Spatial Physicochemical and Metagenomic Analysis of Desert Environment^S

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Investigating bacterial diversity and its metabolic capabilities is crucial for interpreting the ecological patterns in a desert environment and assessing the presence of exploitable microbial resources. In this study, we evaluated the spatial heterogeneity of physicochemical parameters, soil bacterial diversity and metabolic adaptation at meter scale. Soil samples were collected from two quadrats of a desert (Thar Desert, India) with a hot, arid climate, very little rainfall and extreme temperatures. Analysis of physico-chemical parameters and subsequent variance analysis (p -values < 0.05) revealed that sulfate, potassium and magnesium ions were the most variable between the quadrats. Microbial diversity of the two quadrats was studied using Illumina bar-coded sequencing by targeting V3-V4 regions of 16S rDNA. As for the results, 702504 high-quality sequence reads, assigned to 173 operational taxonomic units (OTUs) at species level, were examined. The most abundant phyla in both quadrats were Actinobacteria (38.72%), Proteobacteria (32.94%), and Acidobacteria (9.24%). At genus level, *Gaiella* represented highest prevalence, followed by *Streptomyces*, *Solirubrobacter*, *Aciditerrimonas*, *Geminicoccus*, *Geodermatophilus*, *Microvirga*, and *Rubrobacter*. Between the quadrats, significant difference (p -values < 0.05) was found in the abundance of *Aciditerrimonas*, *Geodermatophilus*, *Geminicoccus*, *Ilumatobacter*, *Marmoricola*, *Nakamurella*, and *Solirubrobacter*. Metabolic functional mapping revealed diverse biological activities, and was significantly correlated with physico-chemical parameters. The results revealed spatial variation of ions, microbial abundance and functional attributes in the studied quadrats, and patchy nature in local scale. Interestingly, abundance of the biotechnologically important phylum Actinobacteria, with large proportion of unclassified species in the desert, suggested that this arid environment is a promising site for bioprospection.

Keywords: Desert, arid soil, spatial heterogeneity, microbial diversity, functional mapping, actinobacteria

Introduction

Desert environments usually encounter water scarcity, high levels of temperature fluctuation, ultraviolet radiation, and oligotrophy [1, 2]. All these factors cause extreme

abiotic stress to the ecosystem process, eventually limiting the vegetation cover in the arid environment. Microorganisms associated these environments drive ecosystem processes and biogeochemical cycling [3, 4], with a minimal contribution from plant systems. With the demonstrated

Physicochemical and Biochemical Analyses

Eight soil samples derived from Q-A and Q-B were air dried in laminar air hood, sieved through 2 mm mesh, and used for physico chemical analysis following standard methods. Twenty grams of sieved soil was taken in a clean 100 ml beaker and 40 ml of distilled water added to make soil suspension (1: 2 ratio, w/v). Electrical conductivity was measured in terms of resistance offered to the flow of current using a conductivity meter and expressed in dSm^{-1} . The water-soluble anions (Cl^- , SO_4^{2-}) and cations (Ca^{2+} , Mg^{2+} , K^+) in the soils were estimated from the filtrate collected after 19 h of shaking with deionised water, according to Bower *et al.* [16].

Organic carbon present in the soil samples was estimated by chromic acid wet digestion method [17]. Total nitrogen in soil was determined after the acid digestion by the micro kjeldahl method [18], whereas mineral N (NO_3^- -N and NH_4^+ -N) were extracted using 2 M KCl and determined using the spectrophotometer [19]. Total P was estimated according to Pemberton [20].

16S rDNA Amplicon Sequencing

Soil DNA was isolated using 500 mg of each soil sample, using HiPurA Soil DNA Purification Kit (HiMedia, India) according to the manufacturer's protocol. The isolated soil DNA was visualized by agarose gel (0.8%, w/v) electrophoresis and quantified using nanodrop spectrophotometer.

