Microbial Diversity in Soil, Sand Dune and Rock Substrates of the Thar Monsoon Desert, India

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Abstract A culture-independent diversity assessment of archaea, bacteria and fungi in the Thar Desert in India was made. Six locations in Ajmer, Jaisalmer, Jaipur and Jodhpur included semi-arid soils, arid soils, arid sand dunes, plus arid cryptoendolithic substrates. A real-time quantitative PCR approach revealed that bacteria dominated soils and cryptoendoliths, whilst fungi dominated sand dunes. The archaea formed a minor component of all communities. Comparison of rRNA-defined community structure revealed that substrate and climate rather than location were the most parsimonious predictors. Sequence-based identification of 1240 phylotypes revealed that most taxa were common desert microorganisms. Semi-arid soils were dominated by actinobacteria and alpha proteobacteria, arid soils by chloroflexi and alpha proteobacteria, sand dunes by ascomycete fungi and cryptoendoliths by cyanobacteria. Climatic variables that best explained this distribution were mean annual rainfall and maximum annual temperature. Substrate variables that contributed most to observed diversity patterns were conductivity, soluble salts, Ca²⁺ and pH. This represents an important addition to the inventory of desert microbiota, novel insight into the abiotic drivers of community assembly, and the first report of biodiversity in a monsoon desert system.

Keywords Cyanobacteria · Desert · Fungi · Sand dune · Soil

Introduction

The desert (dryland) biome occupies over one-third of Earth's land surface, and is characterised by moisture deficit. This is defined by an Aridity Index which reflects the ratio of precipitation to potential evapotranspiration (P/PET) [1]. A P/PET below 1.0 indicates that a location receives less water input via precipitation than is lost through evapotranspiration. Typically semi-arid areas display P/PET of 0.2–0.5, arid areas a P/PET of 0.2–0.05 and hyperarid areas a P/PET below 0.05 [1]. This presents severe challenges to animal and plant life and so microbial communities assume the foremost ecological roles in deserts [2]. Desert soil microbiology has been shown to differ fundamentally from that in other biomes [3], and lithic niches also support unique microbial communities [2, 4]. Biodiversity of desert microbial communities has been relatively well studied (see for example recent reviews: [2, 5, 6]. Soil surfaces may support well-defined biological soil crusts dominated by cyanobacteria, fungi, lichens and mosses, and these have been extensively studied [7]. Similarly the cyanobacterial and lichenized lithic microbial communities of desert pavements and exposed rocks have also received significant recent attention [2, 5, 8]. Relatively less attention has focused on open soils and sand dunes and these have been shown in a few studies to be dominated by a small number of heterotrophic bacterial phyla [9–11].

These microbial communities perform the key geobiological transformations in desert environments [2], as well as regulating physical processes such as dust uplift to the

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atmosphere and soil erosion [12]. Despite this a major gap in our understanding remains concerning the spatial distribution of desert microorganisms. Some studies on lithic communities have indicated the role of macro-climatic drivers such as mean annual precipitation including fog [13–15]. The influence of local scale processes has also been demonstrated, such as spatial self-organisation [15] and biological interactions [16, 17]. There is, however, a relative lack of information on microbial distribution in open soil systems in deserts.

The Thar Desert is a monsoon desert extending over 200,000 km² across northern India and into Pakistan [18]. Topography is dominated by sand dunes and sandy soils, with occasional rocky outcrops. Importantly for desert microbial ecology, this area and monsoon deserts in general are not only largely unstudied but also presents a clearly defined aridity gradient within a single desert system [18] (Fig. 1). This encompasses a range in mean annual precipitation from 100 to 500 mm that falls mostly during the monsoon in July–September each year [18]. The unusual climate of the Thar has very little wind and consequently large (>30 m height) and relatively stable dunes and sandy soils develop [18]. Given the dominance of open soil and sand dunes in this system, we hypothesized that interrogating microbial diversity in these substrates across an aridity gradient from semi-arid to arid and in different substrates would yield novel insight on monsoon desert microbial biodiversity as well as contributing to a wider understanding of spatial patterns in desert microbiology.

