Diversity of culturable methylotrophic bacteria in different genotypes of groundnut and their potential for plant growth promotion

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Abstract

This study aimed at documenting the culturable methylotrophic bacterial diversity across different groundnut genotypes and evaluating their effect on the growth of groundnut. 80 methylotrophic bacterial isolates were obtained from the phyllosphere of 15 groundnut genotypes collected from Tamil Nadu, India. The bacterial isolates were identified through sequencing of the 16S rDNA and were tested for their plant growth-promoting properties. Groundnut seeds were inoculated with methylotrophic bacteria and their effect on growth was evaluated via in vitro and pot experiments. Molecular identification revealed that the isolates belonged to 30 different species. A higher diversity of methylotrophic bacteria at genus and species level was found in groundnut genotype TMV2. Shannon diversity index was the highest in genotype TMV7, followed by VRI2 and TMV2. Similarly, geographical location also influenced the diversity of methylotrophic bacteria. In vitro seed germination assay revealed that methylotrophic isolates enhanced root growth and improved formation of root hair. The radicle length of treated seeds ranged from 2.7 to 8.4 cm. A higher shoot length was observed in the plants from seeds treated with *Methylobacterium radiotolerans* VRI8-A4 (27.3 cm), followed by *Pseudomonas psychrotolerans* TMV13-A1 (26.3 cm) and *Bacillus aryabhatai* K-CO3-3 (23 cm). The findings of this study strongly suggest that beneficial methylotrophic bacteria associated with the phyllosphere of groundnut play a major role in regulating plant growth.

Keywords Groundnut · Phyllosphere · Methylotrophic bacteria · Plant growth-promoting traits

Introduction

Phyllosphere is a unique habitat that is highly influenced by changes in temperature, relative humidity, and UV irradiation. Phyllosphere is considered to contain limited nutrients and the availability of moisture prevents frequent fluctuation on the leaf surface. Phyllosphere harbors a diverse group of prokaryotes, which enhance plant fitness and contribute to various ecosystem functions (Kembel et al. 2014; Valverde et al. 2017). Although bacteria in the phyllosphere are known for their ability to survive and sustain on the leaves, the adaptation mechanisms of survival on the leaf surface are largely unknown (Burch et al. 2016). The availability of nutrients on the leaf surface plays a major role in the successful colonization of phyllosphere microflora. Inorganic ions, sugars, amino acids, and organic acids are hypothesized to leach out of the leaf surface through passive diffusion (Han and Micallef 2016). In addition, plant leaves emit about 0.2–38 µg/g/h methanol (dry mass) as a result of pectin metabolism, which is observed to be higher in young leaves than older leaves (Harley et al. 2007).

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Methylotrophic bacteria can utilize methanol (CH_3OH) as carbon and energy source, for survival on the leaf surface. Many researchers have documented the bacterial diversity in the phyllosphere of major crops such as rice (Mwajita et al. 2013), sunflower (Schauer and Kutschera 2008), soybean (Delmotte et al. 2009), lettuce (Rastogi et al. 2012), and apple (Yashiro et al. 2011). Among various bacterial classes, Proteobacteria are the most prevalent in the phyllosphere. Among Proteobacteria, α -Proteobacteria is more common than γ -Proteobacteria (Bodenhausen et al. 2013; Kembel et al. 2014). Among α -Proteobacteria, methanol utilizing pink-pigmented facultative methylotrophs (PPFM) are abundant in the phyllosphere (Abanda-Nkpawatt et al. 2006). Apart from PPFM, non-pigmented methylotrophic bacteria also inhabit the phyllosphere (Kaparullina et al. 2017). However, the information regarding bacterial diversity, particularly that of methylotrophic bacteria in groundnut phyllosphere, is yet to be reported. India is the second leading world producer of groundnut, behind China, that produces 13 million tons per annum. Groundnut is one of the important oil-seed crops in India and the total production is 6.7 million tons. However, a declining trend in productivity has been observed since 2013 (Economic survey, 2016–2017).

