Curr Microbiol (2011) 62:209–221
DOI 10.1007/s00284-010-9692-4

Bacterial Diversity in the Haloalkaline Lake Elmenteita, Kenya

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Received: 15 January 2010 / Accepted: 6 June 2010 / Published online: 27 June 2010

Abstract Lake Elmenteita is one of the alkaline saline lakes within the Kenyan Rift valley. The lake is situated on the floor of the Kenyan Rift Valley at 1,776 m above sea level and has no direct outlet. The microbial diversity of the lake was investigated using a culture-independent approach. Five different sampling points were selected randomly within the lake. Wet sediments and water samples were collected from each sampling point. In addition, dry mud cake was collected from three points where the lake had dried. DNA was extracted from the samples and the 16S rRNA genes amplified using universal primers for Bacteria. Thirteen clone libraries were constructed using the PCR amplified 16S rRNA genes. A total of 1,663 clones were picked. Representative clones were selected using ARDRA technique for sequencing. 655 partial and non-chimeric clone sequences indicated the presence of 37 orders in the Domain Bacteria. Cyanobacteria were the most abundant clones in terms of numbers whereas members of the phylum Firmicutes group were the second in terms of numbers but the most diverse in terms of genera represented. All clones affiliated to the class *Betaproteobacteria* originated from DNA obtained from the water samples. Analysis using BLAST showed that 93.1% of the sequenced clones had similarity values below 98% to both cultured and as yet uncultured bacteria, resulting in 596 phylotypes. Therefore, it can be concluded that Lake Elmenteita harbours phylogenetically diverse groups of bacteria involved in complex metabolic interactions within the Lake's ecosystem.

Introduction

The soda lakes environments are extremely productive because of high ambient temperatures, high light intensities and unlimited supplies of CO_2 [1]. They are also regarded as naturally eutrophic reservoirs hence they feature considerable microbial diversity [2]. From culture-dependent studies, the soda lakes of the East African Rift Valley have been shown to support a dense and diverse population of aerobic, organotrophic, halophilic, alkaliphilic and alkalitolerant representatives of major bacterial and archaeal phyla [2–5]. A combination of both culture-dependent and culture-independent methods has been used previously to document the microbial diversity of Lake Magadi [6] and some other lakes [7]. Results from the culture-independent studies on Lake Elmenteita pointed towards an underestimation of the microbial diversity [7] when compared to other well-studied lakes such as the Wadi al Natrun in Egypt [8]. Like other environments, Lake Elmenteita has been adversely affected by climatic changes and long periods of drought have led to fluctuation in the water levels. In ecological terms Lake Elmenteita serves as an important refuge for vast flocks of flamingos during the dry seasons. The aim of this study was to assess the microbial diversity of Lake Elmenteita using a culture-independent approach.

Electronic Supplementary Material The online version of this article (doi:10.1007/s00284-010-9692-4) contains supplementary material, which is available to authorized users.

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Materials and Methods

Study Site

Lake Elmenteita is situated 0°27′S, 36°15′E on the floor of the Kenyan Rift Valley at 1,776 m above sea level and has no direct outlet [9]. The region is characterized by a hot, dry and semi-arid climate with a mean annual rainfall of about 700 mm. Due to the high temperatures; there are very high evaporation rates during the drier seasons leading to a reduction in the total surface area. The present size of Lake Elmenteita is roughly 20 km^2 and the depths rarely exceed 1.0 m According to Mwaura [10] the water temperature ranges between 30 and 40°C, the alkalinity of the water is high (1,200 mg $CaCO_3$/l) and the pH is above 9 with a high concentration of carbonates, chlorides and sulphates.

Sampling Site Description and Sample Collection

The sample site, sampling and DNA extraction are described elsewhere [11].

PCR Amplification

Almost full-length 16S rDNA bacterial genes were amplified using the primers bac8f (5′-AG(A/G)GTTTGAT CCTGGCTCAG-3′) and bac1492r (5′-CGGCTACCTTGT-TACGACTT-3′). These primers for PCR amplification of bacteria were based on the fD1 and rP1 primers described by Weisburg et al. [12] and modified by Sørensen et al. [13]. PCR cycling consisted of a 3 min initial pre-incubation step at 94°C followed by 28 cycles of a denaturation step at 93°C for 1 min, a 1 min annealing step at 58°C, and a 1 min elongation step at 72°C and a final extension step at 72°C for 5 min. The PCR mix consisted of 5 µl of 10× PCR buffer [100 mM Tris–HCl (pH 9), primers at a concentration of 0.5 mM, each deoxynucleoside triphosphate at a concentration of 200 mM, 2.0 mM $MgCl_2$, 20 ng of bovine serum albumin (BSA), 0.5 µl of template DNA, and 2.5 U of Taq DNA polymerase (Roche). The volume was adjusted to a final volume of 50 µl with sterile MQ water. The presence of PCR products and their concentration were determined by analysing 5 µl of product on 2% agarose gels after staining with ethidium bromide. The products were compared with a molecular weight marker (Smartladder; Eurogentec). The ladder has a molecular weight ranging from 200 to 10,000 bp and is used for easy quantification as well as size determination of DNA.

Clone Library Construction and ARDRA Screening

These methods were done as previously described [11]. The presence of clone inserts with the expected size was determined by PCR using the primers M13F (5′-GTA AAACGACGGCCAG-3′) and M13R (5′-AGGAAA-CAGCTATGAC-3′). These primers flank the cloning site on the vector. The PCR products were checked on 1% agarose gel. Further screening of PCR fragments was done via ARDRA to determine unique pattern profiles, representatives clones of which were selected for subsequent sequence analysis. A double digestion was done using the restriction enzymes *Eco*NI and *Stu*I (New England Biolabs [NEB], Beverly, Mass.). The fragments were then separated on 2% agarose gel at 37°C for 2 h and 100 V. Representative profiles were picked and the PCR amplicons purified with QIAquick® spin columns (Qiagen, Hilden, Germany).