Materials and Methods

Sampling Site and Sample Collection

The Thar Desert is located in the north-western part of Rajasthan between latitudes 23°3' and 30°12' North and longitudes 63°30' and 70°80' East and was surveyed during October 2010. Sampling sites comprised: semi-arid soil (Jaipur), semi-arid soil and endolith (Ajmer), arid soil and sand dunes (Jaisalmer), arid soil and sand dunes (Jodhpur), according to availability of substrate (Fig. 1). At each site 12 samples were taken randomly from 10 m circular plots at each location (N = 75 samples). All soil samples were recovered aseptically; for soils and sand this was the first 50 mm of soil, directly scooped into sterile 50 ml Falcon tubes. For cryptoendoliths, colonised rock fragments were broken from boulders and placed in 50 ml sterile Falcon tubes. All samples were preserved using Mo Bio Lifeguard™ solution (MO BIO Laboratories Inc., Carlsbad, CA, USA). Samples were stored at ambient temperature during return from the field, until processed in the laboratory.

Soil Geochemical Analysis

A suite of 12 abiotic variables, including pH, soluble salts, total organic carbon, total nitrogen and metals were measured. Soil samples were thawed, air-dried, ground and allowed to pass through <2 mm sieve. Soil pH, electrical

Fig. 1 Map of Thar desert sampling sites, **a** arid and semi-arid zones within India, **b** the arid and semi-arid regions of the Thar Desert

conductivity, soluble salts were determined using soil: water slurry followed by standard potentiometric determination. Total carbon and nitrogen were determined using a thermal conductivity detector at 900 °C. All elemental tests were conducted after air drying and nitric/hydrochloric acid digestion using ICP-MS according to US EPA 200.2.

Recovery and Analysis of Environmental rRNA Gene Phylotypes

Soil samples were homogenised by lightly shaking containers and 50 mg aliquots used to extract environmental DNA using the PowerSoil™ DNA isolation kit following the manufacturer's protocol (MO BIO Laboratories Inc., Carlsbad, CA, USA). PCR amplification of rRNA genes for bacteria [19], archaea [20] and fungi [21] was carried out as previously described. The presence of PCR products was visualised by electrophoresis in 1 % agarose gels, and products were then purified using the GFX™ PCR DNA and gel band purification kit (GE Healthcare, United Kingdom).

PCR amplification was quantified in real-time (applied Biosystems Prism 7000, Foster City, USA) by fluorometric monitoring with SYBR green 1 dye (Invitrogen, Carlsbad, CA, USA). All standard curves were constructed using plasmids from cloned rRNA genes (Qiagen, La Jolla, CA, USA) for archaea, bacteria and eukarya. Quantification of amplicons in each sample was performed in triplicate. Dissociation curves were studied for each run to ensure the threshold cycle (C_t) reflected efficient and specific amplification. The absolute copy numbers of genes was obtained by interpolation from the respective standard curves.

A tRFLP approach was used to estimate phylotype diversity for all samples. This method quantifies sequence variability in small-subunit 16S/18S ribosomal DNA extracted from samples producing a DNA-fingerprint for each of the bacterial, fungal and archaeal assemblages respectively. Restriction digests (*Msp* I for 16S/18S) of FAM-labelled PCR amplicons were subjected to fragment analysis by 3730 Genetic Analyzer; Applied Biosystems). The software Perl and R were used to identify true peaks and bin fragments [22]. Non-metric multidimensional scaling (nMDS) plots of Bray Curtis similarities were made using software PRIMER v6 [23]. The most parsimonious sample from each domain-specific assemblage was then selected for construction of a clone library in order to generate sequence data.

