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16S rRNA Gene Diversity in the Salt Crust of Salar de Uyuni, Bolivia, the World's Largest Salt Flat

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ABSTRACT Salar de Uyuni is a vast, high-altitude salt flat in Bolivia with extreme physico-geochemical properties approaching multiple limits of life. Evidence for diverse halophilic bacteria and archaea was found in its surface and near-surface salt crust using 16S amplicon analysis, providing a snapshot of prokaryotic life.

Salar de Uyuni is the world's largest salt flat, located in the southwestern Altiplano of Bolivia at an altitude of approximately 3,650 m above sea level (1, 2). The geochemical composition of brines and salt crusts (high in lithium, boron, magnesium, potassium, sodium, and chloride) and other physical conditions (high daily temperature fluctuations, UV radiation, and albedo) create a polyextreme environment close to multiple limits of life (3–5). In addition to an understanding of extremophilic life on Earth, characterization of the microbial communities of Salar de Uyuni will contribute to determining the potential habitability of other planets, as well as new biotechnology applications (6–9). To date, however, few microbial genomic studies have been conducted (10–13). This study provides an initial snapshot of microbial diversity in the crust.

Samples were collected in March 2015 from a remote site (20°33′28.58″S, 67°12′29.56″W) in Salar de Uyuni from the surface salt crust (SSC) (BOL4) and at a depth of 5 to 15 cm (near-surface salt crust [NSSC] [BOL3]). DNA was extracted with the PowerLyzer PowerSoil DNA extraction kit (MO BIO Laboratories, Inc., Carlsbad, CA). Library construction and 16S amplicon sequencing of the V3 to V4 region were performed on a MiSeq platform according to the manufacturer's recommendations (Illumina, Inc., San Diego, CA) using the primers Bakt_341F and Bakt_805R (14–16).

Raw reads were processed with mothur (v1.39.5), and sequence data were aggregated with R (v3.4.1) (16–19) (https://www.mothur.org/wiki/MiSeq_SOP). Paired-end sequencing generated 213,999 raw reads (median length, 459 bp [range, 35 to 600 bp]). Reads were assembled with a quality score threshold of 20. Sequences longer than 475 bp and those with ambiguities and homopolymers (>8 bp), as well as chimeras, were removed, and sequences were aligned against the SILVA small subunit (SSU) Ref NR 99 database (v132) (20). Based on analysis using mothur, sequences with at least 97% similarity were binned into operational taxonomic units and classified (using a pseudobootstrap value of 80) against the reference database trimmed to positions 201 to 1000 of the 16S sequence of *Escherichia coli* (GenBank accession number J01859.1). Singletons were removed and only prokaryotic sequences were retained, resulting in 21,636 (SSC) and 3,625 (NSSC) sequences with a median length of 457 bp (range, 419 to 466 bp).

Archaea constituted 13.47% of the SSC sequences and 11.50% of the NSSC sequences, and bacteria constituted 86.53% and 88.50%, respectively. For archaea, all

TABLE 1 Prevalence of archaeal and bacterial 16S amplicons at the phylum and genus levels in the SSC and NSSC of Salar de Uyuni

Sample and taxonomic category[a]	Total no. of sequences	Abundance (%)[b]
SSC (BOL 4)		
Archaea		
Phyla		
Euryarchaeota	2,877	98.73
Nanoarchaeota	37	1.27
Hadesarchaeota	0	0.00
Genera		
Halonotius	1,547	53.09
Halorubrum	542	18.60
Halobellus	276	9.47
Halovenus	202	6.93
Natronomonas	131	4.50
Halococcus	22	0.75
A07HB70	19	0.65
Halodesulfurarchaeum	18	0.62
Halapricum	15	0.51
Halobacterium	12	0.41
Bacteria		
Phyla		
Bacteroidetes	10,906	58.25
Proteobacteria	7,606	40.63
Patescibacteria	66	0.35
Genera		
Salinibacter	9,962	53.21
Acinetobacter	5,181	27.67
Arhodomonas	735	3.93
Brevundimonas	264	1.41
Halofilum	191	1.02
Delftia	142	0.76
Diaphorobacter	132	0.71
Salicola	123	0.66
Fodinibius	75	0.40
Thiohalospira	38	0.20
NSSC (BOL 3)		
Archaea		
Phyla		
Euryarchaeota	389	93.29
Nanoarchaeota	26	6.24
Hadesarchaeota	2	0.48
Genera		
Halodesulfurarchaeum	200	47.96
Halonotius	39	9.35
Halapricum	34	8.15
Halobellus	28	6.71
Halanaeroarchaeum	26	6.24
Halorubrum	19	4.56
Halovenus	8	1.92
Natronomonas	8	1.92
Haloarcula	5	1.20
"*Candidatus* Haloredivivus"	4	0.96
Bacteria		
Phyla		
Proteobacteria	2,365	73.72
Bacteroidetes	375	11.69
Halanaerobiaeota	194	6.05
Genera		
Halorhodospira	968	30.17
Desulfovermiculus	351	10.94
Acinetobacter	314	9.79
Arhodomonas	211	6.58
Salinibacter	200	6.23
Halanaerobium	194	6.05
Thiohalorhabdus	28	0.87
Thiohalospira	23	0.72
Halofilum	19	0.59
Limimonas	15	0.47

[a] The 3 most prevalent phyla and 10 most prevalent genera for each sample are shown.
[b] Abundance was calculated based on the total number of sequences in each domain.

sequences were classified at the phylum level and 96.57% (SSC) and 91.85% (NSSC) at the genus level; 98.73% (SSC) and 93.29% (NSSC) were *Euryarchaeota*, and 1.27% (SSC) and 6.24% (NSSC) were *Nanoarchaeota*. *Hadesarchaeota* were present only in NSSC (0.48%). The most prevalent genera were *Halonotius* in SSC (53.09%) and *Halodesulfurarchaeum* in NSSC (47.96%) (Table 1). For bacteria, 99.71% (SSC) and 93.95% (NSSC) of sequences were classified at the phylum level and 91.36% (SSC) and 73.72% (NSSC) at the genus level. *Bacteroidetes* (58.25% [SSC] and 11.69% [NSSC]) and *Proteobacteria* (40.63% [SSC] and 73.72% [NSSC]) were most prevalent at the phylum level, and *Salinibacter* in SSC (53.21%) and *Halorhodospira* in NSSC (30.17%) were most prevalent at the genus level (Table 1). These findings represent a snapshot of considerable prokaryotic diversity in the largest salt flat on Earth.

Data availability. The 16S rRNA gene amplicon data sets are available at NCBI under SRA accession numbers SRX7011107 (NSSC) and SRX7011108 (SSC).

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