Sequencing

Partial sequences were generated using the primer 518r which targets one of the conserved regions. The reads were manually edited and the sequence data were BLAST (www.ncbi.nlm.nih.gov/BLAST/) analyzed against the GenBank 16S rRNA database. The sequences were then aligned using the CLUSTAL W program against the nearest neighbours [14], and checked for chimeric structures by using the Mallard program [15]. Phylogenetic relationship of the partial sequences was determined using neighbour-joining [16] and maximum-likelihood analyses [17]. These analyses were conducted in MEGA 4 [18]. The evolutionary distances were computed using the Maximum Composite Likelihood method [19]. The resultant tree topologies were evaluated in bootstrap analyses of the Neighbour-joining method based on 1,000 re-samplings [20]. Only representative partial sequences are indicated in dendrograms.

Nucleotide Sequence Accession Numbers

A total of 560 clone sequences that could be properly aligned to the closest neighbours without distorting the phylogenetic trees were deposited in the GenBank under accession numbers FJ764199–FJ764759.

Results

A total of 1,663 clones were picked (sediment samples: 694 clones; water: 644 clones; dry mud: 325 clones). All the clones from sampling sites 1 and 2 were sequenced in order to obtain high coverage of the bacterial diversity. In addition, ARDRA was done for the samples from sites 1 and 2 and these patterns were used as a reference in selecting representative ARDRA profiles from most of the other sites for subsequent clone sequence analyses. In total

Table 1 Summary of phylotypes represented in the 16S rRNA gene clone library

Phyla/class	Order	Dry mud	Sediment	Water
Cyanobacteria	*Chroococcales*	+	+	+
	Nostocales		+	+
	Oscillatoriales	+	+	+
	Stigonematales	+	−	−
Firmicutes	*Bacillales*	+	+	−
	Clostridiales	+	+	+
	Dethiobacter	+	+	−
	Thermoactinomycete	−	+	−
	Thermoanaerobacteria	−	+	−
	Lactobacillales	+		−
Alphaproteobacteria	*Rhizobiales*	+	+	−
	Rhodobacterales	+	+	+
	Spingomonadales	−	+	+
Betaproteobacteria	*Burkholderiales*		−	+
	Methylophilales	−	−	+
Gammaproteobacteria	*Chromatiales*	+	+	+
	Methylococcales	−	+	−
	Thiotrichales	+	+	+
	Xanthomonadales	−	+	−
Deltaproteobacteria	*Bdellovibrionales*	+	+	+
	Desulfovibrionales	−	+	−
	Desulfuromonadales	−	+	−
	Myxococcales	−	+	−
Bacteroidetes	*Bacteroidales*	+	+	+
	Flavobacteriales	+	+	+
	Sphingobacteriales	+	+	+
Chlorobi	*Chlorobiales*	+	+	−
Planctomyces	*Planctomycetales*	−	+	+
Acidobacteria	*Acidobacateriales*	−	+	−
	Solibacteriales	+	+	−
Actinobacteria	*Actinomycetales*	+	+	+
Chloroflexi	*Chloroflexales*	−	+	−
Deinococcus- Thermus	*Deinococcales*	−	+	−
Aquificae	*Aquificales*	+	+	−
Gemmatimonadetes		−	−	+
Spirochetes	*Spirochete*	+	−	−
Verrucomicrobia	*Verrucomicrobiales*	−	+	−
	37	20	30	17

875 clones were selected. Supplementary Table S1 gives a summary of the number of clones picked per site, selected ARDRA profiles that were sequenced per site and the number of non-chimeric sequences per site. Supplementary Fig. S1 shows an example of the range of ARDRA pattern diversity for clones from sample site 2 (ES2-065 to ES2-080), resulting in the formation of 11 unique pattern.

A total of 655 clones gave non-chimeric partial sequences and the affiliation of these clones to different orders of the Domain Bacteria is shown on Table 1. Analysis of the sequences via BLAST showed that 525 (80.15%) sequences were related to as yet uncultured members as recorded in the BLAST database. About 75% of the 655 clones had similarity values between 80 and 97% to both cultured and as yet uncultured microorganisms. Eighty-five clones (13%) had a similarity value of 98%, whereas only 45 clones (6.9%) had similarity values between 99 and 100% to cultured and uncultured

microorganisms, indicating that they may be affiliated to described species. The four dominant phyla determined by in the clone library analyses were the Cyanobacteria (24.4% of sequences), Firmicutes (18.4%), Proteobacteria (16.8%) and Bacteroidetes (17.4%).

A few unique groups were site-specific: clones only retrieved from the hot spring sampling site 2 include members of the orders *Methylophilaceae* (13 clones), *Polyangium* (1 clone), *Thermosynechococcus* (14 clones), Chloroflexi (1 clone) and from the phylum Acidobacteria (7 clones). From water sample W2, only clones related to *Limnothrix* (5 clones) were retrieved. All the 13 clones related to the phylum Actinobacteria were from water of sampling site 1. All the other clones were retrieved from two or more samples.

Predominance of Different Groups

The Phylum Cyanobacteria

As judged from the 160 sequences affiliated to Cyanobacteria, these aerobic phototrophs comprise the most abundant group of microorganisms within the soda lake environment. Four orders of Cyanobacteria were represented with clones originating from members of the genus *Synechococcus* being the most common (Table 2). Cyanobacteria groups reported so far from Lake Elmenteita include *Arthrospira* spp., *A. fusiformis*, *A. arnoldii*, *Synechococcus* spp., *Synechocystis* spp., *Spirulina subtilissima*, *Spirulina subsalsa* and *Pseudanabaena* spp. ([4, 5, 9, 21–24], respectively). Except for the latter taxon members of these genera were also retrieved in this study which increased the range of diversity significantly by reporting the presence of additional 11 genera. Figure 1 shows the phylogenetic affiliation of representative clones to other groups in the phylum.

