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ORIGINAL PAPER

Isolation and characterisation of bacteria from the haloalkaline Lake Elmenteita, Kenya

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Abstract Culture-independent studies show that soda lake environments harbour diverse groups of bacteria and archaea. In this study different enrichment and isolation media were used in an attempt to isolate novel groups of bacteria from Lake Elmenteita. Different media were prepared using filter-sterilised water from the lake. The isolates recovered were purified on tryptic soy agar supplemented with 1% sodium carbonate and 4% sodium chloride. Phylogenetic analysis of 181 partial 16S rRNA gene sequences with excellent quality showed that the majority of the isolates were affiliated to the class *Gammaproteobacteria* and to the genus *Bacillus*. Isolates from the genus *Halomonas* and *Bacillus* constituted 37 and 31% of the total sequenced isolates, respectively. Other groups recovered were related to *Marinospirillum*, *Idiomarina*, *Vibrio*, *Enterococcus*, *Alkalimonas*, *Alkalibacterium*, *Amphibacillus*, *Marinilactibacillus* and the actinobacteria *Nocardiopsis* and *Streptomyces*. Fifty-one different genera were represented with 31 and 15 cultures scoring with their nearest neighbour similarities below 98 and 97%, respectively. Some novel taxa were identified which had not been isolated previously from the soda environment. The results show that the use of different media with varying compositions can help retrieve novel bacterial diversity from the soda lake environment.

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Introduction

Microbial isolates are important in expanding our understanding of bacterial physiology, genetics, and ecology (Palleroni 1997; Zinder and Salyers 2001). Most strains isolated from the Kenyan soda lakes belong to the class *Gammaproteobacterium*, including a large number of aerobic organotrophic strains of the halomonad lineage (Duckworth et al. 1996; Jones et al. 1994). For example, *Alkalimonas delamerensis* (Ma et al. 2004) and the cyanobacteria *Arthrospira fusiformis* and *A. abijatae* (Ballot et al. 2004) were isolated from Lake Elmenteita, while *Anabaenopsis arnoldii* was described in the phytoplankton communities of Lakes Nakuru and Elmenteita (Vareschi 1978; Melack 1988). Several alkaliphilic saccharolytic clostridia were reported to thrive in lakes Elmenteita, Bogoria and Magadi (Jones et al. 1998). Previous attempts at isolation have been based on the alkaline media described by Horikoshi (1991). However, as this medium is rich in organic carbon, heterotrophic microorganisms which grow faster most probably mask other slow growing organisms on a solid medium. The objective of this study was to enrich for and isolate novel groups from Lake Elmenteita for subsequent investigation of their biotechnological potential, using media prepared with lake water.

Materials and methods

Lake Elmenteita is situated at 0°27′S, 36°15′E on the floor of the Kenyan Rift Valley at 1776 m above sea level and has no direct outlet (Melack 1988). The region is

characterised 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 (1999) the water temperature ranges between 30 and 40°C, the alkalinity of the water is high (1200 mg $CaCO_3$/l) and the pH is above 9 with a high concentration of carbonates, chlorides and sulphates. The sampling sites for this study are described elsewhere (Mwirichia et al. 2009).

Enrichment and isolation of microorganisms

The media used for isolation were prepared with water collected from Lake Elmenteita. The water was filtered through a 0.45 μm and then through a 0.22 μm membrane filter (Whatman). The pH of the water from the lake was established to be 8.7. An enrichment strategy was established whereby 14 different liquid media were prepared using the filter-sterilised water collected from the lake. Five additional media were prepared targeting isolation of myxobacteria as described by Shimkets et al. (2006). The various media used in enrichment and isolation are shown in Table 1. Mud and water samples were collected from five different sampling points. Approximately 2 g of mud from each site was used to make a mastermix using filtered and sterilised 50 ml of water from the lake. An aliquot (100 μl) was used to inoculate 5 ml of each of the liquid media and grown for 24 h. These enriched samples were plated onto the respective solid media and allowed to grow for 24 h at 28°C. A second aliquot (100 μl) was plated directly onto solid media and allowed to grow for 48 h at 28°C. Strains obtained were purified on tryptic soy broth supplemented with 15 g Bacto agar (Difco), 3.5% NaCl and 1% $NaCO_3$. The strains were finally stocked in tryptic soy broth supplemented with 3.5% NaCl, 1% $NaCO_3$ and 20% (v/v) glycerol.

