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ORIGINAL PAPER

Changes in the bacterial populations of the highly alkaline saline soil of the former lake Texcoco (Mexico) following flooding

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Abstract Flooding an extreme alkaline-saline soil decreased alkalinity and salinity, which will change the bacterial populations. Bacterial 16S rDNA libraries were generated of three soils with different electrolytic conductivity (EC), i.e. soil with EC 1.7 dS m^{-1} and pH 7.80 (LOW soil), with EC 56 dS m^{-1} and pH 10.11 (MEDIUM soil) and with EC 159 dS m^{-1} and pH 10.02 (HIGH soil), using universal bacterial oligonucleotide primers, and 463 clone 16S rDNA sequences were analyzed phylogenetically. Library proportions and clone identification of the phyla *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Cyanobacteria*, *Bacteroidetes*, *Firmicutes* and *Cloroflexi* showed that the bacterial communities were different. Species and genera of the Rhizobiales, Rhodobacterales and Xanthomonadales orders of the α- and γ-subdivision of *Proteobacteria* were found at the three sites. Species and genera of the Rhodospirillales, Sphingobacteriales, Clostridiales, Oscillatoriales and Caldilineales were found only in the HIGH soil, Sphingomonadales, Burkholderiales and Pseudomonadales in the MEDIUM soil, Myxococcales in the LOW soil, and Actinomycetales in the MEDIUM and LOW soils. It was found that the largest diversity at the order and species level was found in the MEDIUM soil as bacteria of both the HIGH and LOW soils were found in it.

Keywords Clone library · 16S rDNA · Bacterial community · Alkaline-saline soil

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Introduction

The most remarkable examples of occurring alkaline environments are soda deserts and soda lakes (Horikoshi 1999). They are probably the most stable alkaline environments on Earth, with pH ranging from 10.5 to 12.0 depending on the site. The soil of the former lake Texcoco located only a few kilometres from the urban zone of Mexico City, one of the largest cities in the world, is such an alkaline ecosystem. The continued expansion of Mexico City, the artificial flooding of the soil with effluents to vegetate the area so as to avoid dust pollution and pastoral practices have changed the characteristics of soil of the former lake Texcoco (Luna-Guido et al. 2000). The microbial community in this extreme ecosystem will change when soil is flooded and the endemic microorganisms might disappear. The microorganisms adapted to these extreme alkaline saline conditions might have unique metabolic characteristics that still have to be examined.

The soil of the former lake Texcoco is unique. The soil is alkaline with electrolytic conductivities (EC) in saturation extracts from 22 to 150 dS m^{-1}, exchangeable sodium percentages from 76 to 98% and sodium adsorption ratio from 103 to 1718 mM (Beltrán-Hernández et al. 1999). The soil structure is granular in the topsoil and prismatic in the subsoil and the organic matter contents range from 20

to 50 g kg^{-1} dry soil. Natural drainage is poor and a compact ash layer 5–20 cm thick at 16–40 cm depth restricts root growth (Luna-Guido et al. 2001). The soil is formed from volcanic ash deposited in situ in a lacustrine environment and covered recently by colluvial materials without a clear structure and the water content is highly variable fluctuating between saturated and air-dried. It contains a material that is called '*jaboncillo*' or 'moisture soap' with high water retention capacity, olive-green color and unctuous consistence when moist (Gutiérrez-Castorena et al. 2005). The exposed soil is covered with a saline crust, mainly trona. The original size of the lake, more than 140 km^2, exceeded that of other alkaline brines reported in literature (Jones et al. 1967). Its formation started in the Late Pleistocene and continued until the process was interrupted by artificial flooding of the lake starting at the end of the eighteenth century (Zeevaert 1949).

The soil of the former lake Texcoco is not only alkaline, but also extreme saline with EC sometimes above 150 dS m^{-1}. The most well-studied soda lakes are those located in the East African Rift Valley (Duckworth et al. 1996; Rees et al. 2004), in India (Wani et al. 2006), Central Asia (Gorlenko et al. 2004; Ma et al. 2004b; Foti et al. 2008) meromictic Lake Kaiike in Japan (Koizumi et al. 2004) and in North America, Mono Lake (California) (Humayoun et al. 2003; Scholten et al. 2005) and Soap Lake (Washington) (Sorokin et al. 2007). Few studies exist about the microbial community in the remaining lake (Jan-Roblero et al. 2004; Alazard et al. 2007), and only one reported the archaeal community in soil of the former lake (Valenzuela-Encinas et al. 2008). It is thus important to determine the bacterial population in soil of the former lake Texcoco and how flooding has changed it. The objective of this study was to investigate (1) the bacterial diversity in a pristine soil and (2) how changes in soil characteristics due to flooding affected the bacterial community.

Materials and methods

Site description and soil sampling

The sampling sites are located at the former lake Texcoco in the State of Mexico, Mexico (http://www.inegi.gob.mx) (Table 1). Soil samples were collected from three different sites in the season with low rainfall (2006). An area of 1 m^2 was delimited, the first 2 cm of soil were discarded, and the next 10 cm layer was sampled with a hand spade and taken to the laboratory in black polyethylene bags. The samples were 5 mm sieved under aseptic conditions, divided in 250 g sub-samples and stored at –80°C until analyzed.

Metagenomic DNA from soil samples was extracted using a previously reported method (Valenzuela-Encinas et al. 2008). The DNA yield was quantified with the Quantity One software in a Transilluminator 2000 (Gel Doc 2000, BIO-RAD Laboratories Inc., Carlsbad, CA, USA) after 0.8% (w/v) agarose gel electrophoresis, and stained with ethidium bromide solution (0.5 μg ml^{-1}). The DNA extracted was stored at −20°C until used for PCR amplification.

