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A snapshot of microbial communities from the Kutch: one of the largest salt deserts in the World

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Abstract Here we present the first report on the taxonomic diversity of the microbial communities of the saline desert of the Great Rann of Kutch, Gujarat, India, using a metagenomic approach. Seven samples, differing in salinity levels and covering different seasons, were analysed to investigate the dynamics of microbial communities in relation to salinity and season. Metagenomic data generated using whole metagenome sequencing revealed that despite its very high salinity (4.11–30.79 %), the saline desert's microbiota had a rich microbial diversity that included all major phyla. Notably, 67 archaeal genera, representing more than 60 % of all known archaeal genera, were present in this ecosystem. A strong positive correlation (0.85) was observed between the presence of the extremely halophilic bacterium *Salinibacter* and salinity level. Taxonomic and functional comparisons of the saline desert metagenome with those of other publicly available metagenomes (i.e. sea, hypersaline lagoon, solar saltern, brine, hot desert) was carried out. The microbial community of the Kutch was found to be unique yet more similar to the sea biomes followed by hypersaline lagoon.

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Introduction

Hypersaline ecosystems are among the world's most extreme natural environments. They are distributed globally and are represented by a wide range of ecosystem types, such as salt lakes, soda lakes, hypersaline springs, salt flats, playas, solar salterns, and ancient salt deposits (McGenity et al. 2000; Oren 2002; Ventosa et al. 2008). Research on these ecosystems has not only revealed the presence of diverse microbial communities representing all three domains of life (Oren 2002; Ventosa et al. 2008; Caton 2004; Nicholson and Fathepure 2005) but also enzymes with enhanced biotechnological applications (Oren 2002; Caton 2004; Humayoun et al. 2003; Ley et al. 2006; Mesbah et al. 2008). The majority of studies on the various hypersaline areas of the world primarily focus on aquatic communities (Oren 2002; Humayoun et al. 2003; Demergasso et al.2004; Mutlu et al. 2008; Ghai et al. 2011; Narasingarao et al. 2012; Gomariz et al. 2015) and microbial mats (Humayoun et al. 2003; Moune et al. 2003), whereas far fewer attempts have been made to characterise hypersaline soils and sediments (Ventosa et al. 2008; Mesbah et al. 2008; Walsh et al. 2005; Dong et al. 2006; Jiang et al. 2006; López-López et al. 2010). One of the unique and unexplored hypersaline ecosystems in India is the Great Rann of Kutch, located in the Kutch District of Gujarat, India. Stretching over an area of 7505 square kilometres, it is one of the largest salt deserts in the world. It is a huge, desiccated, unbroken, bare surface of dark silt that is encrusted with salt and transforms into a spectacular coastal wetland after the rains. The Rann of Kutch is a large ecotone,

a transitional area between marine and terrestrial ecosystems. This area experiences extreme climatic conditions, with average summer temperatures of approximately 44 °C and highs of 50 °C and minimum winter temperatures approaching or dropping below freezing (McGinley 2008). The hot and hypersaline environment makes the Rann of Kutch a harbour for unique and robust microbial diversity. This unique and dynamically changing ecosystem is represented by shallow, salty marshes during monsoon season; salty mud flats in winter; and dry, crystalline, salty, white desert during summer. Despite the possibility of identifying novel microbes with high economic and industrial potential, there are no detailed reports on the microbial diversity of this region. The Kutch is thought to have originated from marine transgression (Biswas and Deshpande 1970).

Metagenomics is evolving as a potential tool for studying microbial communities using both functional and taxonomic approaches (Tringe et al. 2005; Delmont et al. 2012). Ion torrent PGM has been successfully used by researchers for metagenomic studies (Yergeau et al. 2012; Brown et al. 2013). Analyses of these community genomics overcome the limitations of culture-based methods to reveal community dynamics; additionally, many researchers have successfully commercialised important enzymes and biomolecules for industrial use (Lee and Lee 2013). Analyses of the metagenomic data also provide a comprehensive understanding of microbial survival and adaptation strategies in extreme environments and special ecosystems that are often difficult to measure using biogeochemical or culture-based approaches (Tringe and Rubin 2005; Fierer et al. 2012).

Materials and methods

Site description and sampling

The Great Rann of Kutch is located on the western coast of India and spans 7505 sq km in the state of Gujarat. The mean annual temperature is 45 °C, and the average rainfall is 147 mm (http://imdahm.gov.in/bhj.htm). According to geological history, the Rann of Kutch formed due to marine transgression during the Mesozoic Era (Biswas and Deshpande 1970).

The sampling strategy was primarily based on three parameters: (1) maximum possible reachable locations in the interior desert even without roads, (2) different type of locations varying in salinity levels and (3) areas without human activities so as to capture naturally accruing diversity. Considering these parameters, soil samples were collected from 5 different locations [S1 (23° 48′ 39″ N, 70° 58′ 60″ E), S2 (23° 47′ 33″ N, 71° 0′ 29″ E), S3 (23° 54′ 29″ N, 70° 32′ 16″ E), S5 (23° 50′ 6″ N, 69° 31′ 8″ E) and

S6 (23° 56′ 27″ N, 70° 11′ 18″ E)] in October 2012 along a 150-km transect through areas with various salinity levels to study the influence of salinity on microbial communities. From each site, 5–10 samples were collected and subsequently pooled for metagenomic analysis. Of these, site S6 was selected for a seasonal study, representing the month of October 2012, whereas S4 (23° 56′ 29″ N, 70° 11′ 18″ E) and S7 (23° 56′ 26″ N, 70° 11′ 16″ E) were collected in April (the hottest month) and July (the wettest month) 2013, respectively, from the same site. The sampling sites are shown in Fig. 1. Soil sample collection was performed using an auger with a 3-cm diameter at an average depth of 30 cm. If present, solid salt crusts were removed prior to sampling. Samples were placed into sterile plastic containers and stored at 4 °C until arrival at the laboratory. One portion of each sample was dried and used for physical and chemical analysis, and a second portion was frozen at −80 °C until DNA was extracted.

Physicochemical analysis of samples

The samples were dried at 45 °C until they reached a constant weight and were passed through a 2-mm sieve prior to physicochemical analysis. The pH (Schofield and Taylor 1955) and electrical conductivity (Rhoades 1982) of the soil samples were measured (Table 1).

DNA extraction and sequencing

The DNA extractions were performed using the Power Max Soil DNA Isolation kit (MO BIO Laboratories Inc., Carlsbad, CA, USA). For all seven soil samples, the community DNA was isolated from 5 g of soil using the bead beating and chemical lysis method following the manufacturer's protocol. A Qubit 2.0 Fluorometer (Invitrogen, USA) was used to quantify the DNA. Libraries were prepared using an Ion Express Plus Fragment Library Kit. The metagenomic DNA was sheared into blunt-ended fragments via enzymatic lysis using Ion Shear Plus reagents. The fragmented DNA was ligated to ion-compatible adapters, followed by nick repair to complete the linkage between the adapters and the DNA inserts. Whole-genome sequencing of the metagenomic DNA was performed via Ion Express Template 300 chemistry on a 318 chip using a high-throughput Ion Torrent Personal Genome Machine and an Ion Torrent Server.