Screening for enzymes

Utilisation of various polymers which is an indication of the enzymes produced by an organism was assayed on a basal media containing per litre 1 g yeast extract (Difco), 1 g KH_2PO_4, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.05 g $CaCl \cdot 2H_2O$, 4% NaCl, 1% $NaCO_3$ and 15 g agar. The following substrates were added separately: starch 10 g (Merck),

Table 1 The different media used in this study for enrichment and isolation of bacteria

Medium no.	Medium composition per litre
1.	Tryptic soy agar
2.	10 g starch, 4 g yeast extract, 2 g peptone and 15 g agar
3.	2 g starch, 0. 8 g yeast extract, 0.1 g peptone and 15 g agar
4.	6 ml of 100% glycerol, 1 g arginine, 1 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$ and 15 g agar
5.	0.6 g glucose, 2 g chitin and 14 g agar
6.	2 g chitin and 15 g agar
7.	15 g agar
8.	7.5 g Casamino acids (Difco), 10 g yeast extract (Difco), 3 g Trisodium citrate, 0.3 g $MgSO_4 \cdot 7H_2O$, 2 g KCl, traces of Iron and manganese and 15 g agar
9.	3.75 g KCl, 0.267 g of Ammonium chloride, 0.174 g of K_2HPO_4, 3.7 g MgSO4, 0.5 g Calcium chloride, 0.2 g yeast extract, 0.4 g Peptone and 15 g agar
10.	5.0 g Peptone, 5.0 g yeast extract, 1.0 g K_2HPO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 1 g Glucose and 15 g Agar
11.	5.0 g Peptone, 5.0 g yeast extract, 1.0 g K_2HPO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, 1 g cellulose powder and 15 g agar
12.	5.0 g Peptone, 5.0 g yeast extract, 1.0 g K_2HPO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, 1 g soluble starch and 15 g agar
13.	5.0 g peptone, 5.0 g yeast extract, 1.0 g K_2HPO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, 1 ml olive oil and 15 g agar
14.	5.0 g peptone, 5.0 g yeast extract, 1.0 g K_2HPO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, 1 g xylose and 15 g agar
15.	ECM agar (Shimkets et al. 2006)
16.	WCX agar (Shimkets et al. 2006)
17.	ST21CX agar overlaid with sterile cellulose paper (Shimkets et al. 2006)
18.	Baiting using sterilised soils and sterile rabbit dung (Shimkets et al. 2006)
20.	VY/2 agar and yeast lawns (Shimkets et al. 2006)

carboxymethylcellulose 10 g (Serva, Heidelberg), xanthan 5 g (Sigma) and xylan 2.5 g (Fluka). Esterase/lipase activity was tested using olive oil (domestic grade) at 3 ml/l. These tests were done for all the isolates recovered in this study. After 48 h of growth the plates containing xylan, xanthan and carboxymethylcellulose were flooded with Congo red solution (1 mg/ml) and allowed to stand for 10 min. The dye was then replaced with NaCl (1 M) and subsequently rinsed with distilled water. The plates were observed for halos around the colonies, as indication of positive polymer degradation. The starch-containing plates were flooded with iodine solution (Sigma–Aldrich) and observed for clearings around the colonies. Esterase/lipase activity was indicated by formation of precipitate around the colonies. Chitinase activity was tested by use of 4-methylumbelliferyl *N*-acetyl-β-D-glucosaminide as described by the manufacturer (Sigma–Aldrich).

Molecular identification of the isolated microorganisms

DNA extraction was carried out using the SEQLAB bacteria-DNA-Kit and PCR amplification of the 16S rRNA genes was carried at SeqLab (Göttingen, Germany). Partial gene sequences were obtained using the primer 530 reverse (5′-GKATTACCGCGGCKGCTG-3′). The readings were manually edited and the sequence data was BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) analysed (Altschul et al. 1990) against the GenBank 16S rRNA database. Complete sequences were generated for all those 34 isolates that showed similarity values below 98% in the initial BLAST analysis. Partial and the complete sequences were assembled with the Sequencer software, version 4.1 for Macintosh (Genes Codes, Ann Arbor, MI). Phylogenetic relationship of all the sequences was determined using neighbour-joining (Felsenstein 1985) and maximum-likelihood analyses (Olsen and Woese 1993). These analyses were conducted in MEGA 4 (Tamura et al. 2007). The evolutionary distances were computed using the maximum composite likelihood method (Tamura et al. 2004). The resultant tree topologies were evaluated in bootstrap analyses of the neighbour joining method based on 1000 re-samplings (Felsenstein 1985). Only representative sequences are indicated in the trees.

