbial population in the rhizosphere of a plant is essential to formulate an effective bioinoculant. In this study, the bacterial community present in the rhizosphere of an important oilseed legume, *Arachis hypogaea* (L.) was described with respect to adjoining bulk soil as a baseline control using a 16S rDNA based metagenomic approach. Significantly higher abundance of *Gamma-proteobacteria*, a prevalence of *Bacillus* and the *Cytophaga-Flavobacteria* group of *Bacteroidetes* and absence of the *Rhizobiaceae* family of *Alpha-proteobacteria* were the major features observed in the matured *Arachis*-rhizosphere. The functional characterization of the rhizosphere-competent bacteria was performed using culture-dependent determination of phenotypes. Most bacterial isolates from the groundnut-rhizosphere exhibited multiple biochemical activities associated with plant growth and disease control. Validation of the beneficial traits in candidate bioinoculants in pot-cultures and field trials is necessary before their targeted application in the groundnut production system.

Keywords *Arachis hypogaea* · Rhizosphere · Plant growth promoting rhizobacteria · Bioinoculant · *Proteobacteria* · *Bacillus*

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Introduction

Peanuts are rich sources of important nutrients and bioactive constituents such as arginine-rich proteins, high levels of soluble and insoluble fiber, beneficial fatty acids, vitamins including high folate and vitamin E, phenolics and phytosterols, all of which provide a wide range of health benefits (Kris-Etherton et al. 2008). According to a report of the USDA-Foreign Agricultural Service (2008–2009), China leads in the production of Runner, Virginia, Spanish and Valencia market-type peanuts (*Arachis hypogaea* L.) having a share of about 33% of overall world production, followed by India (18%) and the United States of America (7%). In rain-fed areas of Asia, soil fertility is an important constraint for high pod yields. The majority of the small-scale farmers in these regions are reluctant to invest in chemical fertilizers and other agrochemicals because of the unassured crop returns owing to the high incidence of fungal diseases and unpredictable monsoon (Kishore et al. 2005). Besides, application of inorganic fertilizer debilitates the soil's

physical and chemical status and drastically alters microbial diversity, which is vital to sustain the fertility of soil (Adesemoye and Kloepper 2009; Yang et al. 2000).

As a step towards sustainable agriculture, there is a greater scope for development and popularization of bioinoculants in the groundnut production system. The two most common groundnut plant growth promoting rhizobacteria (PGPR) that enhance biomass, nitrogen and phosphorous uptake and crop-yield are *Pseudomonas* and *Bacillus* sp. (Dey et al. 2004; Saravanakumar and Samiyappan 2007; Kishore et al. 2005). Until recently, most studies aiming for the isolation and identification of PGPR were based on cultivation and phenotypic characterization of bacterial isolates (Prosser et al. 2006). There is growing body of evidence that diverse trophic and functional groups of microorganisms contribute to the establishment and functioning of the microbial community in the rhizosphere (Kent and Triplett 2002). Thus, a polyphasic approach which will provide a reliable and wider identification, as well as a better elucidation of functional attributes of members of the bacterial community, is preferred. Given the economic and nutritional importance of *A. hypogaea* in the Indian sub-continent, the present report utilizes a combination of methods including 16S rDNA based molecular typing, microbial culture techniques, biochemical assays and statistical analyses to characterize the bacterial community in the rhizosphere with respect to that in bulk soil and to decipher the functional attributes related to plant growth promotion and disease control towards systematic reconstruction of a rhizosphere-competent inoculant for the groundnut plant.