Table 2 Cyanobacterial groups represented in the 16S rRNA gene clone library for Lake Elmenteita

Order	Genus	Dry mud	Sediment	Water	Total
Chroococcales	*Cyanobium*	–	–	2	2
	Gloeothece	–	1	–	1
	Microcystis	–	–	1	1
	Synechococcus	3	8	75	86
	Synechocystis	–	–	3	3
Nostocales	*Anabaena*	–	1	2	3
	Nodularia	–	–	2	2
	Nostoc	–	1	5	6
	Calothrix	–	3	4	7
Oscillatoriales	*Arthrospira*	–	3	–	3
	Leptolyngbya	–	14	1	15
	Oscillatoria	6	–	–	6
	Spirulina	1	18	–	19
	Trichodesmium	–	4	1	5
Stigonematales	*Stigonema*	1	–	–	1

The Phyla Firmicutes, Spirochetes, Actinobacteria and Planctomyces

Of the 121 clones affiliated to the phylum Firmicutes, the majority of 85 clones were affiliated to the *Clostridiales*. Also present were clones from the orders *Lactobacillales* (15 clones), *Dethiobacter* (8 clones), *Bacillales* (7 clones), *Thermoactinomycete* (3 clones) and *Thermoanaerobacter* (3 clones). Clones belonging to the order *Clostridiales* were found to be related to the genera *Alkaliphilus*, *Anoxynatronum*, *Clostridium* and *Sporacetigenium*; the closest affiliates of clones were in most cases clones from diverse environments (Fig. 2b). Four clones related to the genus *Bacillus* (similarity values 88–98%) and were from hotspring sediment samples and one clone (ED3-020) was from a dry mud sample. Low G+C isolates from soda lake environments are represented especially by *Bacillus alcalophilus* [25] and *Bacillus clarkia* group, [3]. Clones related to *Alkalibacteria* were similar to *Alkalibacterium olivapovliticus* and clone ED4-015 was related to Isolate WN16 from the geographically neighbouring Lake Nakuru (Fig. 3)

Only two clones belonging to the phylum Spirochetes were detected and they were related to a uncultured spirochete clone WN-FWB from the Wadi An Natrun, Egypt [8]. *Spirochaeta halophila*, a facultative anaerobe, was also previously detected in clone libraries of Wadi Al Natrun, Egypt [8]. The species was fist isolated from lake Eilat in Israel. This indicates that members of this species are present in the soda lakes and probably participate in the sulphur cycle through anaerobic sulphate reduction [26].

Thirteen clones were affiliated to uncultured members of the family *Microbacteriaceae* in the Actinobacteria. All these clones were affiliated to as-yet uncultured bacteria. Within the Planctomyces, 18 clones were retrieved. Five of the clones aligned to the *Pirellula* group, eight to the *Blastopirellula* and one clone formed a deep branch within the *Rhodopirellula*. Clone EW-053 was affiliated to Planctomycete str. 449 [27]. Two clones (ES1-102 and EW2-020) were not affiliated to any cultured planctomycete. Clone ES2-165 was related to a clone retrieved from the Yellowstone National Park.

The Class Alphaproteobacteria

A total of 44 clones were affiliated to members of the class *Alphaproteobacteria.* The most common representatives of

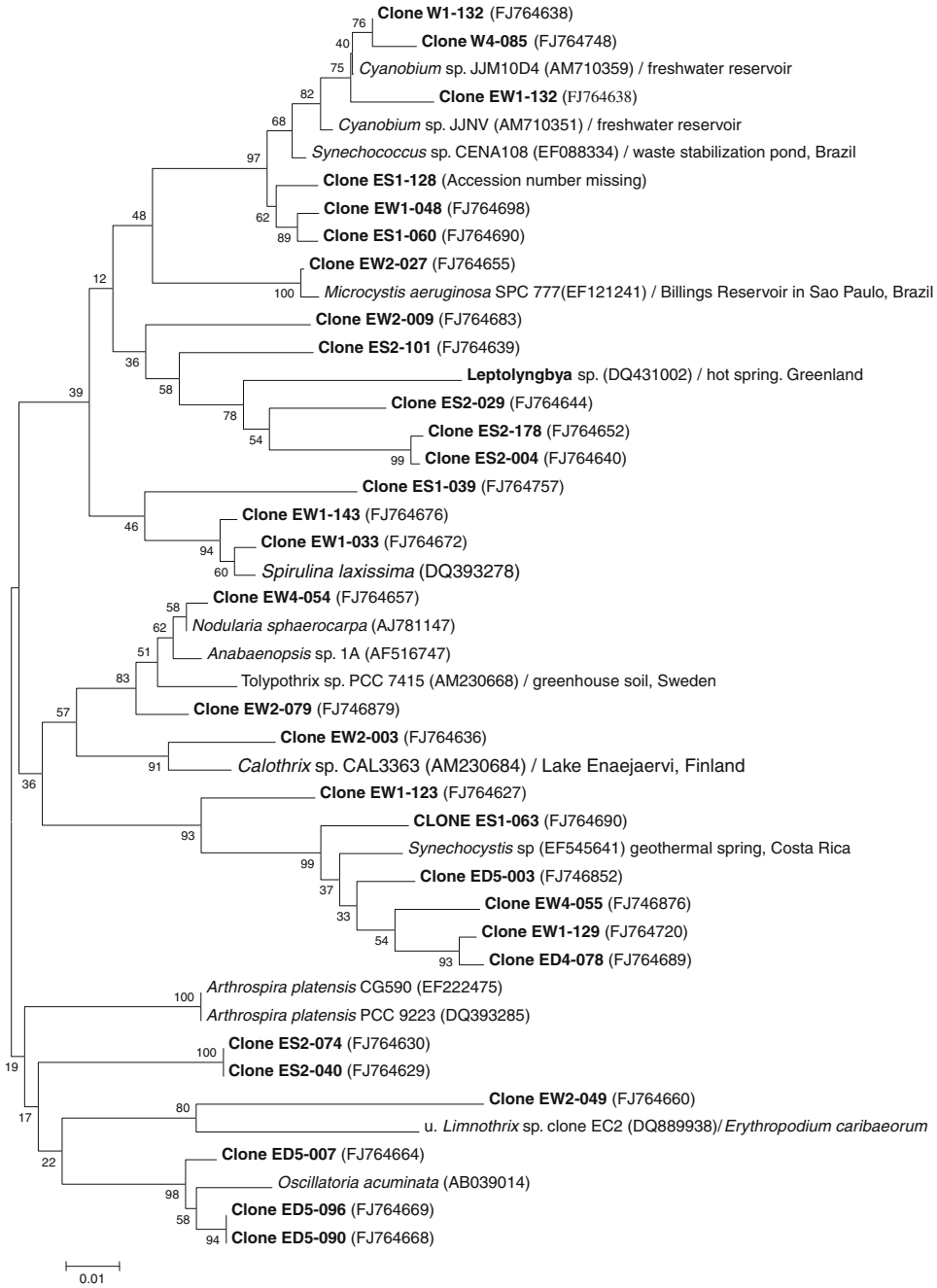

Fig. 1 16S rRNA gene sequence relationships of taxa in the phylum Cyanobacteria. Clones from Lake Elmenteita are indicated in bold font. *ES* wet sediment, *EW* water, *ED* dry mud, *U* uncultured