16S rDNA metagenomic sequencing library preparation involved two PCR reactions, an amplicon PCR and an indexing PCR. In amplicon PCR, V3 region of 16S rDNA was targeted using primers 341F, 5'-CCTACGGGNGGCWGCAG-3' and 785R, 5'-GACTACHVG GGTATCTAATCC-3'. Reaction mixture contained 2.5 μl of soil DNA (5 ng/ μl in 10 mM Tris pH 8.5), 5 μl of 341F primer (1 μM), 5 μl of 785R primer (1 μM), and 12.5 μl of KAPA HiFi Hot Start Ready Mix (2 $\times$) in total volume of 25 μl . Thermal cycle conditions were an initial denaturation at 95°C for 3 min, followed by 25 cycles at 95°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec, and a final extension step at 72°C for 5 min. One micro litre of the PCR product was analysed on Bioanalyzer DNA 1000 chip (Agilent Technologies) to verify the expected size of amplicons (~ 550 bp). The amplified product was purified using AMPure XP beads (Agilent Technologies) to remove free primers and primer dimer species, according to manufacturer's protocol. In a subsequent index PCR, dual indices and Illumina sequencing adapters were attached to product of amplicon PCR, using the Nextera XT Index Kit (Illumina) according to manufacturer's protocol. The resulted 16S rDNA metagenomic sequencing library was cleaned up using AMPure XP beads (Agilent Technologies). One micro litre of 1:50 dilution of the final library was analysed on a Bioanalyzer DNA 1000 chip to verify the expected size~630 bp.

Sequence Processing and Bioinformatics Analysis

The final 16S rDNA metagenomic library was diluted to 4nM using resuspension buffer. An aliquot of 5 μl from each library with unique indices was pooled and sequenced with standard

sequencing oligos on the Illumina MiSeq platform. The raw Illumina reads were subjected to stringent quality control (QC) before considering for bacterial community analysis. Illumina reads with more than 70% of high quality bases with Phred Score greater than 20 were selected using FASTQC Toolkit (Version 0.11.5). A Phred quality score is logarithmically related to the base-calling error probabilities, and is used as a measure to estimate the quality of nucleobases generated by automated DNA sequencing. For instance, Phred Score 20 indicates the probability of one incorrect base call in 100 and represents 99% accuracy of base call in automated sequencing. Subsequently, adapter sequences, primer sequences, and the barcode were removed to generate the QC passed reads.

The QC passed reads were subjected to de-replication (merging of identical reads), de-noising, (trimming to a fixed length, optionally discarding singleton reads) and chimera filtering using UPARSE pipeline [21]. The processed reads were clustered into non-redundant, representative OTUs using UPARSE-OTU algorithm [21], with a 97% cutoff. Sequences that could not be classified to at least a kingdom level were excluded from subsequent analysis. Representatives of operational taxonomic units (OTUs) were determined at a phylum, class, order, genus, and species level. Rarefaction curves were generated using MEGAN5 [22] and compared among the samples. Richness (Chao1), coverage-based richness (ICE and ACE), diversity (Shannon) and evenness were determined using EstimateS [23].

Taxonomy to Functional Mapping of Microbial Community

With intention to probe the microbial metabolic and functional pathways in the microbial community, taxonomic data was automatically mapped with phenotype categories in METAGEN assist [24] using default settings. Percentage of relative abundance of different phenotypes was calculated and a bar chart was plotted.

Statistical Analysis

All quantitative data obtained on soil were analyzed using Minitab 6.0 (Minitab, Inc., State College, Pennsylvania). Non-normal data were normalized by log-transformation. The correlations between each pair of variables were calculated using Pearson's correlation coefficient. Soil physicochemical parameters were then studied by principal components analysis (PCA), visualized with the two principal components. A multivariate data analysis of the OTUs was performed using METAGENassist, a web server for comparative metagenomics [24]. All the OTUs found in two samples were subjected to normalization based on interquartile range (IQR) [25] and log₂-transformation. Both hierarchical clustering and PLS-DA plotting (Partial least squares Discriminant Analysis) of samples were also performed using METAGENassist.

Results and Discussion

Heterogeneity in Physicochemical Parameters

Spatial heterogeneity in physicochemical characteristics

Table 1. Different physicochemical parameters of arid soils averaged over sampling quadrats, Q-A and Q-B.

Quadrates	Physicochemical parameters											
	pH	EC (dSm ⁻¹)	OC (g kg ⁻¹)	TN (g kg ⁻¹)	TP (g kg ⁻¹)	Cl ⁻ (mmol kg ⁻¹)	SO ₄ ²⁻ (mmol kg ⁻¹)*	K ⁺ (mmol kg ⁻¹)*	Ca ²⁺ (mmol kg ⁻¹)	Mg ²⁺ (mmol kg ⁻¹)*	A-N (mg kg ⁻¹)	N-N (mg kg ⁻¹)
Q-A	7.58 ± 0.11	0.28 ± 0.06	5.4 ± 2.8	0.21 ± 0.12	0.02 ± 0.008	20 ± 4.0	44 ± 17.4	13.7 ± 2.9	6.8 ± 1.8	369.7 ± 76.8	1.40 ± 0.32	3.01 ± 0.62
Q-B	7.54 ± 0.03	0.3 ± 0.14	3.9 ± 1.4	0.18 ± 0.15	0.042 ± 0.012	15 ± 4.0	72 ± 7.5	9.25 ± 1.9	7.4 ± 1.7	240.2 ± 29.1	1.19 ± 0.14	2.73 ± 0.26
Overall	7.56 ± 0.08	0.29 ± 0.10	4.7 ± 2.2	0.20 ± 0.13	0.031 ± 0.015	17.5 ± 4.6	58 ± 19.4	11.5 ± 3.3	7.1 ± 1.66	305 ± 87.6	1.29 ± 0.25	2.87 ± 0.46

*Significant difference ($p < 0.05$) among quadrats.

