REVIEW

Brian E. Jones · William D. Grant
Andrew W. Duckworth · Gerald G. Owenson

Microbial diversity of soda lakes

Abstract Soda lakes are highly alkaline extreme environments that form in closed drainage basins exposed to high evaporation rates. Because of the scarcity of Mg^{2+} and Ca^{2+} in the water chemistry, the lakes become enriched in CO_3^{2-} and Cl^-, with pHs in the range 8 to >12. Although there is a clear difference in prokaryotic communities between the hypersaline lakes where NaCl concentrations are >15% w/v and more dilute waters, i.e., NaCl concentrations about 5% w/v, photosynthetic primary production appears to be the basis of all nutrient recycling. In both the aerobic and anaerobic microbial communities the major trophic groups responsible for cycling of carbon and sulfur have in general been identified. Systematic studies have shown that the microbes are alkaliphilic and many represent separate lineages within accepted taxa, while others show no strong relationship to known prokaryotes. Although alkaliphiles are widespread it seems probable that these organisms, especially those unique to the hypersaline lakes, evolved separately within an alkaline environment. Although present-day soda lakes are geologically quite recent, they have probably existed since archaean times, permitting the evolution of independent communities of alkaliphiles since an early period in the Earth's history.

B.E. Jones (✉)
Genencor International BV, PO Box 642, 2600 AP, Delft, The Netherlands
Tel. +31-15-284-3084; Fax. +31-15-284-3076
e-mail: bjones@genencor.com

W.D. Grant · A.W. Duckworth[1] · G.G. Owenson[2]
Department of Microbiology and Immunology, University of Leicester, Maurice Shock Building, University Road, Leicester, LE1 9HN, U.K.

Present address:
[1]Fossil Fuels and Environmental Geochemistry, University of Newcastle, Newcastle upon Tyne, NE1 7RU, UK
[2]Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL, UK

Alkaline environments

Alkaline environments can be placed into several broad categories depending on the nature of the process generating alkalinity. All depend on a continuous process, either microbial or chemical, to maintain an alkaline pH and counter the buffering effect of CO_2, which at a macroscale tend to maintain more a neutral or acidic pH. Soil microbial processes such as ammonification and sulfate reduction rarely lead to stable pHs in excess of pH 10 and may be transient and localized unless unrestricted by factors such as nutrient supply. Human industrial activity by processes such as cement manufacture [$Ca(OH)_2$], mining operations, paper and pulp production (NaOH), and food-processing effluents all generate examples of highly alkaline environments, often in excess of pH 11. Natural geochemical processes such as the weathering of silicate minerals can lead to alkaline water about pH 11 because of $Ca(OH)_2$ (Grant 1992; Grant et al. 1990; Bath et al. 1987). The chemistry of these silicate waters is rather dilute and comparable to that in oligotrophic environments; they have an extremely low buffering capacity, the pH stability being rather limited by the low solubility of $Ca(OH)_2$ (about 10 mM) and exposure to atmospheric CO_2. Carbonate ions are rapidly removed from solution as insoluble calcium or magnesium minerals. In volcanic areas, alkaline hot springs have been reported with pHs to pH 9.5 (Hensel et al. 1997) where alkalinity is probably also generated by decomposition of silicates. Here too, the maintenance of a stable alkaline pH is limited (Jones, personal observations) probably because of exposure to O_2 and outgassing of CO_2.

Soda lakes and soda deserts are naturally occurring alkaline environments. They represent the most stable high-pH environments on Earth, where large amounts of carbonate minerals can generate pH values >11.5. Although these environments are widely distributed, they are often located in inaccessible continental interiors, which has hindered

their detailed and systematic study. The best studied soda lakes are those of the East African Rift Valley, where detailed limnological and microbiological investigations have been carried out over many years (Jones et al. 1994; Grant et al. 1990; Rich 1933; Beadle 1932; Jenkin 1932). Microbial studies of Central Asian soda lakes have also been documented (Zhilina and Zavarzin 1994; Issatchenko 1951).

The genesis of the soda lake environment

The conditions necessary for the formation of a soda lake have much in common with those for the generation of an athalassohaline salt lake, but with the major difference that in a soda lake carbonate (or carbonate complexes) becomes the major anion in solution. The most important contributing factor is a lack of alkaline earth cations (Ca^{2+} and Mg^{2+}) in the surrounding topography, which essentially means an absence of rocks of sedimentary origin. Further circumstances include a shallow depression forming a closed drainage basin with a high marginal relief, having sufficient rainfall to sustain streams entering the basin to produce a standing body of water. In arid zones with high rates of evaporation exceeding inflow, salts accumulate by evaporative concentration (Jones et al. 1994).

In the Rift Valley of Kenya-Tanzania, the graben is composed of Pleistocene alkaline trachyte lavas (high Na^+, low Ca^{2+} and Mg^{2+}). Under the prevailing conditions, groundwater of meteoric origin saturated with CO_2, the molar concentration of HCO_3^-/CO_3^{2-} greatly exceeds that of Ca^{2+}/Mg^{2+}. As a result of evaporation in this arid tropical zone, saturation of the alkaline earth cations is rapidly achieved, and they precipitate out of solution as insoluble carbonates; this leaves Na^+, Cl^-, and HCO_3^-/CO_3^{2-} as the major ions in solution.

The soda lake aerobic environment

One of the noticeable features of many soda lakes is their color. The water may be various shades of green or red because of the massive blooms of microorganisms. This coloring is a reflection of the very high primary productivities associated with these lakes. The almost unlimited supply of CO_2 combined with high ambient temperatures and high daily light intensities in the tropics contribute to making the East African soda lakes among the most productive of the naturally occurring aquatic environments in the world (Melack and Kilham 1974).
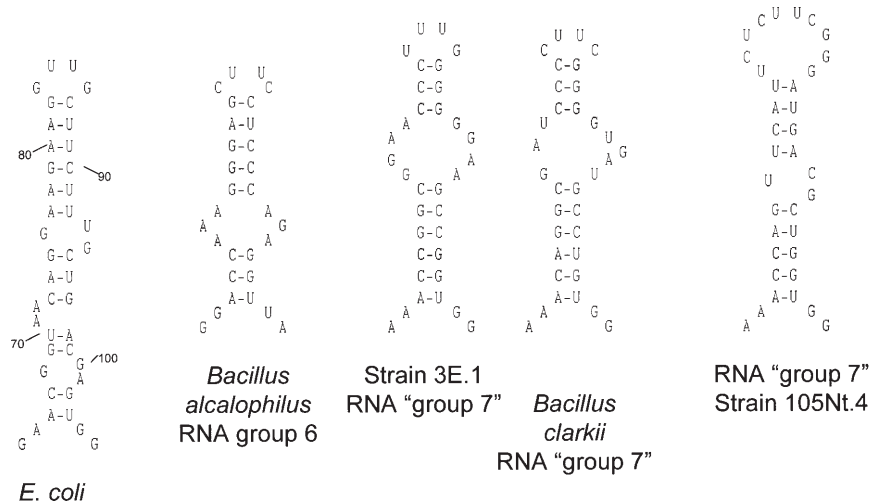
The photosynthetic primary productivity, mainly the result of the dense populations of cyanobacteria, presumably supports the rest of the microbial community. These blooms of cyanobacteria are usually dominated by *Spirulina* spp., but in different lakes and also depending on seasonal factors *Cyanospira* spp. and unicellular forms, which might be *Synechococcus* or *Chroococcus*, may also be common. Not only are the cyanobacteria critical for the fixation of

