Phylogenetic Profiling and Diversity of Bacterial Communities in the Death Valley, an Extreme Habitat in the Atacama Desert

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Abstract The Atacama Desert, one of the driest deserts in the world, represents a unique extreme environmental ecosystem to explore the bacterial diversity as it is considered to be at the dry limit for life. A 16S rRNA gene (spanning the hyper variable V3 region) library was constructed from an alkaline sample of unvegetated soil at the hyperarid margin in the Atacama Desert. A total of 244 clone sequences were used for MOTHUR analysis, which revealed 20 unique phylotypes or operational taxonomic units (OTUs). V3 region amplicons of the 16S rRNA were suitable for distinguishing the bacterial community to the genus and specie level. We found that all OTUs were affiliated with taxa representative of the Firmicutes phylum. The extremely high abundance of Firmicutes indicated that most bacteria in the soil were spore-forming survivors. In this study we detected a narrower diversity as compared to other ecological studies performed in other areas of the Atacama Desert. The reported genera were *Oceanobacillus* (representing the 69.5 % of the clones sequenced), *Bacillus*, *Thalassobacillus* and *Virgibacillus*. The present work shows physical and chemical parameters

have a prominent impact on the microbial community structure. It constitutes an example of the communities adapted to live in extreme conditions caused by dryness and metal concentrations.

Keywords Atacama Desert · Extreme habitat · Bacteria · Extremophiles · 16S rDNA

Introduction

The hyperarid Atacama Desert stretches for more than 1000 km, along the coast of northern Chile from the Pacific Ocean to the western slopes of the Andes [1]. The extreme dryness, salinity, hydrothermalism, oxidizing conditions and toxic metal concentrations make the Atacama Desert a unique ecosystem of extreme environmental settings, inhospitable to most living species [2]. Indeed, these soils are considered Mars-like and offer an ideal setting to investigate the application of life detection systems [3].

Despite the severity of the environmental conditions limit plant and animal life, recent ecological studies have revealed the presence of a relative wide diversity of microorganisms [4]. Broad spectrums of halotolerant, halophilic bacteria and archaea have been recovered from this desert area [5–7].

Traditionally, the estimation of microbial diversity was based solely on culturable microorganisms. However, microscopic observations and mathematical modeling estimates that 99 % of bacteria are non-culturable under standard laboratory conditions [8]. Different studies have been focused on the diversity of uncultured microorganisms in this inhospitable habitat [1, 9, 10].

Studies on biogeochemical cycling in these arid regions are in their infancy. Nevertheless, the evaluation of the

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phylogenetic diversity constitutes the first step in this study. In the present work, our main objective is to document the bacterial diversity of an unvegetated soil at the hyperarid margin of Atacama Desert. For this purpose we performed a physicochemical analysis of collected soil sample and a molecular analysis was conducted through the construction of a 16S rRNA gene library to elucidate the bacterial diversity of microbial community in this extreme unique area of the Atacama Desert.

Materials and Methods

Location, Soil Sampling and Analysis

Sample was collected from soil of the Death Valley, also known as the Mars Valley (latitude 22°55'S, longitude 68°120'W), elevation 100 m above sea level in the Atacama Desert, Chile. The average annual precipitation in this region is 4 mm, with a high UV index. We collected surface soil (0–20 mm) in a sterile plastic container and kept refrigerated during shipping (7 days) until further processed. The pH and temperature of the sample were determined in situ using a pH meter (Hanna Instruments, Woonsocket, RI) and an electronic thermometer (Orion model 290). Concentrations of Na, Ca, K, Fe, Mg, B, As, Mn, Cu and Zn were determined via ICP-OES (Inductively coupled plasma-optical emission spectrometry) using an Iris Advantage spectrometer (Thermo Jarrel Ash Corporation, MA, Franklin, USA). Calcium carbonate (CaCO₃) was determined using an automated continuous flow analyser (Bran + Luebbe III, Germany). Electrical conductivity (EC) was determined in a 1:5 soil/water extract. Total organic carbon (TOC) was analysed by Walkley–Black method [11].

DNA Extraction and PCR Amplification

In this study, two extraction methods were used to obtain the DNA from the soil sample. The Ultra-Clean Mega Soil DNA extraction kit MO BIO Laboratories, Inc., (www.mobio.com) was used based on manufacturer's instructions and CTAB (Cetyl trimethylammonium bromide) method as follows: the suspensions were first heated at 80 °C for 20 min, then incubated with lysozyme (10 mg lysozyme + 5.5 ml Tris–EDTA buffer) at 37 °C for 1 h and finally treated with sodium dodecyl sulfate-proteinase K (10 % SDS 700 µl + proteinase K 60 µl) at 65 °C for 10 min. Afterward the CTAB–NaCl solution (80 µl) was added and the tube was incubated at 65 °C for 10 min. DNA purification and precipitation was performed using phenol–chloroform–isoamylalcohol (25:24:1). Extraction integrity was checked by agarose gel electrophoresis and

quantified spectrophotometrically in a NanoDrop ND 1000 instrument (Thermo Scientific, DE, USA).