Bio-inoculants used by farmers are sometimes incompatible with the plants and hence do not positively impact the plant growth and yield. To overcome this, bio-inoculants should be developed using multi-functional and crop-specific plant growth-promoting bacteria (PGPB) (Sharma et al. 2009). Generally, plants select their own microbiome, and the bacterial populations associated with various plant phenotypes and genotypes are highly variable (Wagner et al. 2016). Methylotrophic bacteria represent an important group of multi-functional PGPB. These methylotrophic bacteria utilize the plant waste product methanol as a carbon and energy source, and enhance plant growth by producing growth hormones such as IAA (Senthilkumar et al. 2009) and cytokinins (Kwak et al. 2014), fix the atmospheric nitrogen (Madhaiyan et al. 2015), and enhance stress tolerance by reducing the stress-related ethylene production (Madhaiyan et al. 2006). Phyllosphere bacteria with several plant growth-promoting traits may also have the ability to enhance plant growth and tolerance to stress. Impact of phyllosphere bacterial strains on plant growth and yield was explained before (Maliti et al. 2005; Tani et al. 2015). This study was undertaken to evaluate the impact of groundnut genotype on phyllosphere cultivable methylotrophic bacterial diversity, and to develop crop-specific bio-inoculants.

Materials and methods

Collection of leaf samples from different groundnut genotypes

Leaf samples from 15 different genotypes of groundnut were collected from the sites in Tindivanam, Vriddhachalam, Coimbatore, Madurai, Karur, and Namakkal in Tamil Nadu, India. At the time of sampling, the plants were at flowering stage (30–40 days). The samples of the same genotype were collected from plants located at least 5 m apart. Five random points were selected for each genotype and five plants were sampled from each point. Five leaves were collected from each of the five plants and were pooled together to make a composite sample. Leaf samples were placed in plastic bags and transferred to the laboratory in an ice box. The list of groundnut genotypes and sampling sites are presented in Table 1 and Fig. S1 (Online Resource).

Isolation of phyllosphere methylotrophic bacteria

Plant leaf samples (3 g) from each genotype were placed in 500-mL Erlenmeyer flasks containing 25 g of glass beads (0.1 cm diameter) and 50 mL of phosphate buffer saline [PBS, containing (g/L) Na_2HPO_4 1.44; KH_2PO_4 0.24; KCl 0.20; NaCl 8.0; pH 7.4], and were agitated at 150 rpm at 30 °C for 2 h. After agitation, appropriate dilutions of the flask contents were plated onto ammonium mineral salt (AMS) agar supplemented with methanol (0.5%; v/v) and cycloheximide (30 $\mu\text{g}/\text{mL}$). The plates were incubated at 30 °C for 4–6 days. Single, well-isolated, and differentiated colonies from the enrichment medium were sub-cultured, purified, and maintained in glycerol stocks.

Molecular identification of methylotrophic bacterial isolates

Bacterial genomic DNA was extracted using a bacterial genomic DNA purification kit (HiMedia, India) by following manufacture's procedure. The universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTACCTTGTACGACTT-3') were used for PCR amplification of the 1.5 kb, full-length 16S rRNA gene. The PCR reaction mixture containing 20 ng of bacterial genomic DNA, 0.3 μL of *Taq* DNA polymerase (New England Biolabs, Gurgaon, India), 2.5 μL of 10 $\times$ *Taq* buffer (Sigma, India), 1.5 mM Magnesium chloride, each dNTPs at a concentration of 0.1 mM, each primer at a concentration of 10 pM was prepared, and the final volume was made up to 25 μL with sterilized deionized water. The thermal cycling conditions were initial denaturation at

Table 1 Sampling location of groundnut leaves and enumeration of methylotrophs from phyllosphere