nitrogen in this environment but they are also producers of O_2. Daytime rates of oxygen production >2 g O_2 m^{-2}h^{-1} have been recorded from *Spirulina* spp. (Melack and Kilham 1974). However, during a 12-month survey of aerobic organotrophic bacterial numbers in relation to cyanobacterial numbers, no correlation was found between secondary productivity and the timing of a cyanobacterial bloom (Jones et al. 1994; Mwatha 1991; Grant et al. 1990). In fact, bacterial numbers were remarkably constant at about 10^5 cfu ml^{-1}, although the dominant types varied. The Rift Valley soda lakes maintain dense populations of nonphototrophic, aerobic organotrophic bacteria, which

Table 1. Taxonomic groups containing prokaryotes isolated from soda lakes (boldface type)

Eubacteria
  Cyanobacteria
    Chroococcales
    Oscillatoriales
      Spirulina spp.
  Firmicutes (gram-positive bacteria)
    Actinomycetes (high G+C gram-positive bacteria)
      Actinomycetales
      Micrococcaceae
      Nocardiform actinomycetes
      Streptomyces
    Low G+C gram-positive bacteria
      Bacillaceae
      Clostridiaceae
      Haloanaerobiales
  Proteobacteria
    Beta subdivision?
    Delta subdivision
    Gamma subdivision
      Ectothiorhodospira
      Halomonadaceae
      Pseudomonas
  Spirochaetales
    Spirochaetaceae
      Spirochaeta
  Thermotogales
    Thermopallium
      Thermopallium natronophilum

Archaea
  Euryarchaeota
    Halobacteriales
      Halobacteriaceae
        Halorubrum
          Halorubrum (Natronobacterium) vacuolatum
        Natrialba
          Natrialba (Natronobacterium) magadii
        Natronobacterium
          Natronobacterium gregoryi
          Unclassified Natronobacterium spp.
        Natronococcus
          Natronococcus amylolyticus
          Natronococcus occultus
          Unclassified Natronococcus spp.
        Natronomonas
          Natronomonas (Natronobacterium) pharaonis
    Methanomicrobiales
      Methanosarcinaceae
        Methanohalophilus
          Methanohalophilus oregonensis
          Methanohalophilus zhilinaeae
          Methanohalophilus sp. Z-7936

Fig. 1. Signature sequences and secondary structure in 16S rRNA of alkaliphilic *Bacillus* spp.

Bacillus alcalophilus RNA group 6

Strain 3E.1 RNA "group 7"

Bacillus clarkii RNA "group 7"

RNA "group 7" Strain 105Nt.4

E. coli

may be somewhat surprising considering the lakes are eutrophic, rather shallow, and presumably oxygen limited. These bacteria may be readily isolated on appropriate media (Horikoshi and Akiba 1982; Grant and Tindall 1980). Viable counts of aerobic organotrophs from a range of dilute lakes indicate 10^5–10^6 cfu ml^{-1} (Mwatha 1991; Grant et al. 1990). Many isolates of organotrophic bacteria obtained from these lakes have been subject to phenotypic (numerical taxonomy), chemotaxonomic, and phylogenetic analysis (Duckworth et al. 1996; Jones et al. 1994). Not surprisingly, most of the isolates are obligately alkaliphilic or at the very least alkali tolerant and represented considerable phylogenetic diversity (Table 1). The majority of gram-negative isolates were members of the gamma subdivision of the Proteobacteria (Woese et al. 1985). Organisms related to but not identical with members of the *Halomonas* group were well represented. These strains are currently undergoing detailed examination because they appear to represent at least two novel genera and several new species within currently accepted taxa. Other gram-negative isolates grouped closely with *Pseudomonas* sensu strictu (rRNA group 1) while other strains formed distinct groups without any close affinity to known taxa, although their closest association was with typical aquatic bacteria such as *Aeromonas*, *Vibrio*, and *Pseudomonas* spp. (Duckworth et al. 1996).

Gram-positive isolates are more diverse, being found in both the high G+C and low G+C divisions. Only two of the high G+C isolates closely identified with established taxa, in this case *Dietzia*, resulting in our proposal of a second species of this genus as *Dietzia natronolimnaios* (Duckworth et al., in press). Other high G+C types are distinct and only loosely associated with known species of *Arthrobacter* and *Terrabacter*. Low G+C isolates were associated with members of the *Bacillus* spectrum, especially *B. alcalophilus*. Recent comparative 16S rDNA sequence analysis of *B. alcalophilus* and some alkaliphilic *Bacillus* soil isolates has established that they form a distinct phylogenetic group (rRNA group 6) within the *Bacillus* radiation

(Nielsen et al. 1994). Some of our isolates were closely related to but not identical to members of the rRNA group 6 bacilli. A second more diverse group of strains were related to the alkaliphilic species *Bacillus agaradherens* and *Bacillus clarkii* (Nielsen et al. 1995), which form the nucleus of another distinct rRNA group ("group 7"). This has been confirmed by signature analysis and the presence of an insertion between positions 70 and 100 (Nielsen et al. 1994) (Fig. 1). However, isolates in this group were phenotypically diverse. Strains isolated from the hypersaline lakes Little Magadi and Natron were in addition highly halophilic, growing well in 30% w/v NaCl. These strains, for example, isolate 105Nt.4, have a different signature sequence, namely a larger terminal loop in the V1 region of the 16S rRNA (Fig. 1). There is some evidence to suggest that the two groups of bacilli are segregated within the soda lake environment. The strains associated with *B. alcalophilus* seem to predominate in shoreline muds and dry foreshore soda soils that are subjected to fluctuating conditions of alkalinity and salinity as water levels change with the seasons. The "group 7" strains, which also have a high Na$^+$ requirement for growth, are seemingly more prevalent in lake waters and sediments where conditions are less variable.