Materials and methods

Fresh, healthy groundnut seeds (AK-1224), obtained from West Bengal State Seed Corporation (Midnapur), were disinfected with 70% ethanol, washed in sterile water and were directly planted in pots containing loam soil of composition 40% sand, 40% silt, 20% clay and of pH 8.17. The soil used was collected from an agricultural field located on the southern fringes of the city of Kolkata, lying in the Gangetic belt of West Bengal, India (22°34′N and 88°24′E). The field was primarily used for commercial production of flowering plants. To manage the soil fertility, the planters grow groundnut and other leguminous plants on a rotational basis. However, there was no report of cultivation of any leguminous plants prior to collection of the soil for plant growth in pots (personal communication with local farmers). Immediately after collection of soil, physicochemical characterization of the soil was performed and organic carbon, available nitrogen, phosphorous and potassium contents were determined to be 0.176%, 121.7, 29.2 and 38.99 kg/h, respectively. Before sowing peanut seeds, the soil was sieved, homogenized and transferred into plastic pots ($12 \times 12 \times 6$ cm; 400 g soil/pot). The seeds were sown in February, 2008 (average maximum and minimum temperatures were 28 and 18°C) and harvested in May, 2008 (average maximum and minimum temperatures were 35 and 25°C). No plant protection methods were used. Initially five surface-sterilized seeds were sown in each pot and twelve such pots were used. After 90 days of sowing, the time required for physiological maturation of *A. hypogaea*, a total of 50 plants were harvested by uprooting them from a depth of 10 cm. The shoot system was cut out using sterile scissors and nodules were separated. The bulk soil was collected by shaking the roots to dislodge the loosely attached soil in 50-ml centrifuge tubes whereas rhizosphere sample was collected by rinsing the roots in phosphate-buffered saline (PBS; 7 g/l NaCl, 0.7 g/l Na_2HPO_4, 0.2 g/l KH_2PO_4; pH 7.2) for 5 min, followed by centrifugation at 7,000 rpm for 5 min at 4°C. Soil representing rhizosphere and bulk samples were analyzed by mixing the soil samples from all the plants and stored at 4 and −20°C for microbiological and molecular analyses, respectively.

Soil DNA was extracted from 100 mg of rhizosphere and bulk soil using a Soil Master DNA Extraction Kit (Epicenter). The bacterial 16S rRNA gene (partial) from the soil DNA was amplified using degenerate primers 27F (5′-AGAGTTTGATCMTGG CTCAG-3′) and 1492R (5′-ACGGYTACCTTGTTA CGACTT-3′), following the PCR protocol described by Zhang et al. (2006). PCR products were purified using a QIAquick Gel Extraction Kit (Qiagen), ligated to TA-vector pTZ57R/T (InsTAclone PCR Cloning Kit, Fermentas) and transformed into *Escherichia coli* DH5-alpha cells. Plasmid DNA was isolated from each clone using the alkaline-lysis method (Birnboim and Doly 1979) followed by amplification of the insert with the 27F and 1492R

primers to confirm integrity of the insert. Four microliter of each PCR product was restriction digested separately with 10 U of *Hha*I and *Rsa*I (Fermentas) following manufacturer's protocol. The digests were electrophoresed in 2% agarose gels and DNA fragment sizes were determined with respect to external DNA-ladder using a TANON gel analysis system (4100). Clones displaying indistinguishable pattern were grouped under a phylotype. DNA sequencing were performed using a BigDye sequence terminator kit v1.1 (Applied Biosystems) with the 27F and a nested 515F (5′-GTGCCAGCMGCCG CGGTAA-3′) primer (Papineau et al. 2005) and an ABI Prism 3100 genetic analyzer. The termini of the 27F and 515F generated sequences of a clone were trimmed by inspecting individual electropherograms by two independent researchers (SH and SSG). Sequence reads generated using primers 27F and 515F for each clone were stitched after identification of overlapping region (at least 50 basepairs overlap). Approximately, 1 kb sequence data was available for each bacterial clone.