Groundnut genotype	Sampling location	Plant habit	Latitude and longitude	Methylotrophs (cfu/g of fresh leaf sample)
TMV2	Tindivanam	Bunch	12°12'20.9"N 79°39'22.9"E	16 × 10 ⁴
TMV7		Bunch	12°13'02.27"N 79°38'20.01"E	1 × 10 ⁴
TMV13		Bunch	12°11'54.01"N 79°38'30.26"E	7 × 10 ⁴
VRI2	Vriddhachalam	Bunch	11°30'32.43"N 79°20'14.68"E	16 × 10 ⁴
VRI6		Bunch	11°30'31.66"N 79°20'16.00"E	4 × 10 ⁴
VRI7		Semi-spreading	11°30'30.83"N 79°20'13.26"E	18 × 10 ⁴
VRI8	Karur	Bunch	11°30'32.06"N 79°20'17.98"E	10 × 10 ⁴
CO 3		Bunch	10°54'49.99"N 78°07'02.78"E	14 × 10 ²
VRI 2		Bunch	10°54'53.73"N 78°08'09.26"E	3 × 10 ³
TMV 7	Namakkal	Bunch	10°55'01.66"N 78°09'15.00"E	10 × 10 ³
TMV 10		Semi-spreading	11°21'05.71"N 77°53'47.89"E	4 × 10 ⁴
CO6		Semi-spreading	11°20'15.52"N 77°54'15.45"E	12 × 10 ⁵
VRIGn 7	Coimbatore	Semi-spreading	11°21'26.60"N 77°53'28.29"E	10 × 10 ⁴
CO7		Bunch	11°01'19.72"N 76°55'39.58"E	21 × 10 ⁴
COGn4		Bunch	11°01'14.79"N 76°55'47.37"E	3.3 × 10 ³
CO6	Madurai	Semi-spreading	11°01'13.94"N 70°55'40.34"E	17 × 10 ⁴
ICGV06146		Bunch	11°01'14.99"N 76°55'45.28"E	83 × 10 ³
ALR 3		Bunch	9°57'52.25"N 78°12'18.49"E	10 × 10 ³
VRI 2		Bunch	9°59'00.58"N 78°12'43.65"E	6.6 × 10 ²
TMV 7		Bunch	9°59'42.09"N 78°15'30.65"E	17 × 10 ³

94 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 57 °C for 1 min, and extension at 72 °C for 2 min, and a final extension at 72 °C for 4 min. PCR products were visualized on 0.8% agarose gel. Sanger sequencing was performed at the Korean Agriculture Culture Collection (KACC), Jeonju, Republic of Korea. The percentage similarity of the isolates with their closest matching standard bacterial strains was evaluated

using eZtaxon (<http://www.ezbiocloud.net/>) (Yoon et al. 2017).

Multifunctional plant growth-promoting traits of methylotrophic bacteria

The methylotrophic bacterial isolates were screened for their plant growth-promoting traits. Production of

indole-3-acetic acid (IAA) was evaluated following the method described by Bric et al. (1991), on Luria–Bertani agar medium supplemented with 5 mM L-tryptophan. The 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity was determined by culturing bacterial isolates on nitrogen-free medium supplemented with 3 mM ACC as nitrogen source (Penrose and Glick 2003). The ability of the isolates to solubilize tricalcium phosphate was evaluated using the National Botanical Research Institute's phosphate (NBRIP) growth medium containing bromophenol blue (Mehta and Nautiyal 2001), wherein a halo zone around the bacterial colonies indicated phosphate solubilization. The solubilization efficiency was calculated using the formula: (solubilization diameter/growth diameter) $\times$ 100 (Anandham et al. 2007). Siderophore production was determined using Chrome Azurol S (CAS) agar plates, wherein the appearance of orange halo zones around the bacterial colonies indicated production of siderophore. Sulfur oxidation was tested using the mineral salts thiosulfate (MST) medium (Anandham et al. 2009) and Zn solubilization by the isolates was tested using the Bunt and Rovira medium supplemented with 0.1% ZnCO₃ (Saravanan et al. 2003).

In vitro seed germination and promotion of the early growth of plants by methylotrophic bacterial isolates

Groundnut seeds (VRI2) were obtained from the Department of Farm Management, Agricultural College and Research Institute, Madurai, Tamil Nadu, India. The surfaces of seeds were disinfected using 70% ethanol for 1 min followed by sodium hypochlorite solution (4%, v/v) for 5 min, and were then rinsed five times with sterile distilled water. Bacterial isolates were cultured for 48 h at 30 °C on nutrient agar supplemented with 0.5% methanol, in Petri plates of 90 mm diameter. The cells were scraped off and resuspended in 20 mL of 0.5% carboxymethylcellulose (CMC), and the surface-disinfected seeds were soaked in this cell suspension for 30 min (Kishore et al. 2005). The inoculated seeds were dried under a flow of sterile air in a laminar air-flow cabinet for 4–5 h before sowing. The inoculated seeds and control seeds (treated with plain 0.5% CMC) were placed on a sterile tissue paper in sterile Petri plates. In addition, another set of seeds was sown in 1.2 kg plastic pots filled with 1 kg field soil. Nutrient content of the soil was 183.02, 39.92, 342, and 4.2 kg/ha of available N, P, K, and S, respectively. Soil pH and EC was 7.1 and 0.21 dS/m, respectively. The seeds in the Petri plate were incubated for 4 days, and radicle length and the number of root hairs formed were measured. The seeds in pots were allowed to grow for 20 days, and then shoot length, root length, and dry weight were measured.