A quite different population of prokaryotes is present in the trona and concentrated brines of the hypersaline lakes of the Magadi-Natron basin. Although cyanobacteria of the genus *Cyanospira* have been isolated from Lake Magadi (Florenzano et al. 1985), cyanobacterial blooms occur only occasionally after extensive rainfall causes dilution of the brine; hence, it is unclear what organisms are responsible for primary production. Red anoxygenic, phototrophic, halophilic *Ectothiorhodospira* spp. have been isolated at Magadi and other soda lakes (Grant and Tindall 1986; Imhoff et al. 1979), but normally the trona crusts are colored red by the presence of large numbers of haloalkaliphilic Archaea. Until recently these obligately halophilic and alkaliphilic Archaea were classified in two genera, *Natronococcus* and *Natronobacterium* (Kamekura

et al. 1997; Grant 1989). Phylogenetic analysis of our more recent isolates indicated they were closely related to these genera but distinct from known species (Duckworth et al. 1996). There are few phenotypic features that can be used to distinguish the strains or the recognized species, but investigations are continuing (S. Grant, personal communication).

Although these results extend our understanding of the diversity of soda lake alkaliphiles, they are clearly biased by the manner of sampling, culture conditions employed, and the choice of isolates for study. Recently, we examined littoral mud and dry soda soils using alternative culture techniques designed to isolate filamentous actinomycetes at pH 10. Numerous spore-forming bacteria with the typical morphology of *Streptomyces* were isolated. These are clearly different from the recently described *Bogoriella caseilytica* (Groth et al. 1997), and preliminary evidence from 16S rDNA sequencing indicates that some strains at least represent new alkaliphilic species of *Streptomyces* (Jones, Meijer, and Van Solingen, unpublished).

The soda lake anaerobic environment

In contrast to aerobic habitats, the anaerobic alkaline environment has received little attention. Because these are very productive environments (Talling et al. 1973; Melack and Kilham 1974) with a concomitant shortage of dissolved O_2, anaerobic processes are bound to play a significant role. Lacustrine muds are black and anoxic, implying the presence of sulfides and the presence of specific anaerobic populations of sulfate-reducing bacteria (SRB). Indeed, geochemists have invoked the presence of sulfate-reducing bacteria to explain the relative depletion of sulfate in the

brines of Lake Magadi (Jones et al. 1977), although sulfate levels of 12.8–17.5 mM in Lake Magadi brines (Jones et al. 1994) would not limit microbial activity. Even so, the distinctive odor of H_2S associated with SRB activity is often not apparent in these sediments (Owenson and Jones, personal observations), presumably because of the high pH (Hao et al. 1996), although sulfide concentrations of 6.2 mM have been recorded (Jones et al. 1994). The sediments also have a sufficiently low redox potential to permit the activity of SRBs; values of −310 to −470 mV were measured in situ (Owenson and Jones, unpublished). Furthermore, there is a substantial if unquantified contribution to primary productivity by anoxygenic phototrophic bacteria of the genus *Ectothiorhodospira*. These bacteria require reduced sulfur compounds as photosynthetic electron donors and clearly play a role in the cycling of sulfur. By evoking the activity of SRBs, the cycle is completed (Fig. 2).

In 1992 we attempted a systematic study of soda lake sediments with the aim of characterizing obligately anaerobic alkaliphiles, especially sulfate-reducing bacteria (Owenson 1997). Anaerobic sediment samples were collected under anaerobic conditions (Duckworth et al. 1996) from five soda lakes in the Kenyan section of the East African Rift Valley. A total of 16 samples were abstracted, 11 from the more dilute "northern" lakes (Elmenteita, Sonachi, Nakuru, and Bogoria), and 5 from the hypersaline Lake Magadi. Previous observations (Jones et al. 1994) suggested that positive enrichments for sulfate-reducing bacteria had been obtained from sediments from Lake Magadi, but no strains were recovered in pure culture. From a total of 161 enrichment cultures performed on the sediment samples, only 16 cultures showed stable sulfate reduction on repeated subculture (Table 2). Anaerobic incubations were performed under a head-space gas phase containing 10% v/v H_2 and 90% v/v N_2; the formation of a black precipitate

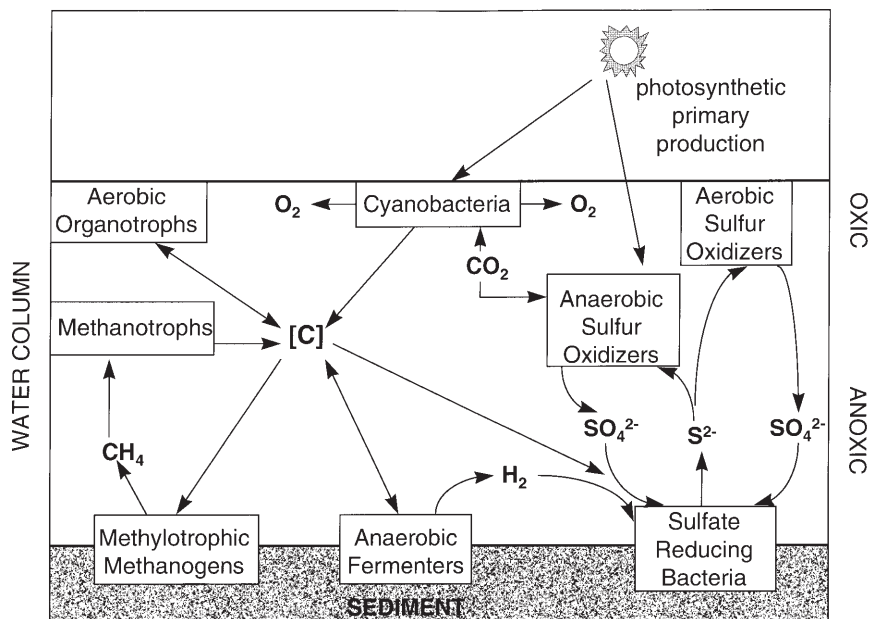**Fig. 2.** A simple model for nutrient cycles in soda lakes

Table 2. Positive enrichment cultures for sulfate-reducing bacteria

Lake	Enrichment substrate					
	Lactate	Acetate	Butyrate	Formate	Fumarate	Ethanol
Elmenteita		+		+	+	+
Nakuru	+		+	+		+
Bogoria	+					+
Sonachi						+
Magadi	+					

Table 3. The origins of obligate anaerobic strains

Isolate	Location	Sample type	Water		
			pH	Temperature (°C)	Conductivity (mS cm^{-1})
E2/B E2/C	Elmenteita	Littoral zone sediment core	10.0	25	25.0
B7/A	Bogoria	Littoral zone (grit and silt) sediment core	11–11.5	34	72.3
B8/A B8/C B8/CFT	Bogoria	Littoral zone sediment core	11	33	59.6
M12/2	Magadi (saltern P1)	Sediment core	10.5	33	33.8
M14/4	Magadi, western causeway	Mud under trona beds	12	32	114.7
M16/4	Magadi, northwestern causeway	Dredged mud	11	34	112.5

of FeS was used as an indicator of H_2S production. Growth was often very slow, typically several months between transfers. A variety of cell morphologies were observed including vibrio-shaped, straight rods, cocci, and cells with a spiral morphology similar to the *Spirochaeta* sp. described by Zhilina et al. (1996a). Attempts to purify the SRBs by plating did lead to the growth of typical black colonies after extended incubation, but subculturing to obtain axenic cultures was unsuccessful, suggesting perhaps that the organisms survived only as members of syntrophic associations. Zhilina and Zavarzin (1994) had more success, reporting the isolation of several organisms from Lake Magadi forming a mixed anaerobic community, one of which was an H_2-utilizing SRB later characterized as *Desulfonatronovibrio hydrogenovorans* (Zhilina et al. 1997).