Phylotype data was used to compare bacterial abundance and diversity in rhizosphere and bulk soils in terms of phylotype richness (Margalef index and Chao1 estimate), diversity (Shannon–Weiner and Simpson's indices), similarity (Sorensen's index) and evenness (Pielou index) using rarefaction analysis (www.biome.sdsu.edu/fastgroup/cal_tools.htm). Binomial proportion testing and independent *t*-tests were performed to calculate the significance of difference of the results between two samples using SPSS for windows version 10.0 (SPSS Inc). Identity of bacterial species in each sequenced clone was deciphered by using the Basic Local Alignment Search Tool (www.ncbi.nlm.nih.gov/BLAST/) and Classifier Tool of Ribosomal Database Project (RDP-II) Release 10 (www.rdp.cme.msu.edu/) after examining for chimeras by the CHIMERA-CHECK online analysis program of RDP-II (http://rdp8.cme.msu.edu/cgis/chimera.cgi?su=SSU) (Han et al. 2009). The phylogenetic affiliation of soil bacteria was done following the classification system proposed by Tindall et al. (2006). Bacterial lineages were defined at the genus level since 16S rDNA sequence analysis may lack the power to uncover species diversity at a finer scale. A total of 155 sequences (140 from metagenomic and 15 from culture-based soil clone libraries) were deposited in GenBank with the accession numbers GU217722–GU217788; GU245895–GU245926; GU269387–GU269410, GQ410441–GQ410442, GQ410548–GQ410549, HQ204321–HQ204332, HQ222600–HQ222601, HQ670722, GQ375136, GQ375143, GQ410444, GQ410448, GQ410453, GQ410478, GQ410492, GQ410501, GQ410504, GQ410516, GQ410533, GQ410553, GQ410555 and HQ326749.

To isolate individual bacterial species, 1 g each of rhizosphere and bulk soil was suspended in 9 ml sterile PBS; centrifuged at 180 rpm for 10 min at 30°C and serially diluted with PBS. One hundred microlitre aliquots from different dilutions were spread onto Luria-agar (HiMedia Laboratories) and Pseudomonas Isolation Agar (Difco Laboratories) and the plates were incubated for 48 h at 30°C (Jensen et al. 2001). Based on the colony characteristics, single colonies were selected and streaked at least three times to allow the isolation of pure colonies. Stock cultures were prepared in Luria-broth containing 25% (v/v) glycerol and stored at −80°C. Routine microbial tests, e.g. Gram-staining, catalase activity and morphology examination were performed. Genomic DNA was extracted from the bacteria following the SDS-CTAB lysis method (Sambrook and Russel 2001). The 16S rRNA gene was amplified, sequenced and assembled using methods described by Han et al. Indole acetic acid (IAA) production by the bacterial isolates in the absence of amended L-tryptophan was assayed colorimetrically (JASCO V-630 spectrophotometer) using the Salkowski reagent as described previously (Ali et al. 2009). The quantity of produced IAA was measured at 530 nm against IAA standards (HiMedia Laboratories). Phosphate-solubilization and siderophore production were qualitatively determined by inoculating the bacteria on to Pikovskaya (PVK) agar medium containing precipitated tri-calcium phosphate (Chaiharn and Lumyong 2010) and chrome azurol S (CAS)-blue agar medium (Schwyn and Neilands 1987), respectively. The presence of a clearing zone around bacterial colonies on PVK medium and a color change (orange/purple) on CAS medium after 3 days of incubation at 28°C were used as the indicator for phosphate-solubilization and siderophore production, respectively.

To assay antifungal activity, 72 h old *Macrophomina phaseolina* fungal spores were seeded at a distance of 4 cm from 24 h grown bacterial colonies

on Potato-dextrose agar medium. Bacterial strain and *M. phaseolina* were grown individually as controls. After 3 days of incubation at 28°C, radial growth of *M. phaseolina* co-inoculated with the bacterium was examined for inhibition of growth. Each experiment for ascertainment of a plant growth promoting trait (PGPT) of an individual bacterial isolate was conducted in triplicate.