Nucleotide sequence accession numbers

The partial sequences of all the isolates that were generated using the primer 530r are deposited in the GenBank under accession numbers between FJ763850 and FJ764198. The sequence reads were between 400 and 593 base pairs. The almost complete sequences of the isolates listed on Table 2 are deposited under the accession numbers FJ764760–FJ764793 and the sequence length is between 1340 and 1606 base pairs.

Results

From a total of 367 isolates obtained in the isolation exercise, 221 isolates were selected for partial sequencing on the basis of colony morphology and for observation of cellular morphology under the microscope. This was done to avoid sequencing several identical isolates from the same media; 181 isolates gave readable sequences of excellent quality representing members of 51 genera in the domain bacteria. Figure 1a, b shows the phylogenetic relationship of representative sequences to the closest neighbours as per BLAST analysis. Due to the large number of closely related isolates in each taxon, only 51 sequences are shown in Fig. 1a, b. However, these represent all the species that were detected in this study.

Though not more than between two and seven different isolates (representing different species) were recovered from any medium, the medium composition seems to have an effect on the taxa recovered. The distribution of genotypes on the different media is shown in Table 2. Members of the genus *Bacillus* were mostly encountered on media 3 and 4 and on media 10–14. Medium 12 only supported growth of bacilli. Halomonads were mostly from media 1 to 10 and 15 to 20. Media 15 to 20 were prepared targeting isolation of myxobacteria. Initial results showed the swarming morphology but after sequencing it was found out that these isolates were related to the genus *Marinospirillum*.

Analysis of the 181 partial sequences showed that 31 isolates had similarities between 97 and 98%, 15 isolates scored between 96 and 97%, while 8 isolates had lower than 96% similarities to their nearest neighbours. The rest of the isolates had similarity values between 99 and 100% to their nearest neighbours. All the 34 isolates that had similarity values of 97% and below were selected for full sequencing. This was to ascertain whether they represented novel phylotypes or not. Results from the 34 isolates that were fully sequenced indicate that some of them may represent novel taxa (Table 3). BLAST analysis of the full sequences indicated that in 26 isolates there was increase in the similarity values, in 6 isolates the values remained the same whereas in 2 isolates (M2-C23 and S-C28) there was a decrease in the similarity values from 100 to 99 and 99 to 98%, respectively. The full sequences confirmed the genera to which the various isolates belonged. It is only in the isolate M16-C8 where there was a change from *Brevibacterium* (partial sequence) to *Vibrio metschnikovii* (full sequence).

Table 2 A summary of the isolates recovered using the different media

	Bacillus	*Entero-coccus*	*Marinilacti-bacillus*	*Halolacti-bacillus*	*Amphi-bacillus*	*Alkali-bacterium*	*Strepto-myces*	*Nocardi-opsis*	*Halomonas*	*Marino-spirillum*	*Idiomarina*	*Alkalimonas*	*Vibrio*	*Nitrincola*	Total number of genera represented in the medium	Total number of species represented in the medium
Medium 1	–	–	–	1	–	–	–	–	1	–	–	–	1	–	3	3
Medium 2	–	1				–	–	–	2	–	3	–	–	1	4	7
Medium 3	3	–	–	–	–	–	–	–	–	–	–	–	–	1	2	4
Medium 4	4	–	–	–	–	–	1	–	2	–	–	–	1	–	4	8
Medium 5	1	–	–	–	–	–	–	–	2	–	–	–	–	–	2	3
Medium 6	–	–	–	–	–	–	–	–	4	–	–	–	–	–	1	4
Medium 7	1	–	–	–	1	–	–	–	1	–	1	1	–	1	6	6
Medium 8	1	–	–	–	–	1	–	–	1	–	1	–	–	–	4	4
Medium 9	1	–	–	–	–	2	–	1	1	–	–	–	1	–	5	6
Medium 10	4	1	–	–	–	–	–	–	1	1	–	–	–	–	3	6
Medium 11	3	–	–	–	–	–	–	–	–	–	–	–	–	–	2	4
Medium 12	5	–	–	–	–	–	–	–	–	–	–	–	–	–	1	5
Medium 13	5	–	–	–	–	–	–	–	–	–	–	–	–	–	2	6
Medium 14	5	–	1	–	–	1	–	–	–	–	–	–	–	–	2	6
Medium 15	–	–	–	–	–	1	–	–	2	1	1	–	–	–	4	5
Medium 16	–	–	–	–	–	1	–	–	2	–	–	–	–	–	1	2
Medium 17	–	–	–	–	–	–	–	–	2	3	–	–	–	–	3	7
Medium 18	–	–	–	–	–	2	–	–	1	–	1	–	–	–	3	3
Medium 20	–	–	–	–	–	1	–	–	2	–	–	–	–	–	1	2