The isolation of chemoorganotrophic alkaliphiles under anaerobic conditions proved to be more tractable. Viable counts on a complex alkaline agar medium indicated 1.85×10^5 to 2.5×10^6 bacteria per ml at 37°C. Enrichments performed on complex substrates such as guar gum (galactomannan; 2:1 mannose:galactose), birchwood xylan, carboxymethyl cellulose, and starch yielded almost exclusively faculatative anaerobes, while the direct plating of sediment or sediment suspensions on agar containing glucose with an appropriate amount of NaCl and incubating under H_2/N_2 provided some obligately anaerobic isolates. Most of the obligate anaerobes could not be maintained in prolonged culture during the course of the study, and only nine strains survived long enough to permit a partial characterization. These nine strains represent only three of the five soda lake basins sampled (Table 3). This poor harvest undoubtedly reflects the technical difficulties associated with culturing obligate anaerobes under a high-salt, high-pH regimen and maintaining strains having unknown growth requirements. An examination of the colony and cell morphology of these strains indicates an apparent diversity of organisms. An analysis of the substrates utilized (using the Biolog system; Biolog, Hayward, CA, USA), fermentation products, and the pH and salt ranges for growth (Table 4) clearly indicated two groups of organisms. The three strains from Lake Magadi are all obligately haloalkaliphilic, requiring at least 12%–16% w/v NaCl for growth, and produce isovaleric acid as a fermentation end product with smaller amounts of isobutyric and acetic acids. The strains from Lakes Elmenteita and Bogoria are less salt tolerant, reflecting the conditions in these lakes, and produce acetate and propionate or butyrate as fermentation products.

Phylogenetic analysis inferred from 16S rDNA sequences served to underline two groups of organisms (Figs. 3 and 4), although all the isolates grouped with the "low G+C mol% division of the gram-positive bacteria." The least halotolerant strains from Elmenteita and Bogoria clustered within "group XI" of the *Clostridium* spectrum

Table 4. Growth properties of obligate anaerobic strains

| Isolate	pH Range (at 4% w/v NaCl)[a]	NaCl range (% w/v)	Substrates utilized	Fermentation products
E2/B	8.5–>10.5	0–8	No data	Acetate, propionate
E2/C	7.5–>10.5	0–4	Sugars	Acetate, butyrate
B7/A	8.5–>10.5	0–8	Amino acids, sugars limited	Acetate, propionate
B8/A	8.5–>10.5	0–8	Sugars	Acetate, propionate
B8/C	8.5–>10.5	0–12	Sugars	Acetate, propionate
B8/CFT	8.5–>10.5	0–12	Sugars	Acetate, propionate
M12/2	9.5–>10.5	16–24	Sugars	Isovalerate, Isobutyrate, acetate
M14/4	9.5–>10.5	12–26	Sugars	Isovalerate, Isobutyrate, acetate
M16/4	8.5–>10.5	12–26	Sugars + amino acids	Isovalerate, Isobutyrate, acetate

[a] Except Magadi isolates: 16% w/v NaCl.
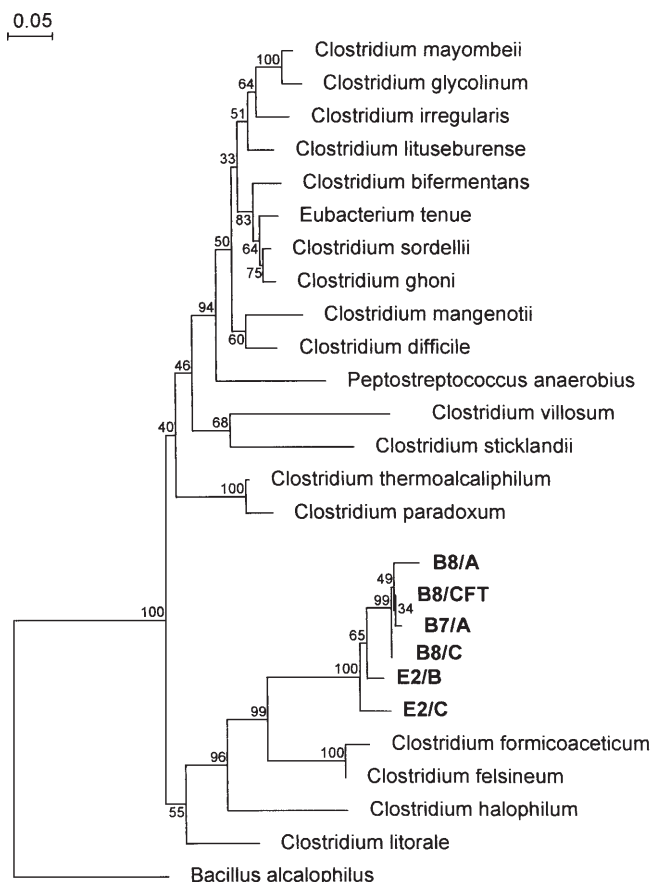
Fig. 3. Phylogenetic tree of the anaerobic soda lake isolates associated with members of the *Clostridium* "cluster XI" (Collins et al. 1994). Evolutionary distances were calculated using the substitution calibration method (Van de Peer et al. 1996), and the topology was inferred using the neighbor-joining method (Saitou and Nei 1987) based on bootstrap analysis of 1000 trees. *Bar*, 0.05 substitutions per nucleotide position

Fig. 4. Phylogenetic tree showing the relationship between haloalkaliphilic isolates and members of the *Clostridium* spectrum (Collins et al. 1994). *Bar*, 0.05 substitutions per nucleotide position