Results and discussion

Two libraries, one with 180 clones from rhizosphere (AFR) and the other with 190 clones from bulk soil (AFB), collected from matured *A. hypogaea* plants, were constructed by integrating a 1.4 kb fragment of the bacterial 16S rRNA gene amplified from soil DNA into the TA vector. Phylotyping was performed by digesting the cloned insert with *Hha*I and *Rsa*I. Seventy-nine and 71 distinct *Hha*I phylotypes were detected from rhizosphere and bulk soil libraries, respectively. The numbers of different *Rsa*I phylotypes were 53 and 46 in rhizosphere and bulk soil libraries, respectively. Rarefaction curves using phylotype frequency data using *Hha*I on rhizosphere and bulk soil samples plateaued at their right hand side, suggesting that the number and distribution of *Hha*I phylotypes captured the diversity of the two specimens nearly completely (Fig. 1a). Twelve *Hha*I and ten *Rsa*I phylotypes were common between the libraries. Therefore, more than 80% of phylotypes were exclusive to each library, as elucidated from low Sorensen's similarity indices between the samples for both enzymes (Table 1). A significantly higher proportion of phylotypes (63%) obtained by *Hha*I restriction digestion from rhizosphere-derived library were represented by single clones compared to those derived from bulk soil (44%); ($\chi^2 = 4.38$, $P = 0.036$). The frequency of the remaining phylotypes varied between 2 and 19 for *Hha*I and 2 and 31 for *Rsa*I with a preponderance of low-frequency phylotypes. The phylotype richness of the rhizosphere bacterial

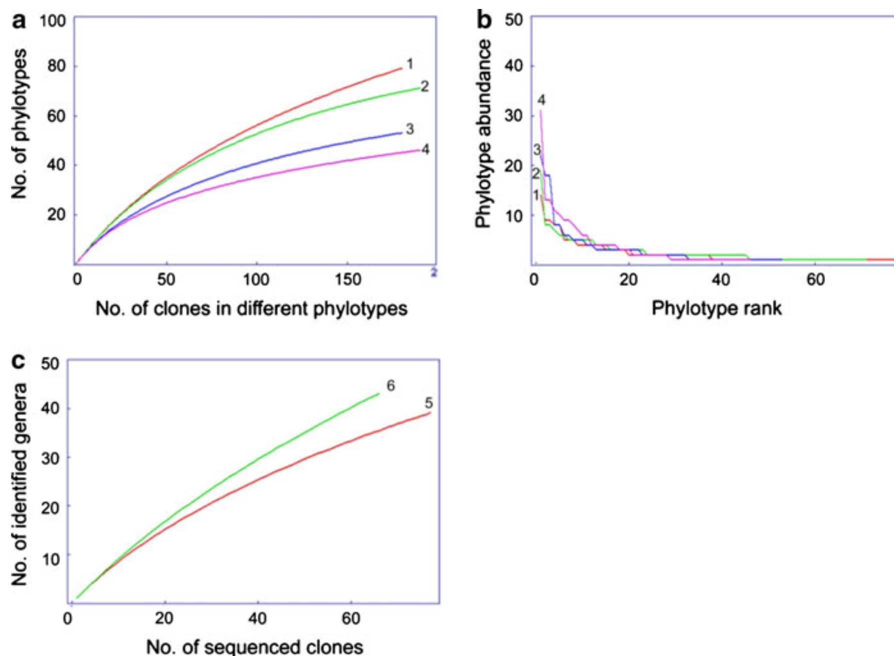

Fig. 1 Diversity and abundance of bacterial population in rhizosphere and bulk soil of *Arachis hypogaea* based on phylotype and sequence data. **a** Rarefaction curves based on phylotype-frequency obtained upon restriction digestion of 16S rDNA clones with *Hha*I (*1* and *2*) and *Rsa*I (*3* and *4*) from rhizosphere (*1* and *3*) and bulk soil (*2* and *4*) libraries of *Arachis hypogaea*, **b** rank-abundance curves based on the abundance of phylotypes obtained upon restriction digestion of 16S rDNA clones with *Hha*I (*1* and *2*) and *Rsa*I (*3* and *4*) from rhizosphere (*1* and *3*) and bulk soil (*2* and *4*) libraries of *Arachis hypogaea*, **c** rarefaction curves based on frequency of the genus detected by 16S rDNA sequencing of clones from rhizosphere (*5*) and bulk soil (*6*) libraries of *Arachis hypogaea*

Table 1 Bacterial distribution, abundance and diversity based on *Hha*I and *Rsa*I restriction analyses of rhizosphere (AFR) and bulk soil (AFB) libraries

