

2.2 Distribution and Diversity of Soda Lake Alkaliphiles

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Genesis of Soda Lakes

Soda lakes and soda deserts are the most stable naturally occurring alkaline environments on earth, where pH values of 10 and above are common. As the name implies, these environments are characterized by large amounts of soda (sodium carbonate, or complexes of this salt), formed by evaporative concentration (Grant 1992; Jones and Grant 2000). Other salts, especially sodium chloride may also concentrate leading to the formation of alkaline saline lakes. Although soda lakes have a worldwide distribution (📍 [Table 2.2.1](#)), they are mainly confined to subtropical latitudes in continental interiors or rain-shadow zones.

Following a very early study by of the Wadi Natrun in Egypt (1898), the likely explanations for a general mechanism promoting alkalinity have been reviewed by a number of authors, notably the work by Eugster and colleagues (Eugster and Hardie 1978; Hardie and Eugster 1970) reviewed by Jones et al. (1994). The simplest theory would suppose the contribution of Na_2CO_3 via vulcanism – at least one active volcano generates a soda-rich lava flow in the southern path of the Rift Valley (Eugster 1970). However, this seems to be an exceptional case and there seems to be universal agreement that the most important feature of such areas is that the surrounding geology is dominated by high Na^+ , low $\text{Mg}^{2+}/\text{Ca}^{2+}$ silicates. The amount of Ca^{2+} in the surrounding geology (and to a lesser extent Mg^{2+}) is critical in determining the final pH of brine. Waters high in Na^+ , Cl^- , and $\text{HCO}_3^-/\text{CO}_3^{2-}$ evaporate down and if the concentration of $\text{HCO}_3^-/\text{CO}_3^{2-}$ greatly exceeds that of any Ca^{2+} and Mg^{2+} , these cations precipitate as insoluble carbonates leaving behind an alkaline brine that develops as a consequence of a shift in the $\text{CO}_2/\text{HCO}_3^-/\text{CO}_3^{2-}$ equilibrium toward CO_3^{2-} , causing the development of a soda (Na_2CO_3) lake with pH values usually between 10 and 11, occasionally >pH12 (Jones et al. 1994). In the presence of significant amounts of Ca^{2+} (and Mg^{2+}), buffering occurs by the constant removal of alkaline carbonates (one of the main buffering systems in the marine environment), leading to a neutral hypersaline lake like the Great Salt Lake, or in the case of very high Mg^{2+} levels a slightly acid, hypersaline lake like the Dead Sea where acidity is generated by the chemistry of precipitation of Mg minerals like sepiolite. 📍 [Figure 2.2.1](#) depicts alternative pathways of brine formation dependant on original ion composition. The precise ion composition depends on the local geology.

The best-studied area where soda lakes have formed is the Kenyan–Tanzanian Rift Valley. The Great Rift Valley running through East Africa is an arid tropical zone where tectonic activity has created a series of shallow depressions. These shallow depressions are often closed basins with no obvious outflow where ground water and streams flowing from the surrounding highlands on the margins of the Rift Valley collect to form semipermanent standing bodies of water. In these zones with high rates of evaporation exceeding inflow, salts accumulate by evaporative concentration (Jones et al. 1994). Here, surrounding high Na^+ trachyte lavas are deficient in both Ca^{2+} and Mg^{2+} . As a result of evaporation in this arid tropical zone, saturation of the alkaline earth cations is rapidly achieved. Concentration of ions leads to Na^+ , Cl^- , and $\text{HCO}_3^-/\text{CO}_3^{2-}$ as the major ions in solution. Total salinities vary with season and range from around 5%(w/v) total salts in the more dilute lakes such as Lake Elmenteita made up mainly from roughly equal amounts of NaCl and Na_2CO_3 at a pH of about 11, to saturated lakes like Lake Magadi at >30% (w/v) total salts, again dominated by NaCl and Na_2CO_3 at pH values approaching 12.