(Collins et al. 1994) (Fig. 3), where they formed a distinct group separate from the previously described alkaliphiles *Clostridium paradoxum* (Li et al. 1993) and *Clostridium thermoalcaliphilum* (Li et al. 1994). A further
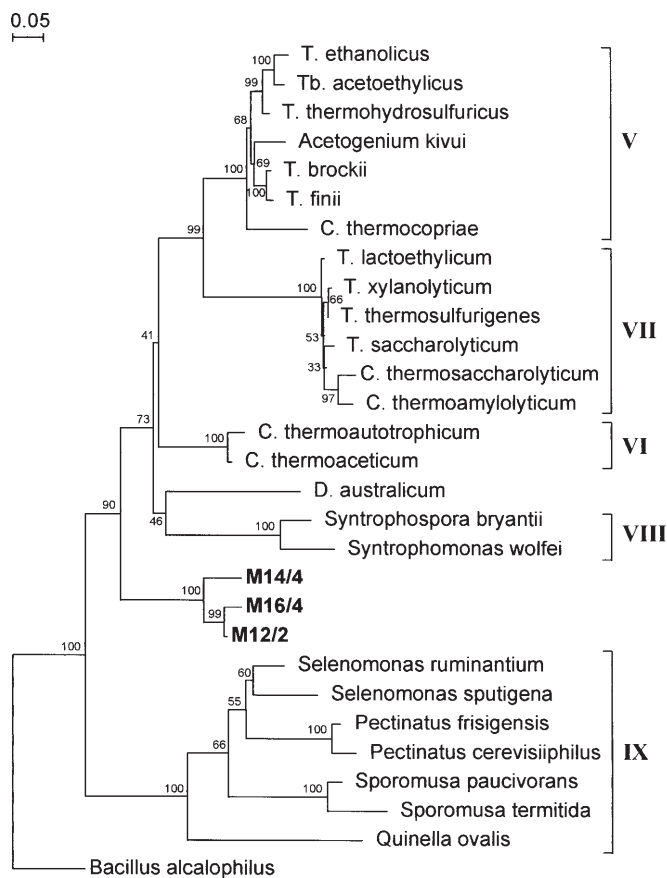
chemoorganotrophic isolate from a SRB enrichment on lactate was also sequenced before the strain was permanently lost. Phylogenetic analysis recovered this strain within "cluster V" of the *Clostridium* group. Although this strain was found to be most closely related to members of the genus *Eubacterium*, sequence similarity levels suggested

that this organism might belong to a new genus (data not shown). The haloalkaliphilic strains isolated from Lake Magadi were clearly different, and detailed phylogenetic analysis placed them between clostridial "clusters VIII and XI" in the scheme of Collins et al. (1994) (Fig. 4), forming a separate, well-defined group with *Clostridium thermoaceticum* and *Clostridium thermoautotrophicum* ("cluster VI") as closest relatives. The evidence is compelling that these strains represent a new genus of obligately anaerobic haloalkaliphiles (Owenson 1997).

The Rift Valley is a volcanically active region, and many of the soda lakes are fed by hot springs at temperatures of 45°–96°C. These are complex habitats, often characterized by a variety of algal mat types whose true nature has never been investigated. These ecosystems provide some interesting gradients of pH, salinity, and temperature. From anaerobic sediments of hot springs at Lake Bogoria, we isolated strictly anaerobic rod-shaped bacteria having a typical outer sheathlike structure and growing optimally at 70°C and pH 9.5. The isolates were gram-negative, fermentative bacteria growing up to 78°C and above pH 10.5 in a low-salts medium containing carbonate (Meijer et al. 1996). 16S rDNA sequencing placed these isolates among the *Thermotogales* but without any close association with known genera (Duckworth et al. 1996). The assignment "*Thermopallium natronophilum*" ("the soda-loving hot cloak") will be proposed to accommodate these strains.

Soda lakes past and present

Although we can try to construct some unifying theory to describe nutrient cycling and the microbial community structure in soda lakes, it can only be superficial (Fig. 2). In the East African Rift Valley there is an obvious difference between the hypersaline lakes of the Magadi-Natron basin with salt concentrations approaching saturation (30% w/v or greater) and the "northern" lakes (Elmenteita, Nakuru, Bogoria, etc.) with salinities of the order of 5% w/v. Soda lakes in other parts of the world may have different chemistry, reflecting local conditions, and these in turn may harbor other specific organisms. However, soda lakes are essentially closed systems, and as Zavarzin (1993) observed should exhibit complete nutrient recycling. The input of carbon and nitrogen into the soda lake ecosystem (if we ignore the obvious contribution by grazing flamingos, etc.) is predominantly through CO_2 and N_2 fixation by photosynthetic cyanobacteria (see Fig. 2). In the oxic zone, a variety of chemoorganotrophs utilize the products of primary production. Many of our aerobic isolates are nutritionally undemanding, producing a wide variety of hydrolytic enzymes, proteinases, cellulases, lipases, amylases, etc. for the recycling of biopolymers. In the anoxic zone some of the elements of nutrient cycling are clear. *Clostridium* spp. use fermentable carbohydrates and amino acids, producing volatile fatty acids (Table 4) (Owenson 1997). Saccharolytic spirochetes perform a similar function, utilizing sugars and a limited range of polysaccharides to produce acetate, lac-

tate, ethanol, and H_2 (Zhilina et al. 1996a). In hypersaline lakes such as Lake Magadi these products may be consumed by the haloalkaliphilic homoacetogen *Natroniella acetogena* (Zhilina et al. 1996b).

Sulfur plays a critical role as interspecies electron carrier. Sulfate-reducing bacteria are a H_2 sink for anaerobic fermentative bacteria and provide sulfide as the electron donor for autotrophic sulfur-oxidizing bacteria, anaerobic *Ectothiorhodospira* spp., and aerobic autotrophs and heterotrophs (Sorokin et al. 1996a,b). Methanogenic bacteria so far identified in soda lakes are obligately methylotrophic and do not use acetate or H_2-CO_2 as energy-yielding substrates (Kevbrin et al. 1997; Lui et al. 1990; Mathrani et al. 1988) but use methanol, methylamines (from betaines?), and dimethyl sulfide, probably derived from the anaerobic degradation of algal mats. However, all the methane is not lost from the system. From Central Asian soda lakes methane-oxidizing bacteria have been isolated (Khmelenina et al. 1997), thus effectively closing the carbon cycle in our soda lake model.