this group are related to members of the genera *Rhodobaca* and *Rhodobacter*. 17 clones were related to *Rhodobaca bogoriensis*. Ten clones were related to *Rhodobacter*, six of them aligning to uncultured members of this genus. Other genera represented include *Mesorhizobium* (clone ES1-022), *Rhodoblastus* (clone ES1-09), *Methylobacterium* (clone ED5-027), *Erythrobacter* (clones EW4-036 and EW2-036). The genera *Sphingomonas*, *Rubrimonas* and *Sandracinobacter* were represented by one clone each. Four clones affiliated to the *Methylococcales* were retrieved from the wet sediments. Several clones in this group especially those from the hotspring (sampling site 2) show phylogenetic affiliation to phylotypes retrieved from similar environments around the world (Fig. 4).

The Class Betaproteobacteria

A total of 38 clones were affiliated to members of the class *Betaproteobacteria* (Fig. 5). All the clones in this group were from the water samples. BLAST analysis showed that

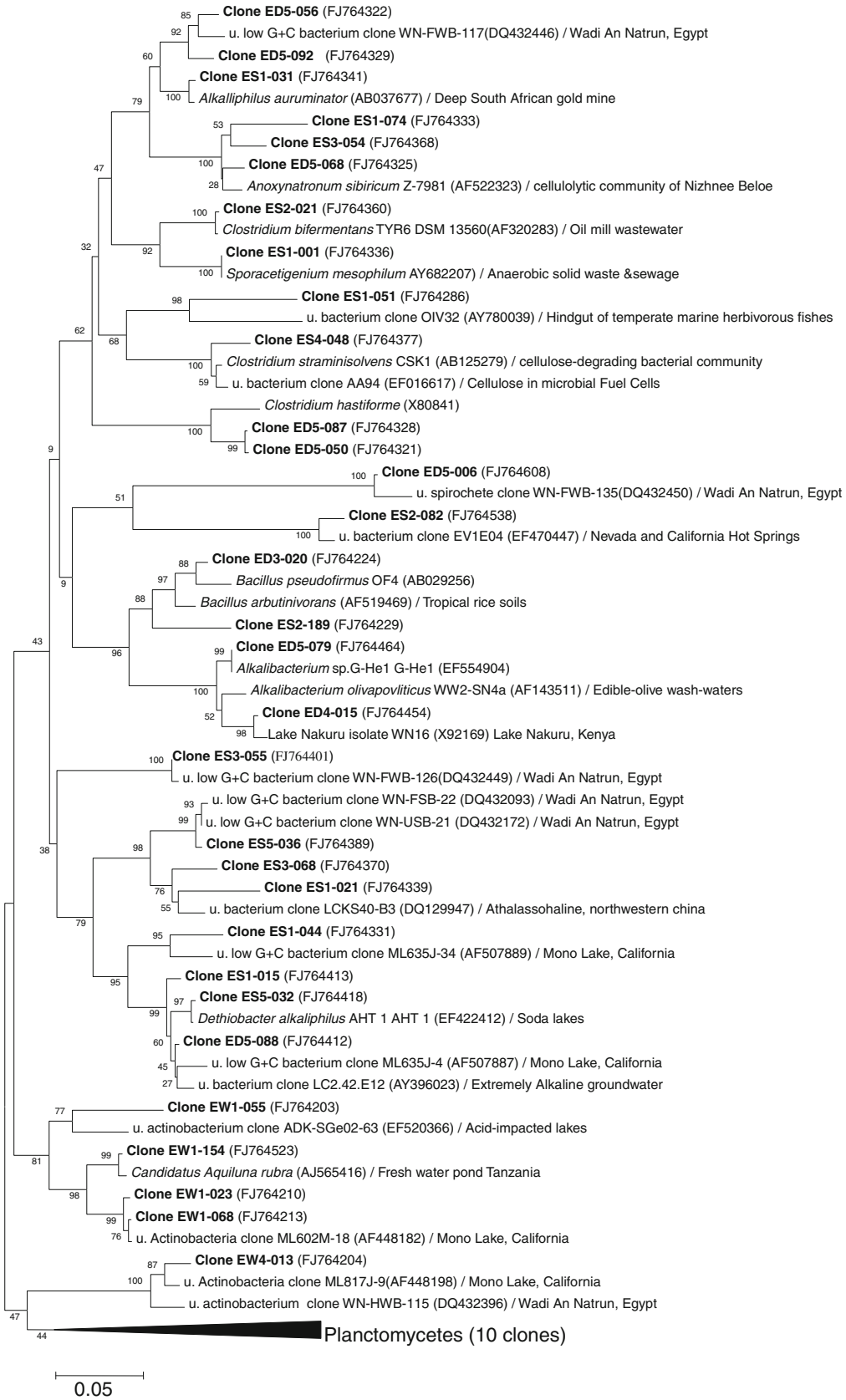

Fig. 2 16S rRNA gene sequence relationships of taxa in the phyla Firmicutes and Actinobacteria. Clones from Lake Elmenteita are indicated in bold font. Abbreviationsas in legend to Fig. 1

