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Assessment of microbial diversity under arid plants by culture-dependent and culture-independent approaches

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In this work, microbial community structure of two distinct arid plants like ker (*Capparis deciduas*) and pearl millet (*Pennisetum glaucum*) was assessed and defined by culture-dependent and culture-independent approaches on the basis of 16S rRNA and random amplified polymorphic DNA (RAPD) analysis. The average Jaccard's similarity coefficient values for cultivated bacteria that is within ker and pearl millet rhizosphere were 0.701 and 0.707, respectively, for non-rhizosphere of ker and pearl millet 0.739 and 0.762, respectively, and for non-cultivable bacteria under ker (0.519) and under pearl millet (0.534). Both culture- dependent and culture-independent methods indicated that in arid crops, microbial diversity is more influenced by soil type rather than plant type and lower Jaccard value for metagenome showed that whole community harbours more diversity because of different microflora than cultivated only. Salinity and temperature tolerance study of bacteria indicated that ker rhizosphere harbours more salinity and temperature tolerant bacteria.

Key words: Random amplified polymorphic DNA (RAPD), ribotyping, Thar Desert, microbial diversity, 16S rRNA.

INTRODUCTION

Soil is a complex habitat where a large number of different microorganisms interact. Soil microbial diversity is an important index of agricultural productivity. Both the plant and soil types influence the microbial diversity of the rhizosphere. Interaction of plants and microorganisms is a result of co-evolution and their balance is important for sustainable agriculture (Smith and Goodmann, 1999; Lau and Lennon, 2011). The influence of perennials as well as annuals in microbial diversity is expected to be more pronounced in harsh climates of the desert. Such studies are scanty for the Thar Desert and most of them pertain to cultivable types, which represent only 1% of the total microbial diversity in the soil and their population may be greatly affected by changes in environmental conditions, hence, failed to envisage the entire population. Until recently, investigators had no idea how accurately cultivated microorganisms represented the overall microbial diversity. The cultivation-dependent approach is limited by the fact that the overwhelming majority of microorganisms present in soil cannot be cultivated under laboratory conditions.

The development of molecular phylogenetics has recently enabled characterization of naturally occurring microbial biota without cultivation. There is a vast amount of information held within the genomes of cultivable and non-cultivable microorganisms, and new methods based

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Abbreviations: RAPD, Random amplified polymorphic DNA; **PM,** pearmillet rhizosphere; **OP,** near pearl millet field; **KR,** ker rhizosphere; **OK,** near ker plantation; **CTAB,** cetyl trimethyl ammonium bromide; **EDTA,** ethylenediaminetetraacetic acid; **SDS,** sodium dodecyl sulfate; **PCR,** polymerase chain reaction; **NB,** nutrient broth.

on analysis of DNA allow investigation of this potential. In recent decades, a number of biological methods based on characterization of microbial DNA or RNA have been developed for reasons of better characterization. These methods are used to identify bacteria and describe bacterial DNA diversity in the case of individual bacteria, or DNA of entire microbial communities. In practice, the use of molecular biological methods includes isolation of the total DNA, amplification and analysis of 16S *rRNA* genes to get material for further analysis. Molecular techniques over the last few decades have revealed an enormous reservoir of unexplored microbes. Among these, *16S rRNA* gene sequencing has been primarily responsible in revealing the status of our lack of knowledge of microbial world such that half of the bacterial phyla recognized so far consist largely of these yet uncultured bacteria (Lozupone and Knight, 2007).

The soil gains importance, especially in saline and drought prone areas. This effect is always more pronounced in the rhizosphere. Hence, the rhizobacteria form a group of the best adapted micro organisms (Hartmann et al., 2008). Soil bacterial communities and the soil processes mediated by bacteria are critical for ecosystems functioning and productivity in arid lands. There is a need to integrate the soil bacterial community into our understanding of ecosystem interactions. The arid-ecosystem inhabiting hardy and woody plants like ker and pearl millet. Ker is distributed as natural wild in the arid and semi-arid regions of north-west India which mainly covers the parts of western Rajasthan and could be improved as a potential plant for horticultural and industrial uses in future (Paroda, 1979).

Pearl millet is used for the present study owing to its increasing worldwide importance as a food and forage crop (Vania et al., 2006). Selection of these crop species for the present study is to help understand if there are some essential rhizobacteria required for its establishment and growth in nature and may also lead to isolation of novel or efficient plant growth promoting rhizobacteria under extreme drought and heat that may further be utilized for enhancing the yield. A very few attempts have been made to study the genetic diversity of the rhizospheric and non-rhizospheric bacteria of pearl millet and ker.

Therefore, the objectives of present study are the isolation and characterization of both culturable and non-culturable microbial diversity under ker and pearl millet through random amplified polymorphic DNA (RAPD) and ribotyping a well as the study of the morphological and physiological properties of isolated bacteria.