About one third of all salt and soda lakes in the world are to be found in an area that stretches from Tibet, throughout Qinghai province to the Inner Mongolia Autonomous Region, although many are unnamed. These lakes are situated in cryoarid zones that experience

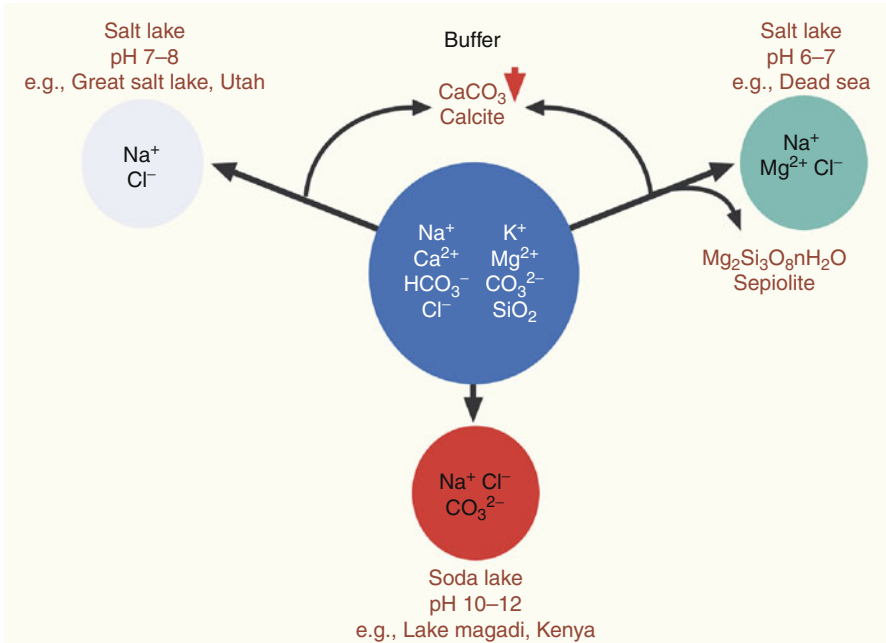
■ **Table 2.2.1**

Worldwide distribution of soda lakes and soda deserts

North America	
Canada	Lake Manito
United States	Alkali Valley, Albert Lake, Lake Lenore, Soap Lake, Big Soda Lake, Owens Lake, Mono Lake, Searles Lake, Deep Springs, Rhodes, Marsh, Harney Lake, Summer Lake, Surprise Valley, Pyramid Lake, Walker Lake
Central America	
Mexico	Lake Texcoco
South America	
Venezuela	Langunilla Valley
Chile	Antofagasta
Europe	
Hungary	Lake Feher
Yugoslavia	Pecena Slatina
Russia	Kulunda Steppe, Tanatar Lakes, Karakul, Araxes plain, Chita, Barnaul, Slavgerod
Asia	
Turkey	Lake Van
India	Lake Lonar, Lake Sambhar
China	Qinghai Hu, Sui-Yuan, Heilungkiang, Kirin, Jehol, Chahar, Shansi, Shensi, Kansu
	Lake Chahannor; Lake Zabuye; Bange Lake; Lake Baer; Lake Wudunao; Lake Hamatai
Africa	
Libya	Lake Fezzan
Egypt	Wadi Natrun
Ethiopia	Lake Aranguadi, Lake Kilotes, Lake Abiata, Lake Shala, Lake Chilul, Lake Hertale, Lake Metahara
Sudan	Dariba lakes
Kenya	Lake Bogoria, Lake Nakuru, Lake Elmentietia, Lake Magadi, Lake Simbi, Lake Sonachi
Tanzania	Lake Natron, Lake Embagi, Lake Magad, Lake Manyara, Lake Balangida, Bosotu Crater, Lakes, Lake Kusare, Lake Tulusia, El Kekhooito, Momela Lakes, Lake Lekandiro, Lake Reshitani, Lake Lgarya, Lake Nduku, Lake Rukwa North
Uganda	Lake Katwe, Lake Mahega, Lake Kikorongo, Lake Nyamunuka, Lake Munyanyange, Lake Murumuli, Lake Nunyampaka
Chad	Lake Bodu, Lake Rombou, Lake Dijikare, Lake Momboio, Lake Yoan
Australia	
Australia	Lake Corangamite, Red Rock Lake, Lake Werowrap, Lake Chidnup

little rainfall and these largely ephemeral sites are charged by occasional rainfall leaching through the surface topography additionally influenced by the evaporative effect of constant wind (Mianping et al. 1993).