Diversity indices and statistical analysis

The Shannon diversity index (H') was calculated using the formula $-\sum(P_i)(\ln P_i)$. The Margalef index of richness (K) was calculated using the formula $K = \log S / \log N$, where S is the number of species and N is the total number of individuals in the sample (Iglesias-Rios and Mazzoni 2014). The data on bacterial diversity and plant growth experiments were subjected to the analysis of variance (ANOVA). Significance at 5% was tested by t test using the SAS package v. 9.1.3.

Results

The diversity of methylotrophic bacteria in the phyllosphere

Among the various groundnut genotypes studied, the total count of methylotrophic bacteria per leaf sample was found to be highest in the leaves of the CO6 genotype (12×10^5 cfu/g of fresh leaf) collected from Namakkal district, while the lowest count was observed in the leaves of the VRI2 genotype (6.6×10^2 cfu/g of fresh leaf) collected from Madurai district (Table 1). A total of 80 methylotrophic bacterial isolates were obtained from 15 groundnut genotypes. Isolated colonies exhibited different colony morphology and pigment production such as pink, yellow, white, and pinkish red.

Phylogenetic tree of the isolates obtained in this study are presented in Fig. 1. Of the 80 methylotrophic bacterial isolates obtained in this study, 29 were pink-pigmented and 51 were non-pink pigmented methylotrophic bacteria. The highest diversity of methylotrophic bacterial genera was observed in TMV2, followed by ICGV06146. Similarly, the genotype TMV2 harbored the highest numbers of methylotrophic bacterial species, followed by VRI2 and TMV10 (Fig. 2a, b). The Shannon and Margalef diversity indices were highest in TMV7, followed by VRI2 and TMV2 (Online Resource Table S1; Fig. 2c, d). The plant habits such as bunch or semi-spreading were not observed to significantly influence the number of genera and species of bacteria (Fig. 2e, f).

Sequencing of 16S rDNA revealed that the isolates belonged to 30 different species (Online Resource Table S2; Online Resource Fig. S2). The methylotrophic bacterium *Methylobacterium populi* was observed in the majority of the groundnut genotypes analyzed in this study (Fig. 3). Among the geographical locations, higher genus and species diversity were observed in samples collected from Tindivanam, followed by Coimbatore, Vriddhachalam, and Namakkal (Online Resource Fig. S3a, b). The Shannon and Margalef diversity index was considerably higher in samples

Fig. 1 Neighbor-joining phylogenetic tree showing methylotrophic bacteria associated with groundnut phyllosphere. The tree was constructed using near full-length bacterial 16S rRNA gene sequences

from Tindivanam, compared to other sampling sites (Online Resource Fig. S3c, d).

Plant growth-promoting properties of the isolates

The isolated methylotrophic bacteria exhibited various plant growth-promoting characters such as ACC deaminase and IAA production, sulfur oxidation, zinc and phosphate solubilization, siderophore production, and catalase activity (Online Resource Table S3; Fig. 4). Out of 80 methylotrophic bacterial isolates, 46% produced catalase and 32.5%

with 1000 bootstraps values. Bootstrap values ≥ 50 (based on 100 replications) are shown at each node. Bar 0.002 estimated nucleotide substitutions per site

produced siderophores. Phosphate and zinc solubilization was exhibited by 41 and 20% isolates, respectively. Sulfur was oxidized by 22.5% of the isolates, while 26.25% of the isolates were positive for ACC deaminase activity and 52.5% were positive for IAA production (Fig. 4).