Restriction enzyme	No. of phylotypes AFR/AFB		Phylotype richness AFR/AFB		Diversity indices AFR/AFB			
	Total	Singleton	Margalef index	Chao1 estimate	Simpson's	Shannon's	Sorensen's similarity	Pielou
*Hha*I	79/71	42/21	14.83/13.35	128/86*	0.974/0.972	4.03/3.95	0.22	0.927/0.924
*Rsa*I	53/46	21/18	10.01/8.58	75/66*	0.950/0.943	3.47/3.30	0.16	0.875/0.866

* indicates difference between the estimates is statistically significant

population was remarkably higher as per Margalef index and Chao1 estimates; ($P = 0.004$). The shallow slopes of the rank-abundance curves (Fig. 1b) and high Pielou indices revealed an even distribution pattern of most bacterial species in rhizosphere and bulk soils. Shannon–Weiner, Simpson's and Pielou diversities were also comparable between the samples (Table 1).

For identification of individual bacterial species present in the rhizosphere and bulk soil of *A. hypogaea*, all *Rsa*I phylotypes and multiple clones representing different *Hha*I sub-classifications from a given *Rsa*I phylotype were selected for sequencing. Altogether 140 clones, 74 clones from rhizosphere and 66 clones from the bulk soil library were sequenced. Based on 16S rRNA gene analysis, the two major bacterial phyla detected in both soils were *Proteobacteria* (43% in rhizosphere and 41% in bulk soil) and *Bacteroidetes* (34% in rhizosphere and 24% in bulk soil). The comparative profile of bacterial species detected in the rhizosphere and bulk soil associated with *A. hypogaea* is presented in Table 2. Few notable observations were: (i) a significant difference in proportion of *Gamma-proteobacteria* between rhizosphere and bulk soil samples (24% in rhizosphere and 6% in bulk soil; $P < 0.0001$), (ii) a complete absence of *Alpha-proteobacteria* belonging to *Rhizobiaceae* family and (iii) a remarkably high abundance of *Cytophaga-Flavobacteria* (*CF*-bacteria) particularly *Flavobacterium* sp. (15%), belonging to *Bacteroidetes* in the rhizosphere.

To utilize the information on bacterial composition of groundnut-rhizosphere towards development of bioinoculants, four PGPTs such as ability to produce IAA and siderophores, to solubilize inorganic phosphates and to exert anti-fungal activity were assayed in individual bacterial isolate. A total of 60 colonies were obtained from rhizosphere soil on Luria (20 colonies) and Pseudomonas-isolation agar (40 colonies) with colony-forming-unit (CFU) in the range of 10^6/g soil. The two major bacterial phyla identified from the rhizosphere by the culture-dependent method were *Proteobacteria* and *Firmicutes*. The species under *Proteobacteria* belonged to the genera *Acinetobacter*, *Aeromonas*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Xanthomonas* (*Gamma-proteobacteria*) and *Achromobacter* (*Beta-proteobacterium*), while those under *Firmicutes* belonged to *Bacillus* and *Staphylococcus* (*Bacilli*) (Table 2). *Agrobacterium* and *Bacillus* were the two genera detected in bulk soil. Thus, *Pseudomonas*, *Bacillus*, *Xanthomonas* and *Agrobacterium* were detected using culture-dependent and independent methods from the same soil source. The very small overlap of bacterial taxa observed between culture-based and culture-independent methods can be attributed to the fact that only 1% of the soil microbes are estimated to be cultivable and only 38% (140 out of 370) of the total clones were sequenced in this study. Rarefaction analysis (Fig. 1c) implicated that 16S rDNA based sequencing revealed the diversity of bacterial population present in *Arachis*-rhizosphere only partially (Zhang et al. 2007).

The majority of the bacterial genera isolated from rhizosphere were found to produce IAA (Table 3). Strains belonging to the genera *Achromobacter*, *Acinetobacter*, *Pseudomonas* and *Staphylococcus* were shown to produce siderophores. *Achromobacter* sp., in addition, could solubilize inorganic phosphates while isolates from the genera *Bacillus*, *Pseudomonas* and *Xanthomonas* demonstrated strong antifungal activity against the plant pathogen *M. phaseolina* which causes crown-rot disease in groundnut plants. Our results implied that *Achromobacter* sp., which showed positive results for three out of four PGPTs examined, is a strong candidate to be used as rhizoinoculant in addition to *Pseudomonas* and *Bacillus* species. The