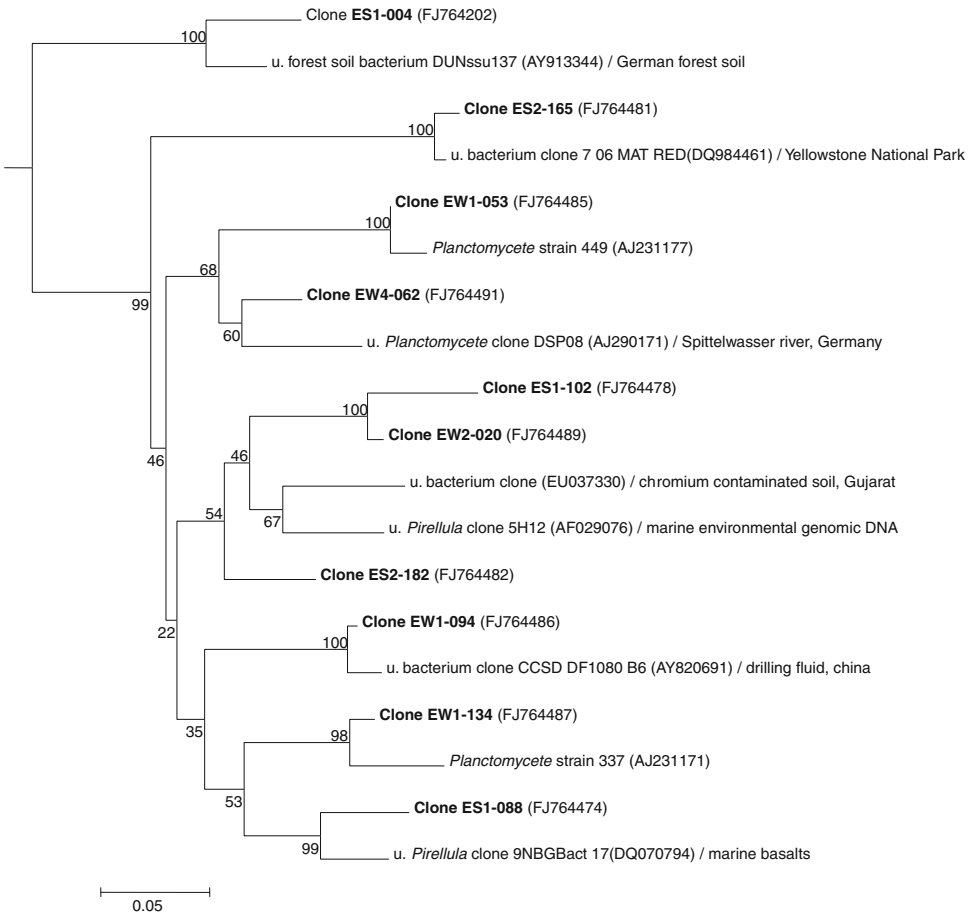

Fig. 3 16S rRNA gene sequence relationships of taxa in the phylum Planctomyces. Clones from Lake Elmenteita are indicated in bold font. Abbreviations as in legend to Fig. 1

two clones from the sampling site 2 were similar to uncultured members of the genus *Macromonas* [28]. Seventeen clones were affiliated to uncultured members of *Hydrogenophaga.* Clone EW2-021 was found to be affiliated to *Aquabacterium citratiphilum* [29]. Others clones were affiliated to the genera *Alcaligenes* and *Lautropia* and the family *Methylophilaceae* was represented by six clones all affiliated to uncultured members of the genus.

The Class Gammaproteobacteria

A total of 20 clones representing various groups were retrieved (Fig. 6). The order *Xanthomonadales* was represented by three clones affiliated to as yet uncultured bacteria. Other genera detected were *Nitrosococcus* (7 clones) and *Thioalkalivibrio* (1 clone). Four clones affiliated to the family *Ectothiorhodospiraceae* were identified. Clone ED5-037 was closely related to *Ectothiorhodospira shaposhnikovii* while clone EW4-003 and EW2-059 were related to *Ectothiorhodosinus mongolicum* isolated from a Mongolian soda lake. Three clones from the hotspring samples were related to *Thiothrix nivea* [30]. This genus comprises filamentous sulphide oxidizing bacteria.

The Class Deltaproteobacteria

Eight clones were affiliated to members of the class *Deltaproteobacteria* (Fig. 6). Clone S2-056 from the hotspring sample was closely affiliated to *Polyangium*. Other taxa include *Bacteriovorax* (EW4-075), *Desulfobulbus* (ED5-036) and *Desulfosarcina* (ES1-119). Clone ES1-134 was closely affiliated to *Desulfonatronum cooperativum* (50) and *D. thiodismutans* [31], the latter being a sulphate reducing bacteria isolated from Mono Lake California. Three clones were affiliated to unclassified Deltaproteobacteria.

Phylum Bacteroidetes, Chloroflexi and Chlorobi

Hundred and fourteen clones belonging to the phyla Bacteroidetes, Chloroflexi and Chlorobi were retrieved (Fig. 7). The *Chitinophaga-Flexibacter* clone sequences were from sediment sample, site 1. All clones in the family