Clone libraries were constructed for PCR amplicons using the TOPO TA Cloning® kit (Invitrogen). Each library comprised 60–200 clones (total N = 1240 clones). Whilst this method has recently been superseded by high-throughput sequencing, it remains a valid approach for microbial diversity assessment where the goal is to identify

the most abundant taxa, and has been proven to correlate well with deeper sequencing efforts in desert soils and rocks that support inherently low diversity [24, 25]. Phylotypes were delineated on the basis of 97 % sequence similarity using the freeware DOTUR [26]. Chimeric sequences were detected using Bellorophon (http://greengenes.lbl.gov/cgi-bin/nph-bel3_interface.cgi) and removed from further analysis. Sampling effort was assessed by the by the calculation of rarefaction curves and estimates of the OTU richness from clone libraries were made using Chao1 with Estimate S [27]. Approximate phylogenetic affiliations were determined by BLAST searches of the NCBI GenBank database (<http://www.ncbi.nlm.nih.gov/>).

Sequences were used to create multiple alignments with reference to selected GenBank sequences using ClustalX v.1.81 [28]. Maximum likelihood analysis was conducted using PAUP* 4.0b8 [29]. Bootstrap values (1000 replications) are shown for branch nodes supported by more than 40 %. All sequences have been deposited in the NCBI GenBank database under accession numbers for bacteria (JQ071624–JQ071724), fungi (JQ071725–JQ071777) and archaea (JQ071778–JQ071814).

Statistical Analysis

Alpha diversity indices (Shannon's Index, Simpsons Diversity Index, Pielou's Evenness) were calculated using untransformed data. Multivariate analysis of diversity data was performed on square-root transformed diversity data, and on non-transformed normalized data for environmental variables. Non-metric multi-dimensional scaling ordinations (NMDS) were used to visualize Bray Curtis Similarities (diversity data) and Euclidean Distances (environmental data). In BEST analyses The BIO-ENV procedure was used to maximize the rank correlation between biotic and environmental data, thereby establishing a ranking (ρ_w) for the effects of environmental variables on diversity. All analyses were performed using Primer v6.1.6 [23]. All results stated as significant have a confidence level of $P < 0.05$ unless stated otherwise.

Results and Discussion

The Thar Desert supported semi-arid and arid regions (Fig. 1). Comprehensive soil geochemical analysis of soils (Table 1) indicated a two-fold drop in C:N ratio, and a fall in total organic content with increasing aridity. All arid soils and sand dunes displayed a pH above neutral, whilst semi-arid soil was slightly acidic. All soils were sandy and had high calcium and ferrous content. The soil chemical analysis strongly suggests the monsoon desert soils of the Thar were less oligotrophic than those of some other hot

Table 1 Geochemical characteristics for soil and sand dune locations in the Thar Desert, India

Climate	Arid soil (Jaisalmer)	Arid soil (Jodhpur)	Arid dune (Jaisalmer)	Arid dune (Jodhpur)	Semiarid soil (Ajmer)	Semiarid soil (Jaipur)
Mean maximum temperature (°C)	43.9	42.8	43.9	42.8	42.3	42.7
Mean minimum temperature (°C)	6.8	8.1	6.8	8.1	9.0	6.9
Mean annual precipitation (mm/year)	31.6	25.9	31.6	25.9	41.4	45.7
Electric conductivity (μS)	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
pH	8.5	8.5	9	8.8	8.1	6.4
Soluble salts (g/100 g)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Ca ⁺ (g/kg)	8800	2800	17,000	5200	3500	1410
Fe ⁺ (g/kg)	12,300	6900	6700	7000	37,000	12,000
Mg ⁺ (g/kg)	2300	2500	2400	2200	10,300	2100
P (g/kg)	290	111	177	146	720	210
K (g/kg)	720	760	570	550	13,100	930
S (g/kg)	<2000	<2000	<2000	<2000	<2000	<2000
Total organic carbon (g/100 g)	0.24	0.09	<0.13	<0.05	0.25	1.24
Total nitrogen (g/100 g)	<0.13	<0.05	<0.13	<0.05	0.06	0.15
C:N ratio	4.1	3.7	1.03	3.2	4	8.2

Table 2 Microbial diversity of soil, sand dune and cryptoendolithic communities in the Thar Desert, India