Table 2 Phylogenetic affiliation of bacterial clones and isolates based on 16S rRNA gene sequencing from the rhizosphere and bulk soil of *Arachis hypogaea*

Representative clones	Closest bacterial affiliation in GenBank	Phylogenetic group (class)	% of identity
Bacterial species from rhizosphere identified using 16S rRNA gene sequencing (AFR)			
AFR-34	Uncultured *Methylocella* sp.	*Alpha-proteobacteria*	92
AFR-2	Uncultured *Hyphomonas* sp.		90
AFR-105	Uncultured *Ralstonia* sp.	*Beta-proteobacteria*	91
AFR-67	Uncultured *Methylibium* sp.		93
AFR-31	Uncultured *Variovorax* sp.		96
AFR-5,66	Uncultured *Janthinobacterium* sp.		93,96
AFR-22,26,27,32,69,102	Uncultured *Pseudomonas* sp.	*Gamma-proteobacteria*	90–99
AFR-9	Uncultured *Cellvibrio* sp.		95
AFR-113	Uncultured *Nitrosococcus* sp.		92
AFR-165	Uncultured *Methylococcus* sp.		93
AFR-6,18,83,84,178	Uncultured *Xanthomonas* sp.		91–96
AFR-154	Uncultured *Gamma proteobacterium*		90
AFR-172	Uncultured *Thioalkalivibrio* sp.		89
AFR-33,108	Uncultured *Legionella* sp.		98
AFR-91,19	Uncultured *Sorangium* sp.	*Delta-proteobacteria*	91
AFR-112,177	Uncultured *Geobacter* sp.		90
AFR-29,68	Uncultured *Pelobacter* sp.		90
AFR-23	Uncultured *Helicobacter* sp.	*Epsilon-proteobacteria*	78
AFR-3,14,43,44,47,62,103,109,124,156,176	Uncultured *Flavobacterium* sp.	*Bacteroidetes (CFB)*	93–96
AFR-74,96,111,137,166	Uncultured *Cytophaga* sp.		88–90
AFR-30,41,54	Uncultured *Chitinophaga* sp.		83
AFR-45,60	Uncultured *Candidatus Amoebophilus* sp.		94
AFR-35	Uncultured *Candidatus Desulforudis* sp.		86
AFR-70	Uncultured *Croceibacter* sp.		86
AFR-10	Uncultured *Pedobacter* sp.		82
AFR-75	Uncultured *Bacillus* sp.	*Firmicutes*	92
AFR-11,21,93	Uncultured *Clostridium* sp.		81–82
AFR-174	Uncultured *Pelotomaculum* sp.		77
AFR-17	Uncultured *Solibacter* sp.	*Acidobacteria*	87
AFR-163	Uncultured *Mycobacterium* sp.	*Actinobacteria*	97
AFR-99	Uncultured *Nocardioides* sp.		92
AFR-81	Uncultured *Tsukamurella* sp.		98
AFR-155	Uncultured candidate division *TM7* bacterium	*Candidate Division*	91
AFR-90	Uncultured *Prochlorococcus* sp.	*Cyanobacteria*	75
AFR-28,48,159	Uncultured *Gemmatimonas* sp.	*Gemmatimonadetes*	88–90
AFR-36,63	Uncultured *Planctomyces* sp.	*Planctomycetes*	83,89
AFR-130,151	Uncultured bacterium *Ellin514*	*Verrucomicrobia*	91,92
Bacterial species from bulk soil identified using 16S rRNA gene sequencing (AFB)			
AFB-96,150	Uncultured *Agrobacterium* sp.	*Alpha-proteobacteria*	95–98
AFB-44,81	Uncultured *Rhizobium* sp.		97,98
AFB-160	Uncultured *Azorhizobium* sp.		89
AFB-23,91,184	Uncultured *Mesorhizobium* sp.		93–98