There is no doubt that in common with the majority of saline sites, soda lakes harbor significant populations of microorganisms. Some of the first studies in modern times were those of (Isachenko 1951) on the Kulunda Steppe Region. Microorganisms that inhabit soda



■ Fig. 2.2.1

Schematic representation of the genesis of hypersaline brines. The *center box* indicates the leaching of minerals by CO_2 -charged waters. Alkaline lake development is dependent on low levels of Ca^{2+} and Mg^{2+} . Neutral lakes develop where Ca^{2+} and Mg^{2+} levels are high. High Mg^{2+} lakes are more acidic due to reactions involving sepiolite precipitation

lakes have to be of necessity alkali-loving or at least alkali-tolerant. The term “alkaliphile” is usually reserved for microorganisms growing optimally or very well at pH values above 9, often with pH optima for growth around 10, showing little, or no growth, at near neutral pH values. Microorganisms found in hypersaline soda lakes generally have an additional requirement for high levels of NaCl – as such, they are known as “haloalkaliphiles.”

Soda Lake Chemoorganotrophs

Soda lakes have high levels of organic matter that supports dense populations of organotrophic prokaryotes. The brines 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, (up to 13,000 cyanobacterial filaments ml^{-1}) (Grant 1992) supports the rest of the microbial community. These blooms of cyanobacteria are usually dominated by *Spirulina*

(*Arthrospira*) 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 (Jones and Grant 2000). Unicellular *Eurhalotheca* types may also be present in hypersaline sites (Mikhodiuk et al. 2008) as well as *Microcoleus chthonoplastis* (Kompantseva et al. 2005). Cyanobacteria are the principal food of the immense flocks of flamingos that inhabit the Rift Valley, one of the most studied soda lake areas.

Not only are the cyanobacteria critical for the fixation of CO₂ and thus generation of carbon for secondary heterotrophs, they also contribute toward fixation of nitrogen in these environments and are also producers of O₂. Daytime rates of oxygen production >2 g O₂ m⁻² h⁻¹ have been recorded from *Spirulina* spp. (Melack and Kilham 1974). However, during a 12-month survey of aerobic heterotrophic bacterial numbers in relation to cyanobacterial numbers no correlation was found between secondary productivity and the timing of a cyanobacterial bloom (Grant et al. 1990; Jones et al. 1994). In fact, bacterial numbers were remarkably constant, although the dominant types varied. These bacteria may be readily isolated on appropriate media (Grant and Tindall 1980). Viable counts of aerobic organotrophs from a range of dilute lakes indicate 10⁵–10⁶ cfu ml⁻¹ (Grant et al. 1990).

The soda lake microbial community contains alkaliphilic representatives of all the major trophic groups of bacteria and archaea. Between these groups, there is cycling of carbon, sulfur, and nitrogen under aerobic and anaerobic conditions present in the lakes. There seems little doubt that in East Africa at least, *Arthrospira platensis*, and *Cyanospira rippkae* are responsible for photosynthetic primary production in dilute lakes. There is also an unquantified contribution to primary productivity made by anoxygenic phototrophic bacteria of the genus *Ectothiorhodospira* (Grant 1992) also seen in other geographical areas (Imhoff et al. 1979; Kompantseva et al. 2005), such as the Transbaikal region and the Wadi al Natrun lakes in Egypt. *Rhodobaca* spp. (Boldareva et al. 2008) and *Rubribacterium* sp. (Boldareva et al. 2009) have also been recorded. It is probable that hypersaline lakes during periods of dilution in any rainy season contain related cyanobacteria and similar anoxygenic phototrophs that may be responsible for primary productivity (Grant 2004).

A survey of those examples of alkaliphiles and haloalkaliphiles brought into culture reveals a remarkable diversity of types, with aerobic alkaliphiles represented in many of the major taxonomic groups of bacteria. Hypersaline environments in particular are relatively low in oxygen due to reduced oxygen solubility (2 ppm in saturated NaCl, compared with 7 ppm in seawater) hence suitable for anaerobic anoxygenic phototrophs and the brines also harbor substantial populations of anaerobic heterotrophs.

The first comprehensive culture survey of soda lake environments that attempted phylogenetic placement of the isolates was that carried out by (Duckworth et al. 1996) who isolated several hundred strains of aerobic, heterotrophic alkaliphilic, and haloalkaliphilic organotrophs from a range of soda lakes in the East African Rift Valley. Phylogenetic analysis revealed many proteobacteria, notably halomonads, plus high and low G + C Gram-positives. Some of these have now been published as novel types, including *Halomonas magadiensis* (Duckworth et al. 2000), *Cellulomonas bogoriensis* (Jones et al. 2005), *Alkalimonas delamerensis* (Ma et al. 2004a), *Dietzia natronolimnaea* (Duckworth et al. 2004). The halomonads (members of the Halomonadaceae like *H. magadiensis*) are probably the most important group of bacterial heterotrophs in both alkaline and neutral hypersaline environments, although other proteobacteria related to pseudomonads and vibrios are also present (Duckworth et al. 1996). Heterotrophic Gram-positive bacteria of both the high G + C (Firmicutes) and low G + C (Actinobacteria) lineages were also readily isolated from hypersaline brines in this study.