Real-time PCR

To enumerate approximal archaeal and bacterial load in all the seven metagenomic samples, standard quantification studies were performed. Two sets of primers targeting the 16S rRNA gene segment of bacteria and that of
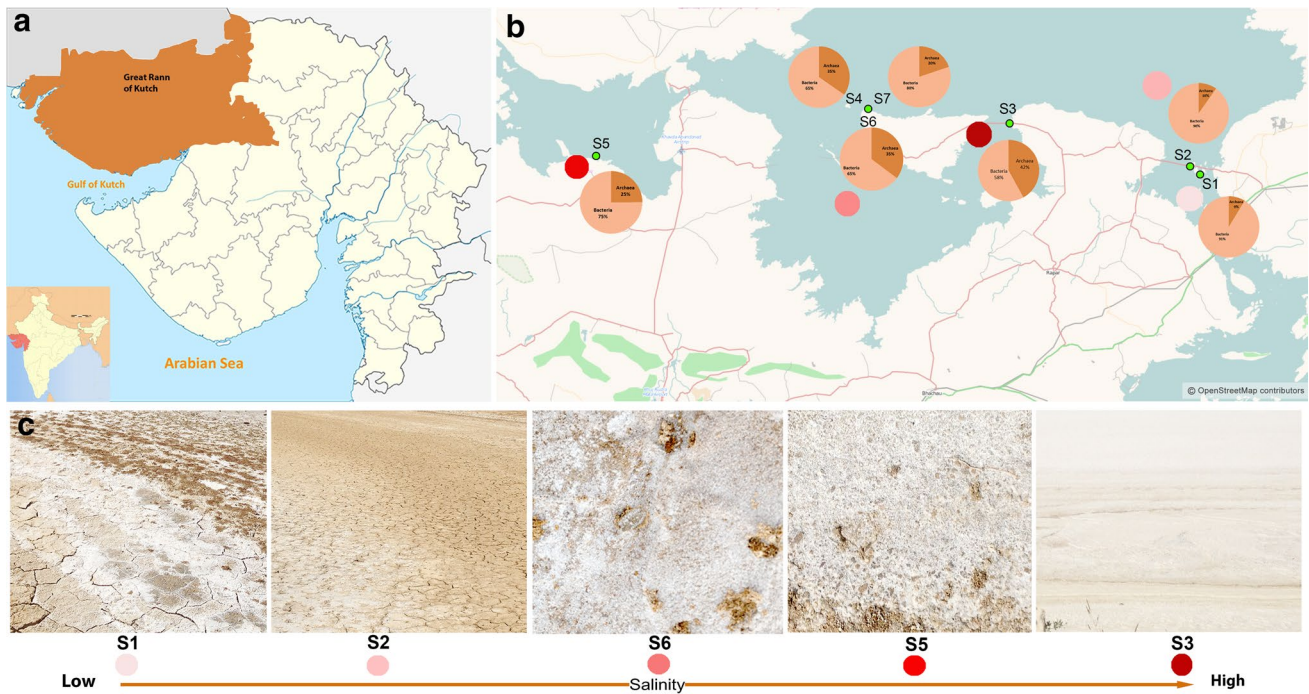
Fig. 1 Map showing Great Rann of Kutch, Gujarat, INDIA and Images of sample collection sites (S1, S2, S3, S4, S5, S6,S7). **a** Map of Gujarat showing Great Rann of Kutch in the inset of India: (http://en.wikipedia.org/wiki/Gujarat#mediaviewer/File:India_Gujarat_loca-tion_map.svg). **b** Geographical location of sample collection sites of Great Rann of Kutch

Table 1 Details of the metagenomes of saline desert of Kutch

Sample ID	GPS coordinates	pH	Electrical conductivity mS/cm	Salinity (%)	Organic carbon mg/kg	Total nitrogen mg/kg
S1	23° 48′ 39″ N, 70° 58′ 60″ E	8	83.8	5.02	15,900	1200
S2	23° 47′ 33″ N, 71° 0′ 29″ E	7.99	68.5	4.11	165,400	4800
S3	23° 54′ 29″ N, 70° 32′ 16″ E	7.84	513.3	30.79	11,800	1200
S4	23° 56′ 29″ N, 70° 11′ 18″ E	8.23	97.5	5.85	12,600	1500
S5	23° 50′ 6″ N, 69° 31′ 8″ E	7.33	328	19.68	11,200	990
S6	23° 56′ 27″ N, 70° 11′ 18″ E	8.6	98	5.88	14,000	1200
S7	23° 56′ 26″ N, 70° 11′ 16″ E	7.18	71.8	4.3	15,200	900

archaea were chosen for the study. The forward primer 5′-AGAGTTTGATCMTGGCTCAG-3′ (positions 27–46) (Lane 1991) and reverse primer 5′-GCTGCCTCCCG-TAGGAGT-3′ (positions 339–354) (Amann et al. 1990) were used for bacterial quantitation, and the forward primer 5′-ACGGGGYGCAGCAGGCGCGA-3′ (positions 344–364) and reverse primer 5′-GWATTACCGCGGCK-GCTG-3′ (positions 519–534) (Yu et al. 2008) were used for archaeal quantitation.

Real-time quantitative PCR was performed on a Step One Real-Time PCR System (Applied Biosystems, USA) using the FGFast SYBR Master Mix (Applied Biosystems). Two different standard curves were prepared from pure cultures of *Staphylococcus haemolyticus* (genome size 2.6×10^6 bp) and *Haloferax alexandrinus* (genome size 3.7×10^6 bp) to determine the bacterial and archaeal load in all soil samples (Jia and Conrad 2009; Yousuf et al. 2012). A Qubit broad range assay was performed to quantify the initial concentration of DNA in the samples, as well as to prepare serial dilution series of both pure cultures. Five dilution series from 10^{-1} to 10^{-5} were prepared for the standards and were subsequently used to quantify the bacterial and archaeal population loads of the metagenomic samples.

Bioinformatics and statistical analysis

Low-quality metagenomic reads were filtered via Ion Torrent Software Suite v3.2. The filtered reads containing 16S rRNA gene sequences were identified via BLASTN

searches against NCBI 16S microbial and archaeal databases with an *e* value cutoff of 1e−5. The taxonomic affiliations were assigned using the Ribosomal Database Project RDP MultiClassifier 1 (Wang et al. 2007) with a 50 % bootstrap confidence cutoff. The RDP MultiClassifier uses an RDP naïve Bayesian Classifier to classify single or multiple files containing 16S rRNA. Additionally, the raw sequence reads of all seven datasets were submitted to the MG-RAST v3.3.7.3 (Meyer et al. 2008) and IMG/M (Markowitz et al. 2012) for downstream analysis. For MG-RAST, the taxonomic identification was based on the top BLAST hit to the M5nr database with an *e* value cutoff of 1e−5 and 60 % identity. The sequences were annotated for functional gene categories against the SEED database using the BLASTX in MG-RAST. The enzymes were identified by annotating reads in the KEGG database and screened against MetaBioME (Sharma et al. 2010) to determine their commercial value. To identify the likely taxonomic source of the stress genes, sequences related to stress genes obtained using MG-RAST (BLASTX) with an *e* value $<10^{-5}$ were selected regardless of alignment length and the percentage of similarity thresholds.

The Alpha diversity was calculated by estimating the Shannon index and evenness using PAST v3 statistical analysis software (Hammer et al. 2001). A non-parametric estimator, chao1, was also calculated to assess the species richness of the samples using PAST v3. The dissimilarity in taxonomic and functional composition between the samples was measured using the Bray-Curtis distance. A principal coordinate analysis (PCoA) implemented in PAST was performed to evaluate the similarity among the various metagenomic datasets (saline desert, hot desert, sea, and hypersaline lagoon) based on the metadata of the taxonomic composition and functional annotation. The captured diversity was determined by generating rarefaction curves in MG-RAST.

Data accessibility

The data are publicly available on MG-RAST and IMG. The submission IDs and direct links to the datasets are provided in Table S1. The DNA sequences from this metagenomic project have been deposited in the NCBI Sequence Read Archive under accession no. S1 (SRX306504), S2 (SRX306503), S3 (SRX519631), S4 (SRX519746), S5 (SRX519747), S6 (SRX519748), and S7 (SRX519749).

Results

This study provides the first report of the microbial diversity of the Kutch saline desert using a metagenomic approach. High-throughput metagenomic sequencing of

environmental DNA isolated from soil samples collected from five locations differing in salinity levels namely S1, S2, S3, S5, S6 was performed. Of these sites, S6 was selected for seasonal study and S4 and S7 are collections of April and July, respectively, from the same site (Fig. 1). This study also describes the prokaryotic taxa present in these areas, their preferential metabolic processes in this saline desert and the effect of seasonal variation on these microbial communities. Kutch metagenomes were compared with the metagenomes of five other biomes that were publicly available in MG-RAST (Table S1). The biomes used for comparison were from hot deserts (Chihuahuan Desert, New Mexico, Mojave Desert, California), seas (Sargasso Sea (Rusch et al. 2007), Puget Sound Estuary, Elba's Sant'Andrea Coast) hypersaline lagoon [the hypersaline lagoon of Mar Menor (Moune et al. 2003)], solar saltern [Chula Vista, California (Legault et al. 2006)], and a saturated brine (Spain).