Table 2 continued

Representative clones	Closest bacterial affiliation in GenBank	Phylogenetic group (class)	% of identity
AFB-46	Uncultured *Novosphingobium* sp.		96
AFB-20	Uncultured *Sphingomonas* sp.		93
AFB-75	Uncultured *Comamonas* sp.	*Beta-proteobacteria*	96
AFB-83	Uncultured *Acidovorax* sp.		94
AFB-39	Uncultured *Variovorax* sp.		97
AFB-41	Uncultured *Methylibium* sp.		95
AFB-170	Uncultured *Lutiella* sp.		96
AFB-28	Uncultured *Cupriavidus* sp.		93
AFB-63,100	Uncultured *Aromatoleum* sp.		91,92
AFB-32,98	Uncultured *Pseudomonas* sp.	*Gamma-proteobacteria*	97
AFB-24	Uncultured *Stenotrophomonas* sp.		97
AFB-178	Uncultured *Alkalilimnicola* sp.		88
AFB-128	Uncultured *Syntrophobacter* sp.	*Delta-proteobacteria*	83
AFB-168	Uncultured *Desulfovibrio* sp.		81
AFB-84,122	Uncultured *Geobacter* sp.		86,92
AFB-87	Uncultured *Desulfonatronospira* sp.		81
AFB-16,56,59,69,77,112,187	Uncultured *Chitinophaga* sp.	*Bacteroidetes (CFB)*	86–90
AFB-8,25,167,180	Uncultured *Cytophaga* sp.		86–87
AFB-186	Uncultured *Sphingobacterium* sp.		93
AFB-15,31	Uncultured *Dyadobacter* sp.		92
AFB-54	Uncultured *Pedobacter* sp.		93
AFB-38	Uncultured *Candidatus Amoebophilus* sp.		84
AFB-19,30,58,60,90	Uncultured *Bacillus* sp.	*Firmicutes*	90–95
AFB-175	Uncultured *Thermoanaerobacter* sp.		81
AFB-124	Uncultured *Carboxydibrachium* sp.		81
AFB-127	Uncultured *Moorella* sp.		84
AFB-51,172	Uncultured *Candidatus Koribacter* sp.	*Acidobacteria*	87,90
AFB-115	Uncultured *Rubrobacter* sp.	*Actinobacteria*	85
AFB-111	Uncultured *Janibacter* sp.		97
AFB-22	Uncultured *Salinispora* sp.		97
AFB-40	Uncultured *Nakamurella* sp.		94
AFB-36	Uncultured *Bifidobacterium* sp.		82
AFB-34	Uncultured candidate division *TM7* bacterium	Candidate division	85
AFB-37	Uncultured *Elusimicrobium* sp.	*Elusimicrobia*	84
AFB-119	Uncultured *Gemmatimonas* sp.	*Gemmatimonadetes*	86
AFB-3,78	Uncultured *Rhodopirellula* sp.	*Planctomycetes*	86
AFB-82	Uncultured *Planctomyces* sp.		87
AFB-21	Uncultured bacterium *Ellin514*	*Verrucomicrobia*	91
AFB-121	Uncultured *Thermodesulfovibrio* sp.	*Thermodesulfovibrio*	83
Cultured bacterial species from rhizosphere in Luria-agar media (RZL)			
RZL-4	*Acinetobacter* sp.	*Gamma-proteobacteria*	98
RZL-17	*Aeromonas* sp.		98
RZL-10	*Enterobacter* sp.		99

Table 2 continued

Representative clones	Closest bacterial affiliation in GenBank	Phylogenetic group (class)	% of identity
RZL-1,3,5	*Pseudomonas* sp.		93
RZL-2,20	*Achromobacter* sp.	*Beta-proteobacteria*	98
RZL-6,7,8,9,11,12,13,15,16,18,19	*Bacillus* sp.	*Firmicutes*	90–99
RZL-14	*Staphylococcus* sp.		98
Cultured bacterial species from rhizosphere in pseudomonas isolation agar media (RZPIA)			
RZPIA-3,31	*Aeromonas* sp.	*Gamma-proteobacteria*	98
RZPIA-23	*Klebsiella* sp.		96
RZPIA-4	*Pseudomonas* sp.		93
RZPIA-5,8,63,79	*Xanthomonas* sp.		96–98
RZPIA-6,7,13,14,15,19,25,28,29,37,38,44,59, 60,62,67	*Achromobacter* sp.	*Beta-proteobacteria*	98–99
RZPIA-9,11,12,16,17,34,35,39,40	*Bacillus* sp.	*Firmicutes*	91–99
RZPIA-55,56,58	*Staphylococcus* sp.		98
Cultured bacterial species from bulk soil in Luria-agar media (BL)			
BL-16	*Agrobacterium* sp.	*Alpha-proteobacteria*	98
BL-10,33,36,59	*Bacillus* sp.	*Firmicutes*	95–99