PCR amplification, construction of 16S rDNA library and sequencing

Samples of purified DNA were used as template for the amplification of bacterial 16S rDNA via PCR. The reaction mixture (25 μl) contained 100 ng of genomic DNA; appropriate primers at 0.5 μM each; dATP, dCTP, dGTP and dTTP each at a concentration of 200 μM; 2.5 mM MgCl; and 1 U of Taq DNA polymerase in the PCR buffer provided by the manufacturer (Invitrogen Life Technologies, Sao Paulo, Brazil). The bacterial specific primers were 46F (5′-GCC TAA CAC ATG CAA GTC-3′) (Yu and Morrison 2004) and 1540R (5′-AAG GAG GTG ATC CAG CCGCA-3′) (Edwards et al. 1989). Amplification conditions included a denaturation step at 93°C for 10 min followed by 25 cycles at 93°C for 1 min, at 57°C for 1 min, at 72°C for 2 min, and a final step at 72°C for 10 min. The amplification was done with a Touchgene Gradient FTGRAD2D (TECHNE DUXFORT, Cambridge, UK). The expected size of the fragments amplified were ~1,500 bp. PCR products were cloned directly into the vector pCR®2.1-TOPO® by using the TOPO TA Cloning Kit (Invitrogen Life Technologies, Carlsbad, CA, USA). Positive clones were detected by the appearance of white colonies in LB plates containing 40 mg ml^{-1} of X-Gal (Invitrogen Life Technologies, Carlsbad, CA, USA) and 50 μg ml^{-1} ampicillin. Recombinant plasmids were isolated from overnight cultures by alkaline lysis (Sambrook et al. 1989) and a restriction analysis with *Eco*RI to detect the insertion was performed. The 16S rDNA sequences were obtained with a 3730X DNA Analyzer (Applied Biosystems, Foster City, CA, USA) using M13 primers.

Molecular identification and phylogenetic analysis

All sequences obtained were checked for chimeras using the CHIMERA CHECK (2.7) online analysis program of the RDP-II database (Cole et al. 2003). The sequences were then subjected to a BLAST search (Altschul et al. 1997) and RDP Analysis Tools of Ribosomal Database Project-II Release 9 (http://rdp.cme.msu.edu/index.jsp) to determine taxonomic hierarchy of the sequences. Multiple alignment analyses were performed with CLUSTAL X (Thompson et al. 1997) selecting related sequences from the NCBI Taxonomy Homepage (TaxBrowser) and Ribosomal Database Project-II databases. The transversion/transition

Table 1 Some characteristics of soil of Texcoco

Site	Localization	EC (dS m^{-1})	pH	Organic C (g kg^{-1} soil)	Inorganic C	Total N	WHC	Clay	Silt	Sand	Textural classification
LOW	19°29′46″N 98°58′01″W	0.68	7.8	32	32	1.74	573	158	56	786	Sandy loam
MEDIUM	19°28′40″N 98°58′10″W	56	10.1	53	11	6.7	740	170	131	699	Sandy loam
HIGH	19°30′47″N 98°59′23″W	159	10.0	24	7.4	1.7	968	537	388	75	Clayey

EC electrolytic conductivity, *WHC* water holding capacity

weighting using the Tamura–Nei model (Tamura and Nei 1993) and the number of bases substitution between each pair of sequences was estimated using program MEGA v. 3.1 (Kumar et al. 2001). Phylogenetic trees were constructed using the Neighbor-joining method and Tamura–Nei model of distance analysis and 500 Bootstrap replications were assessed to support internal branches. Sequences that differed by less than 2.5% were considered to belong to the same phylotype and sequences with similitude percentages below 95% were assigned to the closest family (Rossello-Mora and Amann 2001).

Community richness and composition analysis

Rarefaction, richness, and diversity statistics were also calculated using DOTUR. The input files were in the form of distance matrices generated by using Phylip version 3.6. DOTUR uses the furthest-neighbor method to collapse similar sequences into groups at arbitrary levels of taxonomic similarity and then computes the Shannon, Chao, and ACE statistics for that taxonomic level (Schloss and Handelsman 2005). Operational taxonomic units (OTUs) for community analysis were defined by an 80 and 97% difference in nucleic acid sequences (Rossello-Mora and Amann 2001; Schloss and Handelsman 2005; Mohamed et al. 2008).

Nucleotide sequence accession numbers

The sequences were deposited in the GenBank database and assigned the accession numbers FJ152554–FJ153025.

Results

Site description

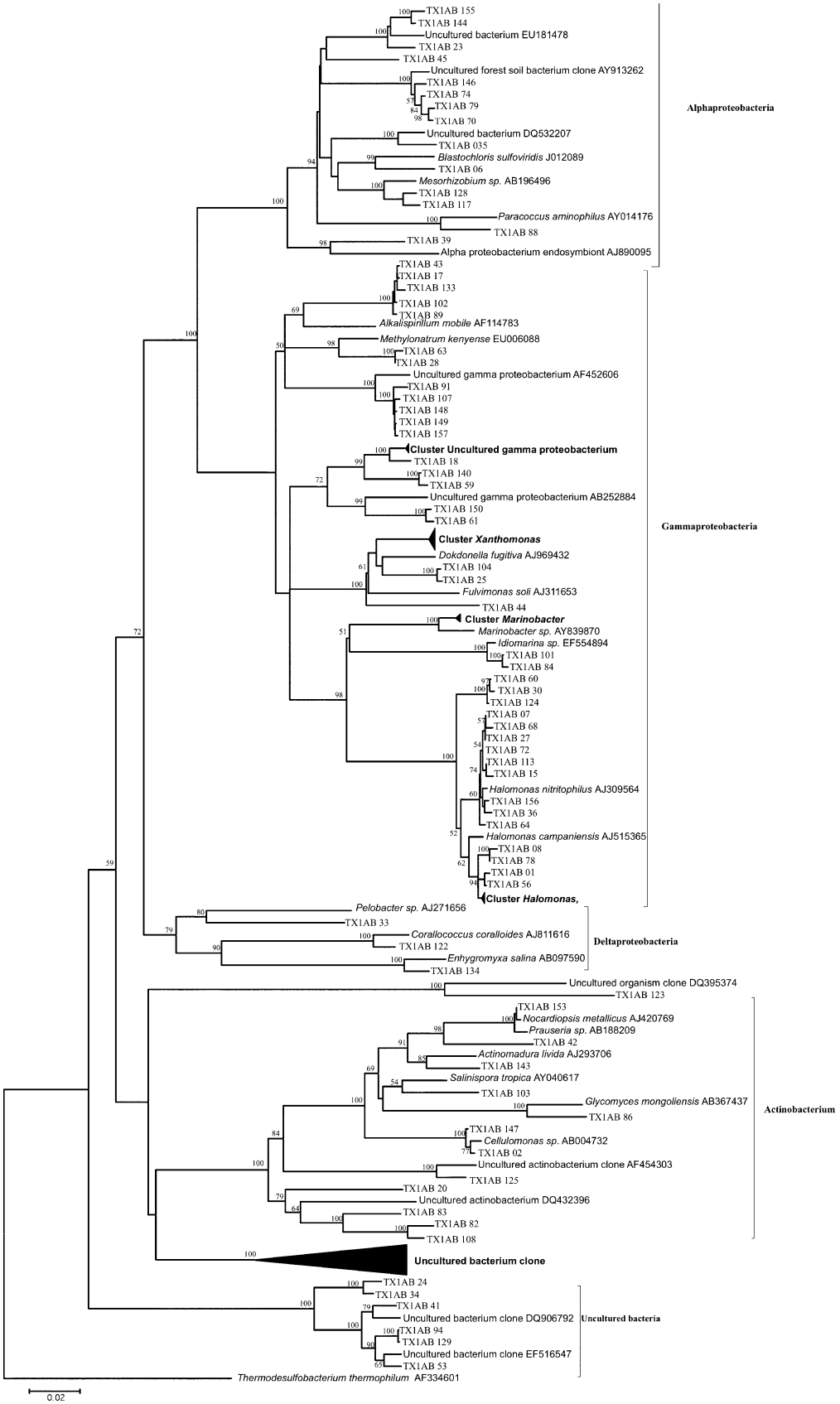
Three soils were collected from the former lake Texcoco and designated according to their salinity and alkalinity, i.e. the soil with electrolytic conductivity (EC) 0.7 dS m^{-1} and pH 7.80 was termed LOW, the soil with EC 56 dS m^{-1} and pH 10.11 or the MEDIUM and the soil with 159 dS m^{-1} and pH 10.02 (Table 1) HIGH.