Especially abundant were members of the low G + C lineage associated with the diverse *Bacillus* spectrum. There were also high G + C relatives of streptomycetes. A more recent culture survey of Lonar soda lake in India revealed a remarkably similar range of aerobic alkaliphiles except that low G + C Gram positives were in a majority. Halomonads were also significant among the Gram negatives (Joshi et al. 2008). A number of other soda lakes at widely different geographical sites have yielded a number of new species including *Streptomyces sodiophilus* from a Chinese lake (Li et al. 2005), *Heliorestis convoluta* from the Wadi al Natrun (Asao et al. 2006), *Roseinatronobacter monicus* from Mono Lake in the USA (Boldareva et al. 2007), further validly named new *Halomonas* spp. from China and Kenya (Boltianskaia et al. 2007), *Bacillus aurianticus* from a Hungarian soda lake (Borsodi et al. 2008), *Nesterenkonia aethiopica* from an Ethiopian soda lake (Delgado et al. 2006), and *Alkalililimnicola ehrlichii* from Mono Lake (Hoeft et al. 2007).

In recent years, the microbial population of several of the Inner Mongolian soda lakes has been subject to quite detailed aerobic culture-based analyses and a similar picture of the heterotrophic aerobes has emerged with examples of proteobacteria such as newly described *Marinospirillum* spp. (Zhang et al. 2002), *Alkalimonas* spp. (Ma et al. 2004a), and *Aquisalimonas* spp. (Márquez et al. 2007). Again, particularly abundant were members of the high G + C *Bacillus* spectrum with new *Bacillus* spp. and the description of many new genera in addition such as *Gracilibacillus* (Carrasco et al. 2006), *Salsuginibacillus* (Carrasco et al. 2007), *Aquisalibacillus* (Márquez et al. 2007), *Sediminibacillus* (Carrasco et al. 2008b), *Amphibacillus haojiensis* (Zhao et al. 2004), and *Halolactibacillus* (Cao et al. 2008). Remarkably, many of these new species and genera from around the world are related to unnamed groups described in the early paper by (Duckworth et al. 1996) on the aerobes in the Rift Valley.

Isolates from soda lakes have proven to have commercial potential in that they secrete many extracellular hydrolytic enzymes, including proteinases, cellulases, and lipases capable of functioning at high pH and possibly high temperature and salt concentration. As such, they are of interest to the detergent industry in particular. Currently, two different cellulases derived from Gram-positive soda lake isolates are marketed for use in laundry and textile processes.

As noted, chemoorganotrophic populations are biochemically very active, hydrolyzing many different polymers, producing sugars and amino acids. These may be used as substrates for the fermentation of simple compounds by anaerobic fermenters. Fatty acids produced by anaerobes may be consumed by other groups such as the alkaliphilic soda lake acetogenic bacteria, including *Natroniella acetigena*, *Thermosyntropha lipolytica* (Svetlitsnyi et al. 1996), and *Tindallia magadiensis* (Zavarzin et al. 1999). Zavarzin, Zhilina, and colleagues have carried out exceptional pioneering studies and continue ongoing work on soda lake anaerobes from a variety of lakes in the former USSR. Viable counts on anoxic soda lake sediments indicate they contain $>10^6$ cfu ml⁻¹ (37°C) chemoorganotrophic alkaliphilic anaerobes especially members of the *Halanaerobiales* and organisms related to other clostridial groups in the low G + C diversion of the Gram-positive bacteria (Jones et al. 1998). These bacteria too, are likely to have a worldwide distribution, for example, *Spirochaeta* spp. have been found at Lake Magadi (Kenya) and Lake Khatyn (Central Asia) (Zhilina et al. 1996). These organisms utilize a wide variety of pentoses, hexoses, and disaccharides producing acetate, lactate, ethanol, and H₂. Obligately anaerobic isolates from the moderately saline lakes were associated with the *Clostridium* group XI. They are phenotypically quite diverse, fermenting a variety of simple sugars or amino acids to acetate and propionate or butyrate and a maximum salt tolerance ranging from 4–12% (w/v). Different isolates were obtained from the hypersaline habitats at Lake Magadi. Phylogenetic analysis also placed these isolates within the *Clostridium* spectrum

but as a separate, well-defined group representing a new genus of obligately anaerobic haloalkaliphiles (Jones et al. 1998).