The 16S ribosomal DNA (16S rDNA) gene was amplified by polymerase chain reaction (PCR) using total DNA as the template and the universal primers designed for Bacteria: the forward primer 16F27 (5'-AGAGTTT GATCMTGGCTCAG-3') and the reverse primer 16R1492 (5'-GGTTACCTTGTTACGACTT-3') [12]. The following programme was used for amplification: one cycle of 94 °C for 5 min, 35 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 2 min, and then an extension step for 20 min at 72 °C. The amplicon from soil sample was purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI) to eliminate non-specific PCR-products.

Clone Library Construction

The purified PCR products were inserted into the pGEM-T Vector system (Promega, Heidelberg, Germany) and transformed into *Escherichia coli* DH5α. The transformed bacteria were plated onto LB agar medium supplemented with 60 µg/ml of ampicillin, 0.03 % X-gal and 0.05 % IPTG, and incubated overnight at 37 °C. White colonies were selected and inoculated in a 96 well plate containing 100 µl of LB with 50 µg/ml of ampicillin and incubated overnight at 37 °C [13]. The pellets were recovered by centrifugation at 3000g for 5 min and used for plasmid preparation using the modified alkaline lysis method described previously [14]. The clones were sequenced using the 338F (5'-ACTCCTACGGGAGGCAGCAG-3') primer for the V3 hyper variable region from the 16S rRNA gene. The nucleotide sequences of the inserts were determined using a DYEnamic dye terminator cycle sequencing kit (GE Healthcare, Brazil) and analyzed by capillary electrophoresis on a MegaBACE 1000 platform (GE Healthcare, Brazil).

Analysis of 16S rRNA Sequences

The 16S rRNA sequences were filtered by quality using dCAS software [15] with Phred quality ≤20. Similarity analysis from sequences, rarefaction curve and indices of diversity were performed using the Mothur applications (Mothur V1.12.2, <http://www.mothur.org>) [16]. To describe the species-level structure of the analyzed sample, the V3 sequences were compared to a database of V3 region sequences excised from the SILVA reference database of full-length rRNA sequences of known taxonomy [17]. All sequences were clustered into operational taxonomic units (OTUs) using modules from the software package Mothur. The strains were identified using the EzTaxon-e server (<http://eztaxon-e.ezbiocloud.net/>) [18] on the basis of 16S rRNA sequence data. The sequence similarity of 97 % was

used as a cutoff for the assignment of OTUs in this study. This cutoff is a commonly used level for comparative analysis in microbial communities [19]. The Mothur software was used to calculate the species richness estimators (Chao1 and ACE) and diversity index (Shannon–Wiener). Nonparametric coverage estimator was used to calculate the coverage obtained for the 16S rRNA V3 datasets using the formula $C = 12 (n_i/N) \times 100$, where N = total number of sequences analysed and n_i = number of sequence that occurred only once among the total number of sequences tested. For the phylogenetic analysis, the featured sequences were compared using the GenBank databases and aligned with the most similar sequences available to construct the phylogenetic trees, using the ARB software package [20]. Evolutionary distance matrices were calculated using the neighbour-joining algorithm [21]. Phylogenetic trees using different methods (distance matrix, maximum parsimony and maximum likelihood) were constructed and compared to elucidate the confidence of local topologies. Bootstrap analyses were performed with 1000 repetitions, and only values higher than 50 % are shown in the phylogenetic tree.

Nucleotide Sequence Accession Numbers

The reported sequences in this study have been submitted to GENBANK database under accession numbers KC210148–KC210392.

Results and Discussion

Site and Soil Characterization

The soil sample was obtained in September 2010 in the Death Valley of the Atacama Desert. This habitat is closely located to copper and lead–zinc mine tailings. The physicochemical characterization of the sample (Supplementary Table 1) indicated that Ca^{2+} , Na^+ and K^+ were the most abundant ions, followed by Fe and Mg^{2+} .

Table 1 Diversity analysis of the 16S rRNA gene clones

	97 %	95 %	90 %
OTUs ^a	20	12	5
Coverage	92.18	95.31	98.04
ACE	232	74	N/C
Chao1	80	26	11
Simpson	0.25	0.36	0.85
Shannon	2.06	1.51	0.36

N/C not calculated

^a Number of unique OTUs defined by using the neighbor algorithm by MOTHUR at 97, 95 and 90 % stringency

The remaining of analyzed elements (B, As, Mn, Cu and Zn) appeared in trace amounts. The pH of the sample was alkaline.

Biodiversity of the Bacterial Clone Library

Recently, Rubin et al. [22] suggested that the DNA extraction protocol used to examine 16S rRNA amplicon data often produced differences on the recovered microbial community structure. For this reason, in this study, we constructed a 16S rRNA library from an Atacama Desert sample, following two different methods described in “Materials and methods” section to increase the evenness of sequencing coverage.

From this library we sequenced 4 plates of 96 wells resulting in 384 clone sequences. 267 sequences out of the 384 yielded good quality using the dCAS software. Only 12 out of the 267 selected sequences were confirmed chimeras using Mothur program. Thus, we used a total of 244 bacterial clones to determine the diversity in this precise area of the desert soil and 20 OTUs were identified.