Bacterial composition

Metagenomic DNA was extracted directly from soil to investigate the diversity of bacteria, and amplified using bacterial 16S rDNA primers. A ~1,500 bp fragment from the 5′ terminus (46–1540 region *Escherichia coli* numbering) was amplified. This section contains the variable regions of the 16S rDNA, which reflects the phylogenetic relationship of corresponding sequences and the degree of diversity in a clone library. The 500 clones obtained, which contained partial 16S rDNA, were sequenced.

α-Subdivision of *Proteobacteria*

The orders of Rhizobiales and Rhodobacterales classified within the *α-Proteobacteria* were found in the three soils (Figs. 1, 2, 3, 4). Overall, 58 clones belonged to the *α-Proteobacteria*: 13 were found in the HIGH, 31 in MEDIUM and 14 in the LOW soil (Fig. 5). Of those found in the HIGH soil, three clones were closely related to the genera *Mesorhizobium* and *Chelatococcus,* both belonging to the Rhizobiales. Eleven clones were closely related to the genera *Amaricoccus*, *Loktanella* and *Rhodobacter* (95.0–97.5% similarity) all grouped within the Rhodobacterales and one clone was grouped within the Rhodospirillales. One clone was closely related to *Natronohydrobacter thiooxidans*, a bacteria belonging to *α-Proteobacteria*, but not yet further classified. In the MEDIUM soil, two clones were closely related to the species *Ochrobactrum grignonense* and *Hyphomicrobium aestuarii* (97.5–100% similarity), one to the genus *Hyphomicrobium,* belonging to the Rhizobiales, and one clone to the genus *Silicibacter* grouped within the Rhodobacterales, three clones of Sphingomonadales were closely related to the species *Kaistobacter terrae*, *Sphingomonas wittichii* and *Sphingomonas melonis*. In the LOW soil, two clones were closely related to the genus


TX1AB 155
TX1AB 144
Uncultured bacterium EU181478
TX1AB 23
TX1AB 45
Uncultured forest soil bacterium clone AY913262
TX1AB 146
TX1AB 74
TX1AB 79
TX1AB 70
Uncultured bacterium DQ532207
TX1AB 035
Blastochloris sulfoviridis J012089
TX1AB 06
Mesorhizobium sp. AB196496
TX1AB 128
TX1AB 117
Paracoccus aminophilus AY014176
TX1AB 88
TX1AB 39
Alpha proteobacterium endosymbiont AJ890095
Alphaproteobacteria
TX1AB 43
TX1AB 17
TX1AB 133
TX1AB 102
TX1AB 89
Alkalispirillum mobile AF114783
Methylonatrum kenyense EU006088
TX1AB 63
TX1AB 28
Uncultured gamma proteobacterium AF452606
TX1AB 91
TX1AB 107
TX1AB 148
TX1AB 149
TX1AB 157
Cluster Uncultured gamma proteobacterium
TX1AB 18
TX1AB 140
TX1AB 59
Uncultured gamma proteobacterium AB252884
TX1AB 150
TX1AB 61
Cluster Xanthomonas
Dokdonella fugitiva AJ969432
TX1AB 104
TX1AB 25
Fulvimonas soli AJ311653
TX1AB 44
Cluster Marinobacter
Marinobacter sp. AY839870
Idiomarina sp. EF554894
TX1AB 101
TX1AB 84
TX1AB 60
TX1AB 30
TX1AB 124
TX1AB 07
TX1AB 68
TX1AB 27
TX1AB 72
TX1AB 113
TX1AB 15
Halomonas nitritophilus AJ309564
TX1AB 156
TX1AB 36
TX1AB 64
Halomonas campaniensis AJ515365
TX1AB 08
TX1AB 78
TX1AB 01
TX1AB 56
Cluster Halomonas,
Gammaproteobacteria
Pelobacter sp. AJ271656
TX1AB 33
Corallococcus coralloides AJ811616
TX1AB 122
Enhygromyxa salina AB097590
TX1AB 134
Deltaproteobacteria
Uncultured organism clone DQ395374
TX1AB 123
TX1AB 153
Nocardiopsis metallicus AJ420769
Prauseria sp. AB188209
TX1AB 42
Actinomadura livida AJ293706
TX1AB 143
Salinispora tropica AY040617
TX1AB 103
Glycomyces mongoliensis AB367437
TX1AB 86
TX1AB 147
Cellulomonas sp. AB004732
TX1AB 02
Uncultured actinobacterium clone AF454303
TX1AB 125
TX1AB 20
Uncultured actinobacterium DQ432396
TX1AB 83
TX1AB 82
TX1AB 108
Actinobacterium
Uncultured bacterium clone
TX1AB 24
TX1AB 34
TX1AB 41
Uncultured bacterium clone DQ906792
TX1AB 94
TX1AB 129
Uncultured bacterium clone EF516547
TX1AB 53
Uncultured bacteria
Thermodesulfobacterium thermophilum AF334601
0.02

◀ **Fig. 1** Phylogenetic relationship between the 16S rDNA sequences obtained from LOW soil of the former lake Texcoco. The tree was constructed with related sequences obtained from NCBI database by using the Neighbor-joining algorithm. *Thermodesulfobacterium thermophilum* (Accession number AF334601) was used as the outgroup. Numbers before branch points represent percentages of bootstrap resampling based on 500 trees. Bootstrap values below 50% are not presented. The *scale bar* represents the expected number of substitutions averaged over all sites analyzed

Mesorhizobium within Rhizobiales, and one to the genus *Paracoccus* within Rhodobacterales. Rhodospirillales were found in the HIGH soil and Sphingomonadales in the MEDIUM soil. The HIGH soil showed more diversity as clones of four different orders of *α-Proteobacteria* were found, three in the MEDIUM soil and only two in the LOW soil (Fig. 5).