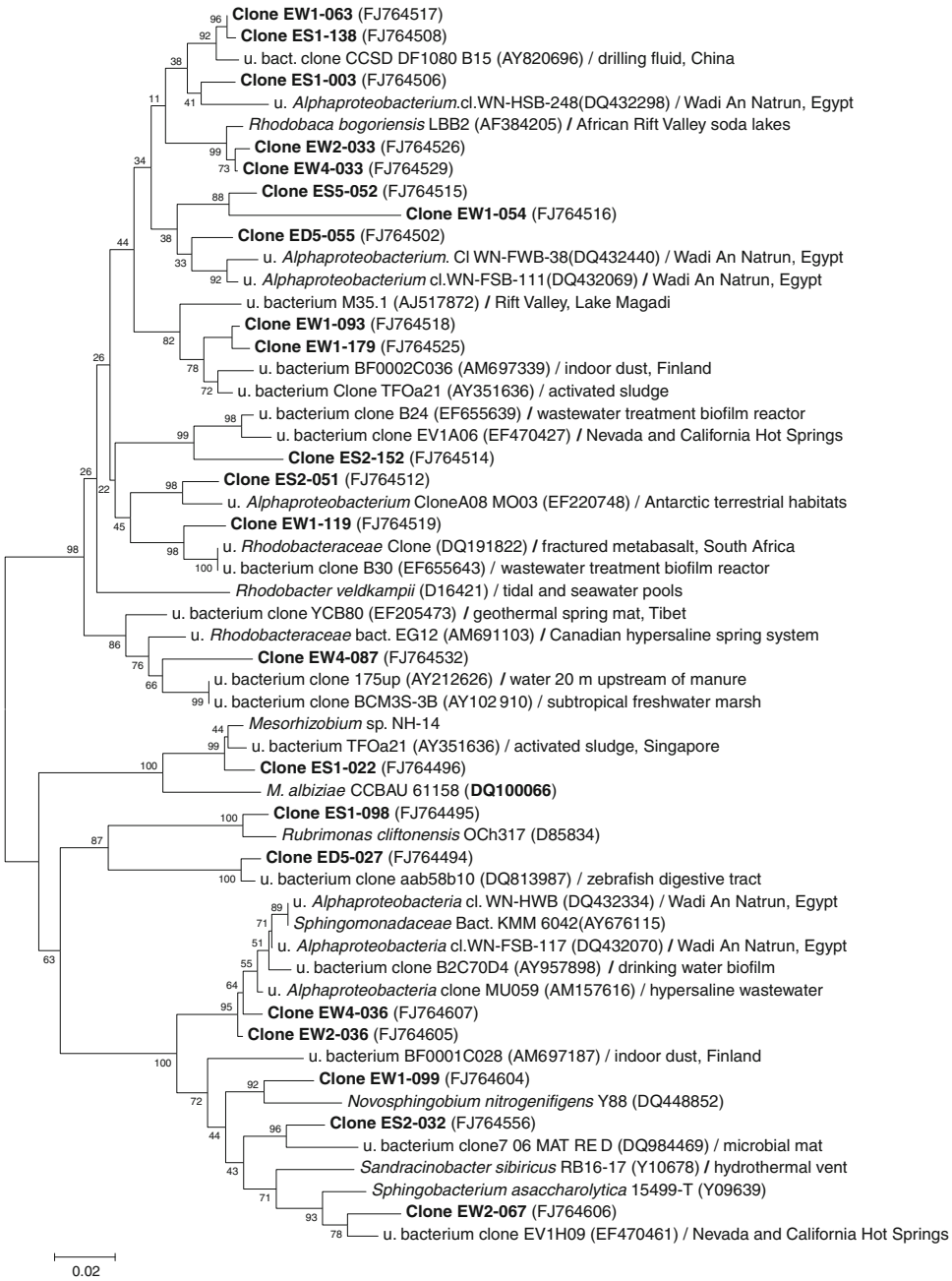

Fig. 4 16S rRNA gene sequence relationships of taxa in the class *Alphaproteobacteria*. Clones from Lake Elmenteita are indicated in bold font. Abbreviations as in legend to Fig. 1

Saprospiraceae aligned to members of *Haliscomenobacter* and all of them were from the hot spring sediment sample. Three clones from the hot spring were affiliated to *Sphingobacteriaceae*. Eight clones from the *Saprospiraceae*, four from *Lewinella* and seven from the phylum Chlorobi formed distinct clades which were only distantly related to other members of their respective groups. Phylogenetic analysis reveals similarity in the habitat from which the clones were retrieved. Three clones were affiliated to Chloroflexi group. "Candidatus Chlorothrix halophilus" is an obligately anaerobic sulphide-dependent phototrophic green nonsulfur bacterium that was cultured from a hypersaline microbial mat [32].

Discussion and Conclusion

The results from this study suggest that the bacterial diversity within Lake Elmenteita is significantly higher than reported before [7]. This is confirmed by the finding that of the 655 clones sequences selected for analysis, 525 (80.2%) were more closely related to uncultured members

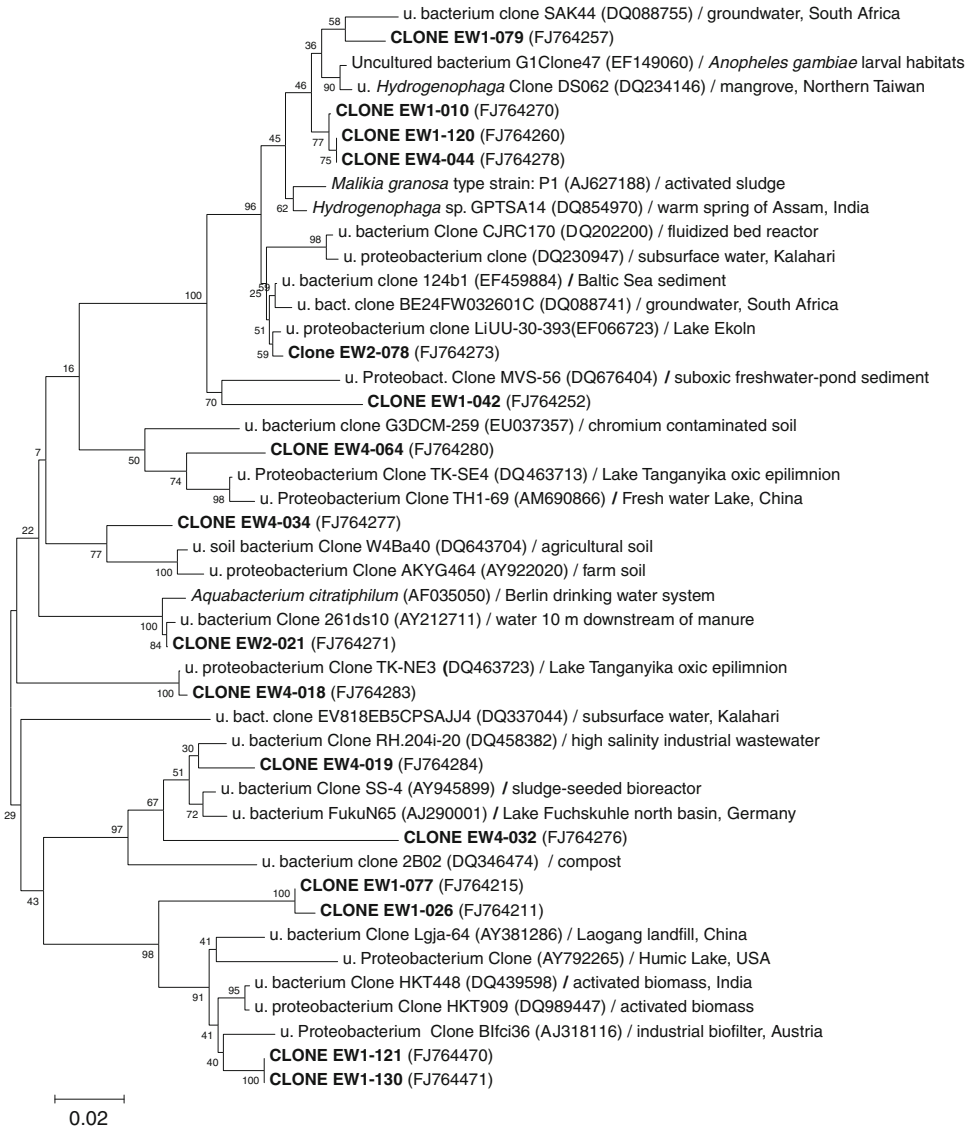

Fig. 5 16S rRNA gene sequence relationships of taxa in the class *Betaproteobacteria*. Clones from Lake Elmenteita are indicated in bold font. Abbreviations as in legend to Fig. 1