Statistical analysis of biodiversity provides interesting insights (Table 1). Mothur program was used to calculate the richness estimators (Chao1 and ACE) and diversity index (Shannon–Wiener). Richness estimators (Chao1 and ACE) were 80 and 232 respectively, using the default setting with a 3 % cut-off in Mothur (Table 1). The Shannon index reflects the reciprocal of Simpson dominance of species, indicating the probability that two randomly selected individuals in the community belong to the same species. Thus, the higher the value, the more dominant species will be and, therefore, less diverse. On the other hand, the Simpson index measures the degree of uncertainty in predicting species that belong to an individual selected at random. Therefore, the lower the value, the less diversity range. Shannon–Wiener and Simpson analysis revealed a low diversity within the clone library (2.06 and 0.25). Similar results have been reported in previous works in the Atacama Desert using V6 region in which ACE, Simpson and Shannon index were 278, 0.14 and 3.0 respectively [10].

The rarefaction curve was calculated in order to know the number of species in a standard sample size (Fig. 1). The curve indicates that the diversity was sampled with good level of confidence and majority of OTUs in the sample were detected but still there is a need of more comprehensive sampling to cover rest of the undiscovered diversity. The composition of the microbial community was deduced from the taxonomic classification of 16S rRNA sequences using Silva database and OTU distribution analysis was observed in the rarefaction curve.

The water contents of Atacama desert soils are subject to day–night variations (e.g., mediated by fog) and that

Fig. 1 Rarefaction curves of observed OTU richness in bacterial 16S rRNA gene library computed with Mothur V1.12.2 [16]

even the most arid regions of the Atacama receive intermittent rainfall; moreover, as may be the case at our low altitude sites, these soils will be subject to variable moisture contents as a result of high water tables controlled by precipitation in the Andes [23]. What is clear, however, is that members of novel taxa can be isolated from these soils using both traditional and not traditional methods, representing promising biotechnology opportunities.

It is well known that chemical composition of the habitats determines its diversity, playing the pH an important role in bacterial diversity, with less diverse communities associated with low pH [24]. The data on the chemical analysis of the Death Valley soil indicated that the sediment sample was alkaline with a pH value of 9.51.

Lozupore and Knight [25] proposed the salinity as the major environmental determinant of bacterial diversity. However, soils presenting pH values higher than 8.5 are also associated with low diversity, being exceptional in nature. Thus it is not clear whether the relationship between bacterial diversity is truly unimodal and more exhaustive studying is needed to comprise which factors mainly affect microorganism distributions and the correlation with biotechnological potentialities of these microorganisms.

Success in culture-independent survey of microorganisms in the Atacama Desert habitat has been diverse [1, 6, 26]. These procedures generally detect the most dominant members of a microbial community and may lose those species in low abundance. However, this type of analysis is necessary for evaluating total population diversity in the varied points of extreme habitats and for predicting functional ecology. On the contrary, culture procedures

have intrinsic biases since only those species, which can grow on the used medium, will be detected [6].

Phylogenetic Analysis of the Bacterial Clones and Dominant Phyla

The present study based on analysis of 16S rRNA gene library revealed Firmicutes as the unique phylum detected. The ability of spores to survive environmental challenges, such as heat, desiccation, presence of organic solvents and oxidizing agents, and UV irradiation, determines the wide variety of habitats that spore-forming Firmicutes colonize [27].

The cloned 16S rRNA gene sequences included 4 different groups of Firmicutes comprising sequences related to species of the genera *Oceanobacillus* (representing 68.9 % of the sequences detected), *Bacillus*, *Thalassobacillus* and *Virgibacillus*, all within the order Bacillales (Table 2). The distribution of clones among the Firmicutes phylum is shown in Fig. 2.

The sequences related to *Oceanobacillus* species were predominant (167 clones). The genus *Oceanobacillus* was first described by Lu et al. [28]. At the time of writing, the genus *Oceanobacillus* comprises 13 species. Members of the genus *Oceanobacillus* are distributed in a wide range of environments including species isolated from deep-sea sediment, mural paintings, freshwater fish, algae, an insect, activated sludge bottom of a mould fermenter food, saline saltern and lakes, dune sand and fermentation liquor [29]. All *Oceanobacillus* clone sequences described in this study were related to halotolerant strains, a consistent result considering the concentration of salt detected in the site sampled. 154 clones presented a sequence similarity (91.1–100 %) to the type species of the genus, *O. iheyensis* [28], an extremely halotolerant and alkaliphilic bacterium able to grow in a wide range of salinity (0–21 %, w/v NaCl) at pH 7.5 and 0–18 % (w/v) at pH 9.5. One possible explanation for the abundance of clones phylogenetically related to *O. iheyensis* derives from the study of its genome. The existence of a large number of osmoprotectant transporters (like Na⁺ and K⁺ transporters, glycine betaine and choline/H⁺ and carnitine/H⁺ symporters) could improve the survival in these saline conditions. Moreover, genes involved in the alkaliphilic phenotype of *O. iheyensis*, could explain the existence of this microorganism in alkaline conditions [30].