New species of *Tindallia* have been reported from Lake Texcoco (Alazard et al. 2007); a clostridial cluster XI relative *Anaerovirgula multivorans* from Owens lake in the USA; *Natronobacillus azotoifgens* (also a N₂ fixer) from several sites including Siberia, Mongolia, and Libya (Sorokin et al. 2008a); *Anaerobacillus alkaliazotrophicus* from Lake Khady in Russia (Zavarzina et al. 2009); and *Clostridium alkalicellum*, *Natrinicola ferrireducens*, and *Alkaliphilus peptidofermens* from Verkhnee soda lake in Russia (Zhilina et al. 2005a, Zhilina et al. 2009a, Zhilina et al. 2009b).

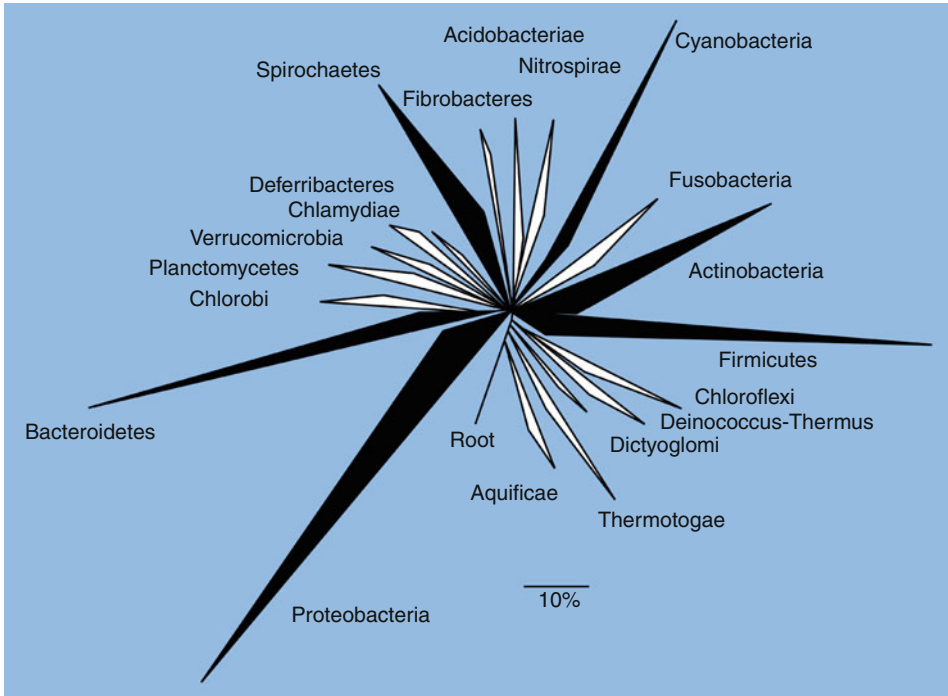
Recent culture studies by Wiegel and colleagues on the Egyptian soda lakes of the Wadi al Natrun have provided new information on the diversity of anaerobes at these sites (Mesbah et al. 2007), including several new genera (*Natronovirga*, *Natrananaerobius*) and the creation of a new family *Natranaerobiaceae* to accommodate these. The majority of these isolates exhibit the additional extremophile trait of moderate thermophily, a reflection of thermal sites in some soda lake environments. These “polyextremophiles” are, however, beyond the scope of this chapter and are considered elsewhere in this volume.