Effect of methylotrophic isolates on seed germination and early growth

The in vitro seed germination experiment demonstrated enhanced groundnut root growth and improved root hair

Fig. 2 Genus (a) and species (b) count, Shannon (c) and Margalef (d) diversity index of methylotrophic bacterial diversity of different groundnut genotype

Methylotrophic bacterial species	Groundnut genotype														
	TMV2	TMV7	TMV13	VRI2	VRI6	VRI7	VRI8	CO 3	TMV 10	CO6	VRI G _n 7	CO7	COG4	ICGV 06146	ALR 3
<i>Methylobacterium thiocyanatum</i>															
<i>Xanthomonas sacchari</i>															
<i>Rhizobium pusense</i>															
<i>Cronobacter malonaticus</i>															
<i>Pseudomonas psychrotolerans</i>															
<i>Paenibacillus mucilaginosus</i>															
<i>Pseudarthrobacter defluvi</i>															
<i>Methylobacterium populi</i>															
<i>Microbacterium barkeri</i>															
<i>Methylobacterium gregans</i>															
<i>Cellulomonas fimi</i>															
<i>Pseudarthrobacter phenanthrenivorans</i>															
<i>Bacillus safensis</i>															
<i>Methylobacterium rhodesianum</i>															
<i>Methylobacterium extorquens</i>															
<i>Bacillus siamensis</i>															
<i>Curtobacterium citreum</i>															
<i>Methylobacterium radiotolerans</i>															
<i>Xanthomonas translucens</i>															
<i>Curtobacterium luteum</i>															
<i>Pantoea dispersa</i>															
<i>Acinetobacter baumannii</i>															
<i>Bacillus aryabhattai</i>															
<i>Pantoea stewartii</i> sub sp. <i>indologenes</i>															
<i>Sphingomonas paucimobils</i>															
<i>Microbacterium hydrothermale</i>															
<i>Paenibacillus humanensis</i>															
<i>Nocardioides zeae</i>															
<i>Sphingomonas pseudosanguinis</i>															
<i>Sphingomonas hankookensis</i>															

Fig. 3 Distribution of methylotrophic bacterial species in different groundnut genotypes. Color fill indicates the presence of species in particular genotype

Fig. 4 Plant growth-promoting traits of the groundnut methylotrophic bacterial isolates

formation by methylotrophic isolates compared to control (Online Resource Table S4; Fig. 5). Radicle length of the treated seeds ranged from 2.7 to 8.4 cm. The highest

radicle length was observed in the seeds treated with *Methylobacterium thiocyanatum* VRI7-A4 (8.4 cm), followed by *Methylobacterium radiotolerans* VRI8-A4 (8.3 cm),

Fig. 5 Groundnut (VRI 2) seed germination and root hair formation. Control groundnut seeds (a) and treated with *M. radiotolerans* VRI8-A4 (b); *P. psychrotolerans* TMV7-6 (c), after 5 days of treatment. Comparison of groundnut seed germination (d). D1, Control; D2, *M. radiotolerans* VRI8-A4; D3, *P. psychrotolerans* TMV7-6

Pseudomonas psychrotolerans K-TMV7-6 (8.2 cm), and *M. populi* TMV7-4 (8.2 cm). In addition, methylotrophic bacterial inoculation enhanced root hair formation, which was not observed in the control seeds. The highest number of root hairs was observed in the seeds treated with *M. populi* TMV7-4 (22 numbers), followed by seeds treated with *P. psychrotolerans* K-TMV7-6 (20.7 numbers) and *M. populi* N-VRIGn7-3 (18.7 numbers). The experiments on early plant growth promotion by the methylotrophic bacteria revealed the enhanced growth of shoots and roots, and higher dry weight (Online Resource Table S4). The highest shoot length was observed in the plants from seeds treated with *M. radiotolerans* VRI8-A4 (27.3 cm), followed by *P. psychrotolerans* TMV13-A1 (26.3 cm) and *Bacillus aryabhattai* K-CO3-3 (23 cm). Similarly, the highest root length was observed in the plants from *P. psychrotolerans* ICGV-5-treated seeds (38.7 cm), followed by *M. populi* N-VRIGn7-4 (38.0 cm) and *Bacillus safensis* TMV13-3 (32.7 cm). The plants from the seeds treated with *Pseudarthrobacter phenanthrenivorans* TMV7-A3 had higher dry weight (2.2 g) compared to other treatments.