Physicochemical characteristics of samples

Table 1 represents the analysis of pH, electrical conductivity, total organic carbon and nitrogen in all collected soil samples. The pH of the soil ranged from 7.18 to 8.6, indicating slight alkalinity. The electrical conductivity of the samples ranged from as low as 68.5 mS/cm in S2 to as high as 513.3 mS/cm in S3, representing a salinity difference of more than 7-fold across samples. The results of the electric conductivity indicate a highly saline environment. The total organic carbon was similar among all samples with a range of 1.1–1.6 %; however, total nitrogen ranged between 0.09 and 0.48 %.

Metagenome data and microbial diversity

Using a shotgun metagenomic approach, a total of 235993491–387783372 bp sequences were obtained with read lengths of 100–159 bp (Table S2). The GC content percentage in the metagenomes ranged from 51 to 60 %. On average, the metagenomes comprised 56–87 % bacteria, 8–40 % archaea, 0.1–2 % eukaryotes, 0.02–1 % viruses and 9–14 % unclassified reads. In prokaryotes, the classifications of the 16S rRNA sequences were compared using the M5nr, IMG/M and NCBI 16S bacterial and archaeal databases. Taxonomic classification and quantification of metagenomic datasets was done against M5nr, and functional classification was done using SEED Subsystems databases on MG-RAST.

Results from MG-RAST showed major prokaryotic phyla were *Proteobacteria* (18.6–47.7 %), *Euryarchaeota* (7.9–40.1 %), *Bacteroidetes* (9.1–18.7 %), *Firmicutes* (5.8–8.4 %), *Actinobacteria* (2.1-5.8 %) and *Cyanobacteria* (2–4.2 %); the minor phyla were *Chloroflexi* (1–5.3 %),

Deinococcus-Thermus (0.5–1.4 %), *Planctomycetes* (1.3–3.9 %) and *Gemmatimonadetes* (1–1.9 %) (Fig. 2).

Amongst *Proteobacteria*, the relative abundance of *Alphaproteobacteria* (5.7–19.4 %), *Gammaproteobacteria* (5.4–17.6 %) and *Deltaproteobacteria* (5.4–11.6 %) was higher than that of *Betaproteobacteria* (1.7–5.1 %), *Epsilonproteobacteria* (0.2–0.5 %) and *Zetaproteobacteria*.

Of the 456 genera identified from the phylum *Proteobacteria*, 135 genera were assigned to *Alphaproteobacteria*, 167 to *Gammaproteobacteria*, 60 to *Deltaproteobacteria* and 75 to *Betaproteobacteria*. At the genera level, *Marinobacter* from class *Gammaproteobacteria*, *Roseobacter* from *Alphaproteobacteria*, *Burkholderia* from *Betaproteobacteria*, and *Geobacter* and *Desulfobacteria* from *Deltaproteobacteria* were abundant in all metagenomes.

Similarly, the phylum *Bacteroidetes*, which was represented by 68 genera, was dominated by *Salinibacter* (0.7–13.8 %) and *Rhodothermus* (0.7–1.7 %). Furthermore, a total of 146 genera were represented in the phylum *Firmicutes*, with the highest percentage of reads from the genera *Bacillus* (0.8–1.2 %).

Another important taxonomic phylum revealed via metagenome analysis was *Actinobacteria*, which exists widely in soil and freshwater and is less prevalent in hypersaline habitats (Goodfellow and Williams 1983; Eiler and Bertilsson 2004). Represented by 93 genera, *Rubrobacter* (0.2–0.7 %) was the most abundant genera within this phylum. Notably, *Cyanobacteria* (with *Synechococcus* as

the dominant genera), *Chloroflexi* and *Planctomycetes* as minor phyla were present in this hypersaline environment.

Archaea constituted 8–40 %, with Euryarchaeota comprising 67 genera that included methanogenic, halophillic and thermophillic archaea. Within Euryarchaeota, the proportion of methanogenic archaea was 4–11 %. The hot hypersaline nature of collection sites justifies the presence of *Halobacteria* and thermophiles.

Taxonomic shifts across salinity gradient

Taxonomic shift was studied for samples collected from different locations but same season October, i.e. S1, S2, S3, S5 and S6. Across the salinity gradient, a clear taxonomic pattern was visible in the abundance profiles of different microbes. The bacteria-to-archaea ratio varied from as low as 1.4:1 in S3 to 10:1 in S1. There was a significant negative correlation ($r = -0.77$) between increased salinity and bacteria abundance; abundance in the high-salinity samples [S3 (52.6 %), S5 (72.9 %)] and S6 (64 %) was lower than that in the lower salinity samples [S1 (87.1 %) and S2 (86.8 %)]. However, the correlation of archaeal abundance with salinity gradient could not be established ($r = -0.60$) suggesting that archaea are more tolerant to salinity as compared to bacteria. At the phylum level, the abundance of *Proteobacteria* and *Verrucomicrobia* was negatively correlated with salinity level ($r = -0.69$ and -0.70, respectively). The relationship between salinity and some
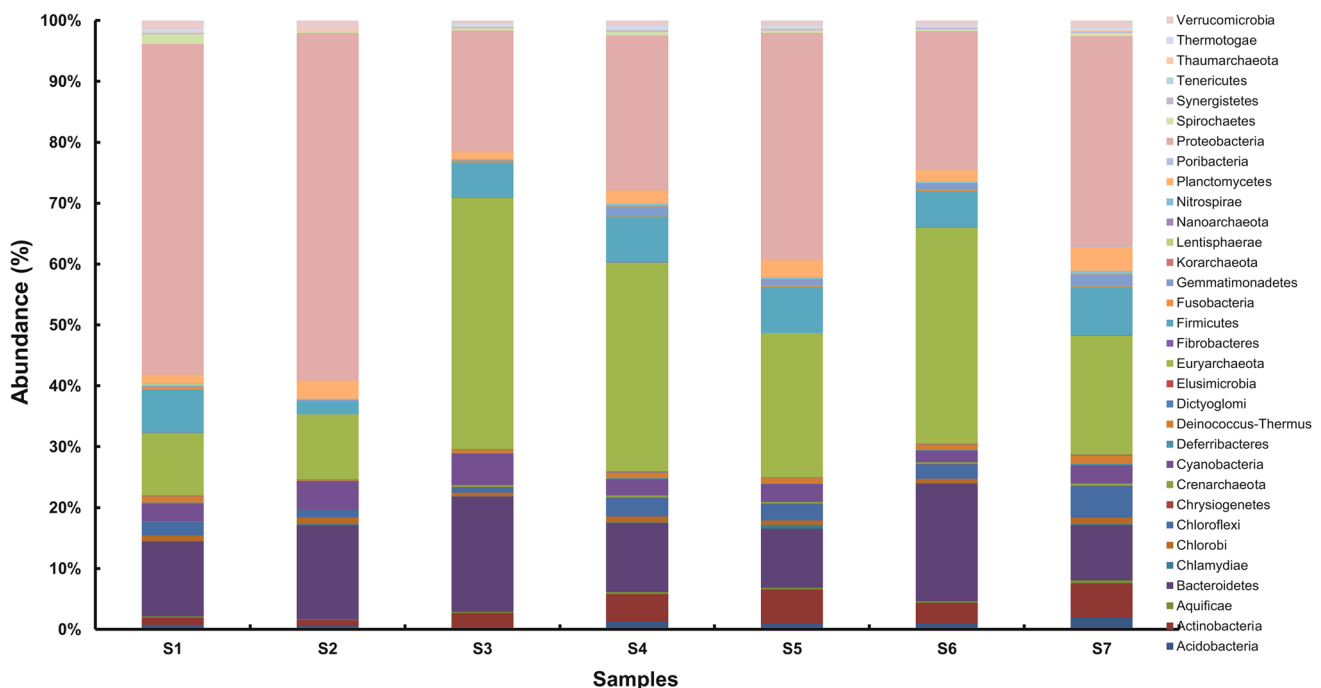

Fig. 2 Taxonomic distribution of bacterial and archaeal phyla of saline desert of Kutch. Relative abundance of reads assigned to bacterial and archaeal phyla in the 7 metagenomes using M5nr database

Actinobacteria, *Chloroflexi* and *Spirochaetes* was non-linear, showing an inverted U-shaped curve, i.e. low abundance at both low and high salinities. The varying abundance of the proteobacterial classes *Alphaproteobacteria*, *Gammaproteobacteria* and *Deltaproteobacteria* could be related to salinity gradient. S3 (higher in salinity) had the lowest percentage of *Betaproteobacteria* and the highest percentage of *Alphaproteobacteria*, whereas S2, with lower salinity, showed inverse results. Similarly, cyanobacterial sequences showed a population decline from low-salinity samples to high-salinity samples. In contrast, a strong positive correlation was observed ($r = 0.85$) between the abundance of *Salinibacter* and the salinity level, i.e. the higher the salinity, the higher the abundance of *Salinibacter*.