Conventional wisdom would suggest that in soda lakes the importance of sulfate-reducing bacteria is likely to be considerable. Geochemists have often implied the presence of microbially mediated sulfate-reducing activity in soda lakes in order to explain the appearance of the black lacustrine sediments or the relative depletion of sulfate in the brines of Lake Magadi for example, but until recently microbiological evidence has been lacking. The black color of the sediments of the Kenyan soda lakes would indicate the presence of sulfide (Jones et al. 1998) but there is often no detectable smell of H₂S presumably because the high pH (>pH 11) retards the escape of S²⁻ as volatile H₂S. A number of initially elusive soda lake sulfate-reducing bacteria have now been brought into culture, including *Desulfonatronospira thiodismutans* (Sorokin et al. 2008a), *Desulfonatronum cooperatum* (Zhilina et al. 2005), *Desulfonatronovibrio hydrogenovorans* (Zhilina et al. 1997), *Desulfonatronum lacustre* (Pikuta et al. 1998), and recent isolates (*Dethiobacter* and *Desulfurivibrio* spp.) reported by Sorokin et al. (2008).

► **Figure 2.2.2** indicates that bacteria isolated from soda lakes are restricted to the most studied evolutionary lines of bacteria, probably a reflection of the culture techniques applied to date. It is probably no accident that the halophilic character coincides with the same lines, a reflection of the usual association between high pH and elevated salt levels in soda lakes.

More recently, studies have tried to analyze the total prokaryote biotope in soda lake environments by extracting DNA and amplifying total 16S rRNA genes, followed by the sequence analyses of the diversity of 16S rRNA clone libraries. Whereas in almost all studies, the bacterial community in both the water and sediments of the lakes was dominated by clones affiliated with the low G+C Gram-type-positive group, Firmicutes-related clones, Proteobacteria, and *Bacteroidetes*, molecular analyses has revealed unexplored biodiversity as judged by the large number of novel bacteria and archaeal 16S rRNA gene sequences that have been detected in East African sites (Grant et al. 1999; Rees et al. 2004), Inner Mongolian sites (Ma et al. 2004b; (Pagaling et al. 2009)), Lake Van in Turkey (Lopez-Garcia et al. 2005), and the Wadi al Natrun (Mesbah et al. 2007).

A detailed biogeographical study of clone sequences from a range of Inner Mongolian lakes and one Argentinean lake has attempted to correlate 16S rRNA sequence diversity with lake chemistry and geography, coming to the conclusion that over short geographical distances, lake chemistry is the determining factor, whereas over large geographical distances, geographical separation becomes important (Pagaling et al. 2009).



■ Fig. 2.2.2

16S rRNA gene sequence tree showing the major bacterial lines of descent. Lines of descent with known soda lake isolates are shown shaded

Soda Lake Haloarchaea

The extremely halophilic, aerobic archaea that require least 1.5 M NaCl for growth are placed in the order Halobacteriales, family Halobacteriaceae, class Haloarchaea (Grant et al. 2001). The current classification of this family is based mainly on three taxonomical characters: 16S rRNA gene sequence, polar lipid composition, and DNA–DNA hybridization (Grant et al. 2001; Oren et al. 1997). Initially classified solely on morphological and biochemical criteria, the group initially comprised only two original genera, *Halobacterium* and *Halococcus*, subsequently expanded to six genera following the adoption of polar lipid analysis as a discriminatory tool (Grant and Larsen 1989). The advent of 16S rRNA gene sequence analyses revolutionized the understanding of the systematics of the group. At the time of writing, the aerobic, extremely halophilic archaea are classified within 28 different genera. Type species of these genera are *Halobacterium salinarum* (Elazari-Volcani 1957), *Halococcus morrhuae* (Kocur and Hodgkiss 1973), *Natronobacterium gregoryi* (Tindall et al. 1984), *Haloarcula vallismortis* (Torreblanca et al. 1986), *Haloferax volcanii* (Torreblanca et al. 1986), *Natronococcus occultus* (Tindall et al. 1984), *Halobaculum gomorrense* (Oren et al. 1995), *Halorubrum saccharovorum* (McGenity and Grant 1995), *Natrialba asiatica* (Kamekura and Dyal-Smith 1995), *Natronomonas pharaonis* (Kamekura et al. 1997), *Halogeometricum borinquense* (Montalvo-Rodríguez et al. 1998), *Natrinema pellirubrum* (McGenity et al. 1998), *Haloterrigena turkmenica* (Ventosa et al. 1999), *Natronorubrum bangense* (Xu et al. 1999), *Halorhabdus utahensis* (Wainø et al. 2000),

Halobiforma haloterrestis (Hezayen et al. 2002), *Halomicrobium mukohataei* (Oren et al. 2002), *Halosimplex carlsbadense* (Vreeland et al. 2002), *Halalkalicoccus tibetensis* (Xue et al. 2005), *Natronolimnobius baerhuensis* (Itoh et al. 2005), *Halovivax asiaticus* (Castillo et al. 2007), *Halostagnicola larsenii* (Castillo et al. 2006), *Haladaptatus paucihalophilus* (Savage et al. 2007), *Halopiger xanaduensis* (Gutiérrez et al. 2007), *Haloplanus natans* (Elevi-Bardavid et al. 2007), *Haloquadratum walsbyi* (Burns et al. 2007), *Halosarcina pallida* (Savage et al. 2008), and *Natronoarchaeum mannanyliticus* (Shimane et al. 2010).

