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RAPD analysis of soil microbial diversity in western Rajasthan

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RAPD analysis of DNA isolated from soils under Ker and associated open areas of Bikaner, Kodamdesar and Nagaur regions of western Rajasthan was carried out using six primers to find out the effect of Ker (*Capparis deciduas*) plants on microbial diversity. The average Jacard's coefficient similarity within Ker samples was less (0.250) than that within adjoining open area (0.337), indicating that the soil under Ker supports more diverse microbes compared to the open areas. Comparatively higher fertility status in terms of NPK and C content under Ker plants was envisaged to support more diverse types of microbes. The soils from the three locations were quite uniform both under Ker (94.53%) and in open area (94.76%).

Keywords: Fertility status, microbial diversity, RAPD analysis, soil.

SOIL microbial diversity is an important index of agricultural productivity[1–3]. Both the plant and soil types influence the microbial diversity of the rhizosphere[4,5]. Interaction of plants and microorganisms is a result of co-evolution and their balance is important for sustainable agriculture. The influence of perennials 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 hence, fail to envisage the entire population. DNA markers, including the RAPDs produced by PCR can be used for the characterization of microorganisms and detection of microbial diversity[6–12] in metagenome, without the need for culturing the same. The present investigation was thus carried out to study microbial diversity under the soils of Ker (*Capparis deciduas*) plant and adjoining open areas at three locations in the hot, arid Thar Desert, Rajasthan. The influence of Ker on fertility status of the soil was also estimated to associate microbial diversity with fertility status of the soils.

Soil samples were collected during June 2006 under Ker and open areas of Bikaner (BKN), Kodamdesar (KODM) and Nagaur (NGR), that have similar soils and vegetation. The samples from Bikaner represented the area that was under cultivation once (about 10 years ago), while the others were from uncultivated fallow barren lands. Cultivated land was included to find out if certain

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Table 1. Primers and bands generated by them through PCR and RAPD analysis

Primer	Sequence (5′ → 3′)	Total no. of bands (*a*)	Total no. of polymorphic bands (*b*)	Polymorphism (*b*/*a* × 100)
OPG-2	GGCACTGAGG	21	21	100
OPG-3	GAGCCCTCCA	17	17	100
OPG-11	TGCCCGTCGT	17	17	100
OPG-12	CAGCTCACGA	13	13	100
OPG-14	GGATGAGACC	14	14	100
OPG-16	AGCGTCCTCC	11	11	100
Total		93	93	100

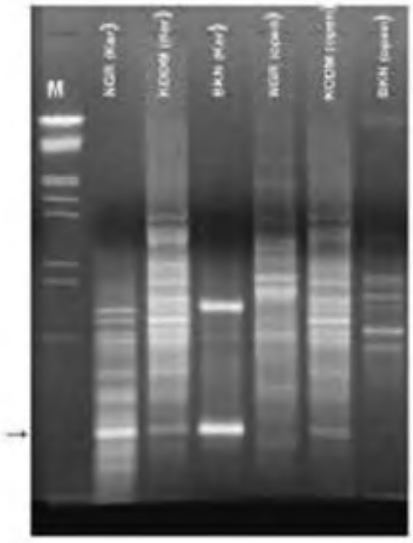

Figure 1. RAPD profile generated by primer OPG-11 using metagenome for the rhizosphere of Ker and adjoining open areas. M, Marker, lambda DNA (*Hin*dIII/*Eco*RI digest).

bacterial types are supported by Ker even with drastic disturbance in soils. Soil samples under Ker and other open areas were collected at a depth of 10–15 cm. 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.

Soil microbial DNA was extracted from 15 g of soil sample using the CTAB method[13] originally suggested for plants, with some modifications. Next 15 g soil sample was well homogenized with 15 ml DNA 2× CTAB DNA extraction buffer (100 mM Tris, 20 mM EDTA, 1.4 M NaCl, 2% CTAB and 2 µl/ml β-mercaptoethanol) supplemented with 1% SDS, in capped polypropylene tubes. A brief sonication treatment (30 s 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, six arbitrary primers (OPG-2, 3, 11, 12, 14 and 16) obtained from Operon Technologies Inc. (Alameda, California) were used. PCR was performed in a final volume of 25 µl containing 10× 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.

RAPD bands were designated on the basis of their molecular size (length of the polynucleotide amplified) determined against λ-DNA *Eco*RI/*Hin*dIII double digest loaded with each primer product. The presence of each band was scored as '1' and its absence as '0'. Faintly visible bands were not scored but a major band corresponding to a faint band was considered for scoring. In order to confirm the presence of bands and determine reproducibility, all the primers were replicated thrice. The details of primers and the bands generated by them through PCR and RAPD analysis are presented in Table 1. A sample photograph of the gel with bands generated with the primer OPG-11 is shown in Figure 1.

The pair-wise association coefficients were calculated from the qualitative data matrix using Jaccard's similarity coefficient. Cluster analysis for the genetic distance was carried out using UPGMA clustering method. The genetic distances obtained from cluster analysis through UPGMA were used to construct the dendrogram (Figure 2), depicting the relationship of the soil DNA fingerprints using the computer program NTSYS-pc, version 2.02 (ref. 14).