MATERIALS AND METHODS

Sample collection

Three soil samples each from rhizosphere of ker and pearl millet along with adjacent non-rhizospheric areas of different location of SKRAU, Bikaner, Rajasthan (71° 54', 74°12' E longitudes and 27° 11', 29° 3'N latitudes) were collected in the month of September that have similar soils and vegetation. The samples from ker rhizosphere represented the area that was under cultivation once (about 10 years ago), while the pearl millet rhizospheric samples were from cultivated lands. Cultivated land was included to find out if certain bacterial types are supported by pearl millet even with drastic disturbance in soils. Samples from each site were well-mixed, air-dried, ground and allowed to pass through a 2.0 mm sieve and stored separately at -80°C for further analysis. Physiochemical parameters of soil were analyzed which showed the soils have electrical conductivity (ECe) 0.30-1.25dS/m, pH ranging from 7.5-8.0 and organic carbon from 0.18-0.45%. All the experiments repeated thrice.

Metagenome profiling

Soil microbial DNA was extracted from 15 g of soil sample using the cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1990) originally suggested for plants, with some modifications. Next 15 g soil sample was well homogenized with 15 ml DNA 2X CTAB DNA extraction buffer (100 mM Tris, 20 mM ethylenediaminetetraacetic acid (EDTA), 1.4 M NaCl, 2% CTAB and 2µl/ml β-mercaptoethanol) supplemented with 1% sodium dodecyl sulfate (SDS), in capped polypropylene tubes. A brief sonication treatment (30s at 15 W (4/10) for 50% active cycles) with titanium microtip was also given using BRAUN LABSONIC U sonicator, followed by incubation at 60°C for 1 h, deproteination by chloroform-isoamylalcohol and precipitation with one-third volume of propanol.

The DNA thus obtained was further purified with fast DNA spin kit (Obigene). Since desert soils are poor in microbial population, this kit recommended for isolation of DNA from small amounts of soil (1 g) directly, was used to purify DNA isolated from larger amounts of soil. In order to develop RAPD profiles, ten arbitrary primers (OPG-2, 3, 11, 12 and so on) obtained from Operon Technologies Inc. (Alameda, California) were used. Polymerase chain reaction (PCR) was performed in a final volume of 25 µl containing 10X assay buffer, 1.0 unit of *Taq* DNA polymerase (Bangalore Genei), 200 µM each of dNTPs (Fermentas), 10 pmol/ reaction of random primers and 50 ng template DNA. A thermal cycler (Biometra) was programmed for the initial denaturation step (94°C) of 5 min, followed by 44 cycles of 1 min denaturation along with 1 min primer annealing (37°C) and 2 min primer extension (72°C), followed by the 7 min primer extension (72°C) step. Amplicons were resolved by electrophoresis on 1.2% agarose gel (Himedia) containing 0.5 µg/ml ethidium bromide and run for 3-3.5 h at 100 V with cooling.

The 16S *rRNA* gene was amplified with bacterial specific universal primers P1 (5' AGAGTTTGATCCTGATCCTGGCTCAG 3') and P2 (5' TACCTTGTTACGACTT 3'). PCR reaction was performed in final volume of 25 µl containing 2.5 µl 10X assay buffer with $MgCl_2$ (Bangalore Genei), 0.19 µl of *Taq* polymerase (3 U/µl), 2.0 µl dNTPs, 1 µl primer-1 (10pM/µl) (OPERON TECHNOLOGIES), 16.3 µl deionised water and 2.5 µl template DNA (25 ng/µl). The PCR was performed for 35 cycles in 'Thermocycler' (Bio metra). The PCR was set with initial denaturation step of 5 min at 94°C, followed by 35 PCR cycles (denaturation at 94°C for 1 min, a primer annealing at 48°C for 1min, and primer extension at 72°C for 2 min) with a final extension of 7 min at 72°C. The PCR products, were analyzed on 1% agarose gel (Himedia, molecular grade), prepared in 1X TBE buffer containing 0.5 µg/ml of ethidium bromide. The gel was viewed under UV trans-illuminator and photographed by digital camera (Gel Doc S- Mini Bis Bioimaging. System, USA). Amplified PCR products (5-10 µl) were digested singly overnight at 37°C with 5 U of two different restriction enzymes *viz*; *Dpn*II and *Alu*I (New England Biolabs). Restricted fragments (10 µl) were analyzed on horizontal gel electrophoresis in 3.0% agarose (Sisco Research laboratories