of the Domain Bacteria than to described species. Interesting is the occurrence of clones representing members of the class *Betaproteobacteria* which have not been previously reported from the East African soda lakes [7] or the other soda lakes such as Wadi al-Natrun in Egypt [8]. The functional role of members of this phylum in the soda lake environment needs to be further investigated preferably using a culture-dependent approach. The dominance of Cyanobacteria concurs with earlier reports that the less alkaline lakes such as Lake Elmenteita are usually dominated by dense blooms of Cyanobacteria while the hypersaline lakes occasionally support blooms of both cyanobacteria and alkaliphilic anoxygenic phototrophs belonging to the genera *Halorhodospira* and *Ectothiorhodospira* [4, 5, 21, 33, 34]. Members of the latter genus have also been identified, though in small numbers only.

Primary productivity is driven by several groups within the soda lake environment. The Cyanobacteria form the dominant group as shown by their diversity. Organic matter is also produced by anoxygenic phototrophic purple bacteria of the family *Ectothiorhodospiraceae* [2] as well as the order *Chromatiales* [35]. Eight clones affiliated to the order *Chromatiales* were retrieved from the water, dry mud and wet sediment samples. The alkaliphilic *Rhodobaca bogoriensis*, originally isolated from Lake Bogoria in Kenya, is capable of both phototrophic and chemotrophic growth and is a representative of purple nonsulfur bacteria described from soda lake environments [36]. The filamentous green nonsulfur bacteria are among the most abundant organisms in both oxic and anoxic layers of microbial mats from a variety of environments [37–40]. The occurrence of clones from both *Chlorobi* and

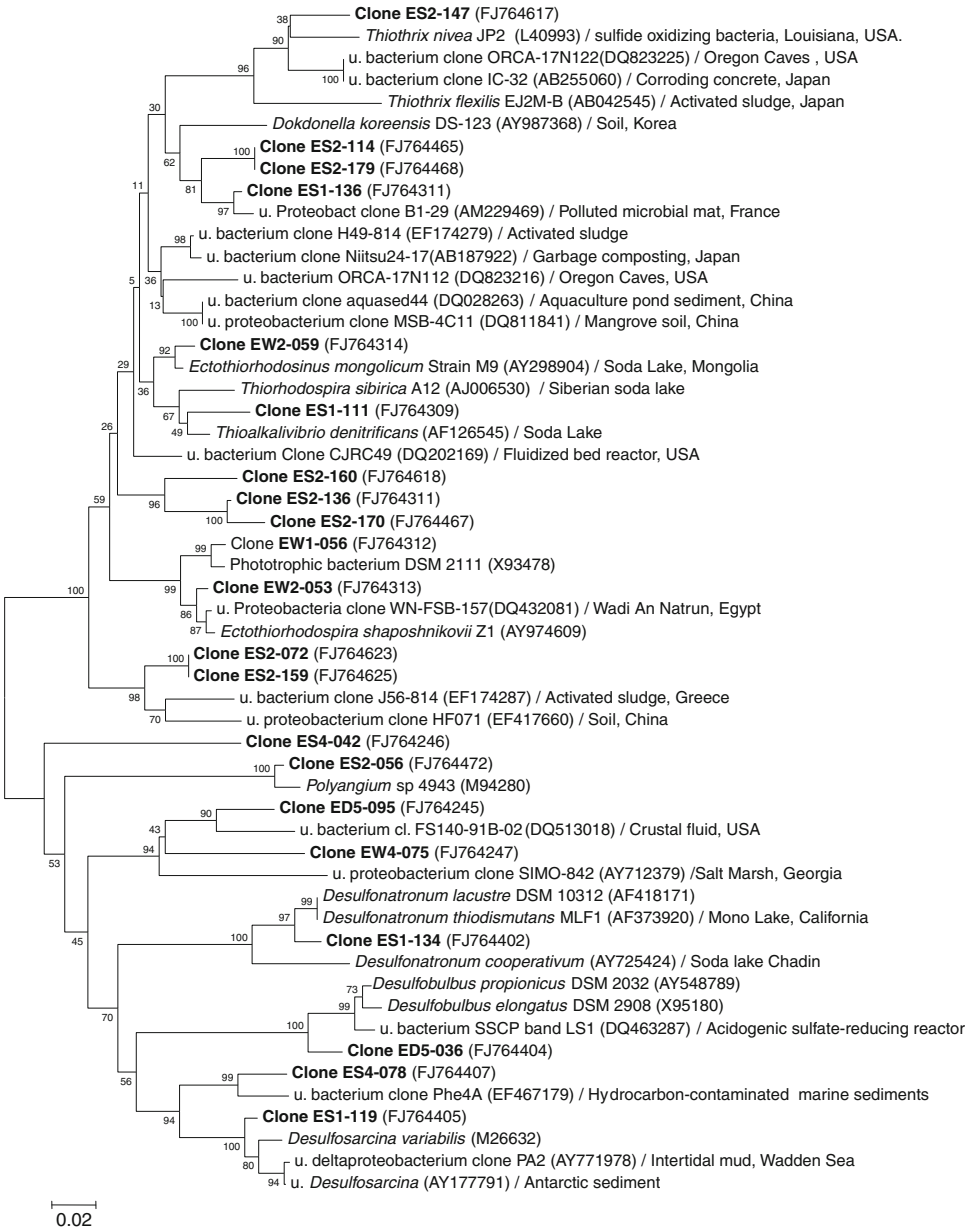

Fig. 6 16S rRNA gene sequence relationships of taxa in the class *Gammaproteobacteria* and *Deltaproteobacteria*. Clones from Lake Elmenteita are indicated in bold font. Abbreviations as in legend to Fig. 1

Chloroflexi shows that these groups are present and contribute to primary production in the Lake.