8 clones were related (91.0–99.3 % similarity) to *O. profundus* [31], a halotolerant facultatively alkaliphilic bacterium, growing in the NaCl range of 0–14 % (w/v), with optimum at 1–3 % (w/v) NaCl. Clone sequences related to the 16S rRNA sequence of *O. picturae* [32] with similarities ranging from 94.36 to 98.84 % were found in the study (5 clones) having optimal growth with a NaCl

Table 2 Summary of taxonomic assessment of representative 16S rRNA sequenced clones from library of the Atacama Desert

Clone number	Closest BLAST match (GenBank accession number)	% similarity range	Number of clones	Source of isolation of closest reference strain
<i>Ata_4a_2a_G08</i>	<i>Oceanobacillus iheyensis</i> strain HTE831 (BA0002)	91.18–100.00	154	Deep-sea sediment (Iheya Ridge, Japan)
<i>Ata_1b_002a_F05</i>	<i>Oceanobacillus profundus</i> strain CL-MP28 (DQ386635)	91.01–99.33	8	Sediment core-sample (Ulleung Basin of the East Sea, Korea)
<i>Ata_1b_002a_C05</i>	<i>Oceanobacillus picturae</i> strain R-5321 (AY616012)	94.36–98.84	5	Biofilm formation of mural painting (Carmona, Spain)
<i>Ata_1a_002a_A04</i>	<i>Thalassobacillus devorans</i> strain G-19.1 (AJ717299)	94.22–99.64	32	Hypersaline habitat (sourthen Spain)
<i>Ata_1a_001b_A05</i>	<i>Bacillus halmapalus</i> strain DSM 8723 (X76447)	91.47–96.20	5	Soil sample (Haga, Tochigi, Japan)
<i>Ata_1a_001a_D02</i>	<i>Bacillus marisflavi</i> strain TF-11 (NR_025240)	92.01–99.82	27	Sea water (Yellow Sea, Korea)
<i>Ata_4a_02a_E12</i>	<i>Bacillus subtilis</i> subsp. <i>subtilis</i> strain DSM 10 (NR_027552)	97.23–97.37	2	Mangrove forests (Andaman, India)
<i>Ata_3a_1a_D03</i>	<i>Bacillus niabensis</i> strain 4T19 (NR_043334)	95.78	1	Cotton-waste composts for muchroom (Korea)
<i>Ata_3b_1b_C12</i>	<i>Bacillus sonorensis</i> strain NRRL B-23154 (NR_025130)	87.57–98.91	3	Sonoran desert (Arizona)
<i>Ata_1a_001a_F01</i>	<i>Virgibacillus chiguensis</i> strain Halo30-9 (NR_044086)	98.46–98.61	2	Chigu saltern (southern Taiwan)
<i>Ata_3b_1b_D10</i>	<i>Virgibacillus halodenitrificans</i> strain DSM 10037 (NR_042967)	91.00	1	Marine solar saltern of the Yellow Sea (Korea)

concentration of 5–10 %, according to the description. This species was first described as *Virgibacillus picturae* [33] and subsequently reclassified as *Oceanobacillus picturae* [32]. The wide range of alkaline growth of *Oceanobacillus* spp. precludes the presence of these microorganisms in this habitat.

32 clones closely related to the moderately halophilic phenol degrading bacterium *Thalassobacillus devorans* [34] were also detected (94.2–99.6 % similarity). This species has been also isolated in a recent study achieved in Atacama Desert to determine the population of cultivable extremophilic hydrolytic bacterial community [7].

Species of the genus *Bacillus* are widely distributed and include a variety of phenotypically heterogeneous bacteria exhibiting a wide range of nutritional requirements, physiological and metabolic diversities [35]. Aerial distribution of the dormant spores probably explains the occurrence of *Bacillus* in most habitats. Indeed, the habitat analyzed would favor the occurrence of spore formation as a protective mechanism to cope with such hyperarid conditions [36]. Several alkaliphilic *Bacillus* species have been isolated from soil samples showing halophilic characteristics [37]. In the present study, 38 clones were resembled to the genus *Bacillus*, including isolates related to alkaliphilic species, being the moderately halophilic bacterium *Bacillus marisflavi* [38] broadly represented in the ribosomal library (27 clones showed more than 92 %

sequence similarity). 5 clones presented more than 91 % relation to *Bacillus halmapalus* [39]; 3 clones showed more than 87 % similarities to a species isolated from a desert soil, *Bacillus sonorensis* [40]. Three other clones were most closely related to *Bacillus subtilis* subsp. *subtilis* (97.2–97.3) (2 clones) [41] and one clone presented similarity (95.78 %) to *Bacillus niabensis* [42].