	Arid sand dune (JSD)	Arid soil (Jb)	Semiarid soil (LL)	Semiarid endolith (A)
<i>Alpha diversity</i>				
<i>Bacteria</i>				
Shannon Index	2.8	4.9	4.5	2.9
Simpson Diversity Index	0.9	1	1	0.9
Pielou's Evenness	0.8	0.97	0.96	0.8
<i>Eukarya</i>				
Shannon Index	1.5	2.6	2.6	2.4
Simpson Diversity Index	0.7	0.9	0.9	0.9
Pielou's Evenness	0.7	0.8	0.8	0.8
<i>Archaea</i>				
Shannon Index	2.1	1.7	2	1.6
Simpson Diversity Index	0.8	0.8	0.8	0.7
Pielou's Evenness	0.8	0.9	0.8	0.7
<i>qPCR: copy number [relative abundance in % (standard deviation C_i)]</i>				
Archaea	0.07×10^5 [0.68 (0.59)]	0.23×10^5 [2.46 (0.67)]	1.27×10^5 [0.29 (0.14)]	0.23×10^5 [0.28 (0.47)]
Bacteria	0.23×10^5 [22.35 (0.48)]	8.7×10^5 [95.82 (0.10)]	439×10^5 [99.2 (0.06)]	80×10^5 [98.99 (0.40)]
Eukarya	0.81×10^5 [76.97 (0.18)]	0.16×10^5 [1.72 (0.98)]	2.3×10^5 [0.51 (0.56)]	0.58×10^5 [0.73 (0.55)]
<i>Bacteria</i>				
No. of clones	100	200	200	100
No. O.T.U (97 % cutoff)	42	155	113	39
Chao1 richness	79.4	469.6	153.4	78.8
Average blast similarity	96 %	95 %	96 %	96 %
<i>Eukarya</i>				
No. of clones	100	100	100	100
No. O.T.U (97 % cutoff)	8	25	29	24
Chao1 richness	7.3	24.3	37.5	29.3
Average blast similarity	98 %	96 %	97 %	97 %
<i>Archaea</i>				
No. of clones	60	60	60	60
No. O.T.U (97 % cutoff)	14	7	12	9
Chao1 richness	15.3	6.2	11.4	8.5
Average blast similarity	97 %	98 %	98 %	98 %

Table 2 continued

	Arid sand dune (JSD)	Arid soil (Jb)	Semi-arid soil (LL)	Semi-arid endolith (A)
<i>% Relative abundance of multi-domain community</i>				
Cyanobacteria	0	0	1	64
[Chroococcidiopsis sp.]	–	–	0	[32]
[Oscillatoriales]	–	–	0	[30]
[Pleurocapsales]	–	–	0	[0]
[Unknown cyanobacteria]	–	–	[1]	[2]
Acidobacteria	0	5	3	0
Actinobacteria	14	5	40	5
Alphaproteobacteria	<1	10	19	6
Bacteroidetes	0	1	6	0
Betaproteobacteria	0	1	5	2
Chloroflexi	<1	26	4	2
Deltaproteobacteria	0	4	4	0
Firmicutes	2	9	6	1
Gammaproteobacteria	<1	7	2	5
Gemmatimonadetes	<1	5	4	0
Nitrospirae	0	1	0	0
Planctomycetes	<1	2	4	0
Thermobaculum	0	1	0	0
Verrucomicrobia	1	1	1	0
Unknown bacteria	4	21	3	15
Ascomycota	75	1	<1	1
Basidiomycota	2	<1	<1	<1
Chytridiomycota	0	<1	<1	0
Fungi Incertae sedis	0	0	0	<1
Alveolata	0	<1	<1	<1
Amoebozoa	0	<1	0	<1
Ichthyosporea	0	<1	0	0
Metazoa	1	<1	<1	<1
Rhizaria	0	<1	<1	0
Crenarchaeota	1	2	<1	<1
Euryarchaeota	<1	<1	<1	0
Thaumarchaeota	0	0	<1	0
Unknown archaea	<1	<1	<1	<1

deserts. For example, in the driest Atacama Desert soils the total organic content ranged from 200 to 700 µg/g, and in the Sahara near Abu Simbel, Egypt were 1700 µg/g [10, 30] an order of magnitude less than in Thar. The BEST analysis revealed that the most influential combination of variables on microbial diversity were mean annual precipitation, maximum annual temperature, electrical conductivity, soluble salts, pH and Ca²⁺ content in soils (BEST $p_w = 0.321$). This strongly indicates that macroclimate influence is driven by water availability and upper temperature limits. Substrate variables related to salinity and pH indicate osmotic stress rather than nutrient stress is a major driver.