EC: electrical conductivity, OC: organic carbon, TN: total nitrogen, TP: total phosphorus, A-N: ammonical nitrogen, N-N: nitrate nitrogen.

was revealed in the arid sampling sites, quadrates A (Q-A) and quadrates B (Q-B) (Table 1). Overall vegetation cover was very less in the experimental area, and observed as 12% and 9% in Q-A and Q-B, respectively. Soil pH and EC varied from 7.54 to 7.58 and 0.28 to 0.3 dS m⁻¹, respectively. As expected and reported elsewhere [8], the pH was positively correlated with Mg²⁺. Correlation among different soil parameters is summarized in Table S1.

The OC concentration was insignificantly higher in Q-A (5.4 ± 2.8 g kg⁻¹) than Q-B (3.9 ± 1.4 g kg⁻¹). This difference roughly correlates with relatively dense vegetation cover in Q-A than Q-B, and suggested greater substrate availability for microbial metabolism in quadrates Q-A. Total forms of nutrients such as total nitrogen, total phosphorus, ammonical N (A-N) and nitrate N (N-N) showed no significant differences among the two quadrates. The nitrogen content is less in both quadrats (0.18 to 0.21 g kg⁻¹) with negligible difference between Q-A and Q-B. This deficiency is, however, made up to an extent by the presence of both ammonical N and nitrate N. The total phosphorus content was 0.02 ± 0.008 and 0.042 ± 0.012 g kg⁻¹ in Q-A and Q-B, respectively. The overall values of C and P in the experimental site were higher than the values reported for other deserts, as well as for soil, sand dune and rock substrates in other sites of the Thar Desert [26]. Therefore, this type of desert soil is reasonably fertile if moisture is not deficient [27].

In the experimental site, K⁺, SO₄²⁻, and Mg²⁺ were the most variable parameters, showed significant differences between the two quadrates, and suggested their contribution to soil heterogeneity in local scale. It agrees well with a recent report describing the contribution of soluble anions (Cl⁻, SO₄²⁻) and cations (Ca²⁺, K⁺) in the heterogeneity of different quadrats studied at Cuatro Ciénegas, Mexico [8]. However, significant variation was not observed for Ca²⁺ in

the current study at Thar Desert. Among the quadrats, Q-A exhibited the high value of Cl⁻, K⁺, and Mg²⁺ (20 ± 0.4, 13.7 ± 2.9, and 369.7 ± 76.8 mmol kg⁻¹, respectively), while Q-B showed high value of SO₄²⁻ and Ca²⁺ (72 ± 7.5 and 7.4 ± 1.7 mmol kg⁻¹, respectively).

The PCA of the physicochemical parameters was performed to further explore their spatial heterogeneity among the two quadrats (Fig. 2). The first principal component (PC 1) explained 36.74%, while the second principal component (PC 2) explained 28.30% of the total variation in the soil parameters among quadrates. The

Fig. 2. PCA analysis: loading plot of soil physicochemical parameters (EC = electrical conductivity, OC = organic carbon, TP = total phosphorus, TN = total nitrogen, AN = ammonical nitrogen, NN = nitrate nitrogen).

Table 2. Estimators of sequence library, sequence, coverage, evenness and diversity.

Richness estimates and diversity indices	Q-A	Q-B
QC passed reads	3,98,170	3,04,334
Processed reads	33418	28168
Number of OTUs	173	170
Chao1 (richness)	174.03	173
ACE (coverage-based richness)	178.54	173
Shannon (Diversity)	4.33	4.43

variables associated with PC1 were pH, Mg^{2+} , K^+ , Cl, N-N, OC, and EC. PC 2 was mainly related to soil nutrients such as TN, and TP, along with SO_4^{2-} .