Some moderately halophilic, spore-forming bacteria were originally assigned to the genus *Bacillus* but have been reclassified within new genera. One example of such independent lineage is a group comprising the genera *Alkalibacillus* and the genus *Virgibacillus* [43]. In this work, two out of the 3 clones assigned to the *Virgibacillus* genus showed sequence similarity to the species *Virgibacillus chiguensis* [44] showing a high sequence similarity (98.4–98.6 %) and the other clone was phylogenetically assigned to *Virgibacillus halodenitrificans* (91 % sequence similarity) [45].

Although soil bacterial diversity, as estimated by phylotype richness and diversity (Shannon index) [46] varied across ecosystem types, a predominance of Firmicutes clones and abundance of *Bacillus* species were presented in studies on alkaliphilic diversity in distinctive habitats as lakes in Mongolia and lake Van in Turkey [47].

Given the size of the Atacama Desert it is not surprising that it contains a wide range of ecological niches. Changes in soil microbiology along Atacama transect are correlated

Fig. 2 Phylogenetic tree of the 16S rRNA gene clones obtained from the Atacama Desert sample. The tree was constructed based on the neighbor-joining method using 11 selected representative sequences and the reference bacterial sequences (GeneBank database). Bootstrap analyses were performed with 1000 repetitions and only values higher than 50 % are shown. The scale bar indicates the distance in substitutions per nucleotide. The clone code included “Ata” for Atacama Desert and the corresponding number of 16S rRNA gene library

with water availability and therefore, microbial diversity, community structure and rates of metabolic activity are clearly distinct [48]. Previous studies performed in Atacama Desert by using culture-independent techniques differed in the predominant phyla detected. Demergasso et al. [5] analyzed the planktonic bacterial and archaeal assemblages in lakes of northern Chile area, resulting dominated by Cytophaga–Flavobacterium–Bacteroidetes (CFB) phyla and few Proteobacterias. Drees et al. [1] reported a predominance of bacteria from the *Gemmatimonadetes* and *Planctomycetes* phyla. In contrast, Connon et al. [6] detected Actinobacteria, Proteobacteria and Firmicutes based on 16S rRNA gene clone libraries and PLFA analyses, being the dominant group the Actinobacteria comprising 94 % of the 16S rRNA gene clones, while the PLFA data indicated Proteobacterias the most prevalent. Lester et al. [49] determined Actinobacteria, Proteobacteria and Firmicutes, although PLFA were dominated by Proteobacteria. Recently, a study at the hyperarid margin of the Atacama Desert revealed communities with unique bacterial diversity marked by high abundances of novel *Actinobacteria* and

Chloroflexi and low levels of *Acidobacteria* and *Proteobacteria* phyla [15].

In conclusion this work elucidates the existence of unique bacterial profile associated with the alkaline soil sample from the Atacama Desert. On the basis of the present data, the best-adapted group in the screened area in Atacama Desert seems to be Firmicutes with *Oceanobacillus*, *Bacillus* and *Thalassobacillus* as the predominant genera detected. As spores remain viable for long periods of time, Firmicutes can survive long distance transportation by climatic or biological agents, leading to a higher dispersion rate. We do not presume that the bacteria that we have detected are indigenous to the Atacama Desert, but however, our data revealed that novel bacteria taxa could be isolated from these soils, representing promising biotechnological opportunities. There are several studies concerning the biotechnological applications of products synthesized by bacterium isolated from the Atacama Desert as antimicrobial agents, carotenoids or pigments, with many biological functions that can be exploited in the medicine, health foods, the foods industry, and cosmetic formulations [36, 50]. Overall, the

Atacama Desert constitutes an extraordinary source of bioactive products synthesized by novel species. However, a lot of questions concerning the ecological role of microorganisms in this area remained to be answered. This work provides a framework for further studies of these evidently important habitats.

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Conflict of interest None.