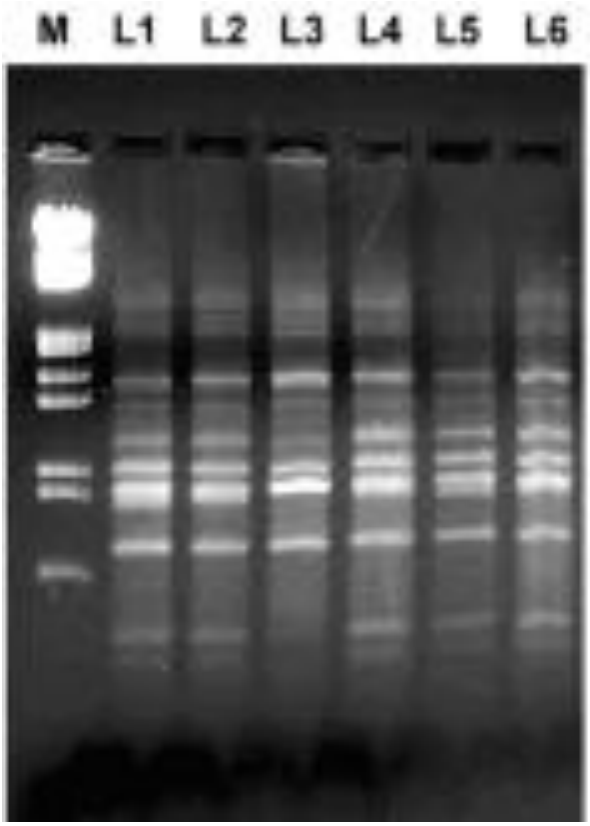

Figure 1. RAPD profile generated by primer OPG-11 using metagenome for rhizosphere of Ker and Pearl millet. M, Marker- Lambda DNA/*Eco R* I digest; L1-L3, pearl millet rhizospheric metagenome; L4-L6, Ker rhizospheric metagenome.

Pvt. Ltd) in 1X TBE buffer and electrophoresed at 75 volts for 3 h and visualized after ethidium bromide (0.5 µg/ml) staining.

The presence or absence of particular DNA fragment was converted into binary data and the pair wise genetic similarties among two genotypes under study was determined using Jaccard's coefficient (Jaccard, 1908). Cluster analysis for the genetic distance was then carried out using UPGMA (unweighted pair group method with arithmetic mean) clustering method. The genetic distances obtained from cluster analysis through UPGMA were used to construct the dendrogram, depicting the relationships of the genomes using computer program NTSYS pc version 2.02 (Rohlf, 1998).

Morphological and physiological properties of bacterial isolates

For the isolation of rhizospheric and non-rhizospheric bacteria, 10 g soil from each sample was added to 90 ml of 0.85% saline water and vortexed for 30 min on a mechanical rotary shaker and serially diluted up to 10^{-4} to 10^{-6} and 100 µl of each suspension were plated on plain nutrient agar (NA) and incubated at 37°C temperature for 24 to 48 h. Bacterial cultures were maintained in paired nutrient slants and stored at 4°C till further use (Rouatt and Katznelson, 1961). These bacterial isolates were examined under the stereo microscope (Leicca Int., Germany) for their shape and colony colour and tested for gram staining. The intrinsic resistance of the rhizospheric and non-rhizospheric bacterial isolates against salinity and temperature was evaluated by observing the growth on nutrient agar medium (Yildirim et al., 2008).

Genetic diversity of bacterial isolates

Genomic DNA of the sixty four rhizospheric and non-rhizospheric bacterial isolates was isolated from 24 h old culture grew in nutrient broth (NB) medium using a modified method of DNA isolation (Hill et al., 1972; Pierre et al., 1991). PCR and ribotyping analysis were done as described above.

RESULTS AND DISCUSSION

Standardization of DNA isolation from soil

Genomic DNA of pure quality is the prime requisite for molecular studies. Soil community DNA isolation is cumbersome because of organic matter and humus present in the soil. They also co-precipitate with DNA in any standard protocols and interfere with PCR reactions. Initially, standard CTAB method resulted in lower yield (0.53 and 1.26 µg/g) and inferior quality DNA ($A_{260/280}$ 1.0 and 1.36). Enzymatic lysis involving lysozyme also yielded low quantity (0.73 and 0.86 µg/g) and quality DNA ($A_{260/280}$ 1.2 and 1.3) for two samples. Addition of anionic detergent, SDS along with CTAB yielded maximum DNA (1.53 and 1.4 µg/g) in both the samples but was of inferior quality ($A_{260/280}$ 1.35 and 1.4). A sonication step was incorporated in this method to increase the yield and to improve the quality of DNA. Even though, the sonication treatment increased the yield, the quality remained inferior which was evident from ratio of $A_{260/280}$ values ranging from 1.06 to 1.22. Therefore, an additional purification step using Fast DNA Soil Kit (Qbiogene Fast DNA spin kit) was attempted. After this purification, the DNA yield declined and ranged from 3.45 to 4.05 µg/g of soil, but the quality improved ($A_{260/280}$ ranged from 1.56 to 1.72). Incorporation of SDS along with CTAB resulted in higher yield and relatively moderate purity (1.4 to 1.53). Similar results have been reported by several workers (Chen et al., 2010; Siddhapura et al., 2010).