Discussion

Bacteria inhabiting plant phyllosphere are studied to a lesser extent than rhizosphere inhabitants. Several researchers have isolated and characterized the plant growth-promoting bacteria associated with the rhizosphere, and the beneficial effect of these bacteria is well documented (Lee et al. 2016; Yan

et al. 2017; Pfeiffer et al. 2017). In contrast, interest on phyllosphere microflora is mainly focused on plant pathogens, and their colonization and pathogenicity have been documented rather than plant growth promotion (Maciel et al. 2014; Hubbard et al. 2015; Meena et al. 2017). Few studies have focused on phyllosphere bacteria beneficial to plants (Meena et al. 2012; Madhaiyan et al. 2015).

In this study, the methylotrophic bacterial populations in groundnut leaves of different genotypes were found to vary from 10^2 to 10^4 cfu/g of fresh leaf. In accordance with these findings, Yashiro et al. (2011) also reported significant variation in the phyllosphere bacterial population across different genotypes of apple. There is ample evidence suggesting that plant genotype may play a crucial role in determining the structure of phyllosphere microbial communities (Correa et al. 2007; Bodenhausen et al. 2017). In addition, bacterial populations of 10^3 – 10^6 cfu/g and 0 – 10^3 cfu/g of leaf sample were reported on red clover and winter wheat, respectively (Omer et al. 2004). Similarly, the influence of genotype on the phyllosphere microbiome of *Boechera stricta* has recently been documented (Wagner et al. 2016). The developing microbial population must relate to a large extent to the phenotypic characteristics of the plant, which is ultimately controlled by its genetic make-up. Likewise, ‘hot-spots’ of microbial growth associated with specific sites on the leaf would also be expected to be under the influence of plant genetics. In this study, hot-spots on groundnut leaves for the growth of methylotrophic bacteria were observed in the native genotypes of Tindivanam (TMV genotypes) and Vriddhachalam (VRI genotypes).

The population of leaf methylotrophic bacteria was similar across the sampling locations. However, significant variations were observed in the number of genera, species, and diversity indices across the sampling locations. Margalef index (*K*) specifically refers to the species richness among the plant genotype and sampling location. In this study, *K* varied across plant genotype and geographical locations. Higher *K* was observed in TMV7 (3.6), followed by VRI2 (3.4) and TMV2 (2.8) genotypes. A study by Raja et al. (2008) reported significantly different Margalef indices for the PPFM populations of different host plants. Shannon diversity indices of bacteria in the phyllosphere of chestnut were also reported to be significantly different across sampling locations (Valverde et al. 2017). The different Shannon diversity indices observed among methylotrophic bacteria in the groundnut phyllosphere, collected from different geographical locations in this study, corroborated with the previously reported findings. Wagner et al. (2016) suggested that the leaf exudates and phenotypes of plant leaves might play important roles in the selection of phyllosphere bacteria.

The higher bacterial diversity in Tindivanam, Vriddhachalam, and Coimbatore indicates suitable environmental

conditions for microbial growth and activity. These regions have lower annual mean temperature and receive higher rainfall than the other sampling locations (Karur, Namakkal, and Madurai). Due to the favorable environmental conditions, groundnut leaf stomata might be opened for a long time, leading to a higher placement of the nutrients and methanol emitted through stomata, which in turn could facilitate higher methylotrophic bacterial diversity. The findings of the bacterial diversity among different genotypes and geographical locations may confirm that Tindivanam and Vriddhachalam are the hot-spots for methylotrophic bacterial study.

In this study, PPFM were found in all the groundnut genotypes collected from the different geographical locations, which is in agreement with previous reports. This indicates that the PPFM are ubiquitous inhabitants of groundnut plant leaves. Kaparullina et al. (2017) isolated some colorless and yellow-pigmented methylotrophic bacteria from the genera of *Methylophilus*, *Methylobacillus*, *Hansschlegelia*, *Methylopila*, *Xanthobacter*, and *Paracoccus*, in addition to PPFM, from the phyllosphere of herbs, shrubs, and trees. Similarly, we have also isolated several non-pigmented methylotrophic bacteria from the groundnut phyllosphere. This clearly indicates that a diverse group of methylotrophic bacteria inhabits the groundnut leaf surface. In addition, the methylotrophic property of *P. psychrotolerans*, *Cellulomonas fimi*, and *Nocardioides zeae* has been described for the first time in this study, which expands our knowledge on these taxa.