Alkaliphiles are not just confined to soda lakes but may be found almost anywhere, even in deep-sea trenches and in soils whose bulk pH measurements would not suggest the presence of alkaliphiles (Horikoshi 1991). However, it is equally clear that some organisms are unique to soda lakes, especially the haloalkaliphiles found in the hypersaline lakes such as Lake Magadi. While we suggest that extremely halophilic alkaliphiles are confined to the hypersaline alkaline lakes, this is clearly not the case for the halotolerant and alkali-tolerant alkaliphiles found in the less saline soda lakes. Some of the species found in the Kenyan soda lakes, or their close relatives, have been found in different environments around the world. In particular, common endospore-forming alkaliphilic gram-positive bacteria, which are currently assigned to the genus *Bacillus*, are fairly ubiquitous. They may even turn up in man-made alkaline environments. For example, an alkaliphile isolated from olive-processing effluent was most closely related to an unclassified "low G+C gram-positive" bacterium isolated from Lake Nakuru (N.J. Russell, personal communication). We can probably assume that many of the nonexacting alkaliphiles have spread beyond the borders of their soda lake environs. From an experience of the Rift Valley situation it is not difficult to suggest mechanisms for dispersal. In this arid region, dust storms are common and great whirlwinds carry the soda dust vast distances. Moreover, the soda lakes are not entirely closed systems. They suffer considerable disturbance from wildlife, in particular, the vast flocks of wading flamingos that feed on *Spirulina* and return their feces to the nutrient cycle. These birds nest in the most inaccessible parts of Lake Natron and fly up and down the Rift Valley in search of lakes with cyanobacterial blooms on which to feed and freshwater to drink. They even penetrate the olive-growing regions of the Mediterranean. The Rift Valley is also a popular route for migratory birds that spend the summer in northern Europe and thus contribute to the spread of some alkaliphiles.

The apparent uniqueness of some of the soda lake prokaryotes implies that they must have evolved in an alka-

line, saline environment. Although soda lakes may be relatively uncommon, being confined to specific geographic regions (Zavarzin 1993; Grant and Tindall 1986), they are not insignificant because they comprise more than 80% of all inland waters by volume (Hammer 1986). It is obvious that soda lakes must have existed throughout the geological record. Even so, evaporites are ephemeral and are quickly recycled and unless deposits are large may go unrecognized. Present-day soda lakes are geologically very recent. Lake Magadi along with Lake Natron must be the oldest of the Rift Valley lakes since they are in the final active evolutionary phase of trona (sodium sesquicarbonate) production. The more dilute "northern" lakes are still in a much earlier phase of soda lake evolution. Lake Magadi in its present form is no older than 10000 years (in evolutionary terms a very short time span), but there were precursors to Lake Magadi. During the Middle Pleistocene, 800000 years ago, the area was covered by Lake Olorongo, a vast deep lake that covered a much larger area than the present Lake Magadi (Eugster and Hardie 1978). At times the water of Lake Olorongo was quite fresh; at other times in its history it had a pH of 9.5, probably with a chemistry similar to the present-day lakes to the north (Elmenteita, Nakuru, etc.). So, when we attempt to explain the diversity in the soda lake environment we may need to take into account their relatively short life span. Certainly, if our aim to describe diversity in terms of microbial communities and their interactions, we do need to relate these to the available nutrient and energy sources, the chemical and physical nature of the environment, and the range over which these may fluctuate. For example, essential cations (e.g., Mg^{2+}) become sequestered and microbes must evolve efficient means for their uptake. NH_4^+ as a nitrogen source would be limited by becoming volatile NH_3 at high pH, and levels of 0.3–5.4 mM total (Kjeldahl) nitrogen recorded in 20 water samples recovered from a variety of soda lake locations suggests concentrations can fluctuate enormously (Jones, unpublished).

One of the largest fossil soda lakes is the Green River formation in Utah and Wyoming (USA), created from two lakes (Lake Gosiute, Lake Uinta) covering an area of 100000 km^2 during the Eocene (55 to 36 million years ago) (Eugster and Hardie 1978). Over a period of more than 12 million years, sediments as much as 3 km thick were laid down together with trona in vast quantities. Underlying the trona beds are carbonaceous oil shales rich in kerogen, which would seem to indicate that organic matter does accumulate in soda lakes and that recycling is not complete. Sulfides are common in the oil shales, pointing to sulfate-reducing bacteria activity in the anaerobic muds. The trona deposits are mined commercially, and mine brine samples have yielded alkaliphilic and haloalkaliphilic Bacteria and Archaea, although the isolates have not been characterized in any detail (Grant, unpublished).

Fossil soda lakes of even greater age have been inferred from secondary characteristics. The 2.3-billion-year-old Ventersdorp Formation in South Africa, which dates from the Late Archean to Early Proterozoic era, shows evidence of processes similar to those presently going on in Lake Magadi (Karpeta 1989). More important to these arguments are the presence of stromatolites in the Precambrian Ventersdorp strata (Buck 1980). The layered rock columns known as stromatolites are fossilized cyanobacterial mat communities thought to have been laid down in shallow alkaline seas in arid zones. Modern equivalents of Precambrian stromatolites have been recorded in the East African Rift Valley (Casanova 1986), Lake Tanganyika (Kempe and Kazimerczak 1989), Lake Van (Kempe et al. 1991), and Lake Salda (Russell 1996), two alkaline lakes in Turkey. Cyanobacteria have evolved little in their long history. Indeed, there is little basis in morphology to distinguish extant cyanobacteria from their Proterozoic counterparts (Schopf 1992). *Spirulina*, a coiled cyanobacterium common in Rift Valley soda lakes, has an apparent parallel in *Heliconema*, a fossil cyanobacterium (Schopf 1992). The earliest evidence of stromatolites and cyanobacteria date back 3.5 billion years, perhaps only half a billion years after life began (Miller 1992).

Zavarzin (1993) has argued convincingly that alkaline soda lakes are ancient prokaryotic communities. Because there is considerable phylogenetic diversity among soda lake prokaryotes (see Table 1), which our work confirms and extends, and that the metabolic diversity this represents is sufficient to maintain an autonomous microbial community, Zavarzin speculates that continental (soda) lakes are the origin of prokaryotic diversity. Certainly, many of the problems assigning branching orders to 16S rDNA sequences arise because many of the major taxa diverged from each other over a relatively short time span. Arguably, this was the prokaryotic equivalent of the "Cambrian explosion," the sudden appearance of many different animal phyla in the fossil record (Runnegar 1992). The reconstruction of the paleoclimate is problematic (Miller 1992), and even if we discount the theory that the Archaean oceans were soda oceans (Kempe and Degens 1985), we may accept that the weathering of volcanic rocks on the megacontinents of the late Archaean era could lead to the conditions necessary for the formation of vast inland soda lakes. We have shown that alkaliphiles are not monophyletic, being found in most of the major prokaryotic groups (although, perhaps significantly, not in the Crenarchaeota, the extreme thermophiles) as separate lines of descent (Table 1). If the "Precambrian explosion" of prokaryotic diversity did take place in alkaline and probably saline environments (even if life itself began elsewhere), the hypothesis raises important questions about the nature of early biochemistry and ancestral lineages.