The haloalkaliphilic archaea are a distinct physiological group of haloarchaea due to their obligate alkaliphily (Grant and Larsen 1989). These archaea have been found in hypersaline alkaline habitats such as soda lakes and soda soils at many different geographical sites, for example, Lake Magadi in Kenya ((Tindall et al. 1980; Tindall et al. 1984); Mwatha and Grant, 1993; (Duckworth et al. 1996; Kanai et al. 1995)), the Wadi Natrun in Egypt (Morth and Tindall 1985; Soliman and Trüper 1982), Owens Lake in California (Morth and Tindall 1985), soda lakes in China, Inner Mongolia, and Tibet (Feng et al. 2005; Itoh et al. 2005; Tian et al. 1997; Wang and Tang 1989; Xu et al. 1999; Xu et al. 2001; Xue et al. 2005), soda solonchak soils in Russia (Zvyagintseva and Tarasov 1987), and a soda lake in India (Upasani and Desai 1990).

As might be expected, the isolates from alkaline soda lakes, as well as having a requirement for high levels of salt, have an additional requirement for high pH in growth media (haloalkaliphilic), usually growing between pH 8.5 and 11.0 with an optimum at pH 9.5–10.0, whereas those haloarchaea from neutral sites generally have pH optima for growth between pH 6 and pH 8 (haloneutrophilic). The extremely alkaline conditions would appear to favour the use of sodium ions rather than protons as the coupling ion in energy generation, but analysis of the genome sequence of the haloalkaliphilic haloarchaeon *Natronomonas phaoronis* clearly identifies protons as the coupling ion between respiratory chain and ATP synthesis (Falb et al. 2005).

Haloalkaliphilic haloarchaea were initially assigned to the then new genera *Natronobacterium* and *Natronococcus* described by (Tindall et al. 1984) following a study of Lake Magadi in Kenya, after earlier reports (Soliman and Trüper 1982; Tindall et al. 1980) of red halophiles at Kenyan and Egyptian alkaline hypersaline sites. Apart from the obligate requirement for high pH shown by the haloalkaliphilic strains, unlike neutrophilic haloarchaea, these organisms also exhibited very low requirements for Mg^{2+} , a consequence of living in alkaline brines essentially devoid of Mg^{2+} that has been removed by precipitation as insoluble $MgSO_4$ as the alkaline brine developed. Several of the other haloarchaeal genera referred to earlier exclusively harbor haloalkaliphilic types (often genera with *Natrono* prefixes), whereas other genera, notably *Halorubrum*, *Natrialba*, and *Halobiforma* have haloalkaliphilic soda lake representatives together with haloarchaea that are have pH optima for growth in the neutral region derived from neutral sites. ▶ [Table 2.2.2](#) lists those validly published haloalkaliphilic species that have been isolated from soda lakes. It should be noted that several of the neutrophilic haloarchaea are capable of some growth at quite alkaline pH despite originating in neutral pH saline environments. The recently described *Natronoarchaeum mannanyliticum* is also clearly a haloalkaliphile despite having been isolated from salt made from seawater at a salt making works in Japan (Shimane et al. 2010).

The climax population in sodium-dominated hypersaline lakes at the point of halite (NaCl) precipitation is almost always comprised of haloarchaea. Dense blooms of these organisms color neutral (and alkaline) hypersaline lakes and salterns bright red due to carotenoid production (▶ [Fig. 2.2.3](#)). In neutral hypersaline lakes, there is also some variable color contribution from bacteria of the genus *Salinibacter* and the eukaryotic alga *Dunaliella salina*