β-Subdivision of *Proteobacteria*

Only in the MEDIUM soil were clones found that belonged to the *β-Proteobacteria* (Figs. 2, 4). Of these, three clones were closely related to the Burkholderiaceae family and one clone was closely related to species *Leptothrix ginsengisoli*. One clone belonged to *β-Proteobacteria* but could not be classified within an order.

γ-Subdivision of *Proteobacteria*

The order of Xanthomonadales, classified within *γ-Proteobacteria*, was found in the three soils. Overall, 96 clones belonged to *γ-Proteobacteria* and 47 were found in the HIGH soil, 11 in the MEDIUM and 38 in LOW soil. Of those found in the HIGH soil, 15 clones were closely related to the species *Pantoea agglomerans,* belonging to the Enterobacteriales. One clone, which was closely related to the genus *Idiomarina*, grouped within the Alteromonadales. Five clones were closely related to the genus *Halomonas* and one clone was closely related to the species *Halomonas desiderata* within Oceanospirillales. One clone was closely related to the genus *Xanthomonas* and one within Xanthomonadales. Six clones were closely related to the species *Natronocella acetinitrilica, Methylonatrum kenyense* and *Alkalilimnicola ehrlichei*, belonging to the Ectothiorhodospiraceae family. In the MEDIUM soil, three clones were closely related to the species *Rhizobacter dauci*, *Pseudomonas putida* and *Pseudomonas vancouverensis*, belonging to the Pseudomonadales, and nine clones to the genus *Aquicella* grouped within the Legionellales. Two clones were closely related to the species *Lysobacter enzymogenes* within Xanthomonadales. In the LOW soil, fifteen clones were closely related to the genus *Xanthomonas* belonging to the Xanthomonadales, two to the species *Halomonas nitritophilus,* twenty-five to the *Halomonas* genus grouped within the Oceanospirillales, nine to the genus *Marinobacter* and two to the *Idiomarina* grouped within the Alteromonadales. Thirteen clones were closely related to the Chromatiales. The HIGH soil showed more diversity as clones of eight different orders of *γ-Proteobacteria* were detected, five in the MEDIUM and only four in the LOW soil.

Actinobacteria

Actinobacteria were found in the MEDIUM and LOW soils and 21 clones belonged to that phylum (Fig. 4). Nine were found in the MEDIUM soil and 12 in the LOW soil. Of these, two clones were closely related to *Streptomyces griseoruber* and *Arthrobacter oxydans* and one clone was closely related to genus *Cellulomonas.* Seven clones belonging to *Actinobacteria* phylum could not be classified in an order.

Firmicutes, Acidobacteria and other groups

Clones belonging to *Firmicutes* were found only in the HIGH soil and three were related to the genus *Alkaliphilus* (Figs. 3, 4). Clones belonging to *Acidobacteria* were found only in the MEDIUM soil. *δ-Proteobacteria* were found in the HIGH and LOW soils, while *Cyanobacteria* and *Cloroflexi* only in the HIGH soil. Clones of Desulfuromonadaceae family were found in the HIGH and LOW soils, while clones of the Myxococcaceae family only in the LOW soil. Both families belong to *δ-Proteobacteria*. *Oscillatoria amoena* and other Oscillatoriales related clones belonging to *Cyanobacteria* were found in the LOW and HIGH soils.

Uncultured bacterial clones

An important amount of bacteria reported as non-cultivable was found in the three soils, i.e. 28% in the LOW soil, 18% in the MEDIUM and 46% in the HIGH soil. Those clones were related closely to uncultured bacteria mostly found in forest soils.

Effect of salinity on community composition

Rarefaction curves at the estimated phylum level (distance = 0.20) reached saturation for all libraries (Fig. 6). Rarefaction curves at the estimated species level did not reach saturation (distance = 0.02). The richness was high at all sites. However, the bacterial species richness in MEDIUM soil was greater than HIGH and LOW soils indicated by the inclines in of rarefaction curves (Fig. 6). Measures of diversity and richness were calculated using DOTUR (Table 2).

Diversity and richness was higher in the MEDIUM soil than in the HIGH and LOW soils.

Discussion

Site description

The soil of the former lake Texcoco is a unique ecosystem. The allophanic soil is a flooded former lakebed with pH reaching 10.2. Salt crusts are formed on the soil surface resulting in an electrolytic conductivity up to 159 dS m^{-1}. Microorganisms in this environment have to adapt to changing soil conditions such as water logging during the rainy season and dry conditions during the rest of the year. Earlier studies have shown that even under these conditions microorganisms are active although certain soil processes are inhibited, i.e. nitrification, urease activity, decomposition of cellulose and glucose, or altered, i.e. the assimilatory reduction of NO_3^- is not inhibited by NH_4^+ and large amounts of NO_2^- are produced (Vega-Jarquin et al. 2003; Conde et al. 2005). Identifying the microorganisms in soil of the former lake Texcoco is a first step in understanding their role in the earlier mentioned processes (Valenzuela-Encinas et al. 2008). Flooding with effluents to vegetate this barren land so as to avoid dust pollution in Mexico City has reduced pH and salt content but also changed bacterial populations, to what extent is yet unknown. Determining bacterial populations in soil with different times of flooding will show how reduction in pH and salt affected composition of soil microbial communities.

Comparing soil phylotypes

The phylotypes found in the LOW, MEDIUM and HIGH soil libraries were different. In the LOW and HIGH soils, most of clones of *γ-Proteobacteria* were related to microorganisms previously found in soda lakes and/or in soil. *Natronocella acetinitrilica*, *Methylonatrum kenyense* and *Alkalilimnicola ehrlichei* found in the HIGH soil, belong to the Chromatiales order. Isolates of Chromatiales found in the HIGH soil have been obtained from marine, hypersaline and haloalkaline environments and require or prefer saline and alkaline growth conditions (Imhoff and Süling 1996; Tourova et al. 2007). *Halomonas* belongs to the order of Oceanospirillales; mostly marine and moderately halophilic microorganisms that are phenotypically diverse (Arahal and Ventosa 2006). The *Idiomarina* genus, belonging to the order of Alteromonadales, is strictly aerobic and chemoorganotrophic, isolated from seawater and hypersaline habitats (Ivanova et al. 2000; Martínez-Cánovas et al. 2004; Choi and Cho 2005). Other microorganisms, such as *Xanthomonas* belong to a group of bacteria with diverse physiological characteristics and phytopathological specializations (Goncalves and Rosato 2002) while *Pantoea agglomerans* is a facultative anaerobe formerly named *Enterobacter agglomerans*. It is a ubiquitous member of the Enterobacteriaceae that is found in plants and feces of humans and animals (De Champs et al. 2000; Francis et al. 2000).