A major hurdle to our complete understanding of soda lake microbial communities is our ability to culture the microorganisms. At present our comprehension is based on organisms isolated offsite in a distant laboratory. These may not even be dominant microorganisms. Indeed, we have evidence that if experiments are conducted in situ then a different spectrum of organisms is isolated (Mwatha 1991). Recently, we attempted to circumvent this problem by a molecular approach. Soda lake water was filtered onsite through a series of membrane filters. The different colors of the filters indicated that some size fractionation of the organisms was achieved. From the filters DNA was extracted

and transported back to the laboratory. Attempts were made to amplify by PCR 16S rRNA genes using both bacterial and archaeal primers and to clone the products for sequence determination. The preliminary results are remarkable. From the clear, warmwater (48°C, pH 10.5) springs at Lake Magadi an archaeal signal was obtained, hinting at a hitherto uncultured thermoalkaliphile. From the Magadi salterns, which by conventional culturing techniques would appear to be a virtual monoculture of *Natronobacterium*, only archaeal primers were successful in generating the 16S rRNA gene product. Phylogenetic analysis of ten clones indicated that nine of these cloned 16S rRNA genes are distinct from those of cultivated haloalkaliphiles and other halobacteria, forming three groups on the periphery of the halobacterial branch of the Euryarchaeota. One cloned gene shows only 75% similarity to all known archaeal sequences, representing a new distinct archaeal line (Grant et al. 1998). While these studies are only at an early stage and will contribute to our understanding of soda lake diversity, there remains the challenge to obtain these organisms in culture so we may study their physiology. Only then will we understand their importance and contribution to these fascinating extreme environments.

References

Bath AH, Christofi N, Neal C, Philip JC, Cave MR, McKinley IG, Berner U (1987) Trace element and microbiological studies of alkaline groundwaters in Oman, Arabian Gulf: a natural analogue for cement pore waters. Report Fluid Processes Res. Group, Brit. Geol. Surv. FLPU 87-2

Beadle LC (1932) Scientific results of the Cambridge expedition to the East African lakes 1930. IV. The water of some East African lakes in relation to their flora and fauna. J Linn Soc Zool 38:157–211

Buck SC (1980) Stromatolite and oil deposits within the fluvial and lacustrine sediments of the Precambrian Ventersdorp supergroup of South Africa. Precambrian Res 12:311–320

Casanova J (1986) East African rift stromatolites. In: Frostick LE, Renaut RW, Reid I, Tiercelin JJ (eds) Sedimentation in the African rifts. Geol Soc Spec Publ No. 25. Blackwell, Oxford, pp 201–210

Collins MD, Lawson PA, Willems A, Cordoba JJ, Fernandez Garayzabal J, Garcia P, Cai J, Hippe H, Farrow JAE (1994) The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. Int J Syst Bacteriol 44:812–826

Duckworth AW, Grant WD, Jones BE, Van Steenbergen R (1996) Phylogenetic diversity of soda lake alkaliphiles. FEMS Microbiol Ecol 19:181–191

Eugster HP, Hardie LA (1978) Saline lakes. In: Lermann A (ed) Lakes: chemistry, geology and physics. Springer-Verlag, New York, pp 237–293

Florenzano G, Sili C, Pelosi E, Vincenzini M (1985) *Cyanospira rippkae* and *Cyanospira capsulata* (gen. nov. and spp. nov.): a new filamentous heterocystous cyanobacteria from Magadi Lake (Kenya). Arch Microbiol 140:301–306

Grant S, Grant WD, Jones BE, Kato C, Li L (1998) A novel archaeal lineage from an East African saltern. Abstracts, International congress extremophiles '98, Yokohama, Japan, p 156

Grant WD (1989) *Natronobacterium, Natronococcus*. In: Staley JT, Bryant MP, Pfennig N, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 3. Williams & Wilkins, Baltimore, pp 2230–2233

Grant WD, Tindall BJ (1980) The isolation of alkalophilic bacteria. In: Gould GW, Corry JCL (eds) Microbial growth and survival in extremes of environment. Academic Press, London, pp 27–36

Grant WD, Tindall BJ (1986) The alkaline, saline environment. In: Herbert RA, Codd GA (eds) Microbes in extreme environments. Academic Press, London, pp 22–54

Grant WD, Jones BE, Mwatha WE (1990) Alkaliphiles: ecology, diversity and applications. FEMS Microbiol Rev 75:255–270

Grant WD (1992) Alkaline environments. In: Lederberg J (ed) Encyclopedia of microbiology, vol 1. Academic Press, San Diego, pp 73–80

Groth I, Schumann P, Rainey FA, Martin K, Schuetze B, Augsten K (1997) *Bogoriella caseilytica* gen. nov., sp. nov., a new alkaliphilic actinomycete from a soda lake in Africa. Int J Syst Bacteriol 47:788–794

Hammer UT (1986) Saline lake ecosystems of the world. Junk, Dordrecht

Hao OJ, Chen JM, Huang L, Buglass RL (1996) Sulfate-reducing bacteria. Crit Rev Environ Sci Technol 26:155–187

Hensel R, Matussek K, Michalke K, Tacke L, Tindall BJ, Kohlhoff M, Siebers B, Dielenschneider J (1997) *Sulfophobococcus zilligii* gen. nov., spec. nov. a novel hyperthermophilic archaeum isolated from hot alkaline springs of Iceland. Syst Appl Microbiol 20:102–110

Horikoshi K (1991) Micro-organisms in alkaline environments. Kodansha, Tokyo and VCH Verslagsgesellschaft mbH, Weinheim

Horikoshi K, Akiba T (1982) Alkalophilic microorganisms. Springer, Berlin Heidelberg New York

Imhoff JF, Sahl HG, Soliman GSH, Trüper HG (1979) The Wadi Natrûn: chemical composition and microbial mass developments in alkaline brines of eutrophic desert lakes. Geomicrobiol J 1:219–234

Issatchenco BL (1951) Chlorous, sulphate and soda lakes of the Kalunda steppe and the biogenic processes in them. Selected works, vol 2. Academia Naukova, Leningrad, pp 143–162

Jenkin PM (1932) Reports of the Percy Sladen expedition to some Rift Valley lakes in Kenya in 1929. VII. Summary of the ecological results with special reference to the alkaline lakes. Ann Mag Nat Hist Ser X 8:133–181

Jones BE, Grant WD, Collins NC, Mwatha WE (1994) Alkaliphiles: diversity and identification. In: Priest FG, Ramos-Cormenzana A, Tindall BJ (eds) Bacterial diversity and systematics. Plenum, New York, pp 195–229

Jones BF, Eugster HP, Rettig SL (1977) Hydrochemistry of the Lake Magadi basin, Kenya. Geochim Cosmochim Acta 41:53–72

Kamekura M, Dyall-Smith ML, Upsani V, Ventosa A, Kates M (1997) Diversity of alkaliphilic halobacteria: proposals for transfer of *Natronobacterium vacuolatum*, *Natronobacterium magadii*, and *Natronobacterium pharaonis* to *Halorubrum*, *Natrialba*, and *Natronomonas* gen. nov., respectively, as *Halorubrum vacuolatum* comb. nov., *Natrialba magadii* comb. nov., and *Natronomonas pharaonis* comb. nov., respectively. Int J Syst Bacteriol 47:853–857