For fertility analysis of the soils, three replicates of each soil sample drawn from 0 to 15 cm depth were collected, air-dried, ground, passed through 2 mm sieve and

Table 2. Nutritional analysis of different soil samples

Sample	Nitrogen (kg/ha)	Phosphorus (kg/ha)	Potassium (kg/ha)	Organic carbon (kg/ha)
NGR (Ker)	80	14.0	139.0	4840
KODM (Ker)	85	14.7	138.2	4620
BKN (Ker)	86.2	15.4	140.7	4180
NGR (Open)	72	13.1	116.1	3520
KODM (Open)	75.8	12.6	125.6	3520
BKN (Open)	75.0	11.5	108.7	3300
Mean	79.0	13.5	128.0	4106
CV	7.3	10.7	10.5	14.6
CD (5%)	5.2	1.3	12.0	0.03

Table 3. Jaccard's average similarity coefficient of soils generated by UPGMA analysis

Sample	NGR (Ker)	KODM (Ker)	BKN (Ker)	NGR (Open)	KODM (Open)	BKN (Open)
NGR (Ker)	1.000					
KODM (Ker)	0.324	1.000				
BKN (Ker)	0.230	0.197	1.000			
NGR (Open)	0.342	0.371	0.327	1.000		
KODM (Open)	0.366	0.462	0.263	0.397	1.000	
BKN (Open)	0.343	0.314	0.302	0.314	0.300	1.000

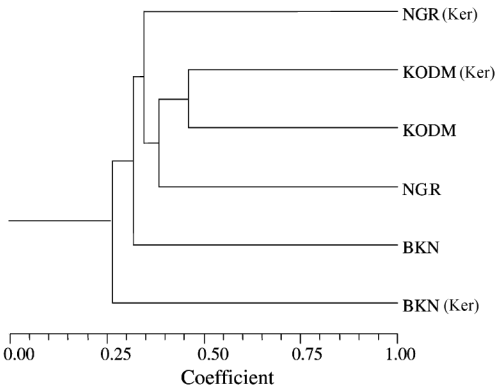

Figure 2. Dendrogram showing relationship among six samples of soil generated by UPGMA method based on RAPD analysis.

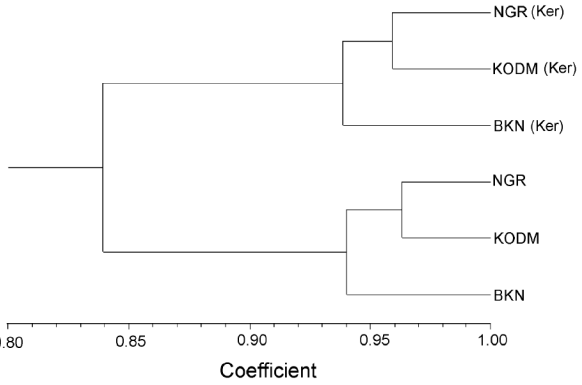

Figure 3. Dendrogram showing relationship among six samples of soil generated by UPGMA method based on fertility analysis.

stored for further analysis. These soil samples were analysed for organic carbon[15], phosphorus[16], available potassium[17] and nitrogen[18] content using standard procedures (Table 2).

The genetic similarities based on RAPD patterns have been presented in the form of Jaccard's similarity coefficient in Table 3. The average genetic similarity obtained was 0.323 with a range from 0.230 {NGR (Ker) and BKN (Ker)} to 0.462 {KODM (Ker) and KODM (Open)}. The lesser values of similarity index indicate high diversity for soil microbes in this region. The average similarity within Ker samples (0.250) was less than within adjoining open area (0.337), indicating that the soil under Ker supports more diverse microbes compared to the open areas. The average similarity between the two groups, i.e. samples under Ker and open area was more (34.3%, i.e. 65.7% diversity) compared to within the two types of soil groups, indicating that soil under Ker supported more common microbes than the respective open spaces. Studies pertaining to diverse plant species also supported the effect of plants on the microbial population[19]. The clustering of samples from Bikaner, shown to be highly diverse, joining the group at a higher level of diversity, supports the fact that the area was under cultivation and that the other areas were never cultivated. This indicates that cultural practices, including fertilizer application change the microbial diversity pattern in the soil[20]. Soil DNA samples under Ker plants were found to have diverse banding

Table 4. Manhattan's average dissimilarity coefficient converted into similarity values by subtracting from one among soils based on fertility status

Soil sample	NGR (Ker)	KODM (Ker)	BKN (Ker)	NGR (Open)	KODM (Open)	BKN (Open)
NGR (Ker)	1.000					
KODM (Ker)	0.959	1.000				
BKN (Ker)	0.920	0.957	1.000			
NGR (Open)	0.855	0.840	0.843	1.000		
KODM (Open)	0.875	0.861	0.864	0.963	1.000	
BKN (Open)	0.814	0.799	0.802	0.942	0.938	1.000

pattern among themselves than among soils of the associated open area.

Manhattan's dissimilarity coefficient analysis based on fertility status was carried out after standardization of the data using NTSYS-pc, version 2.02 (Table 4). The average similarity coefficient was 88.2%, with a range 79.9–96.3%. The open area tended to be more dissimilar over regions with respect to fertility status compared to the soil under Ker due to the addition of organic matter by the plant. Though clustering of soils based on fertility status did not correlate with RAPD-based clustering, the Bikaner sample remained the most diverse, as it was from a cultivated area. However, this was not due to higher fertility level of the Bikaner sample. Nevertheless, it may be taken as an indication that cultivation brings about some physical and chemical changes, which in turn affect the microbial diversity and this cannot be reversed even after cultivation is suspended.

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