Samples with higher salinity (S3, S5) and moderate salinity S6 were dominated by various species of *Euryarchaeota*, including the genera *Haloarcula*, *Haloquadratum*, *Halogeometricum*, *Haloferax*, *Natronomonas*. *Salinibacter* were the dominant bacterial genera in S3, S5 and S6. Apart from *Salinibacter*, *Rhodothermus* and *Gemmatimonas* were highly abundant genera in S5 and S6 whereas *Rhodothermus* and *Desulfohalobium* in S3 (Table S3). S1 and S2 exhibited a higher abundance of bacterial species compared with archaeal species. Pigment-producing organisms from the genera *Rhodobacter*, *Roseobacter*, *Rhodopseudomonas*, *Rhodopirellula*, and *Jannaschia*, among others, as well as halophillic bacteria from the genera *Salinibacter*, *Marinobacter*, *Zunongwangia*, and *Chromohalobacter* were more abundant in both samples, whereas sulphate-reducing organisms from genera such as *Desulfobacterium*, *Desulfococcus* and *Desulfatibacillum* were abundant in S1.

Seasonal shifts in microbial communities

Initial sampling was performed on 31st October 2012, from which (based on highest saline site accessible during all seasons) S6 (23° 56′ 27″ N, 70° 11′ 18″ E) was selected for seasonal study. Because April is the hottest month, with temperatures averaging 41 °C and peaking at 49.5 °C, sample S4 (23° 56′ 29″ N, 70° 11′ 18″ E) was collected on 28th April 2013. Similarly, because July is the wettest month with an average rainfall of 147 mm, sample S7 (23° 56′ 26″ N, 70° 11′ 16″ E) was collected on 27th July 2013. The bacteria-to-archaea ratios were the same in both October and April, i.e. 1.8:1, but showed a significant change in July, reaching 4:1. *Euryarchaeota* and *Bacteroidetes* were abundant in April and October with percent abundance (April—*Euryarchaeota* 33.7 %, *Bacteroidetes* 11.3 %) (October—*Euryarchaeota* 34.4 %, *Bacteroidetes* 18 %) due to the extremely hot and dry climate but significantly decreased to 19.5 and 9.11 %, respectively, in July. It was observed that methanogenic archaea increased by 1.6-fold in the month of July as compared to April while

non-methanogenic archaea decreased by 1.02-fold. *Proteobacteria*, *Chloroflexi*, *Cyanobacteria*, *Firmicutes*, *Verrucomicrobia*, *Actinobacteria*, *Deinococcus-Thermus*, *Chlorobi*, *Gemmatimonadetes*, *Acidobacteria* and *Planctomycetes* were the abundant phyla during July (the wet season). The abundance of the bacterial genera *Rhodothermus*, *Gemmatimonas*, *Planctomyces* was high in the month of July. Although clear seasonal community shifts were observed at the phylum and genus levels, the dominant microorganisms remained constant across all seasons.

Diversity estimates

To evaluate the extent of diversity captured, a rarefaction curve was computed for all seven datasets based on the number of reads and species (Fig. 3). As shown in Fig. 3, S5, S3 and S6 appear to be less diverse compared with S1 and S2 and reach a near-constant phase. The bacterial and archaeal diversity were further estimated using the Shannon index, as well as an evenness and non-parametric richness estimation (chao1 richness) (Table 2). The alpha diversity, species richness and evenness of the taxa varied across all metagenomes. These results indicated that amongst the five location-based datasets, S5 had the highest bacterial and archaeal diversity (Shannon indices of 6.4 and 3.0, respectively), S3 had the lowest value for bacteria (Shannon diversity index of 5.05) and S2 (2.56) was least diverse in archaea. In terms of bacterial richness, S1 (3192) had the highest chao1 value and S3 (2311) had the lowest. Similarly, archaeal richness was highest in S6 (422) and lowest in S2 (156). In samples exhibiting seasonal variations, S4 (April) had the highest bacterial diversity (6.2) and richness (2990). Archaeal richness was higher in S6 (October).

Functional profile of the saline desert of the Kutch

The major functional gene categories obtained were related to core metabolic functions, such as protein metabolism (9.5–10.9 %), carbohydrate metabolism (6.4–10.2 %), amino acids and derivatives (5.0–8.8 %) and vitamin and pigment metabolism (3.9–6.8 %) (Fig. 4). Additionally, a fraction of sequences (4.3 %) showed similarities to genes involved in the degradation and metabolism of xenobiotics. These sequences constituted 0.5–2.8 % of the overall metabolic distribution in which the degradation of chlorocyclohexane, chlorobenzene, benzoate and nitrotoluene was the most prevalent.

On average, stress response gene categories constituted 2.1–4.1 % of the total functional gene categories. The stress gene categories were dominated by oxidative stress (45.1–58.4 %), osmotic stress (15.4–20.5 %) and heat shock (12.4–28.2 %) (Fig. 5), indicating the essential features of survival strategies in this complex eco-geographic niche.
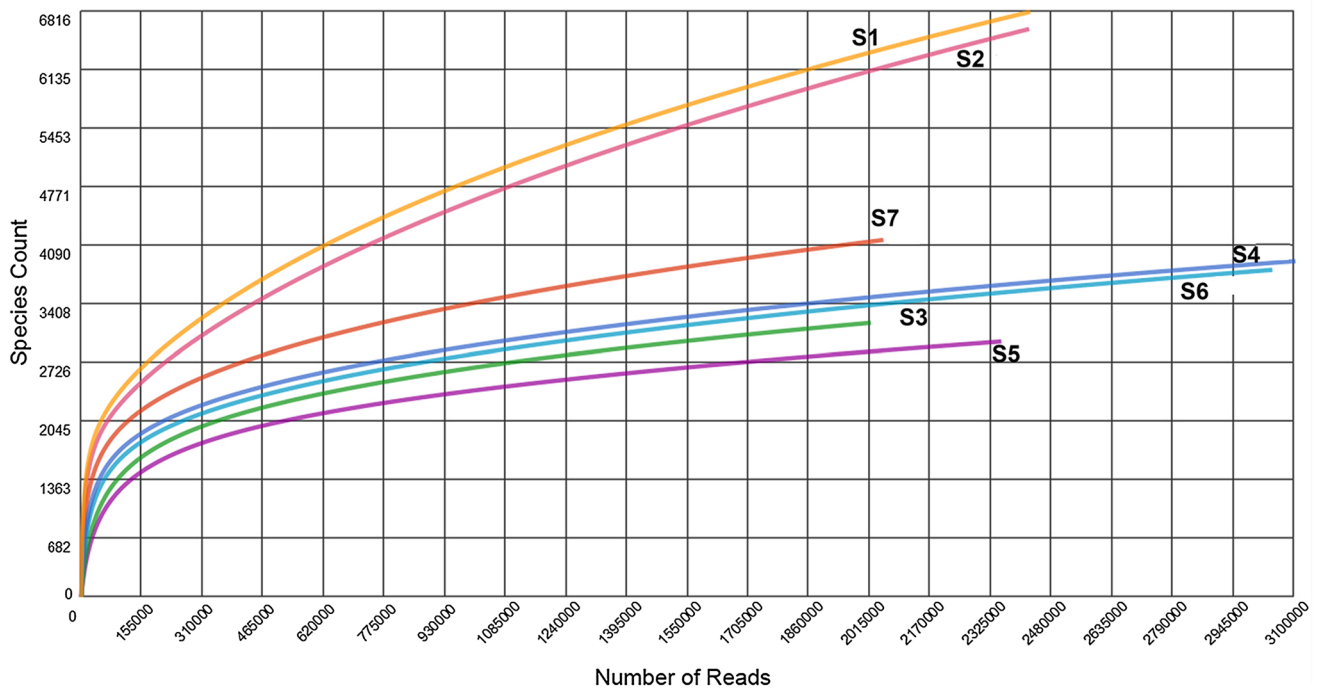
Fig. 3 Rarefaction analysis for the observed species. The rarefaction curves for samples S3, S4, S5, S6 and S7 reached the near-plateau phase representing good sampling depth; however, S1 and S2 did not reach a clear asymptote