A number of aerobic chemoorganotrophic, alkaliphilic isolates obtained from several soda lakes in East Africa have been studied in detail [3, 41]. Organotrophic bacteria of the Actinobacteria phylum, i.e., *Bogoriella caseilytica* [42] and *Cellulomonas bogoriensis* [43] have also been described from Lake Bogoria in Kenya. Others are members of the genus *Dietzia natronolimnaea* [16], the genera *Arthrobacter* and *Terrabacter* [3] from Lake Oloiden, Kenya. The phylum Planctomyces probably contribute to the mineralization of organic material created by phototrophic primary producers. Saccharolytic spirochetes utilize sugars and a limited range of polysaccharides to produce acetate, lactate, ethanol and hydrogen under anaerobic conditions [2]. Two haloalkaliphilic strains *Spirochaeta alkalica* and *S. africana* have been isolated from Lake Magadi, and an alkaliphilic species *S. asiatica* from Lake Khatyn, Central Asia [44]. The *Bacilli* are aerobic or facultatively spore forming heterotrophs whereas clostridia are anaerobic fermentors and some groups are sulphate reducers. Other heterotrophic groups detected in the clone libraries were from the phylum Bacteroidetes. The orders *Sphingobacteriales* and *Flavobacteriales* consist mainly of aerobic heterotrophic organisms while strains of order *Bacteroidales* are all anaerobic [45, 46].

The occurrence of clones affiliated to the orders *Desulfobacterales*, *Desulfovibrionales* and *Desulfomonadales* and the genus *Desulfitibacter* confirms the fact that the microbial sulphur cycle seems to be one of the most active

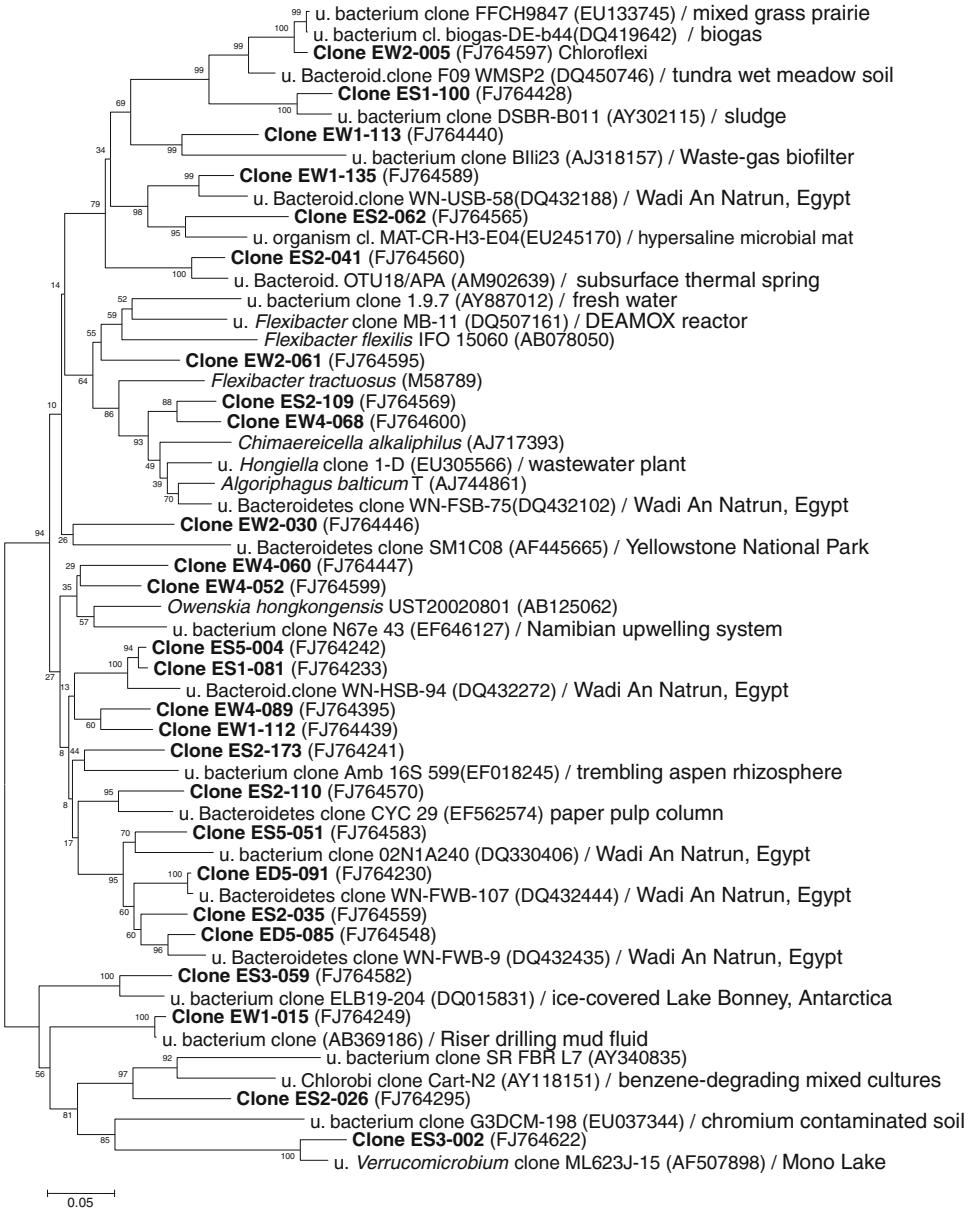

Fig. 7 16S rRNA gene sequence relationships of Taxa in the phylum Bacteroidetes, Chlorobi and Chloroflexi. Clones from Lake Elmenteita are indicated in bold font. Abbreviations as in legend to Fig. 1

in the soda lakes with anaerobic phototrophic purple sulphur bacteria and sulphate reducing alkaliphiles as the main actors [47]. It has been proposed that sulphate reduction is responsible for generating alkaline conditions as a result of transformation of sulphate to sulphide [2]. The genus *Methylobacterium* contains strictly aerobic, facultatively methylotrophic bacteria that are able to grow on C1 compounds more reduced than carbon dioxide as the sole carbon and energy sources [48]. In spite of the limitations of the molecular approach such as differential DNA extraction, primer selectivity, and variable rRNA gene copies [49], the approach remains as an effective method to identify the most prominent microorganisms in a natural environment. From the data obtained in this study it can be concluded that the microbial communities within the soda lakes are complex and major trophic groups are represented. Novel approaches for enrichment and isolation will further deepen our understanding of the roles played by the different groups within the ecosystem.

Acknowledgement This study was supported by DAAD within a PhD scholarship (Sandwich model). The work was done at the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen), Braunschweig.

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