References

- Drees KP, Neilson JW, Betancourt JL, Quade J, Henderson DA, Pryor BM, Maier RM (2006) Bacterial community structure in the hyperarid core of the Atacama Desert, Chile. *Appl Environ Microbiol* 12:7902–7910. doi:[10.1128/AEM.01305-06](https://doi.org/10.1128/AEM.01305-06)
- Stivaletta N, Barbieri R, Billi D (2012) Microbial colonization of the salt deposits in the driest place of the Atacama Desert (Chile). *Orig Life Evol Biosph* 42:187–200. doi:[10.1007/s11084-012-9289-y](https://doi.org/10.1007/s11084-012-9289-y)
- Bagaley DR (2006) Uncovering bacterial diversity on and below the surface of a hyper-arid environment, the atacama desert, Chile. Thesis. Louisiana State University, USA
- Azua-Bustos A, González-Silva C, Arenas-Fajardo C, Vicuña R (2012) Extreme environments as potential drivers of convergent evolution by exaptation: the Atacama Desert coastal range case. *Microbiology* 3:426–435. doi:[10.3389/fmicb.2012.00426](https://doi.org/10.3389/fmicb.2012.00426)
- Demergasso C, Casamayor E, Chong G, Galleguillos P, Escudero L, Pedrós-Alió C (2004) Distribution of prokaryotic genetic diversity in athalassohaline lakes of the Atacama desert Northern Chile. *FEMS Microbiol Ecol* 48:57–69. doi:[10.1016/j.femsec.2003.12.013](https://doi.org/10.1016/j.femsec.2003.12.013)
- Connon SA, Lester ED, Shafaat HS, Obenhuber DC, Ponce A (2007) Bacterial diversity in hyperarid Atacama Desert soils. *J Geophys Res* 112:1–9. doi:[10.1029/2006JG000311](https://doi.org/10.1029/2006JG000311)
- Moreno ML, Piubeli F, Bonfa MRL, García MT, Durrant LR, Mellado E (2012) Analysis and characterization of cultivable extremophilic hydrolytic bacterial community in heavy-metal contaminated soils from the Atacama Desert and their biotechnological potentials. *J Appl Microbiol* 113:550–559. doi:[10.1111/j.1365-2672.2012.05366.x](https://doi.org/10.1111/j.1365-2672.2012.05366.x)
- Babalola OO, Kirby BM, Le Roes-Hill M, Cook AE, Cary SC, Burton SG, Cowan DA (2009) Phylogenetic analysis of active bacterial populations associated with Antarctic Dry Valley mineral soils. *Environ Microbiol* 3:566–576. doi:[10.1111/j.1462-2920.2008.01809.x](https://doi.org/10.1111/j.1462-2920.2008.01809.x)
- Dorador C, Vila I, Remonsellez F, Imhoff JF, Witzel KP (2010) Unique clusters of Archaea in Salar de Huasco, an athalassohaline evaporitic basin of the Chilean Altiplano. *FEMS Microbiol Ecol* 2:291–302. doi:[10.1111/j.1574-6941.2010.00891.x](https://doi.org/10.1111/j.1574-6941.2010.00891.x)
- Neilson JW, Quade J, Ortiz M, Nelson WM, Legatzki A, Tian F, La Comb M, Betancourt JL, Wing RA, Soderlund CA, Maier RM (2012) Life at the hyperarid margin: novel bacterial diversity in arid soils of the Atacama Desert, Chile. *Extremophiles* 16:553–566. doi:[10.1007/s00792-012-0454-z](https://doi.org/10.1007/s00792-012-0454-z)
- Walkley A, Black IA (1934) An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci* 37:29–38
- Lane DJ (1991) 16S/23S rRNA sequencing. In Stackebrandt E, Goodfellow M (eds) *Nucleic acid techniques in bacterial systematics*. Modern microbiological methods. Wiley, New York, pp 115–148
- Shivaji S, Reddy GSN, Aduri RP, Kutty R, Ravensschlag KA (2004) Bacterial diversity of a soil sample from Schirmacher Oasis, Antarctica. *Cell Mol Biol* 50:525–536
- Vettore AL, Silva FR, Kemper EL, Arruda P (2001) The libraries that made SUCEST. *Genet Mol Biol* 24:1–7. doi:[10.1590/S1415-47572001000100002](https://doi.org/10.1590/S1415-47572001000100002)