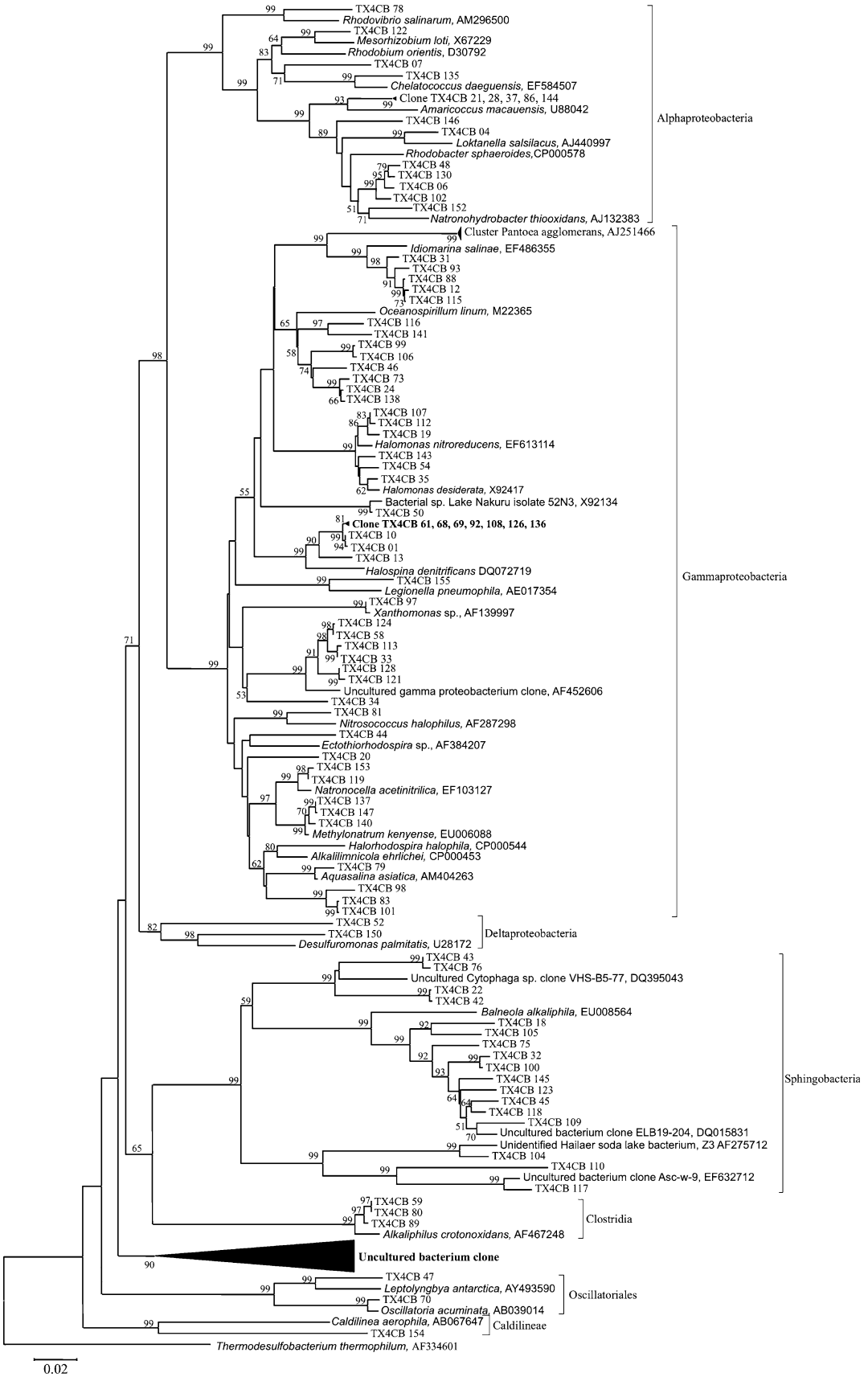
All phylotypes found in the MEDIUM soil are typical for soil and water environments. *Lysobacter* species grouped in order Xanthomonadales typically found in soil and water, are bacterial control agents of fungal diseases (Sullivan et al. 2003; Yu et al. 2007). *Pseudomonas putida* and *Pseudomonas vancouverensis* are grouped in the order Pseudomonadales, a group of species with nutritional versatility (Ait Tayeb et al. 2005). Strains of the *Aquicella* genus grouped in the order Legionellales require neutral pH for growth and have been isolated from water (Santos et al. 2003).

Organisms found in the LOW soil were related to microorganisms previously found in extreme environments. *Halomonas*, *Idiomarina* and the *Ectothiorhodospira* clade are related to organisms usually found in soda lakes and were similar to clones found in the HIGH soil. Members of the genus *Marinobacter* are extremely halotolerant and have been isolated from marine environments, saline soil and coastal hot springs (Gauthier et al. 1992; Yoon et al. 2004).

In contrast to the *γ-Proteobacteria*, most of the clones of the *α-Proteobacteria* found in the LOW and HIGH soils were previously described or cultivated from soil or activated sludge, but one was found in a soda lake (Maszenan et al. 1997). It has to be remembered that the soil was flooded with effluents of a wastewater treatment plant, which might explain the presence of microorganisms normally found in wastewater. *Chelatococcus* and *Mesorhizobium* are Rhizobiales and they can reduce atmospheric dinitrogen when living in symbiosis with leguminous plants (Kaneko et al. 2000; Kersters et al. 2006). Studies so far indicate that cells of *Chelatococcus* are ubiquitously distributed in aquatic environments, and their number increases with both increasing eutrophication and temperature (Egli and Auling 2005). *Amaricoccus, Rhodobacter* and *Loktanella* belong to the order of Rhodobacterales,

Fig. 2 Phylogenetic relationship between the 16S rDNA sequences obtained from MEDIUM soil of the former lake Texcoco. The tree was constructed with related sequences obtained from NCBI database by using the Neighbor-joining algorithm. *Halalkalicoccus tibetensis* (Accession number AF435112) was used as the outgroup. Numbers before branch points represent percentages of bootstrap resampling based on 500 trees. Bootstrap values below 50% are not presented. The *scale bar* represents the expected number of substitutions averaged over all sites analyzed ▶