Metagenome profiling using PCR and 16S rRNA

Molecular characterization of the soil samples from rhizospheric regions of pearl millet and ker with ten RAPD markers was done. The total number of bands amplified varied from 11 (OPG- 16) to 21 (OPG-2). The size of amplified fragments ranged from 100 to 3530 bp (Figure 1) and predominantly within the range of 325 to 3500 bp. Average number of bands per primer were 14.3. The number of polymorphic bands ranged from 10 (OPG-16) to 19 (OPG-2) producing a total of 86 polymorphic bands which accounted for 92.4% average polymorphism and an average amplification of 14.3 bands per primer.

Table 1. Jaccard's average similarity coefficient generated by UPGMA analysis for different soil metagenome.

Parameter	KR1	KR2	KR3	PMR1	PMR2	PMR3
KR1	1.000					
KR2	0.494	1.000				
KR3	0.505	0.602	1.000			
PMR1	0.462	0.473	0.483	1.000		
PMR2	0.483	0.602	0.473	0.526	1.000	
PMR3	0.516	0.548	0.483	0.623	0.559	1.000

KR, ker rhizosphere; PMR, pearl millet rhizosphere.

The Jaccard's similarity coefficient ranged from 0.462 [PMR-1 (BKN) and KR-1 (BKN)] to 0.623 [PMR-1 (BKN) and PMR-3 (BKN)] with an average of 0.522 for RAPD patterns (Table 1). The average similarity within ker (0.519) and pearl millet (0.534) rhizosphere was almost the same. The clustering pattern also revealed that the two major clusters had 49.8% similarity.

Metagenome from pearl millet rhizosphere presented higher genetic similarity (54.5%) that could be expected from complex metagenome. One major cluster comprising of three pearl millet samples (PMR-1, PMR-2 and PMR-3) and two of ker samples (KR-2 and KR-3) with 51.4% similarity coefficient indicated that there could be some common microflora in the metagenome. KR-1 branched as separate shoot from the major cluster with 50.2% dissimilarity. The conserved 16S *rRNA* gene analysis is also a preferred molecular tool to elucidate the phylogenetic relationship among bacteria as it provides unambiguous data which is reproducible across laboratories (Drancourt et al., 2000). The 16S *rRNA* gene was amplified with bacterial specific universal primers and gel electrophoresis of undigested PCR products revealed that all isolates produced a single band of about 1500 bp (Figure 5). Same results were obtained by Sudhir et al. (2009). Single digestion of this fragment with tetra-cutter *Alu*I resulted into distinct banding pattern including four to six bands with molecular sizes ranging from 130 to 450 bp with six distinct restriction pattern in all the samples analyzed. The application of UPGMA clustering has shown the existence of one major cluster when restriction profiles of amplified 16S rRNA obtained with *Alu* I. The correlated profiles have been grouped together with different soil samples exhibiting identical ARDRA profiles. Major cluster consisted rhizospheric soil metagenome of both the plant species *viz*; ker and pearl millet and non-rhizospheric soil metagenome of pearl millet. Major cluster further sub divided into a number of sub clusters, within rhizospheric soil metagenome of pearl millet 100% similarity was found due to the closeness in the banding pattern obtained after restriction with *Alu*I and it showed 75% similarity with non-rhizospheric soil metagenome of pearl millet. Both rhizospheric and non-rhizospheric soil metagenome of ker showed 80% percent similarity (Figure 2).

Morphological and physiological properties of bacterial isolates

Morphological study of bacterial isolates indicated that out of 64 isolates, 53 were found to be gram negative whereas, 11 stained were gram positive (Table 2). Salinity and temperature tolerance study of bacterial isolates showed that most of the bacterial isolates (rhizospheric as well as non-rhizospheric) were less halophilic. Bacterial population was similar in both rhizospheric and non-rhizospheric soil samples at 37°C without supplementation of NaCl in the medium but highest bacterial population was found under ker rhizosphere when nutrient agar medium was supplemented with 10% percent NaCl at 50°C temperature. It indicated that ker rhizosphere harbours more salinity and temperature tolerant bacteria (Table 3). The phylogeny of bacterial cultures has been explored mainly using morpho-logical characters especially to define and identify bacterial taxa but only morphological characters could not be reliable attributes to all members of the same morpholo-gical species (Lakshmanan et al., 2003).

Bacterial diversity study using RAPD and 16S rRNA from rhizosphere of pearl millet and ker along with respective non-rhizospheric areas

Molecular characterization of the bacterial isolates from rhizosphere and non-rhizosphere regions of pearl millet with 10 RAPD markers indicated that 8 primers produced 74 scorable amplicons of sizes ranging between 350 to 2700 bp with average polymorphism 82.4% and resolving power of 9.25 bands per primers. The Jaccard's similarity coefficient values within pearl millet rhizospheric bacterial isolates varied from 0.318 [PM-10 (bacterial isolate from pearl millet rhizosphere) and PM-16 (bacterial isolate from pearl millet rhizosphere)] to 0.954 [PM-8 (rhizospheric) and PM-9 (rhizospheric)] with an average of 0.707. Within non-rhizospheric bacterial isolates, it varied from 0.500 [OP-5, OP-15; OP-1, OP-5 (bacterial isolate from near pearl millet field)] to 1.000 [OP-7 and OP-10 (non-rhizospheric isolates)] with an average of 0.762. The average similarity between two groups was 0.650. Cluster