Sequence Data and Diversity Estimates

In this study, a total of 702,504 16S rDNA high-quality reads with average read length 301 bp were generated from the desert DNA samples. Rarefaction curves (Fig. S1) of both samples tended to approach the saturation plateau, revealing the depth of the experiments and a greater coverage of the bacterial community associated with the desert environment [28]. After the stringent quality filtration using UPARSE metagenome pipeline, 61,585 (8.76%) reads were assigned to 173 OTUs. Samples wise sequence reads,

and OTUs along with various diversity estimates are summarized in Table 2. The studied desert samples showed relatively higher and similar diversity, with closer Shannon values for both Q-A and Q-B. These diversity estimates are consistent with previous studies of the Thar Desert at different locations [26].

Bacterial Communities in the Desert Environment

Bacterial community profiling of extreme and stressful environments in local scales is crucial for interpreting broader ecological patterns. Especially, extreme hot and cold deserts characterized by high spatial heterogeneity [8], possess endemic microbial communities [13, 29, 30] that are thought to play key roles in ecosystem processes [31]. To test the bacterial heterogeneity in a local scale, as it represents excellent model systems for investigating microbial adaptation and responses to environmental stressors [13], bacterial community data of Thar Desert soils was generated through 16S rDNA deep sequencing using Illumina platform.

The taxonomic composition of Q-A and Q-B was collectively studied first to understand the general bacterial diversity in the studied desert environment (Fig. 3A). Our analysis indicated that two phyla, namely Actinobacteria (38.7%), and Proteobacteria (32.9%) are most abundant, followed by moderately abundant Acidobacteria (9.24%), and less abundant Bacteroidetes (3.46%), Firmicutes (3.46%), and Planctomycetes (3.46%). At the class level, 38.15% and

Fig. 3. Diversity and relative abundance of different bacteria in the arid experimental site (A), and relative difference in Q-A and Q-B (B).

26.01% of the total sequences represented the Actinobacteria and Alphaproteobacteria, respectively, followed by Acidobacteria Gp16 (3.46%). At the bacterial family level, an unclassified family affiliated to Actinobacteria was most abundant (15.6%), followed by Gaiellaceae (6.35%) and Streptomycetaceae (5.78%). At genus level, the *Gaiella* (6.35%), *Streptomyces* (5.2%) and *Solirubrobacter* (4.62%) showed relatively higher abundance, followed by array genera with 3.4 to 0.5% among the population.

Abundance of different bacterial phyla in the studied sample is consistent with previous studies of both hot and cold desert soils [31, 13]. Particularly, abundance of Actinobacteria and Proteobacteria has been widely reported, with limited variations according to climatic difference and specific niches [26, 31, 32]. A recent real-time quantitative PCR-based approach by Rao *et al.* [26], revealed abundance of Actinobacteria and Proteobacteria in semi-arid soils collected from the other sites of the Thar Desert. Similarly, metagenome analysis [31] of hot Namib Desert (Namibia) and cold Miers Valley (Antarctica), also witnessed the abundance of Actinobacteria and Proteobacteria, while some hypoliths are dominated by Cyanobacteria as well. In the present study, Cyanobacteria are a relatively minor contributor to total bacterial diversity, as visible hypoliths were not found in the study site.

The phylum Actinobacteria constitutes one of the largest phyla and was systematically studied due to its unique biotechnological potential [13], especially in production of drug leads [33]. Consequently, environments that have abundance of Actinobacteria capture the attention of natural products researchers [12, 34–36]. In the present study, abundance of Actinobacteria is 38.7%, and is comprised of several species affiliated to both unclassified classes and well-known biotechnologically important genus *Streptomyces*. The relative abundance of different genera affiliated to Actinobacteria was showed in Fig. S2. Ultimately, it is a suggestive sign of suitability of the desert environment for bioprospection studies, notably focused on Actinobacteria, as witnessed by recent reports [13].

Spatial Heterogeneity of Bacterial Community in the Desert Environment

Our knowledge of the structure, composition and physiology of biotic components in arid soils has improved in the recent past [2, 8, 37]. But, the spatial heterogeneity of bacteria at local scales is largely unaddressed despite having ecological significance. In this study heterogeneity in diversity of bacteria was studied at local scale, and the notable difference was found in the abundance rather than

the diversity (Fig. 3B).

Partial least squares discriminant analysis (PLS-DC) was performed and the plot (Fig. S3) was generated to illustrate bacterial community variation between the quadrats. First component accounted for 93.1% of variation, and indicated that overall differences were related to the abundance of specific OTUs rather than their presence or absence, as observed in a different study on a soil-borne bacterial structure [38]. This observation suggested that adjacent experimental quadrats harbor similar (but not equal) bacterial communities and the variations were controlled by many bacterial taxa. This result was also complemented by the heat map analysis (Fig. S4). An underlying reason behind this difference may be the spatial heterogeneity observed in soil physicochemical parameters at local scale as found in a different desert experimental site [8].