The values in the table indicate not only the presence but also the number of different species per genera represented in each medium

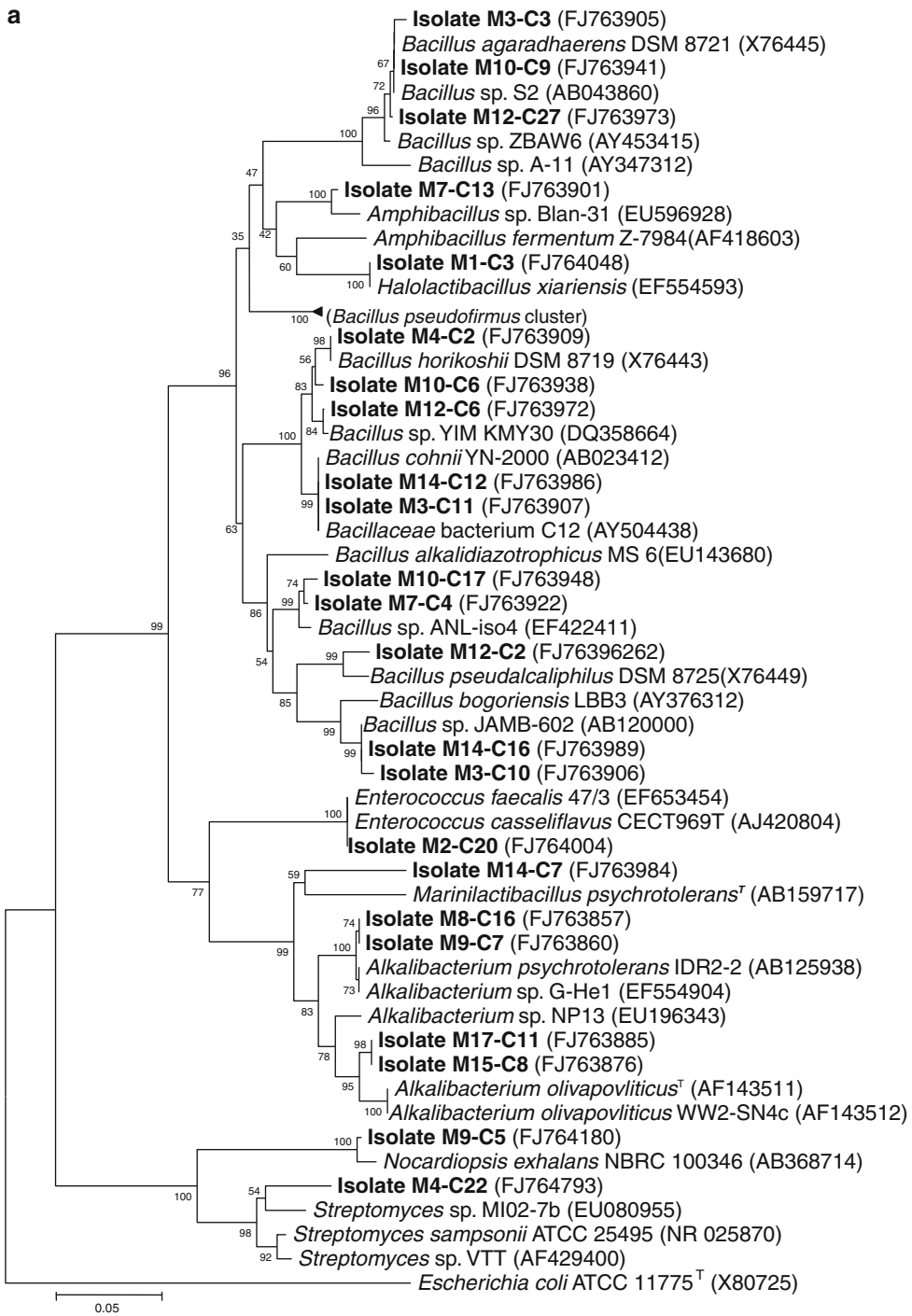
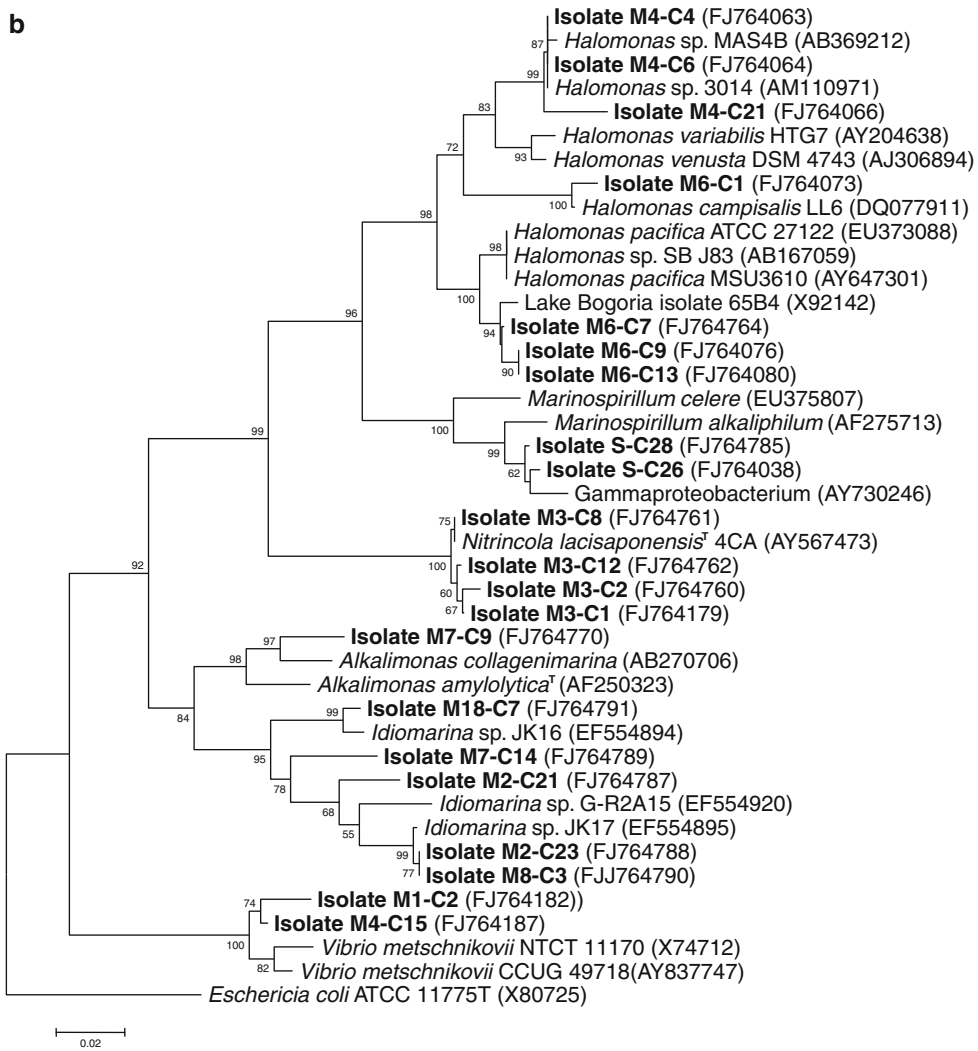


Fig. 1 **a** Phylogenetic affiliation of the isolates from this study to other members of 2 phyla; firmicutes and Actinobacteria. Isolates from this study are indicated in *bold font*. Only bootstrap values above 50 are shown. **b** Phylogenetic affiliation of the isolates from this study to other members of the class *Gammaproteobacterium*. Isolates from this study are indicated in *bold font*. Only bootstrap values above 50 are shown