The genes for oxidative stress were overrepresented in low- and moderate-salinity samples (S1, S2, S6) and underrepresented in high-salinity samples (S3, S5), whereas the genes for osmotic stress were overrepresented in high-salinity samples. In the higher saline samples, most of the oxidative and heat stress genes belonged to *Bacteroidetes* and *Halobacteria* (Table 3). The most abundant osmotic stress tolerance genes were related to choline, betaine and glucan biosynthesis. Most of the genes encoding the high-affinity choline uptake protein *BetT* belonged to *Gammaproteobacteria* and *Alphaproteobacteria*. However, the choline sulphatase gene belonged to representatives of *Halobacteria*. The heat shock *dnaK* gene cluster was present, which indicates that heat shock proteins may be induced given high temperature and salt stress (Kilstrup et al. 1997). *Bacteroidetes* and *Halobacteria* contributed to the majority of the heat shock protein chaperones (*dnaK* and *dnaJ*).

Taxonomic comparison with other biomes

Furthermore, the saline desert dataset was compared with the hot desert, sea, hypersaline lagoon, solar saltern and brine datasets to examine differences in the compositions of the microbial communities. A PCoA plot generated based on taxonomic (phylum) abundance using the Bray-Curtis distance showed a variance of 34.1 % on PCoA1 and 18.7 % on PCoA2. Salinity may be an important environmental factor in determining the level of similarity between different microbial communities, as suggested by the clustering of samples by salinity (Fig. 6). The saline desert samples (S1, S2, S4, S6 and S7) clustered with the Sargasso Sea (Open Sea), Puget Sound (estuary) and Elba, Sant'Andrea (coast) samples, whereas the S5 sample clustered with the hypersaline lagoon biome (L1, L2) and S3 was between the lagoon and sea biomes.

Although the overall microbial community structures of the hypersaline, sea and Kutch metagenomes were similar (as depicted in the PCoA plot), the proportion of *Halobacteria* was substantially higher in the Kutch metagenome. However, the similarity of the Kutch and sea metagenomes was based on the similar proportions of *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Methanobacteria* and *Halobacteria* (Fig. 7). The Kutch metagenome had the highest proportion of *Halobacteria*, and this characteristic resembled those of the saturated brine and solar saltern. Other similar phyla in the solar saltern, brine and Kutch metagenomes include *Gammaproteobacteria*, *Clostridia*, *Flavobacteria*, *Methanomicrobia* and *Bacillus*. However, the former two are dominated by *Halobacteria* (nearly 90 % of the entire microbial population) compared with the Kutch metagenome in which (despite maximal occurrence of *Halobacteria*) their proportion is approximately 50 %. The Kutch metagenome harboured microbes found not only in the sea or hypersaline metagenomes but

Diversity indices	S1 (bacteria)	S1 (archaea)	S2 (bacteria)	S2 (archaea)	S3 (bacteria)	S3 (archaea)	S4 (bacteria)	S4 (archaea)	S5 (bacteria)	S5 (archaea)	S6 (bacteria)	S6 (archaea)	S7 (bacteria)	S7 (archaea)
Shannon index	6.3	3.073	5.91	2.56	5.05	2.95	6.2	2.991	6.44	3	5.69	2.909	4.97	3.322
Evenness	0.2151	0.0935	0.2225	0.0952	0.1391	0.0673	0.1972	0.5773	0.3142	0.1019	0.1225	0.04958	0.3979	5773
Chao1	3192	252	2386	156	2311	345.9	2990	381.6	2400	258.9	2871	422.4	594.3	61.6

Table 2 Alpha diversity indices of the bacterial and archaeal communities of seven saline desert metagenomes

also those found in hot deserts such as *Actinobacteria*, *Alphaproteobaceria*, *Betaproteobaceria*, *Gammaproteobaceria* and *Bacillus*. The differences were visualised in terms of the higher abundance of *Alphaproteobacteria*, *Gammaproteobacteria* and *Halobacteria* in the Kutch metagenome compared with those in the hot desert. These results reflect the unique microbial diversity in the Kutch saline desert, the diversity of which is most similar to the sea biome.

Functional comparison with other biomes

To assess the functional relatedness to other metagenomes, the functional profile of the Kutch metagenome was compared with the datasets of the hot desert, sea, hypersaline lagoon, solar saltern and brine metagenomes. The PCoA plot generated using the Bray-Curtis distance achieved a high level of variance explanation with values of 46.3 % for the first axis and 29.3 % for the second (Fig. 8). The plot showed that S1, S2, S3, S4, S6 and S7 clustered with the sea biome. This is because many of the gene categories have similar abundance levels. However, S5 clustered with the solar saltern and hypersaline lagoon. This correlation was statistically validated by calculating the Pearson correlation coefficient. The correlation coefficient values of Kutch saline desert biome with other biomes were sea ($r = 0.95$), hypersaline lagoon ($r = 0.96$), hypersaline brine (0.91), solar saltern (0.89) and hot desert ($r = 0.84$) biomes. A comparison of stress response gene categories revealed that the oxidative stress genes were highest in the brine, followed by the hypersaline lagoon. A similar abundance of oxidative stress genes was found in the solar saltern and saline desert biomes.

Quantification of archaeal and bacterial loads using qPCR

The standard curves generated from known amounts of *Staphylococcus haemolyticus* and *Haloferax alexandrinus* DNA were used to predict bacterial and archaeal loads in the seven metagenomic samples. The amplification efficiencies of the bacterial and archaeal standard curves were 102.6 % (slope −3.23) and 103.9 % (slope −3.25), respectively, with an R2 value of 0.99, which indicated a successfully designed qPCR assay.