Uncultured alpha proteobacterium, DQ453806
TX5A 161
TX5A 140
TX5A 82
Rhodopseudomonas cryptolactis, AB087718
Methylosinus acidophilus, DQ076754
TX5A 94
TX5A 64
TX5A 146
TX5A 77
TX5A 133
TX5A 59
TX5A 95
Uncultured alpha proteobacterium AB252931
TX5A 158
Ochrobactrum grignonense, AJ242581
TX5A 110
TX5A 87
TX5A 131
TX5A 141
TX5A 124
Pedomicrobium manganicum, X97691
TX5A 17
Hyphomicrobium sulfonivorans, AF538931
TX5A 147
Hyphomicrobium aestuarii, Y14304
TX5A 11
TX5A 104
TX5A 105
TX5A 52
Silicibacter pomeroyi, AF098491
TX5A 48
TX5A 29
Uncultured bacterium, DQ833466
TX5A 28
Kaistobacter terrae, AB258386
TX5A 27
TX5A 130
Sphingomonas wittichii, AB021492
Sphingomonas herbicidovorans, AB042233
Sphingomonas melonis, AB055863
TX5A 78
TX5A 100
TX5A 97
Uncultured sludge bacterium, AF234730
TX5A 57
Alphaproteobacteria
TX5A 10
TX5A 16
Psucimonas lemoignei, AB021375
TX5A 70
Leptothrix ginsengisoli, AB271046
TX5A 109
Rhizobacter dauci, AB297965
Uncultured beta proteobacterium, EF612410
TX5A 84
Betaproteobacteria
TX5A 19
TX5A 88
Lysobacter enzymogenes, AY947529
Pseudomonas putida, AY918067
TX5A 25
TX5A 89
Pseudomonas vancouverensis, AJ011507
Cluster Pantoea agglomerans, AJ251466
TX5A 128
Aquicella siphonis, AY359283
TX5A 106
TX5A 108
Gamma proteobacterium, AB174844
TX5A 47
TX5A 154
TX5A 62
Uncultured bacterium, AY221036
Gammaproteobacteria
Acidobacteria
TX5A 129
Uncultured bacterium, AB252959
TX5A 49
TX5A 79
TX5A 22
TX5A 18
TX5A 159
Cluster I
TX5A 142
TX5A 55
TX5A 152
Uncultured soil bacterium, AY493916
TX5A 96
TX5A 61
TX5A 101
Uncultured bacterium, AB252957
TX5A 122
TX5A 150
Uncultured bacterium, AB240321
Uncultured bacterium, EF516211
TX5A 15
TX5A 112
Uncultured bacterium, EF515894
TX5A 85
Cluster II
TX5A 098
Uncultured bacterium, DQ906907
TX5A 60
Uncultured Gemmatimonadetes, EF612389
TX5A 139
TX5A 42
TX5A 33
TX5A 114
TX5A 36
TX5A 30
Uncultured soil bacterium, AY493975
TX5A 40
Uncultured bacterium, AJ581612
TX5A 120
Cluster III
TX5A 76
Streptomyces griseoruber, AB184064
TX5A 115
Arthrobacter oxydans, X83408
Actinobacteria
Uncultured bacterium, DQ129371
TX5A 119
TX5A 26
TX5A 118
Uncultured bacterium, EF494371
TX5A 90
Cluster IV
TX5A 024
Uncultured bacterium, DQ404672
Uncultured bacterium, DQ906853
TX5A 136
TX5A 41
Uncultured bacterium, EF632946
Cluster V
Halalkalicoccus tibetensis, AF435112
0.05


TX4CB 78
Rhodovibrio salinarum, AM296500
TX4CB 122
Mesorhizobium loti, X67229
Rhodobium orientis, D30792
TX4CB 07
TX4CB 135
Chelatococcus daeguensis, EF584507
Clone TX4CB 21, 28, 37, 86, 144
Amaricoccus macauensis, U88042
TX4CB 146
TX4CB 04
Loktanella salsilacus, AJ440997
Rhodobacter sphaeroides,CP000578
TX4CB 48
TX4CB 130
TX4CB 06
TX4CB 102
TX4CB 152
Natronohydrobacter thiooxidans, AJ132383
Alphaproteobacteria
Cluster Pantoea agglomerans, AJ251466
Idiomarina salinae, EF486355
TX4CB 31
TX4CB 93
TX4CB 88
TX4CB 12
TX4CB 115
Oceanospirillum linum, M22365
TX4CB 116
TX4CB 141
TX4CB 99
TX4CB 106
TX4CB 46
TX4CB 73
TX4CB 24
TX4CB 138
TX4CB 107
TX4CB 112
TX4CB 19
Halomonas nitroreducens, EF613114
TX4CB 143
TX4CB 54
TX4CB 35
Halomonas desiderata, X92417
Bacterial sp. Lake Nakuru isolate 52N3, X92134
TX4CB 50
Clone TX4CB 61, 68, 69, 92, 108, 126, 136
TX4CB 10
TX4CB 01
TX4CB 13
Halospina denitrificans DQ072719
TX4CB 155
Legionella pneumophila, AE017354
TX4CB 97
Xanthomonas sp., AF139997
TX4CB 124
TX4CB 58
TX4CB 113
TX4CB 33
TX4CB 128
TX4CB 121
Uncultured gamma proteobacterium clone, AF452606
TX4CB 34
TX4CB 81
Nitrosococcus halophilus, AF287298
TX4CB 44
Ectothiorhodospira sp., AF384207
TX4CB 20
TX4CB 153
TX4CB 119
Natronocella acetinitrilica, EF103127
TX4CB 137
TX4CB 147
TX4CB 140
Methylonatrum kenyense, EU006088
Halorhodospira halophila, CP000544
Alkalilimnicola ehrlichei, CP000453
TX4CB 79
Aquasalina asiatica, AM404263
TX4CB 98
TX4CB 83
TX4CB 101
Gammaproteobacteria
TX4CB 52
TX4CB 150
Desulfuromonas palmitatis, U28172
Deltaproteobacteria
TX4CB 43
TX4CB 76
Uncultured Cytophaga sp. clone VHS-B5-77, DQ395043
TX4CB 22
TX4CB 42
Balneola alkaliphila, EU008564
TX4CB 18
TX4CB 105
TX4CB 75
TX4CB 32
TX4CB 100
TX4CB 145
TX4CB 123
TX4CB 45
TX4CB 118
TX4CB 109
Uncultured bacterium clone ELB19-204, DQ015831
Unidentified Hailaer soda lake bacterium, Z3 AF275712
TX4CB 104
TX4CB 110
Uncultured bacterium clone Asc-w-9, EF632712
TX4CB 117
Sphingobacteria
TX4CB 59
TX4CB 80
TX4CB 89
Alkaliphilus crotonoxidans, AF467248
Clostridia
Uncultured bacterium clone
TX4CB 47
Leptolyngbya antarctica, AY493590
TX4CB 70
Oscillatoria acuminata, AB039014
Oscillatoriales
Caldilinea aerophila, AB067647
TX4CB 154
Caldilineae
Thermodesulfobacterium thermophilum, AF334601
0.02