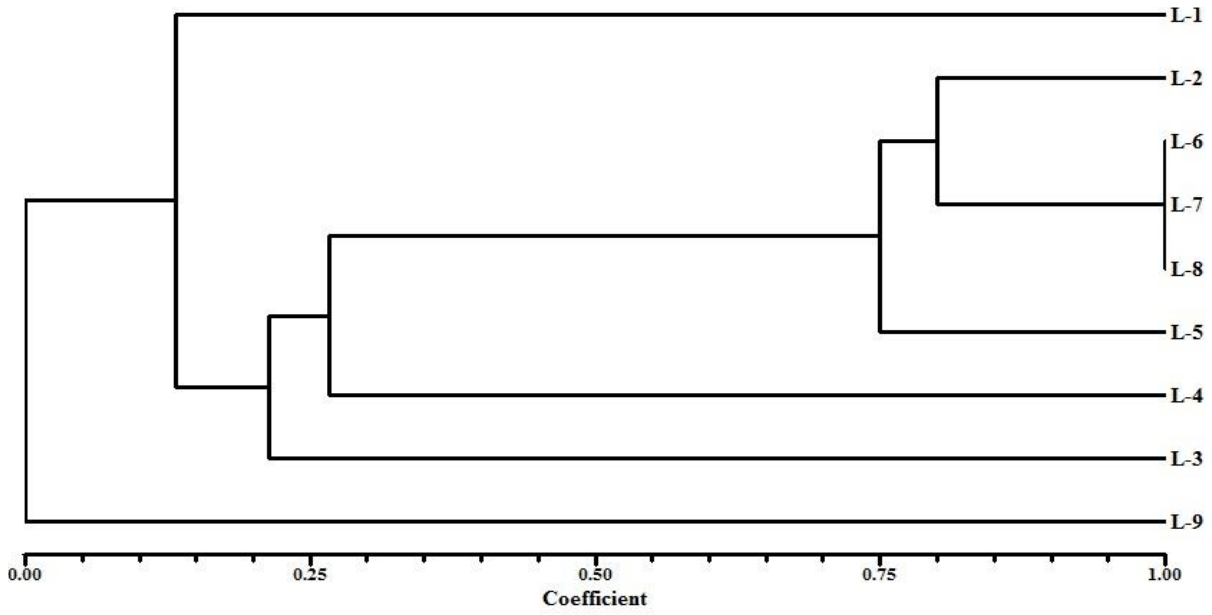

Figure 2. Cluster analysis of rhizospheric and non-rhizospheric community genome using restriction enzyme *Alu* I. L1, OK ; L2- L4, KR; L5, OP; L6-L8, PMR; L9, control; KR, ker rhizosphere; PMR, pearl millet rhizosphere; OP, non-rhizospheric area near pearl millet; OK, non rhizospheric area near ker.

analysis of thirty two bacterial isolates of pearl millet resulted in a distinct clustering of isolates into two major clusters with 57% genetic similarity (Figure 3).

Genetic diversity study of the bacterial isolates from rhizosphere and non-rhizosphere regions of ker with 10 RAPD markers indicated that 8 primers produced 63 scorable bands. The size of amplified fragment ranged from 300 to 3500 bp most lying between the size ranges of 400 to 3000 bp with 71.4% average polymorphism, with an average amplification of 7.9 bands per primer. Genetic similarity estimates based on RAPD data by Jaccard's coefficient analysis generated similarity coefficient matrix for ker samples, which ranged within rhizospheric bacterial isolates of ker from 0.370 [KR-13 (bacterial isolate from pearl millet rhizosphere) and KR-15 (bacterial isolate from ker rhizosphere)] to 0.926 [KR-3,KR-4; KR-5, KR-6; KR-6,KR-7;KR-5, KR-11;KR-6, KR-11 and KR-7, KR-11(ker rhizospheric)] with an average of 0.701 for RAPD. Within non-rhizospheric bacterial isolates, similarity coefficient varied from 0.370 [OK-9 and OK-13 (isolate near ker plantation)] to 1.000 [OK-7 and OK-10 (non-rhizospheric isolates)] with an average of 0.739. The average similarity coefficient between two groups was 0.628. Cluster analysis resulted in a dendrogram comprising of two clusters with 49.2% genetic similarity (Figure 4).

RAPD based detection of genetic polymorphism has been successfully used for inter and intra-specific genetic diversity of microbial communities of the soil across the tropics and sub tropics (Harry et al., 2001; Patel and Behra, 2011; Sharma et al. 2008)). Results of molecular characterization revealed that there is considerable variation between the isolates of rhizospheric and non-rhizospheric region and also, within group variation were evinced. These results are at par with the results earlier reported in the genetic diversity studies of bacterial isolates of rhizospheric regions (Babalola et al., 2002; Gajbhiye et al., 2010).