BLAST analysis of the partial sequences shows that 109 isolates (60%) belonged to the class *Gammaproteobacteria*. Among these, 68 isolates were affiliated to the genus *Halomonas*, 20 isolates were affiliated to microorganisms belonging to the genus *Marinospirillum* while 15 isolates were related to *Idiomarina* species. Several isolates from each taxon were full sequences to ascertain their affiliation (Table 3). The isolate (M7-C9) was affiliated to the genus *Alkalimonas* and the almost complete sequence shows that the closest neighbour is *Alkalimonas delamerensis* (98%), the type strain of which was previously isolated from Lake Elmenteita. Interestingly, five isolates from the genus *Vibrio* were closely related to the marine *Vibrio metschnikovii*.

Only two actinobacterial isolates were recovered and fully sequenced. The first one (M9-C5) was related to *Nocardiopsis exhalans* while the second (M4-C21) was from the genus *Streptomyces* and it had 96% similarity to

Fig. 1 continued



Streptomyces caelestis. The strain had an optimum growth temperature of 39°C and an optimum pH of 8.4.

From the phylum firmicutes, two isolates affiliated to the genus *Enterococcus* were recovered. The genus *Enterococcus* contains bacterial species associated with animals and plants. A total of 58 isolates from the genus *Bacillus* was recovered and this makes it the second most common group after the halomonads. In this study seven isolates were recovered which closely related to *Alkalibacterium olivoapovliticus* strain WW2-SN4a (AF143511), *A. olivoapovliticus* strain WW2-SN4c (AF143512), *A. psychrotolerans* (AB125938), *Alkalibacterium* sp. G-He1 (EF554904) and *Alkalibacterium* sp. NP13 (EU196343). One isolate (M7-C13) from the genus *Amphibacillus* was recovered and it had a 97% similarity to *Amphibacillus sediminis*. A single isolate (M14-C7) with 94% BLAST similarity to *Marinilactibacillus* sp. was recovered. The genus *Marinilactibacillus* (Ishikawa et al. 2003) has only two described species and both are from the ocean.

Enzyme assay

Of the 181 isolates screened, 67 were able to utilise between 1 and 5 of the substrates screened. The phylogenetic affiliation of these isolates was as follows: *Bacillus* (39), *Halomonas* (12), *Vibrio* (5), *Alkalibacterium* (4), *Enterococcus* (2), *Idiomarina* (2) and *Alkalimonas*, *Halolactibacillus* and *Streptomyces* had 1 isolate each. Only *Bacillus* species were able to utilise carboxymethylcellulose. Among the *Bacillus*, isolates M13-C22, M14-C13, M14-C3, M10-C9 AND M13-C14 were able to utilise xanthan, xylan, starch, olive oil and carboxymethylcellulose. The highest chitinase activity was recorded among the *Vibrio* species.

Discussion

The use of different media prepared using water from the lake could have helped retrieve novel taxa not detected in