In the low saline samples (S1 and S2), the total bacterial loads (2.56×10^6 and 2.88×10^5 copies/gram, respectively) were higher than those of archaea (1.24×10^4 and 2.5×10^4 copies/gram, respectively). The samples with highest salinity, i.e. S3 and S5, had the highest archaeal populations: 39 % (1.61×10^5 copies/gram of soil) and
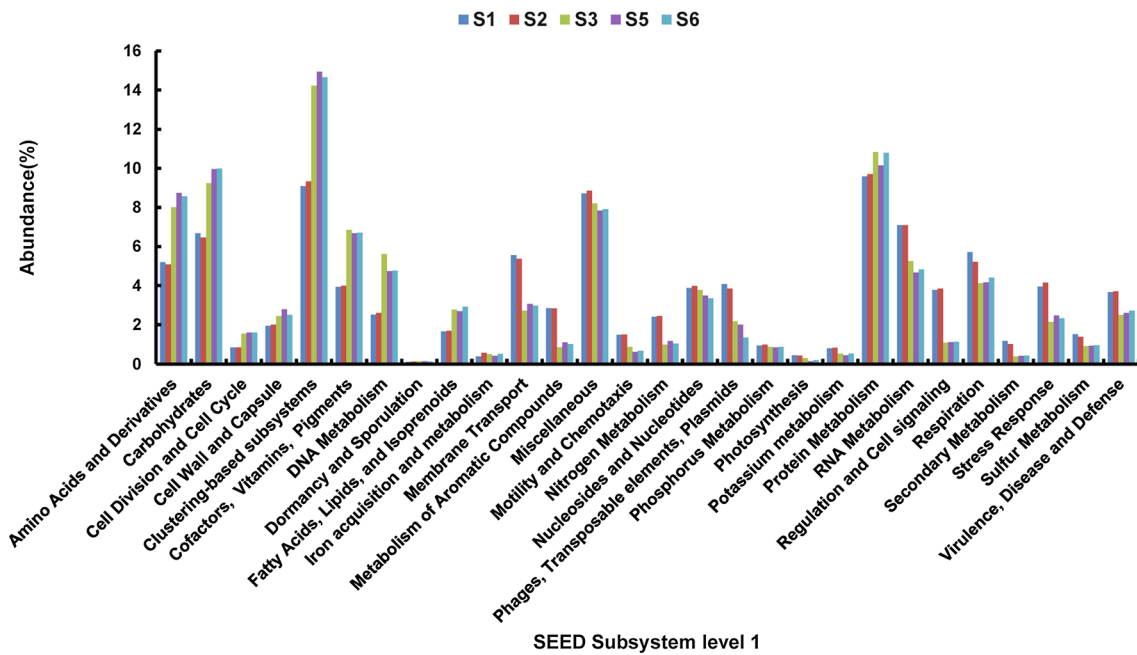
Fig. 4 Relative abundance of functional gene categories. Relative abundances of major functional gene categories in SEED subsystem hierarchy at level 1 obtained from five location-based samples (S1, S2, S3, S5 and S6)
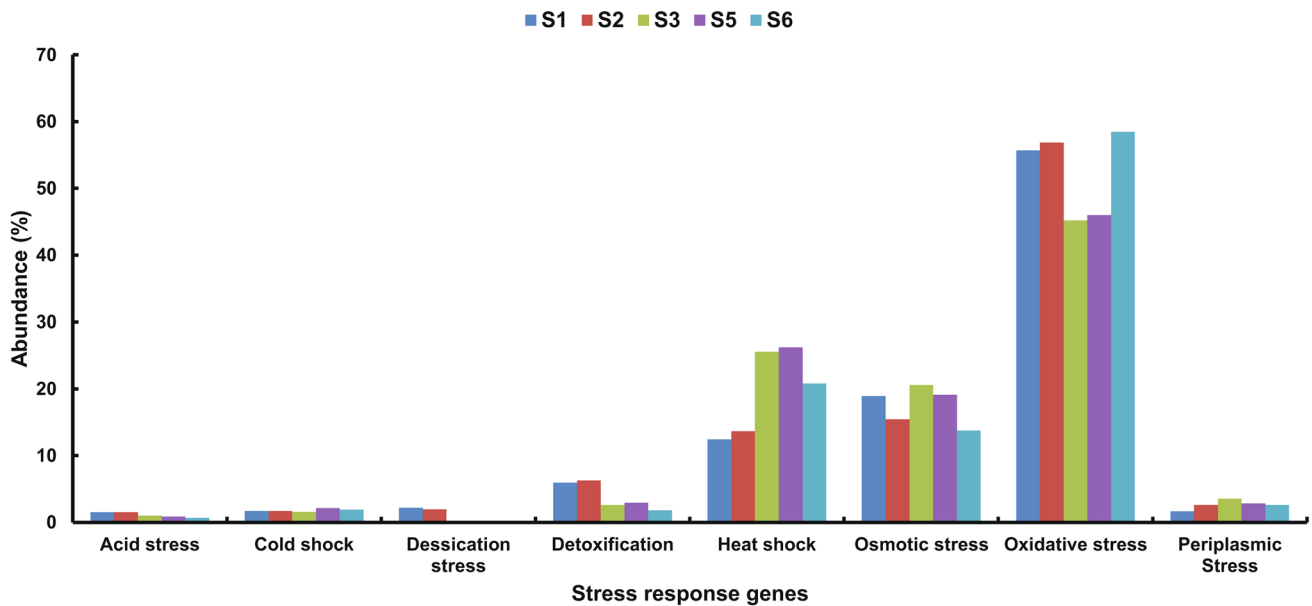

Fig. 5 Relative abundance of stress response gene categories. Relative abundances of genes related to stress response in SEED subsystem obtained from five location-based samples (S1, S2, S3, S5 and S6). *Bar graph* represents percentage of abundance

22 % (2.19 × 10^5 copies/gram of soil), respectively, of the seven samples (Table 4).

As shown in Table 4, seasonal variations in bacterial and archaeal populations were also observed, with a higher total archaeal load in S4 (April) and S6 (October) and the lowest in S7 (July).

Discussion

The Rann of Kutch in the western part of India is a seasonal salt marsh and one of the world's largest saline deserts. There are no data available on the microbial community in this unique location, baring few individual bacterial isolates

Table 3 Taxonomic source of the genes implicated in response to oxidative, osmotic and heat shock stress

Stress type	Protein or subsystem name	Phyla (no. of hits) found in saline desert metagenomes				
		S1	S2	S3	S5	S6
Oxidative stress	Catalase	*Deltaproteobacteria* (52)	Flavobacteria (52)	Bacteroidetes (92)	Halobacteria (19)	Halobacteria (114)
	Peroxidase	*Deltaproteobacteria* (32)	*Alphaproteobacteria* (30)	Halobacteria (25)	Bacteroidetes (13)	Bacteroidetes (98)
	RNA polymerase sigma factor RpoD	*Alphaproteobacteria* (39) *Gammaproteobacteria* (29)	Flavobacteriia (50) Bacteroidetes (38)	Bacteroidetes (91)	Bacteroidetes (17)	Bacteroidetes (132) *Gammaproteobacteria* (19)
	NAD-dependent glyceraldehyde-3-phosphate dehydroge	–	Flavobacteriia (50)	Bacteroidetes (92) Halobacteria (24)	Bacteroidetes (13) Halobacteria (10)	Halobacteria (68)
Heat shock	Chaperone protein DnaK	Halobacteria (25) *Deltaproteobacteria* (15)	*Deltaproteobacteria* (18) *Alphaproteobacteria* (12)	Bacteroidetes (29) Halobacteria (20)	Bacteroidetes (5) Halobacteria (19)	Bacteroidetes (56) Halobacteria (123)
	Chaperone protein DnaJ	Halobacteria (9) *Alphaproteobacteria* (14)	Bacteroidetes (13) Halobacteria (5)	Bacteroidetes (10) Halobacteria (8)	Bacteroidetes (9) Halobacteria (14)	Bacteroidetes (33) Halobacteria (28)
	Translation elongation factor LepA	*Deltaproteobacteria* (16)	Bacteroidetes	Bacteroidetes (34)	Bacteroidetes (9)	Bacteroidetes (72)
Osmotic stress	Sarcosine Oxidase	*Alphaproteobacteria* (182)	*Alphaproteobacteria* (286) Actinobacteria (141)	–	*Gammaproteobacteria* (9)	–
	Choline sulphatase	Halobacteria (205) *Deltaproteobacteria* (56)	–	Halobacteria (240)	Actinobacteria (16) *Alphaproteobacteria* (9)	Halobacteria (206)
	High-affinity choline uptake protein BetT	*Alphaproteobacteria* (68) *Delta-proteobacteria* (63)	*Alphaproteobacteria* (68) *Deltaproteobacteria* (63)	*Deltaproteobacteria* (144) Halobacteria (44)	*Gammaproteobacteria* (16) *Alphaproteobacteria* (16)	*Gammaproteobacteria* (103) *Alphaproteobacteria* (103) Actinobacteria (103) Flavobacteriia (103)
	glucan synthase	*Deltaproteobacteria* (32)	*Gammaproteobacteria* (167)	–	*Deltaproteobacteria* (4)	*Deltaproteobacteria* (14)
	ABC transporter	*Alphaproteobacteria* (182) Bacteroidetes (141)	*Deltaproteobacteria* (141) *Alphaproteobacteria* (564) *Gammaproteobacteria* (141)	*Gammaproteobacteria* (28)	Actinobacteria (6) Bacteroidetes (6)	Actinobacteria (82) Bacteroidetes (82) Bacilli (82)

(Thomas et al. 2012). This, along with the extremities of temperature, limited rainfall and high salinity, makes this region inhospitable for life. However, microbes exhibit a profound ability to survive in extreme environments. This study is the first to report on microbial diversity across variable salinities in the Kutch using metagenomics, a tool for the study of microbial diversity in unexplored microbial communities (Andrew et al. 2012). Studying a metagenome provides the added advantage of exploring minor phyla and functional attributes of the microorganisms within the ecosystem.