◄ **Fig. 3** Phylogenetic relationship between the 16S rDNA sequences obtained from HIGH soil of the former lake Texcoco. The tree was constructed with related sequences to obtained from NCBI database by using the Neighbor-joining algorithm. *Thermodesulfobacterium thermophilum* (Accession number AF334601) was used as the outgroup. Numbers before branch points represent percentages of bootstrap resampling based on 500 trees. Bootstrap values below 50% are not presented. The *scale bar* represents the expected number of substitutions averaged over all sites analyzed

characterized by a heterogeneous metabolism. The *Loktanella* genus is moderately halotolerant and chemoheterotrophic and was isolated from microbial mats in Antarctic lakes (Van Trappen et al. 2004). *Rhodobacter* is photosynthetic and was isolated from aquatic sediments (Ramana et al. 2008). *Amaricoccus* is a chemoheterotroph, generally found in activated sludge (Maszenan et al. 1997; Kong et al. 2002). Another clone was "*Natronohydrobacter*" an unidentified strain commonly found in alkaline saline environments (Humayoun et al. 2003; Mesbah et al. 2007).

In the MEDIUM soil, *Ochrobactrum grignonense*, an aerobic bacteria isolated from soil samples (Lebuhn et al. 2000), and *Pedomicrobium* and *Hyphomicrobium*, found in aquatic environments (Ridge et al. 2007), are appendaged bacteria which reproduce by budding and have a dimorphic life cycle involving non-motile prosthecate mother cells and motile swarmer cells (Rainey et al. 1998). These bacteria play an important role in the bacterial community structure of activated wastewater sludge, and novel species have recently been isolated from soil, swamp sludge and

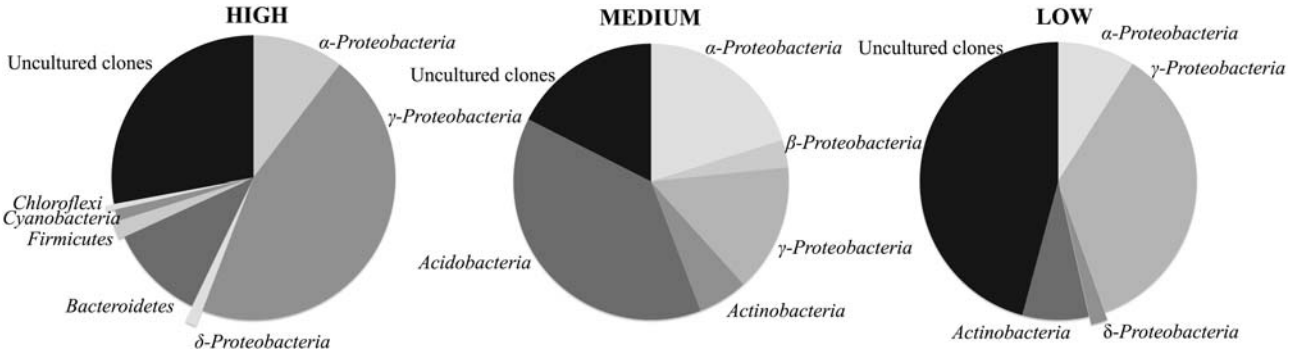
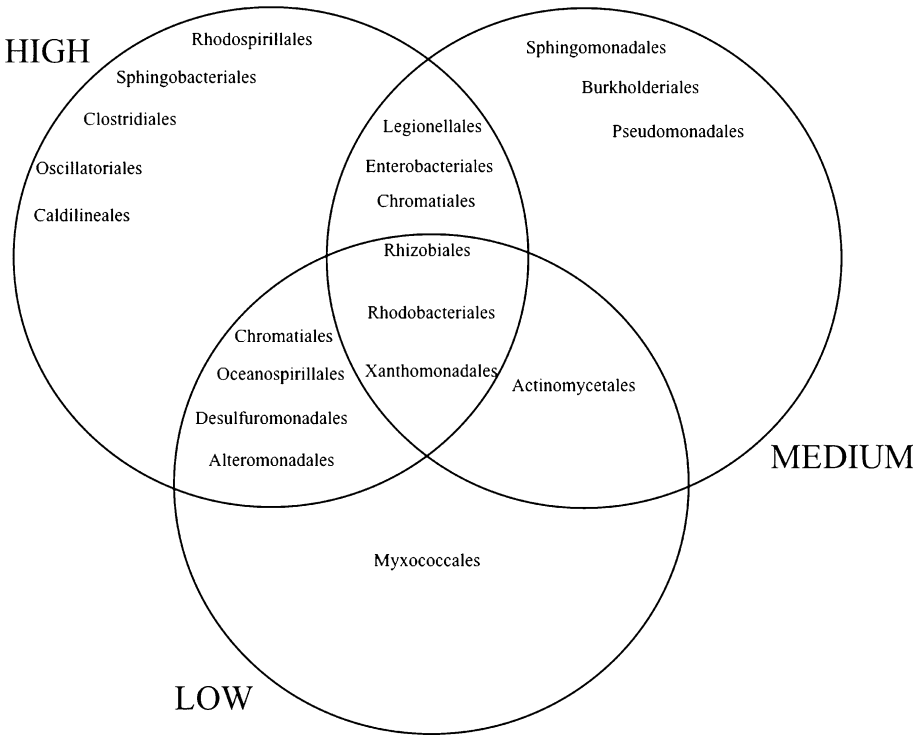


Fig. 4 Comparison of the bacterial community composition in three soils collected from lower (LOW), middle (MEDIUM) and upper (HIGH) salinity and alkalinity as revealed by 16S rDNA library. Sectors indicate the relative percentage calculated from the total

Fig. 5 Comparison of the distribution of bacterial orders in three soils collected from lower (LOW), middle (MEDIUM) and upper (HIGH) salinity and alkalinity as revealed by 16S rDNA library



freshwater (Xie and Yokota 2004). *Silicibacter* are moderately halophilic bacteria isolated from a silica-rich geothermal lake and seawater (González et al. 2003). No information is available in the literature about the genus *Kaistobacter*, but it has been found by 16S rRNA gene based phylogenetic analysis in soil samples and from a radioactive site (Asker et al. 2007; Bernard et al. 2007; Shrestha et al. 2007). *Sphingomonas wittichii* was isolated from river water and *Sphingomonas melonis* is phytopathogenic (Yabuuchi et al. 2001; Buonaurio et al. 2002).