The bacterial isolates were clustered into two distinct clusters with few exceptions based on the habitat as rhizospheric and non-rhizospheric which substantiates the moderately broad distribution of genetic variability. Similarity indices of high values were obtained within rhizospheric samples invariably in pearl millet and ker samples. This also suggests that there are similar organisms in the rhizosphere regions of pearl millet and ker which could be probably because both crops belong to arid-ecosystem. The study was in concordance with the reports on rhizospheric bacterial isolates of ker, maize, sorghum and cotton (Singh et al., 2004; Sharma et al., 2005).

Both culture- dependent and culture-independent methods indicated that in arid crops, microbial diversity is more influenced by soil type rather than plant type and lower Jaccard value for metagenome showed that whole community harbours more diversity because of different microflora than cultivated only.

Conclusion

The analysis of the community by comparing the isolates not only contributes to the general understanding about the diversity but also provides a large collection of organisms that could be further characterized and utilized.

Table 2. Morphological characteristics of bacterial isolates, isolated from rhizosphere of pearl millet *(Pennisetum glaucum*), ker (*Capparis deciduas*) and their respective non-rhizospheric areas.

Isolate	Gram stain	Shape of bacteria	Colony colour on nutrient agar	Isolate	Gram stain	Shape of bacteria	Colony colour on nutrient agar
PM-1	-ve	Rods	Yellow brown	KR-1	-ve	Rods	Creamy
PM-2	-ve	Rods	Light pink	KR-2	-ve	Short rods	Yellow
PM-3	-ve	Rods	Off white	KR-3	-ve	Rods	Off white
PM-4	-ve	Rods	White	KR-4	+ve	Rods	White
PM-5	-ve	Rods	Light green	KR-5	-ve	Short rods	Yellow brown
PM-6	-ve	Rods	Yellow	KR-6	-ve	Rods	Yellow
PM-7	-ve	Rods	Off white	KR-7	-ve	Rods	Off white
PM-8	-ve	Rods	Yellow	KR-8	-ve	Rods	Yellow
PM-9	-ve	Rods	White	KR-9	+ve	Rods	White
PM-10	-ve	Rods	Creamy	KR-10	-ve	Curved rods	Creamy
PM-11	+ve	Rods	Creamy	KR-11	+ve	Rods	Creamy
PM-12	-ve	Cocci	Creamy	KR-12	-ve	Cocci	Off white
PM-13	-ve	Rods	Yellow	KR-13	-ve	Rods	Yellow
PM-14	-ve	Rods	Off white	KR-14	-ve	Rods	White
PM-15	-ve	Cocci	Yellow	KR-15	-ve	Cocci	Yellow
PM-16	+ve	Rods	Yellow brown	KR-16	-ve	Rods	Creamy
OP-1	+ve	Rods	White shiny	OK-1	+ve	Rod	Off white
OP-2	+ve	Rods	White	OK-2	-ve	Short rods	Light pink
OP-3	-ve	Cocci	Yellow	OK-3	-ve	Short rods	White
OP-4	-ve	Rods	Light pink	OK-4	-ve	Cocci	Yellow
OP-5	-ve	Rods	White	OK-5	-ve	Rods	Creamy
OP-6	-ve	Rods	Yellow	OK-6	-ve	Curved rods	Yellow brown
OP-7	-ve	Rods	Light green	OK-7	-ve	Rods	Light brown
OP-8	-ve	Rods	Creamy	OK-8	-ve	Rods	Creamy
OP-9	+ve	Rod	Off white	OK-9	+ve	Rods	White shiny
OP-10	-ve	Short rods	Light pink	OK-10	+ve	Rods	White
OP-11	-ve	Short rods	White	OK-11	-ve	Cocci	Yellow
OP-12	-ve	Cocci	Yellow	OK-12	-ve	Rods	Light pink
OP-13	-ve	Rods	Creamy	OK-13	-ve	Rods	White
OP-14	-ve	Curved rods	Yellow brown	OK-14	-ve	Rods	Yellow
OP-15	-ve	Rods	Light brown	OK-15	-ve	Rods	Light green
OP-16	-ve	Rods	Creamy	OK-16	-ve	Rods	Creamy

Pm, Pearmillet rhizosphere; op, near pearl millet field; kr, ker rhizosphere; ok,near ker plantation.

Table 3. Physiological characteristics of bacterial isolates, isolated from rhizosphere of pearl millet, ker and their respective non-rhizospheric areas.