- Guo Y, Ribeiro JMC, Anderson JM, Bour S (2009) dCas: a desktop application for cDNA sequence annotation. *Bioinformatics* 9:1195–1196. doi:[10.1093/bioinformatics/btp129](https://doi.org/10.1093/bioinformatics/btp129)
- Schloss PD, Westcott SL, Ryabin T (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* 23:7537–7541. doi:[10.1128/AEM.01541-09](https://doi.org/10.1128/AEM.01541-09)
- Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, Peplies J, Glöckner FO (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucl Acids Res* 21:7188–7196. doi:[10.1093/nar/gkm864](https://doi.org/10.1093/nar/gkm864)
- Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA Gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* 62:716–721. doi:[10.1099/ijs.0.038075-0](https://doi.org/10.1099/ijs.0.038075-0)
- Nam YD, Jung M-J, Roh SW, Kim MS, Bae JW (2011) Comparative analysis of Korea human gut microbiota by bar-coded pyrosequencing. *PLoS One* 7:e22109. doi:[10.1371/journal.pone.0022109](https://doi.org/10.1371/journal.pone.0022109)
- Ludwig W, Strunk O (2004) ARB: a software environment for sequence data. *Nucl Acids Res* 4:1363–1371. doi:[10.1093/nar/gkh293](https://doi.org/10.1093/nar/gkh293)
- Ludwig W, Strunk O, Klugbauer S, Klugbauer N, Weizeneberger M, Neumaier J, Bächleiner M, Schleifer K-H (1998) Bacterial phylogeny based on comparative sequence analysis. *Electrophoresis* 19:554–568. doi:[10.1002/elps.1150190416](https://doi.org/10.1002/elps.1150190416)
- Rubin BER, Sanders JG, Hampton-Marcell J, Owens SM, Gilbert JA, Moreau CS (2014) DNA extraction protocols cause differences in 16S rRNA amplicon sequencing efficiency but not in community profile composition or structure. *Microbiol Open*. doi:[10.1002/mbo3.216](https://doi.org/10.1002/mbo3.216)
- Okoro CK, Brown R, Jones AL, Andrews BA, Asenjo JA, Goodfellow M, Bull AT (2009) Diversity of culturable actinomycetes in hyper-arid soils of the Atacama Desert, Chile. *Antonie Van Leeuwenhoek* 95:121–133. doi:[10.1007/s10482-008-9295-2](https://doi.org/10.1007/s10482-008-9295-2)
- Baati H, Guermazi S, Amdouni R, Gharsallah N, Sghir R, Ammar E (2008) Prokaryotic diversity of a Tunisian multipond saltern. *Extremophiles* 12:505–518. doi:[10.1007/s00792-008-0154-x](https://doi.org/10.1007/s00792-008-0154-x)
- Lozupore CA, Knight R (2007) Global patterns in bacterial diversity. *PNAS* 27:11436–11440. doi:[10.1073/pnas.0611525104](https://doi.org/10.1073/pnas.0611525104)
- Bull AT, Asenjo JA (2013) Microbiology of hyper-arid environments: recent insights from the Atacama Desert, Chile. *Antonie Van Leeuwenhoek* 6:1173–1179. doi:[10.1007/s10482-013-9911-7](https://doi.org/10.1007/s10482-013-9911-7)
- Galperin MY (2013) Genomic diversity of spore-forming firmicutes. *Microbiol Spectr* 1:TBS-0015-2012. doi:[10.1128/microbiolspectrum.TBS-0015-2012](https://doi.org/10.1128/microbiolspectrum.TBS-0015-2012)
- Lu J, Nogi Y, Takami H (2002) *Oceanobacillus iheyensis* gen. nov., sp. nov. In Validation List no. 85. Validation of publication of new names and new combinations previously effectively published outside the IJSEM. *Int J Syst Evol Microbiol* 52:685–690. doi:[10.1099/ijs.0.02358-0](https://doi.org/10.1099/ijs.0.02358-0)
- Hirota K, Hanaoka Y, Nodasaka Y, Yumoto I (2013) *Oceanobacillus polygona* sp. nov., a facultatively alkaliphile