We used real-time quantitative PCR to estimate relative abundance of phylotypes for all domains. By calibrating our PCR individually against archaea, bacteria and eukaryal amplicons we were able to establish absolute and

relative abundance for each domain in each sample. The results were striking and for all sample groups the standard deviations were low (Table 2). Overall, it illustrated that all soils and cryptoendoliths were dominated by bacteria > fungi > archaea. Conversely, sand dunes were all dominated by fungi > bacteria > archaea. Community composition as defined by tRFLP analysis revealed samples clustered according to habitat type (i.e. soils, sand dunes, endoliths) rather than location (Fig. 2). Differences between soil communities were distinct between arid and semi-arid locations (bacteria: ANOSIM, Global $R = 0.622$, $P < 0.001$, $n = 75$; fungi: ANOSIM, Global $R = 0.44$, $P < 0.001$, $n = 75$; archaea: ANOSIM, Global $R = 0.49$, $P < 0.001$, $n = 75$).

To further characterize the communities we constructed clone libraries based on near full-length 16S and 18S rRNA gene sequences. The interpolation of clone library

Fig. 2 Nonmetric multi-dimensional scaling plot Bray Curtis similarities for **a** bacterial, **b** eukaryal and **c** archaeal, rRNA gene phylotypes recovered from arid soil, arid sand dune, semi-arid soil and semi-arid cryptoendolith in the Thar desert. *Dashed line* represent statistically significant groupings (bacteria: ANOSIM, Global R = 0.622, $P < 0.1$, $n = 75$; eukarya: ANOSIM, Global R = 0.44, $P < 0.1$, $n = 75$; archaea: ANOSIM, Global R = 0.49, $P < 0.1$, $n = 75$)

Fig. 3 Diversity and relative abundance of bacteria (a), eukarya (b) and archaea (c) from arid soil (Jaisalmer); arid sand dune (Jaisalmer); semi-arid soil (Ajmer) and semi-arid endolith (Ajmer) of the Thar Desert

sequence data with tRFLP fragments allowed an estimate of relative abundance for all phylotypes across all domains for each sample (Table 2; Fig. 3). We determined phylogenetic identity for all recoverable phylotypes (Figs. 4, 5,

6). All operational taxonomic units (O.T.U) were assigned phylogenetic identity based on near full-length rRNA sequence and spanned 19 phyla. Sand dune communities were dominated by ascomycete fungi and actinobacteria,

Fig. 4 Phylogenetic relationships among bacterial partial 16S rRNA phylotypes recovered from the Thar desert. Sequence code prefix denotes location: JB, arid soil (Jaisalmer); JSD, arid sand dune (Jaisalmer); LL, semi-arid soil (Ajmer); A, semi-arid endolith

(Ajmer). NCBI Genbank accession numbers are given for each sequence generated in this study. Tree topologies are supported by bootstrap values >50 % for 1000 replications. Scale bar = 0.3 nucleotide changes per position

whereas soil communities were almost exclusively bacterial and largely comprised actinobacteria, alpha proteobacteria and chloroflexi. The semi-arid soils and cryptoendoliths supported bacterial photoautotrophs (cyanobacteria) but they were not recovered from arid substrates. The cryptoendolithic community of the semi arid region was dominated by cyanobacteria although they displayed very low abundance in soil. Diversity estimates indicated that overall soils were consistently more biodiverse than sand dune or cryptoendolith communities (Table 2). Furthermore the eukaryal sequence data also indicated *Amoeba*, *Metazoa* and *Alveolata* signatures in soils, suggesting protists and micro-invertebrates are present as part of the community, and interestingly the presence of ‘exotic’ fungi including nematode trapping fungi and desert truffles (Fig. 6).