Despite the highly saline conditions, a diverse and rich microbial community is present in this otherwise inhospitable region. This species richness agrees with the report of Wang et al. 2007, who found an increase in microbial richness with increasing salinity in freshwater and saline systems. In general, the samples were dominated by *Proteobacteria*, *Bacteroidetes*, and *Firmicutes*, which was also reported by Hollister et al. (2010) who studied the microbial community structure of hypersaline soils and sediments. Additionally, this study identified the dominance
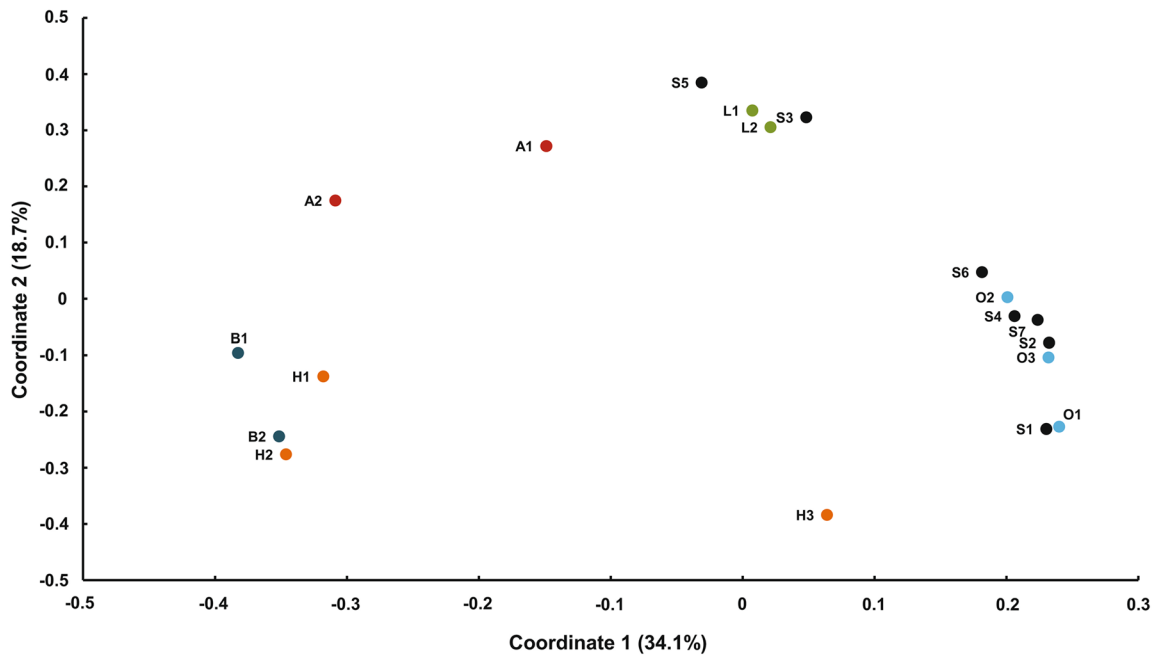
Fig. 6 PCoA plot of the relative abundance of taxonomic (Phylum) profiles of the selected biomes. Principal coordinate analysis using Bray-Curtis distance of the selected biomes. Comparative sites include hot desert *H1*, *H2* (Chihuahuan desert, New Mexico), *H3* (Mojave desert, california); sea *O1* (Puget Sound estuary) *O2* (Elba Sant Andrea coast) *O3* (Sargasso sea); saturated brine *B1*, *B2* (Spain); solar saltern *A1*, *A2* (Chula vista, CA); hypersaline lagoon *L1*, *L2* (Marmenor, murcia) datasets. *S1*, *S2*, *S4*, *S6* and *S7* cluster with *O1*, *O2* and *O3* whereas *S3* and *S5* cluster with hypersaline lagoon *L1*, *L2* metagenomes

of *Euryarchaeota*, which reflects the extremities of this ecosystem.

The results further suggest that the abundance of major phyla, as well as the structuring of the bacterial community, appear to be influenced by salinity, with *Salinibacter* dominating the higher salinity region, whereas *Marinobacter*, *Rhodothermus* and *Rhodobacter* dominated the lower salinity region. The overall community structure was dominated by *Haloarcula*, *Halogeometricum*, *Natronomonas* and *Halobacterium*. *Haloarchaeales* was the most active population in the degradation of organic matter in hypersaline environments (Gasol et al. 2004). These results agree with other reports recognizing salinity as a key environmental factor for globally structuring bacterial communities (Lozupone and Knight 2007). Here, the dominant *Bacteroidetes* species *Salinibacter* may play a key role in the carbon cycle due to their ability to degrade long chain carbon, which may be available in the form of animal and plant remains (McGenity and Gramain 2010).

The variations in archaeal and bacterial load were enumerated via qPCR analysis, which is increasingly used as a molecular method to quantify the microbial load in complex microbiomes (Klieve et al. 2003; Tajima et al. 2001; Keshri et al. 2013). Quantitation results perceived highest archaeal population loads in high saline soil samples.

qPCR was also able to capture seasonal shifts in bacterial and archaeal population.

At the functional level, the abundance of carbohydrate and DNA metabolism genes could be attributed to core genes that are solely responsible for cell survival and propagation in all organisms, whereas the abundance of amino acid metabolism genes may be because of amino acid-based solutes, which are commonly used by bacteria for osmoregulation (Fierer et al. 2012). Higher levels of alanine, aspartate and glutamate may have been due to the continuous biosynthesis of acidic amino acids, which are required to maintain a cationic environment in hypersaline systems (Ventosa et al. 1998). The presence of xenobiotic degradation genes corroborates reports in which halophiles have been used to degrade xenobiotic compounds (Bertrand et al. 1990; Oren et al. 1992; Ward and Brock 1978).

The shift in diversity was also recognised at the functional level. In an ecosystem as extreme as the Kutch, major differences were observed in oxidative and osmotic stress response genes. Higher levels of oxidative stress response genes in areas of lower salinity were unexpectedly observed, which may be because of the wider niche availability for both halotolerant and limnotolerant species. This includes organisms that adapt to the environment and survive at the limit of their tolerance.
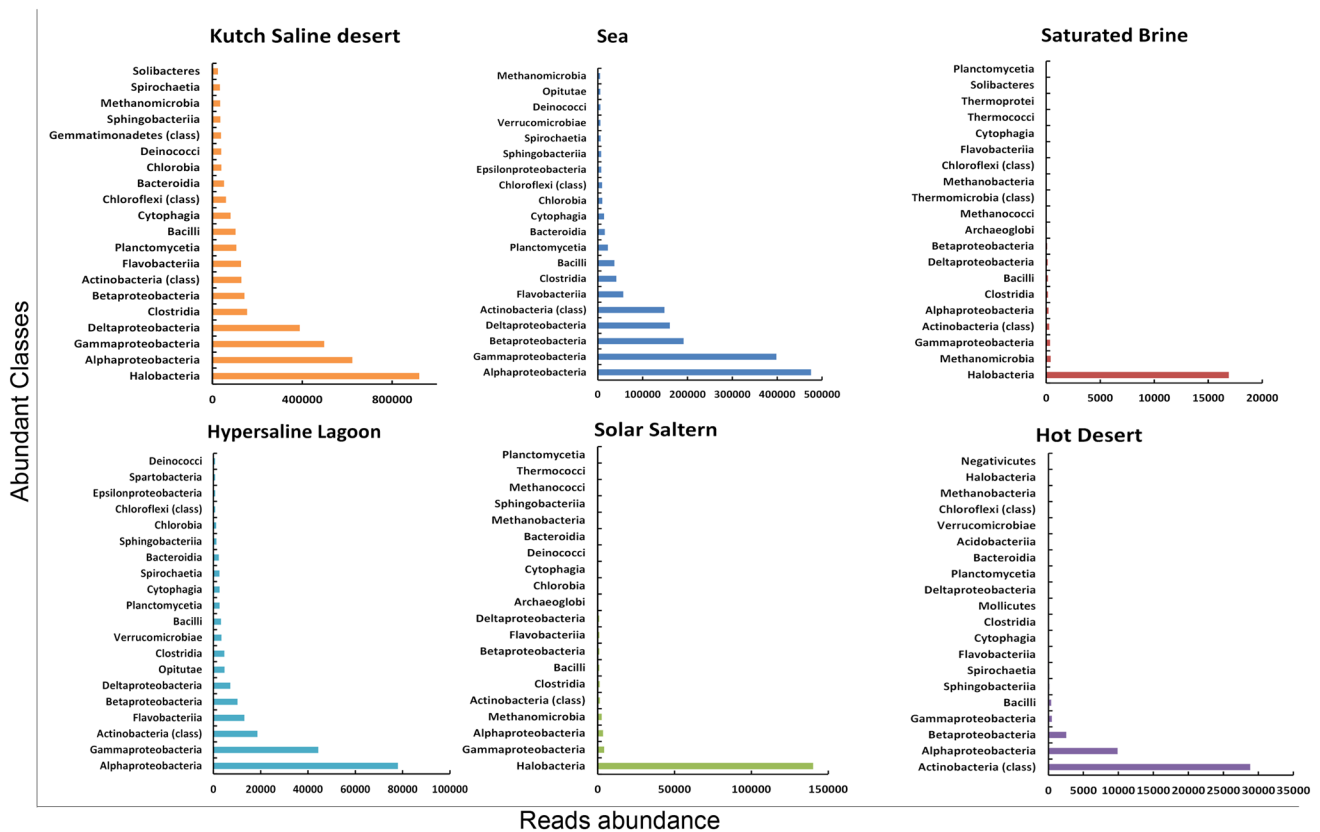
Fig. 7 *Stacked bar* representing distribution of various classes in saline desert and other saline and desert biomes. *Stacked bar* showing taxonomic distribution for all retrieved sequences