Table 3 Plant-growth-promoting attributes of the bacterial isolates from rhizosphere of *Arachis hypogaea*

Bacterial species (isolate ID/GenBank accession number)	IAA production (μg/ml)	Phosphate solubilization	Siderophore production	Antifungal activity
Achromobacter sp. (RZPIA-62/HQ204322)	3.3 ± 0.17	+	+	–
Acinetobacter sp. (RZL-04/HQ204331)	4.2 ± 0.20	–	+	–
Aeromonas sp. (RZPIA-31/HQ204324)	3.2 ± 0.25	–	–	–
Bacillus sp.				
(RZL-09/HQ222601)	26.0 ± 1.8	–	+	–
(RZPIA-11/HQ204328)	2.08 ± 0.66	+	+	–
(RZL-12/HQ222600)	1.5 ± 0.4	–	–	–
(RZPIA-34/HQ204325)	1.24 ± 0.58	–	–	+
Enterobacter sp. (RZL-10/HQ204332)	25.6 ± 0.66	–	–	–
Staphylococcus sp. (RZPIA-56/HQ204326)	0.77 ± 0.05	–	+	–
Klebsiella sp. (RZPIA-23/HQ204321)	10.6 ± 1.5	–	–	–
Pseudomonas sp. (RZPIA-04/HQ204329)	2.4 ± 0.40	–	+	+
Xanthomonas sp. (RZPIA-63/HQ204323)	0.56 ± 0.67	–	–	+

+ indicates the presence of the phenotype and – indicates the absence of the phenotype in a bacterial isolate. Data represent the means ± standard error of three independent observations

detection of *Xanthomonas* sp., one of the most ubiquitous groups of plant-associated bacterial pathogens in the rhizosphere of *A. hypogaea* is not surprising (Kremer et al. 1990). The very high abundance of *Gamma-proteobacteria* in the rhizosphere and the presence of different PGPT in bacterial isolates belonging to *Pseudomonas*, *Klebsiella* and *Enterobacter* strengthen the idea that association of these species in the groundnut-rhizosphere is beneficial for plant growth (Ibáñez et al. 2009). The very high abundance of *CF*-bacteria in the *Arachis*-rhizosphere was presumably due to their ability to degrade complex organic exudates thus contributing to the turnover of carbon, nitrogen and phosphorous

(Forsberg et al. 1981). Our inability to detect any *Bradyrhizobia* from the rhizosphere of groundnut plants was unexpected and may be attributed to following factors. First, the incubation period of 48 h used to isolate the soil bacteria might be inadequate for growth of rhizobial strains. Moreover, the agricultural field from which the soil was collected lacked immediate groundnut cultivation record. Thus, it remains possible that different results would be obtained if soil and plants were analyzed from a field with groundnut growing history.

To conclude our results show, that although soil harbours a diverse bacterial population, a characteristic pattern of microbiota, many of which display plant growth promoting activities, is associated with the rhizosphere of *A. hypogaea*. Validation of beneficial traits in the candidate microbial inoculants in pot-cultures and field trials is necessary before their application in the groundnut production system. Finally, we acknowledge that though 16S rDNA sequence analysis is a useful tool to classify bacterial lineages, additional data using other fingerprinting techniques, that show a high degree of intraspecies discrimination power, such as multilocus sequencing typing (MLST), RNA-based fluorescence in situ hybridization, or cultivation targeting and nutrient utilization profiling are necessary for unequivocal assessment of bacterial phylogenies in these environmental samples.

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Conflict of interest The authors declare that they have no conflict of interest.

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