Table 3 BLAST results of the 34 isolates that were fully sequenced

ID	Nearest neighbour in BLAST	%	Length in Bp	Accession No.
M1-C6	*Bacillus pseudofirmus*	98	1550	FJ764768
M4-C7	*Bacillus pseudalcaliphilus*	98	1361	FJ764769
M8-C14	*Bacillus pseudofirmus*	98	1551	FJ764772
M8-C11	*Bacillus* sp.	99	1549	FJ764771
M9-C3	*Bacillus* sp.	98	1552	FJ764773
M10-C8	*Bacillus pseudalcaliphilus*	98	1606	FJ764774
M10-C17	*Bacillus* sp.	98	1551	FJ764775
M13-C24	*Bacillus pseudofirmus*	98	1584	FJ764776
M14-C6	*Bacillus pseudofirmus*	98	1559	FJ764777
M14-C16	*Bacillus hemicellulolyticus*	98	1570	FJ764778
M2-C21	*Idiomarina* sp.	96	1542	FJ764787
M2-C23	*Idiomarina* sp. JK 17	99	1538	FJ764788
M7-C14	*Idiomarina* JK 17	97	1539	FJ764789
M8-C3	*Idiomarina* JK 17	99	1539	FJ764790
M18-C7	*Idiomarina* JK16	98	1425	FJ764791
M15-C1	*Marinospirillum alkaliphilum*	97	1519	FJ764782
M18-C5	*Marinospirillum alkaliphilum*	97	1521	FJ764783
S-C25	*Marinospirillum alkaliphilum*	97	1522	FJ764784
S-C28	*Marinospirillum alkaliphilum*	98	1523	FJ764785
S-C30	*Marinospirillum alkaliphilum*	97	1538	FJ764786
M3-C2	*Nitrincola lacisaponensis*	97	1407	FJ764760
M3-C8	*Nitrincola lacisaponensis*	98	1350	FJ764761
M3-C12	*Nitrincola lacisaponensis*	98	1445	FJ764762
M4-C11	*Alkalibacterium indicireducens*	99	1606	FJ764766
M8-C6	*Alkalibacterium indicireducens*	99	1545	FJ764767
M7-C10	*Marinilactibacillus piezzotolerans*	94	1544	FJ764779
M14-C7	*Marinilactibacillus piezzotolerans*	94	1544	FJ764780
M4-C21	*Halomonas* sp.	98	1498	FJ764763
M4-C22	*Streptomyces caelestis*	96	1564	FJ764793
M6-C7	Lake Bogoria isolate	98	1340	FJ764764
M7-C9	*Alkalimonas delamerensis*	97	1379	FJ764770
M7-C13	*Amphibacillus* sp.	97	1571	FJ764765
M16-C8	*Vibrio metschnikovii*	98	1386	FJ764781
M9-C5	*Nocardiopsis exhalans*	99	1552	FJ764792

These isolates had similarity values below 98% in the initial BLAST analysis. The *M* in the ID represents the medium whereas the *C* represents the colony morphology. *S* was for colonies with a swarming morphology

the soda lake before. Of the sequenced isolates, 77 isolates had similarity values ranging 94–98% to cultured members of the domain bacteria. The media composition seemed to have an effect on the recovery of different groups. Most of the *Bacillus* (40 isolates) species were from media 10 to 14. This could have been due to the various carbon sources in these media which supported the proliferation of heterotrophic groups. Only halomonads were recovered on media 6 and 20. The halomonads were recovered in 14 of the 19 media, mostly media that were low in organic carbon. Swarming morphology characteristic of the myxobacteria was observed in several media. However, partial sequencing indicated that the isolates were related to members of the genus *Marinospirillum* notably *M. Alkaliphilum*.

In terms of diversity, the majority of the isolates were from the class *Gammaproteobacteria* and the *Bacillus* group within the firmicutes. This concurs with earlier reports that the majority of Gram-negative isolates and culture-independent bacterial clones retrieved from soda lakes belong to the class *Gammaproteobacteria* (Jones et al. 1994, 1998; Ma et al. 2004), including strains related closely to typical aquatic bacteria such as *Aeromonas* and *Pseudomonas* (Duckworth et al. 1996), moderate halophiles from the *Halomonas/Deleya* group, and marine bacteria, e.g. *Marinobacter* (Rees et al. 2004). Species belonging to the family *Halomonadaceae* are ubiquitous and have been isolated from seawater, estuarine water, hypersaline soils, and bodies of hypersaline water,

including Antarctic lakes, the Dead Sea, and several soda lakes of the Rift Valley, Kenya. They are aerobic, and some strains have the capacity for facultative anaerobic growth in the presence of nitrate. Members of *Halomonadaceae* have been shown to be of biotechnological importance in the production of compatible solutes as well as extracellular compounds such as exopolysaccharides and enzymes, and their use in environmental bioremediation processes (Nakayama et al. 2000; Garcia et al. 2004; Llamas et al. 2006; Wu et al. 2008).

The helical Gram-negative bacteria isolates retrieved here differed from the genus *Marinospirillum* in the physiological, biochemical, DNA G + C content and phylogenetically. These could also represent a new genus whose members differ from those of the genus *Marinospirillum*. Within the genus *Idiomarina* (Ivanova et al. 2000), 16 species are now validly published after the reclassification of the members of the genus *Pseudidiomarina* (Jean et al. 2009; Taborda et al. 2009). Although some isolates in this study were affiliated to the genus *Idiomarina* according to BLAST results, phylogenetic analysis of the full sequences indicates that they form a distinct cluster and hence could belong to a novel genus (data not shown).