The oxidative stress response is one of the important lines of defence against any abiotic stress. Higher levels of oxidative stress response genes may play an important role in the survival of tolerant organisms against a variety of stresses in this ecosystem. However, the metagenomes from regions of highest salinity were dominated by the osmotic tolerance genes. This may be due to the dominance of microbes with an adaptive mechanism for salt, i.e. halophiles, and fewer salt-tolerant microbes. Thus, most of the functional shift observed was in accordance with the taxonomical shift; this finding was similar to that obtained by Fierer et al. (2012).

Another interesting feature of the Kutch is the seasonal variation in the landscape, which exists as a dry or wet region during different parts of the year. During the dry season, the entire area is dried, leaving behind a salt-encrusted land; with the onset of the rainy season, water begins to stagnate on its surface. The effects of these climatic conditions on the composition of the microbial community were studied. *Euryarchaeota* were abundant in the months of April and October due to the extremely hot and dry climate. *Chloroflexi*, *Cyanobacteria* and *Planctomycetes* were abundant in July, which may be attributed to the conversion

of the desert into a shallow, brackish wetland during the wet season, making a favourable habitat for these phyla. *Cyanobacteria* may be the photosynthetic energy source of the ecosystem, providing organic carbon.

Despite seasonal variations to the extent that the entire landscape of the region changes every few months, the diversity of the microbes is not significantly affected. These results contradict the well-known theory of IDH (intermediate disturbance hypothesis) in which increased disturbance produces less diversity (Catford et al. 2012). However, the results are in accordance with an alternative hypothesis proposed by Denslow (Denslow 1985) stating that the species diversity is maximum in a disturbance-mediated coexistence. The alternative hypothesis is based on the fact that species generally adapt to the level of disturbance in their ecosystem through evolution (whether the disturbance is of high, intermediate or low level). The fact that this study was conducted on a seasonal site also indicates the reproducibility of the experimental method employed and hence the utility of metagenomic data in studying ecology. Such studies will also form a basis for devising culturing strategies, collection times, etc., for studying culturable diversity from such an extreme environment.
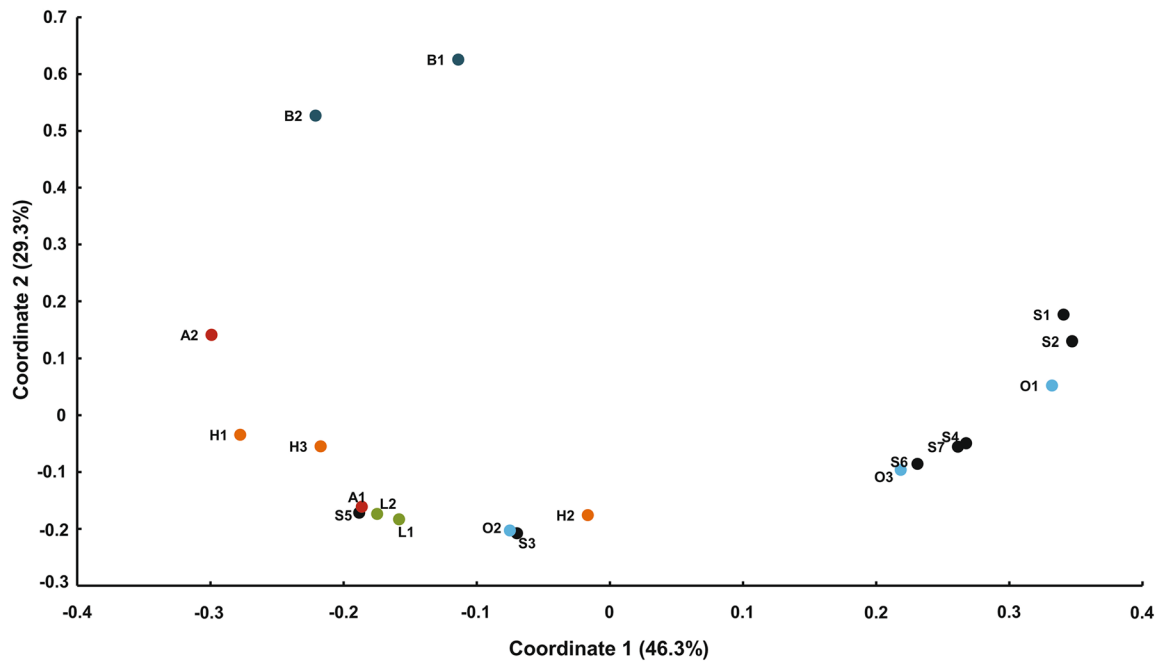
Fig. 8 PCoA plot of functional SEED Subsystem level 1 of the selected biomes. Functional community composition of the saline desert metagenomes (*S1*, *S2*, *S3*, *S4*, *S5*, *S6*, *S7*) and other saline, hypersaline and desert environments, based on principal coordinate analysis of the relative abundances of all MG-RAST subsystems. Principal coordinate analysis using Bray-Curtis distance of the selected biomes. Comparative sites include hot desert *H1*, *H2* (Chi-huahuan desert, New Mexico), *H3* (Mojave desert, california); sea *O1* (Puget Sound estuary) *O2* (Elba Sant Andrea coast) *O3* (Sargasso sea); saturated brine *B1*, *B2* (Spain); solar saltern *A1*, *A2* (Chula vista, CA); hypersaline lagoon *L1*, *L2* (Marmenor, murcia) datasets. Functional diversity patterns amongst biomes shows *S1*, *S2*, *S4*, *S6* and *S7* cluster with *O1* and *O2* whereas *S3* and *S5* cluster with *O2*, *L1* and *L2*

Table 4 Enumeration of bacterial and archaeal population using qPCR

Sample ID	Bacterial load	Archaeal load
S1	2.56×10^6	1.25×10^4
S2	2.88×10^5	2.55×10^4
S3	9.58×10^5	1.61×10^5
S4	3.67×10^5	8.73×10^4
S5	2.19×10^5	5.45×10^4
S6	5.68×10^5	1.45×10^5
S7	5.09×10^4	2.93×10^4

The dynamic ecosystem and seasonality observed in The Great Rann of Kutch are the result of significant geomorphic processes caused by sea level changes, climatic fluctuations and tectonic movements during the Mesozoic to Holocene Epochs of the Cenozoic Era (McGinley 2008; Biswas and Deshpande 1970). Hence the metagenome of the Great Rann of Kutch was compared with those of other biomes from hot desert, hypersaline lagoon, sea, solar saltern and brine ecosystems. The Kutch represents an ecosystem with a seasonal landscape that alternates between wetland and saline desert, whereas oceans and deserts are stable ecosystems with relatively few fluctuations. In the present investigation, the microbial community of the Kutch was unique yet observed to be similar to that of the sea biome followed by hypersaline lagoon.

This study also presents the first snapshot of a microbial community from a previously uncharacterised ecosystem. Further exploring this region will enrich our knowledge about its microbial ecology as well as the taxonomic and functional attributes at work.

Compliance with ethical standards

Conflict of interest The author(s) declare no competing financial interests

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