Isolate	Effect of varying NaCl conc.			Effect of varying temp.			Isolate	Effect of varying NaCl conc.			Effect of varying temp.		
	5%	10%	15%	40°C	45°C	50°C		5%	10%	15%	40°C	45°C	50°C
PM-1	+	+	+	+++	+	+	KR-1	+	+	+	++	+	+
PM-2	+	+	+	++	+	+	KR-2	+	+	+	++	+	+
PM-3	+++	+	+	++	+	+	KR-3	+++	+	+	+++	+	+
PM-4	+	+	+	++	+	+	KR-4	+	+	+	++	+	+
PM-5	+++	+	+	++	+	+	KR-5	+++	+	+	+++	+	+
PM-6	+	+	+	++	+	+	KR-6	+	+	+	++	+	+
PM-7	+++	+	+	++	+	+	KR-7	+++	++	+	+++	+	+
PM-8	++	+	+	++	+	+	KR-8	+	+	+	++	+	+

Table 3. Contd

PM-9	++	+	+	++	+	+	KR-9	+	+	+	++	+	+
PM-10	+++	+	+	++	+	+	KR-10	+++	+	+	+++	+	+
PM-11	+++	++	+	+++	++	+	KR-11	+++	+++	+	+++	+++	++
PM-12	+++	++	+	+++	++	+	KR-12	+++	+++	+	+++	++	+
PM-13	+++	+	+	+++	++	+	KR-13	+++	+	+	+++	++	+
PM-14	+++	+	+	++	+	+	KR-14	+++	+	+	++	+	+
PM-15	+++	++	+	+++	++	+	KR-15	+++	+++	+	+++	+++	+
PM-16	+++	++	+	+++	++	+	KR-16	+++	+++	+	+++	++	+
OP-1	+++	+++	++	+++	++	++	OK-1	++	+	+	+++	+	+
OP-2	++	+	+	++	+	+	OK-2	++	+	+	+++	+	+
OP-3	++	+	+	++	+	+	OK-3	+	+	+	++	+	+
OP-4	+	+	+	++	+	+	OK-4	+	+	+	++	+	+
OP-5	++	+	+	++	+	+	OK-5	++	+	+	+++	+	+
OP-6	+++	+++	+	+++	+++	+	OK-6	+	+	+	++	+	+
OP-7	++	+	+	++	+	+	OK-7	++	+	+	+++	+	+
OP-8	+++	+++	+	+++	+++	+	OK-8	+	+	+	++	+	+
OP-9	++	+	+	+++	+	+	OK-9	+++	+++	++	+++	++	++
OP-10	++	+	+	+++	+	+	OK-10	++	+	+	++	+	+
OP-11	+	+	+	++	+	+	OK-11	++	+	+	++	+	+
OP-12	+	+	+	++	+	+	OK-12	+	+	+	++	+	+
OP-13	++	+	+	+++	+	+	OK-13	++	+	+	++	+	+
OP-14	+	+	+	++	+	+	OK-14	+++	+++	+	+++	+++	+
OP-15	++	+	+	+++	+	+	OK-15	++	+	+	++	+	+
OP-16	+	+	+	++	+	+	OK-16	+++	+++	+	+++	+++	+

+. No growth; ++, slight growth; +++, good growth.

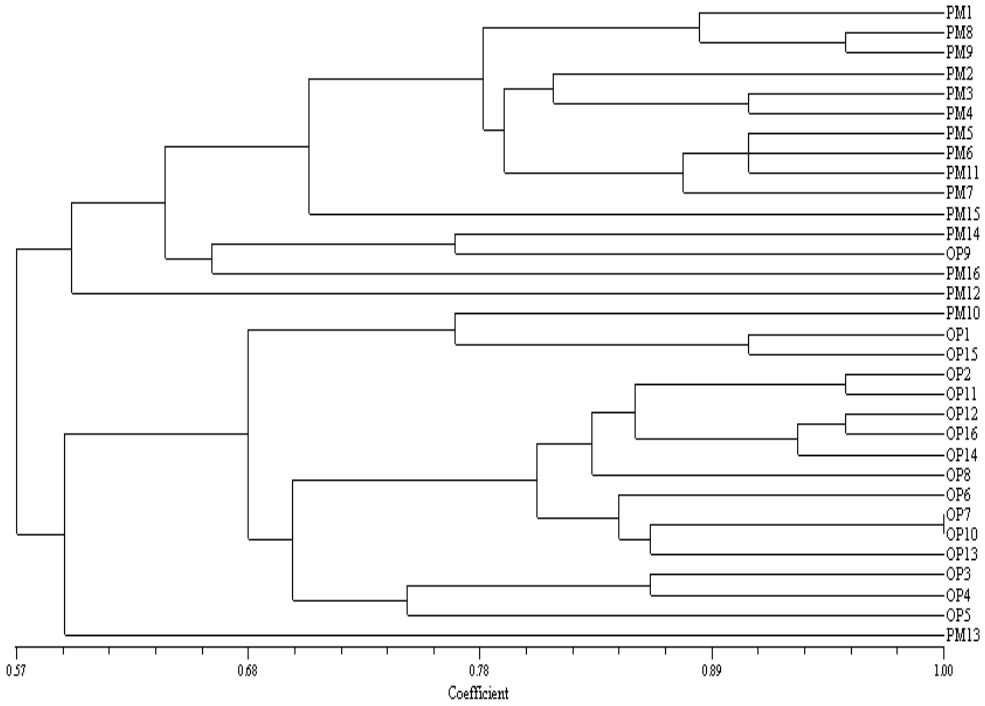

Figure 3. Dendrogram showing relationship among thirty two bacterial isolates from rhizospheric and non-rhizospheric pearl millet soil samples generated by UPGMA analysis based on RAPD.