- isolated from indigo fermentation fluid. *Int J Syst Evol Microbiol* 63:3307–3312. doi:[10.1099/ijs.0.048595-0](https://doi.org/10.1099/ijs.0.048595-0)
30. Takami H, Takaki Y, Uchiyama I (2002) Genome sequence of *Oceanobacillus iheyensis* isolated from the Iheya Ridge and its unexpected adaptive capabilities to extreme environments. *Nucl Acids Res* 30:3927–3935. doi:[10.1093/nar/gkf526](https://doi.org/10.1093/nar/gkf526)
 31. Kim YG, Choi DH, Hyun S, Cho BC (2007) *Oceanobacillus profundus* sp. nov., isolated from a deep-sea sediment core. *Int J Syst Evol Microbiol* 57:409–413. doi:[10.1099/ijs.0.64375-0](https://doi.org/10.1099/ijs.0.64375-0)
 32. Lee JS, Lim JM, Lee KC, Lee JC, Park YH, Kim CJ (2006) *Virgibacillus koreensis* sp. nov., a novel bacterium from a salt field, and transfer of *Virgibacillus picturae* to *Oceanobacillus* as *Oceanobacillus picturae* comb. nov. with emended descriptions. *Int J Syst Evol Microbiol* 56:251–257. doi:[10.1099/ijs.0.63734-0](https://doi.org/10.1099/ijs.0.63734-0)
 33. Rodriguez-Diaz M, Swings J, De Vos P (2003) *Virgibacillus carmonensis* sp. nov., *Virgibacillus necropolis* sp. nov. and *Virgibacillus picturae* sp. nov., three novel species isolated from deteriorated mural paintings, transfer of the species of the genus *Salibacillus* to *Virgibacillus*, as *Virgibacillus marismortui* comb. nov. and *Virgibacillus salexigens* comb. nov., and emended description of the genus *Virgibacillus*. *Int J Syst Evol Microbiol* 53:501–511. doi:[10.1099/ijs.0.02371-0](https://doi.org/10.1099/ijs.0.02371-0)
 34. García MT, Gallego V, Ventosa A, Mellado E (2005) *Thalassobacillus devorans* gen. nov., sp. nov., a moderately halophilic, phenol degrading, Gram-positive bacterium. *Int J Syst Evol Microbiol* 55:1789–1795. doi:[10.1099/ijs.0.63560-0](https://doi.org/10.1099/ijs.0.63560-0)
 35. Xu D, Côté JC (2003) Phylogenetic relationships between *Bacillus* species and related genera inferred from comparison of 3' end 16S rDNA and 5' end 16S-23S ITS nucleotide sequences. *Int J Syst Evol Microbiol* 53:695–704. doi:[10.1099/ijs.0.02346-0](https://doi.org/10.1099/ijs.0.02346-0)
 36. Myasnik M, Manasherob R, Ben-Dov E, Zaritsky A, Margalith Y, Barak Z (2001) Comparative sensitivity to UV-B radiation of two *Bacillus thuringiensis* subspecies and other *Bacillus* sp. *Curr Microbiol* 43:140–143. doi:[10.1007/s002840010276](https://doi.org/10.1007/s002840010276)
 37. Nogué Y, Takami H, Horikoshi K (2005) Characterization of alkaliphilic *Bacillus* strains used in industry: proposal of five novel species. *Int J Syst Evol Microbiol* 55:2309–2315. doi:[10.1099/ijs.0.63649-0](https://doi.org/10.1099/ijs.0.63649-0)
 38. Yoon JH, Kim IG, Kang KH, Oh TK, Park YH (2003) *Bacillus marisflavi* sp. nov. and *Bacillus aquimaris* sp. nov., isolated from sea water of a tidal flat of the Yellow Sea in Korea. *Int J Syst Evol Microbiol* 53:1297–1303. doi:[10.1099/ijs.0.02365-0](https://doi.org/10.1099/ijs.0.02365-0)
 39. Nielsen P, Fritze D, Priest FG (1995) Phenetic diversity of alkaliphilic *Bacillus* strains: proposal for nine new species. *Microbiology* 141:1745–1761. doi:[10.1099/13500872-141-7-1745](https://doi.org/10.1099/13500872-141-7-1745)
 40. Palmisano MM, Nakamura LK, Duncan KE, Istock CA, Cohan FM (2001) *Bacillus sonorensis* sp. nov., a close relative of *Bacillus licheniformis*, isolated from soil in the Sonoran Desert, Arizona. *Int J Syst Evol Microbiol* 51:1671–1679. doi:[10.1099/00207713-51-5-1671](https://doi.org/10.1099/00207713-51-5-1671)
 41. Nakamura LKL, Roberts MS, Cohan FM (1999) Relationship of *Bacillus subtilis* clades associated with strains 168 and W23: a proposal for *Bacillus subtilis* subsp. *subtilis* subsp. nov. and *Bacillus subtilis* subsp. *spizizenii* subsp. nov. *Int J Syst Bacteriol* 49:1211–1215. doi:[10.1099/00207713-49-3-1211](https://doi.org/10.1099/00207713-49-3-1211)
 42. Kwon SW, Lee SY, Kim BY, Weon HY, Kim JB, Go SJ, Lee GB (2007) *Bacillus niabensis* sp. nov., isolated from cotton-waste composts for mushroom cultivation. *Int J Syst Evol Microbiol* 57:1909–1913. doi:[10.1099/ijs.0.64178-0](https://doi.org/10.1099/ijs.0.64178-0)
 43. Porwal S, Lal S, Cheema S, Kalia VC (2009) Phylogeny in aid of the present and novel microbial lineages: diversity in *Bacillus*. *PLoS One* 4:e4438. doi:[10.1371/journal.pone.0004438](https://doi.org/10.1371/journal.pone.0004438)
 44. Wang CY, Chang CC, Ng CC, Chen TW, Shyu YT (2008) *Virgibacillus chiguensis* sp. nov., a novel halophilic bacterium isolated from Chigu, a previously commercial saltern located in southern Taiwan. *Int J Syst Evol Microbiol* 58:341–345. doi:[10.1099/ijs.0.64996-0](https://doi.org/10.1099/ijs.0.64996-0)
 45. Yoon JH, Oh TK, Park YH (2004) Transfer of *Bacillus halodenitrificans* Denariáz et al. 1989 to the genus *Virgibacillus* as *Virgibacillus halodenitrificans* comb. nov. *Int J Syst Evol Microbiol* 54:2163–2167. doi:[10.1099/ijs.0.63196-0](https://doi.org/10.1099/ijs.0.63196-0)
 46. Hill TCJ, Walsh KA, Harris JA, Moffett BF (2003) Using ecological diversity measures with bacterial communities. *FEMS Microbiol Ecol* 43:1–11. doi:[10.1111/j.1574-6941.2003.tb01040.x](https://doi.org/10.1111/j.1574-6941.2003.tb01040.x)
 47. López-García P, Kazmierczak J, Benzerara KS, Guyot F, Moreira D (2005) Bacterial diversity and carbonate precipitation in the giant microbialites from highly alkaline lake Van, Turkey. *Extremophiles* 9:263–274. doi:[10.1007/s00792-005-0457-0](https://doi.org/10.1007/s00792-005-0457-0)
 48. Crits-Christoph A, Robinson CK, Barnum T, Fricke WF, Davila AF, Jedynak B, McKay CP, Diruggiero J (2013) Colonization patterns of soil microbial communities in the Atacama Desert. *Microbiome* 1:28. doi:[10.1186/2049-2618-1-28](https://doi.org/10.1186/2049-2618-1-28)
 49. Lester ED, Satomi M, Ponce A (2009) Microflora of extreme arid Atacama Desert soils. *Soil Biol Biochem* 39:704–770. doi:[10.1016/j.soilbio.2006.09.020](https://doi.org/10.1016/j.soilbio.2006.09.020)
 50. Namitha KK, Negi PS (2010) Chemistry and biotechnology of carotenoids. *Crit Rev Food Sci Nutr* 8:728–760. doi:[10.1080/10408398.2010.499811](https://doi.org/10.1080/10408398.2010.499811)