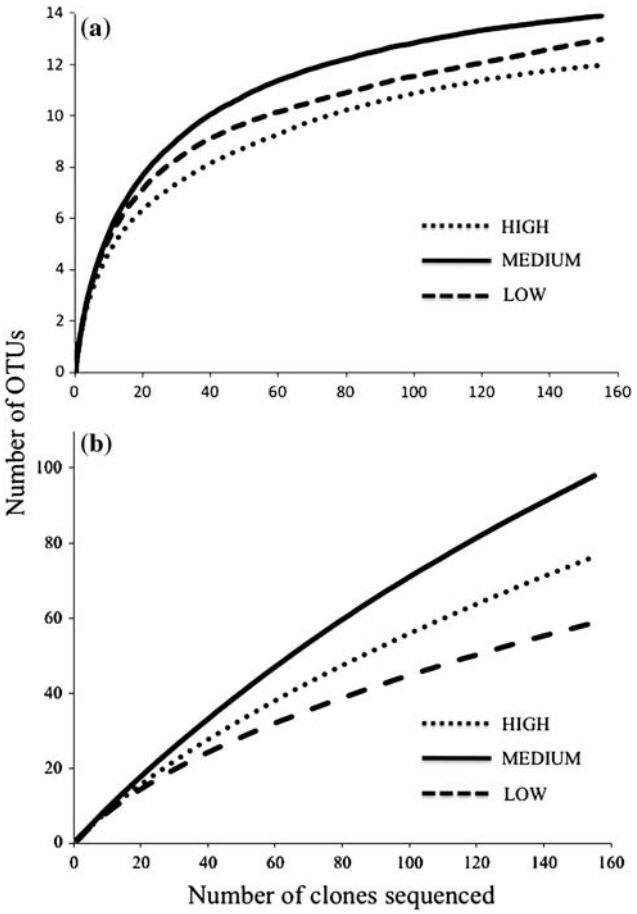


Fig. 6 Rarefaction curves at **a** phylum (distance = 0.20) and **b** species level (distance = 0.02). Bacterial richness in HIGH, MEDIUM and LOW soils is indicated by the slopes in of rarefaction curves

In the LOW soil, *Mesorhizobium* are bacteria isolated from soil and rhizosphere (Kaneko et al. 2000). They form nitrogen-fixing symbioses with leguminous plants. *Paracoccus* is a member of the order Rhodobacterales endowed with substantial metabolic versatility (La et al. 2005).

Firmicutes were only found in the LOW soil. Bacteria from genus *Alkaliphilus* are anaerobic and alkaliphilic heterotrophs isolated from methanogenic environments, deep mines and leachate ponds (Takai et al. 2001; Cao et al. 2003; Ye et al. 2004). Low and high abundancies of *Firmicutes* have been reported in similar saline environments (Humayoun et al. 2003; Ma et al. 2004a, b; Abed et al. 2007; Jiang et al. 2007).

Bacteroidetes were only found in the LOW soil. Seventeen clones were identified, belonging to bacterial groups of remarkable phenotypic diversity, with aerobic or anaerobic lifestyles, living in ecosystems as diverse as those of the oral cavity, gut, soil or aquatic systems (Vingadassalom et al. 2005). *Bacteroidetes* together with *Proteobacteria* were frequently found to be the dominant bacterial phyla in marine ecosystems (Stevens et al. 2005).

Actinobacteria were only found in the MEDIUM and LOW soils. *Actinobacteria* include some of the most common soil bacteria, playing an important role in decomposition of organic materials, such as cellulose.

Acidobacteria were only found in the MEDIUM soil. They have often been found in molecular ecological studies of soils. More than 30% and even 50% of the sequences obtained in 16S rDNA clone libraries from soil belonged to this phylum (Quaiser et al. 2003). These bacteria might thus be important contributors to ecosystems functioning (Eichorst et al. 2007).

Effect of salinity on community composition

In the HIGH soil, the dominant populations belonged to the α-, γ-*Proteobacteria*, bacteriodetes and some to the Cyanobacteria. β-*Proteobacteria* were found in MEDIUM soil.

Table 2 Diversity and predicted richness of LOW, MEDIUM and HIGH from the former lake Texcoco as estimated by the Shannon diversity index and Chao1 and ACE richness estimators computed using DOTUR

Site	Number of clones sequenced	Distance	Number of of OTUs[a]	Chao1 value	ACE value	Shannon index
LOW	155	0.2	59	101	143	3.52
		0.02	13	19	17	1.92
MEDIUM	161	0.2	101	239	227	4.34
		0.02	14	14	15	2.05
HIGH	156	0.2	77	183	224	3.85
		0.02	12	12	14	1.70

[a] Operational taxonomic units

Benlloch et al. (2002) reported similar results for lakes with 22 and 8% salinity. Changes in salinity might affect microorganisms in two different ways. They adapt to the changed salinity or they are replaced by microorganisms that are adapted to the changed conditions (Wu et al. 2006). Jiang et al. (2007) reported a combination of both mechanisms as found for soil of the former lake Texcoco. The phylotype replacement mechanism appears to operate at the phylum level as changes of major phylum/groups were observed with increased salinity. However, at the species-level, gradual evolution and adaptation appears to take place. Jiang et al. (2007) found multiple clones and isolated sequences from both low- and high-salinity samples clustered together that were closely related to the genus *Halomonas*. Many species within this genus have a wide range of salinity tolerance. Different species of *Halomonas* were found in soil of the former lake Texcoco. On the average, members of the phylum *Actinobacteria* make up 13% of the soil bacterial community (Janssen 2006). The amount of *Actinobacteria* was 12% in the MEDIUM and LOW soils, but the high salinity and alkalinity strongly inhibited them as none were found in soil the HIGH soil.

The amount of clones used was sufficient to reveal all phyla present in the samples, but not the amount of species as indicated by the rarefaction curves (Mohamed et al. 2008). Further sampling from the other three libraries may have revealed more diversity at the species level (Mohamed et al. 2008). Values for number of OTU's, Chao1, ACE and Shannon index indicated a greater diversity and richness in the MEDIUM soil than in the HIGH and LOW (Table 2). This could be explained by the fact that bacteria adapted at both high and low salinity could be found in soil with medium salinity.

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