Phyllosphere bacteria benefit from methanol produced by the leaves, by means of methylotrophy. However, methanol is not the only carbon compound to be emitted from the leaf and utilized by methylotrophic bacteria (Sy et al. 2005). Sugars leached out of the leaf surface are also utilized by phyllosphere bacteria for survival and colonization (Mercier and Lindow 2000). In addition to sugar utilization, bacteria have also developed other survival mechanisms such as UV resistance (Yoshida et al. 2017) and biofilm formation and aggregation (Morris et al. 1997) for better survival in the phyllosphere. Siderophore production by the bacteria is another adaptation mechanism for survival in low-nutrient environments such as leaf surface.

The methylotrophic isolates obtained in this study exhibited multiple plant growth promoting traits such as IAA, siderophore, ACC deaminase, nitrogen fixation, sulfur oxidation, and solubilization of insoluble minerals. Mineral solubilization efficiency of phyllosphere bacteria was also confirmed by Jayashree et al. (2011). The leaf-associated diazotrophic bacterial community of rainforests plays a vital role in nitrogen fixation in plants (Madhaiyan et al. 2015). The ability of phyllosphere bacteria to synthesize auxins has recently been reported (Kaparullina et al. 2017). In addition to the use of PGPB as bio-inoculants, several phyllosphere bacteria may also be considered as potential candidates to be bio-inoculants.

The information on the utilization of phyllosphere bacteria as bio-inoculants is scarce, and very few commercial products are available on the market. Leaf-inhabiting *Methylobacterium* species affected a significant increase in the plant dry biomass and chlorophyll content of *Jatropha* (Madhaiyan et al. 2015). Likewise, in our study, groundnut early plant growth was significantly improved by pigmented and non-pigmented methylotrophic bacteria. *Methylobacterium* spp. isolated from rice plant significantly improved the germination percentage and seedling length of red pepper and tomato (Madhaiyan et al. 2006; Ryu et al. 2006). IAA production by methylotrophic bacteria is directly correlated with increased root and shoot length. Recently, IAA-producing phyllosphere methylotrophic bacteria (*Methylobacterium extorquens* MM2) isolated from mustard leaves have been reported to improve tomato shoot and root length compared to control (Subhaswaraj et al. 2017). This study has also found IAA-producing methylotrophic bacteria to influence the growth of groundnut plants. Bacteria habitat on the phyllosphere directly influences the growth and yield of the plants. A recent report by Batool et al. (2016) provides that the bacteria habitat on the high-yielding wheat varieties exhibit superior plant growth-promoting activity than that of the phyllosphere bacteria of low-yielding wheat variety. In the present study, higher shoot and root length were observed in plants treated with *P. psychrotolerans* TMV13-A1 (26.3 cm) and *P. psychrotolerans* ICGV-5 (38.7 cm) cultures. Methylotrophic bacteria isolated from rice phyllosphere improve better ripening of rice seeds, and increased the size of barley grains compared to the control treatment (Tani et al. 2012, 2015).

Conclusions

The severe environmental fluctuations challenge the survival of bacteria on the phyllosphere. The different groups of bacteria inhabit the leaf surface, particularly the pink pigmented and non-pink pigmented methylotrophic bacteria. The findings of this study highlight the cultivable methylotrophic bacterial diversity in the phyllosphere of different groundnut genotypes. The plant growth-promoting properties and early plant growth promotion by the phyllosphere bacteria confirm their importance in plant interaction. Multiple plant growth-promoting traits exhibiting bacterial isolates from groundnut phyllosphere may be used as crop-specific bio-inoculants. In future, elucidation of the molecular mechanisms by which plants control microbial populations in the phyllosphere would be valuable. In addition, experiments related to the manipulation of phyllosphere microbiome via plant genotype may provide exciting opportunities in the management of applied aspects of phyllosphere microbiology, such as the survival and activity of beneficial microbes.

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Compliance with ethical standards

Conflict of interest The authors declare that we have no competing interests.

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