The two isolates of the genus *Nocardia* recovered in this study were identical phylogenetically though the morphology was different. Therefore they could represent a new species from the soda lake environment. The closest neighbour in BLAST analysis was *Nocardiopsis exhalans* (Peltola et al. 2001). High G + C Gram-positive bacteria isolated from the Kenyan soda lakes so far belong to the genus *Dietzia*, *Arthrobacter* and *Terrabacter* (Duckworth et al. 1996, 1998; all the three being from Lake Oloiden). *Bogoriella caseilytica* (Groth et al. 1997) and *Cellulomonas bogoriensis* (Jones et al. 2005) have also been described from Lake Bogoria in Kenya. So far no isolate from the genus *Streptomyces* has been described from the East African soda lake environment. The single isolate recovered in this study could represent a novel genus. Members of the genus *Streptomyces* are involved in the biodegradation of various polymers abundant in soil owing to their ability to produce extracellular enzymes.

Bacillus species are among the most commonly found aerobic, eubacterial alkaliphiles both in soda lakes and in less selective environments (Horikoshi and Akiba 1982; Krulwich and Guffanti 1983; Guffanti et al. 1980, 1986; Takami et al. 1999). The *Bacilli* were the second group after the *Halomonas* in terms of diversity. The *Bacilli* are grouped into two clusters of alkaliphiles and alkaline-tolerant isolates based on physiological and biochemical characteristics as well as DNA base composition, hybridisation, and 16S rDNA analyses (Fritze et al. 1990; Nielsen et al. 1994, 1995).

The genus *Alkalimonas* was first proposed by Ma et al. (2004) to describe two novel alkaliphilic microorganisms described from Kenyan and Mongolian soda lakes, respectively. The single isolate from the genus *Alkalimonas* isolated was closely related to *Alkalimonas delamerensis* isolated from the same lake (Ma et al. 2004). These strains can be readily distinguished from close phylogenetic relatives by being alkaliphilic and halophilic meaning they are well suited for survival in soda lake conditions (Ma et al. 2004).

Members of the genus *Alkalibacterium* are found in the soda lake and in this study seven isolates were recovered. An isolate designated as WN16 from Lake Nakuru was isolated in a previous study by Duckworth et al. (1996). The genus *Amphibacillus* was first proposed by Niimura et al. (1990) and the genus currently comprises four recognised species, *Amphibacillus xylanus* (Niimura et al. 1990), *Amphibacillus fermentum*, *Amphibacillus tropicus* (Zhilina et al. 2001) and *Amphibacillus sediminis* (Sun-Young et al. 2007). The single isolate recovered in this study could therefore represent a new species within the same genus.

The genus *Marinilactibacillus* has only two described species and both are from the ocean. Isolation and taxonomic studies of lactic acid bacteria from marine environments to date are few and have generally been confined to those from cultured fish (Ringø and Gatesoupe 1998; Gatesoupe 1999). The single isolate recovered was 94% similar to *Marinilactibacillus piezzotolerans*. The low similarity value indicates that the isolate could represent a novel genus of lactic acid bacteria from the soda lake.

The name *Vibrionaceae* was formally proposed (Véron 1965) as a convenient grouping for fermentative bacteria that have polar flagella and a positive oxidase reaction. The isolates recovered in this study were affiliated to *Vibrio metschnikovii*, isolated from the ocean. Many factors probably govern their distribution, but four of the most important are: the particular animal or plant hosts, temperature, salinity, and depth below the surface for the species that are found in the ocean (Simidu and Tsukamoto 1985).

Microbial communities in natural alkaline environments such as soda lakes have attracted attention as a possible source of novel enzymes and metabolites for use in biotechnology. Microbes are a preferred source of enzymes since they are cheaper to produce and their enzyme content is more predictable and controllable (Adams and Kelly 1995; Plummer and Tarentino 1991). Phylogenetic analysis showed that some of the isolates retrieved belong to novel groups not reported in the soda lakes before.

Conclusion

The generic affiliation of cultured microbes reflects the sampling methods and culture conditions used in the

isolation process and this has a major effect on the range of types encountered in the laboratory. Those methods that match most closely the physiology of members of a microbial community will be enriched and subsequently isolated. The use of different media helped retrieve novel groups not reported before from the soda lake environments. Some of the media used also targeted detection of useful extracellular enzymes produced by the alkaliphiles.

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