Physiological and Biological Mapping of Desert Bacterial Community

In order to evaluate the physical and biological attributes of the desert bacterial community, automated taxonomy-to-phenotype mapping was done. Among the observed bacterial community, 20% to 32% belonged to mesophiles (Fig. 4A), followed by 7% to 10% of thermophiles, and 6% to 8% of psychrophiles. Abundance of mesophilic bacteria may be attributed to vast temperature fluctuations in Bikaner [15], and correlates well with different reports of mesophilic bacterial load in arid environments [26, 39]. These mesophilic bacteria were represented mainly by aerobic (Fig. 4B) and free-living bacteria (Fig. 4C), as vegetation was scarce and sparsely found in the current study sites, as with other arid environments [8].

In the further mapping for biological attributes, predominance of Gram-positive (Fig. 5A) and spore-forming bacteria (Fig. 5B) was observed in quadrats Q-A and Q-B. It reveals the specific bacterial adaptation according to the stress conditions in the desert. Indeed, desert bacteria were resisting the extreme conditions either by forming desiccation-resistant spores or tolerating low water potential as vegetative cells [40].

Bacterial communities associated with nutrient-limited arid soils play key roles as the drivers of energy input and biogeochemical processes [6, 8, 11, 41]. Based on energy and carbon requirements (mode of nutrition), bacterial taxa associated with the experimental sites were categorized into as many as 11 bacterial groups (Fig. 5C). The major nutritional types were heterotroph, autotroph, and chemo organotroph in both quadrats. Despite this, significant variations were found in the relative abundance of minor

Fig. 4. Mapping of desert bacterial community to different physiological characteristics, growth temperature (A), respiration (B), and interactions with other organisms (C). Inner and outer ripples represent Q-A and Q-B, respectively.

nutritional groups, and strongly attested the nutritional heterogeneity in the desert soil in the local scale.

Metabolic Implications

Metabolic activities were predicted based on the taxonomy-to-phenotype mapping results. A total of 27 types of bacterial metabolic activities (Fig. 6) were identified among

Fig. 5. Mapping of desert bacterial community to different biological characteristics, cell membrane type (A), sporulation (B), and nutritional types (C). Inner and outer ripples represent Q-A and Q-B, respectively.

the bacterial communities of the study site. This number includes nitrogen fixation, ammonia oxidizer, dehalogenation, sulphate and sulfur reduction, nitrite reduction, sulfide oxidation and several bioremediation activities (aromatic hydrocarbons degrader, xylan degrader, chitin degrader, sulfur oxidizer, cellulose degrader, and lignin degrader). Similar taxonomy-to-phenotype mapping has

Fig. 6. Microbial metabolic functions encoded in bacterial communities from experimental sites.

recently been performed for gut microbial communities of termites [42]. The bacteria mineralizing organic nutrients were very rich in the desert soil, and capable of running key biogeochemical cycles. Recently, Vikram *et al.* [32] described schemes of biogeochemical cycling pathways based on functional capacity dictated by functional marker genes [43] in a hyper-arid desert soil niche community. Even though the present study is based on automated taxonomy-to-phenotype mapping, bacterial types were found to be accorded with that deduced-by-functional-genes based approach [32].

In conclusion, microbial communities are key drivers of ecosystem services in arid environments. On the other hand, the arid ecosystems are characterized by high spatial heterogeneity. Together, these features of arid ecosystems result in complex ecological events that are largely unaddressed, while considered being under the control of microbial communities and their association with nutrients and other physicochemical gradients in arid soils. The experimental quadrats of the current study showed statistically significant variation of anionic and cationic content and ultimately the microbial community distribution and relative abundance rather than the diversity. The functional implications of the bacterial communities associated with the desert soil were studied through an automated taxonomy-to-phenotype mapping approach, results revealed selective abundance of bacterial taxa relevant to physicochemical abiotic conditions. Overall, the variability of physicochemical properties and microbial

relative abundance and the metabolic predicted implications of this arid ecosystem are likely to exist in most soil ecosystems, and needs to be considered when making ecological inferences. Interestingly, the relative abundance of Actinobacteria, including large proportion of unclassified species in the Thar Desert suggested that this arid environment is suitable for bioprospection of bioactive natural products. Moreover, these Actinobacteria are suitable models for studying and understanding abiotic, stress-alleviating mechanisms in arid ecosystems.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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