Karpeta WP (1989) Bedded cherts in the Riegat Formation, Hartbeesfontain, South Africa: a late Archean to early Proterozoic magadiitic alkaline playa lake deposit? South Afr J Geol 92:29–36

Kempe S, Degens ET (1985) An early soda ocean. Chem Geol 53:95

Kempe S, Kazimerczak J (1989) Calcium carbonate supersaturation and the formation of *in situ* calcified stromatolites. In: Ittekot V, Kempe S (eds) Facets of modern biogeochemistry. Springer, Berlin Heidelberg New York

Kempe S, Kazimerczak J, Landmann G, Konuk T, Reimer A, Lipp A (1991) Largest known microbialites discovered in Lake Van, Turkey. Nature (Lond) 349:605–608

Kevbrin VV, Lysenko AM, Zhilina TN (1997) Physiology of the alkaliphilic methanogen Z-7936, a new strain of *Methanosalsus zhilinaeae* isolated from Lake Magadi. Microbiology 66:261–266

Khmelenina VN, Kalyuzhnaya MG, Starostina NG, Suzina NE, Trotsenko YA (1997) Isolation and characterization of halotolerant alkaliphilic methanotrophic bacteria from Tuva soda lakes. Curr Microbiol 35:257–261

Li Y, Mandelco L, Wiegel J (1993) Isolation and characterization of a moderately thermophilic anaerobic alkaliphile, *Clostridium paradoxum* sp. nov. Int J Syst Bacteriol 43:450–460

Li Y, Engle M, Weiss N, Mandelco L, Wiegel J (1994) *Clostridium thermoalcaliphilum* sp. nov., an anaerobic and thermotolerant facultative alkaliphile. Int J Syst Bacteriol 44:111–118

Liu Y, Boone DR, Choy C (1990) *Methanohalophilus oregonense* sp. nov., a methylotrophic methanogen from an alkaline, saline aquifer. Int J Syst Bacteriol 40:111–116

Mathrani IM, Boone DR, Mah RA, Fox GE, Lau PP (1988) *Methanohalophilus zhilinae* sp. nov., an alkaliphilic, halophilic, methylotrophic methanogen. Int J Syst Bacteriol 38:139–142

Meijer D, Van Steenbergen R, Jones BE (1996) Isolation and characterisation of a novel alkaliphilic and thermophilic bacterium from the soda lake environment belonging to the order *Thermotogales*. Abstracts, International conference on thermophiles '96, Athens, GA, USA

Melack JM, Kilham P (1974) Photosynthetic rates of phytoplankton in East African alkaline, saline lakes. Limnol Oceanogr 19:743–755

Miller SL (1992) The prebiotic synthesis of organic compounds as a step toward the origin of life. In: Schopf JW (ed) Major events in the history of life. Jones and Bartlett, Boston

Mwatha WE (1991) Microbial ecology of Kenyan soda lakes. PhD thesis, University of Leicester, UK

Nielsen P, Fritze D, Priest FG (1995) Phenetic diversity of alkaliphilic *Bacillus* strains: proposal for nine new species. Microbiology (UK) 141:1745–1761

Nielsen P, Rainey FA, Outtrup H, Priest FG, Fitze D (1994) Comparative 16S rDNA sequence analysis of some alkaliphilic bacilli and establishment of a sixth rRNA group within the genus *Bacillus*. FEMS Microbiol Lett 117:61–66

Owenson GG (1997) Obligately anaerobic alkaliphiles from Kenya soda lake sediments. PhD thesis, University of Leicester, UK

Rich F (1933) Scientific results of the Cambridge expedition to the East African lakes 1930. VII. The algae. J Linn Soc Zool 38:249–275

Runnegar B (1992) Evolution of the earliest animals. In: Schopf JW (ed) Major events in the history of life. Jones and Bartlett, Boston

Russell MJ (1996) The generation of hot springs of sedimentary ore deposits, microbialites and life. Ore Geol Rev 10:199–214

Saitou N, Nei M (1987) The neighbour joining method – a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Schopf JW (1992) The oldest fossils and what they mean. In: Schopf JW (ed) Major events in the history of life. Jones and Bartlett, Boston

Sorokin DY, Lysenko AM, Mitushina LL (1996a) Isolation and characterisation of alkaliphilic heterotrophic bacteria which oxidize reduced sulfur compounds to tetrathionate. Microbiology 65:326–338

Sorokin DY, Van Steenbergen R, Robertson LA, Jones BE, Kuenen JG (1996b) Isolation and characterisation of alkaliphilic chemolithotrophs from soda lakes. Abstracts, 1st international congress on extremophiles, Estoril, Portugal

Talling JF, Wood RB, Prosser MV, Baxter RM (1973) The upper limit of photosynthetic productivity by phytoplankton: evidence from Ethiopian soda lakes. Freshwater Biol 3:53–76

Van de Peer Y, Van der Auwera G, De Wachter R (1996) The evolution of stramenopiles and alveolates as derived by "substitution rate calibration" of small ribosomal subunit RNA. J Mol Evol 42:201–210

Woese CR, Weisburg WG, Hahn CM, Paster BJ, Zablen LB, Lewis BJ, Macke TJ, Ludwig W, Stackebrandt E (1985) The phylogeny of purple bacteria: the gamma subdivision. Syst Appl Microbiol 6:25–33

Zavarzin GA (1993) Epicontinental soda lakes as probable relict biotopes of terrestrial biota formation. Microbiology 62:473–479

Zhilina TN, Zavarzin GA (1994) Alkaliphilic anaerobic community at pH 10. Curr Microbiol 29:109–112

Zhilina TN, Zavarzin GA, Rainey F, Kevbrin VV, Kostrikina NA, Lysenko AM (1996a) *Spirochaeta alkalica* sp. nov., *Spirochaeta africana* sp. nov., and *Spirochaeta asiatica* sp. nov., alkaliphilic anaerobes from the continental soda lakes in Central Asia and the East African rift. Int J Syst Bacteriol 46:305–312

Zhilina TN, Zavarzin GA, Detkova EN, Rainey FA (1996b) *Natroniella acetigena* gen. nov. sp. nov., an extremely haloalkaliphilic, homoacetic bacterium: a new member of *Haloanaerobiales*. Curr Microbiol 32:320–326

Zhilina TN, Zavarzin GA, Rainey FA, Pikuta EN, Osipov GA, Kostrikina NA (1997) *Desulfonatronovibrio hydrogenovorans* gen. nov., sp. nov., an alkaliphilic, sulfate-reducing bacterium. Int J Syst Bacteriol 47:144–149