Phylotypes recovered in this study may be regarded as a ‘typical’ desert soil community, and have also been recorded in other deserts. These include cyanobacteria belonging to the genera, *Pseudanabaenaceae*, *Chroococcidiopsis*, *Phormidium*, *Microcoleus* [13, 25, 31–33]; heterotrophic bacterial phylotypes belonging to the genera *Rubrobacter*, *Arthrobacter*, *Sphingomonas*, *Chloroflexus*, *Roseiflexus*, *Pontibacter* [9, 34], fungal phylotypes belonging to, *Mattitolomyces*, *Ascobolus*, *Chaetomium*, *Preussia*, *Chaetothyriales*, *Rhodotorula* [7, 35]; and archaeal phylotypes belonging to *Methanosarcina* and *Thermotoplasma* [36]. The key finding that fungi dominate sand dunes whereas bacteria dominate soils is of interest. A closer examination of phylotypes recovered in our study suggests that fungal taxa in sand dunes are likely to be genera with heavily melanised taxa such as

Fig. 5 Phylogenetic relationships among fungal partial 18S rRNA phylotypes recovered from the Thar desert. Sequence code prefix denotes location: JB, arid soil (Jaisalmer); JSD, arid sand dune (Jaisalmer); LL, semi-arid soil (Ajmer); A, semi-arid endolith

(Ajmer). NCBI Genbank accession numbers are given for each sequence generated in this study. Tree topologies are supported by bootstrap values >50 % for 1000 replications. Scale bar = 0.7 nucleotide changes per position

Scolecobasidium [37] or those adapted to xeric habitats such as *Wallemia* [38]. The fungal assemblage included *Chaetothyriales* phylotypes, indicating a fungal genera belonging to *Eurotiomycetes*, known for its melanisation, and rock inhabiting nature which protects it from UV and solar radiation [39]. Changes in dominance of fungi and bacteria in soil are often correlated with pH [40], although in this study negligible difference (soil pH 8.8, Sand pH 9.0) was recorded. Instead the difference may reflect broader adaptation of fungi to the dune environment, possibly due to their filamentous growth form in a relatively unstable substrate.

Many of the bacterial and fungal taxa recovered in our study displayed high similarities to biological soil crust (BSC) organisms [7]. A limitation of our study is that well developed biological soil crusts were not encountered during this study. These are a common feature of deserts worldwide and several of the soil phylotypes recovered in our study are known to be key in crust development. Our study sites were without well-developed soil crusts, but the data indicates the

soils nonetheless retain a potential reservoir of recruitment for crust development. The actinobacterial phylotypes largely affiliated to the genus *Rubrobacter*, a noted radiation (desiccation) tolerant desert bacterium [41]. Similarly the Chloroflexi and alpha proetobacterial phylotypes affiliated with common desert taxa [31, 42] and those from other extreme environments [43]. Whilst the archaea comprised a very small part of the overall community, phylotypes indicated desert-adapted taxa such as *Methanosarcina*, a stress-tolerant methanogenic archaeon known for survival of desiccation stress [36].

Overall we have demonstrated that soils and sand dunes support a fundamentally different microbial community in the monsoon desert of the Thar. We have not investigated whether these communities are dynamic or remain relatively static seasonally but this would be an interesting avenue for future research. Given the increasing pressure on this region from subsistence agriculture, and the detrimental effects this has on soil stability [12], it is timely to have documented that a

Fig. 6 Phylogenetic relationships among archaeal partial 16S rRNA phylotypes recovered from the Thar desert. Sequence code prefix denotes location: JB, arid soil (Jaisalmer); JSD, arid sand dune (Jaisalmer); LL, semi-arid soil (Ajmer); A, semi-arid endolith

(Ajmer). NCBI Genbank accession numbers are given for each sequence generated in this study. Tree topologies are supported by bootstrap values >50 % for 1000 replications. Scale bar = 0.08 nucleotide changes per position

reservoir of microbial taxa implicated in development of stabilizing soil crusts are present.

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