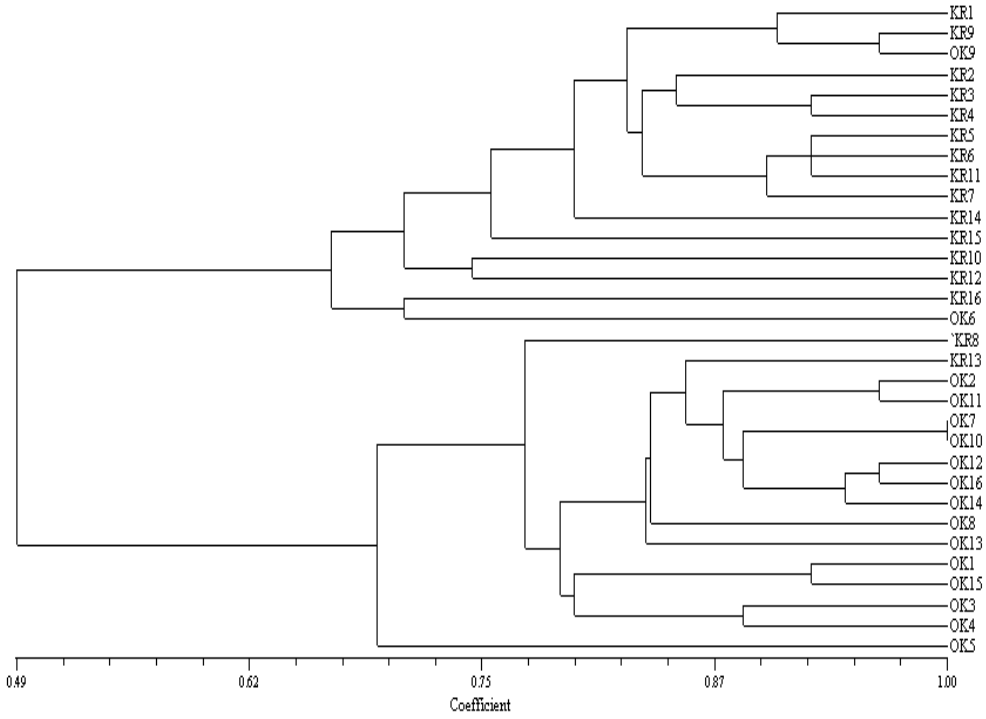

Figure 4. Dendrogram showing relationship among thirty two bacterial isolates from ker rhizosphere and non-rhizosphere soils generated by UPGMA analysis based on RAPD.

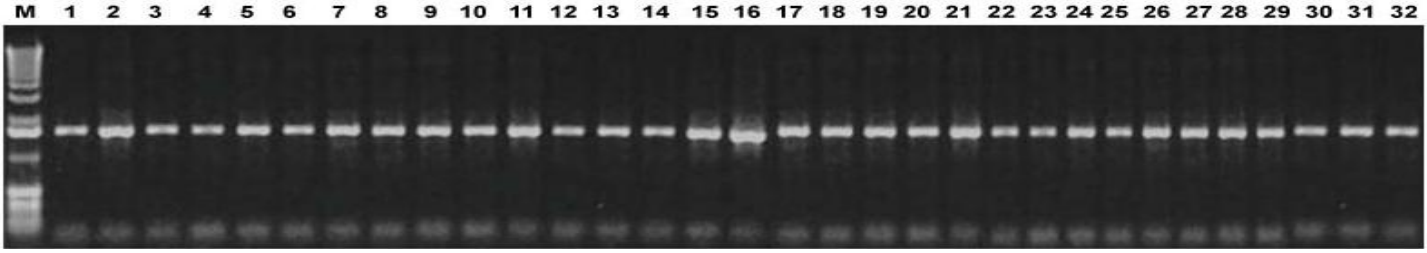

Figure 5. Agarose gel profile of 16S rDNA amplification of pearl millet and ker rhizospheric bacterial isolates. M, Marker-Lambda DNA/*Eco R* I digest; Lanes 1- 16: rhizospheric soil bacterial isolates of pearl millet (*(Pennisetum glaucum*) field; 17-32, rhizospheric soil bacterial isolates of ker (*Capparis deciduas*).

Both culture- dependent and culture-independent methods indicated that in arid crops, microbial diversity is more influenced by soil type rather than plant type. Further, this research covered a small area of arid region but could be utilized for whole desert region crops and is suitable to assess microbial diversity.

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