■ Table 2.2.2

Soda lake haloarchaea

Species	Original location	Reference
<i>Natronococcus occultus</i>	Lake Magadii, Kenya	(Tindall et al. 1984)
<i>Natronococcus amylolyticus</i>	Lake Magadii, Kenya	(Kanai et al. 1995)
<i>Natronobacterium gregoryi</i>	Lake Magadii, Kenya	(Tindall et al. 1984)
<i>Natronomonas pharonis</i>	Lake Magadii, Kenya	(Tindall et al. 1984) Kamekura et al. 1997
<i>Natrialba magadii</i>	Lake Magadii, Kenya	(Tindall et al. 1984) (Kamekura et al. 1997)
<i>Natrialba hulunbeirensis</i>	Hulunbeir Province, Inner Mongolia	(Xu et al. 2001)
<i>Natrialba chahannaoensis</i>	Lake Chahannor, Inner Mongolia	(Xu et al. 2001)
<i>Natronolimnobius baerhuensis</i>	Lake Baer, Inner Mongolia	(Itoh et al. 2005)
<i>Natronolimnobius innermongolicus</i>	Lake Baer, Inner Mongolia	(Itoh et al. 2005)
<i>Natronorubrum bangense</i>	Bange Lake, Tibet	(Xu et al. 1999)
<i>Natronorubrum tibetense</i>	Bange Lake, Tibet	(Xu et al. 1999)
<i>Halorubrum vacuolatum</i>	Lake Magadii, Kenya	(Mwatha and Grant 1993) (Kamekura et al. 1997)
<i>Halorubrum alkaliphilum</i>	Xinjiang Province, China	(Feng et al. 2005)
<i>Halorubrum luteum</i>	Lake Chahannor, Inner Mongolia	(Hu et al. 2008)
<i>Halorubrum tibetense</i>	Lake Zabuye, Tibet	(Fan et al. 2004)
<i>Halalkalicoccus tibetensis</i>	Lake Zabuye, Tibet	(Xue et al. 2005)
<i>Halobiforma nitratireducens</i>	Lake Chahannor, China	(Hezayen et al. 2002)

(Elevi Bardavid et al. 2008), but there is no good evidence for the presence of these or similar organisms in alkaline hypersaline sites. Haloarchaeal blooms in neutral solar salterns are known to promote crystallization of halite and almost certainly also in alkaline salterns. It is possible that the cells may serve as templates in the nucleation of halite crystals and their subsequent development (Lopéz-Cortés and Ochoa 1998). In neutral salterns, observations of halite crystallization show that haloarchaea become entrapped within the crystals, leaving behind a so-called bittern brine dominated by $MgCl_2$ and KCl , which does not support significant growth of haloarchaea, although it is not actually toxic (Norton and Grant 1988).

Haloarchaea are organotrophic (Grant et al. 2001) and presumed to develop to high densities in hypersaline sites by deriving carbon and cell protein from phototrophic primary productivity by other salt-loving organisms. Despite apparently inhospitable conditions, salt and soda lakes are extremely productive environments (particularly soda lakes, presumably because of unlimited access to CO_2 for photosystems via the $HCO_3^-/CO_3^{2-}/CO_2$ equilibrium). Phototrophic productivity is probably greatest during periods of dilution, since most of the recorded phototrophs, with the possible exception of *Dunaliella* spp. are unable to grow significantly at saturation point for $NaCl$. In neutral environments, haloarchaea probably derive most of their carbon as glycerol from preceding blooms of *D. salina* that occur as halite



■ Fig. 2.2.3

Soda crust at lake Magadi, Kenya

saturation approaches. In alkaline sites, the role of *Dunaliella* is taken by haloalkaliphilic cyanobacteria, notably *Spirulina* and *Cyanospira* spp. (Grant and Jones 2000). Hypersaline environments are relatively low in oxygen due to reduced oxygen solubility (2 ppm in saturated NaCl, compared with 7 ppm in seawater) and the brines may also harbor substantial populations of anaerobic phototrophic bacteria of the genus *Halorhodospira* that may be significant producers of biomass from time to time (Grant 2004).

Direct molecular analysis of both alkaline and neutral hypersaline brines by 16S rDNA amplification of environmental DNA, preparation of gene libraries, followed by sequence determinations of individual 16S rRNA genes, has revealed novel haloarchaeal lineages that have yet to be brought into culture (Caton et al. 2009; Grant et al. 1999; Mwachia et al. 2010; Ochsenreiter et al. 2002; Valenzuela-Encinas et al. 2008) together with clones that affiliate with isolates in culture. In diverse alkaline saline environments including Lake Texcoco (Mexico), an unnamed soda lake in Nevada (USA) and Lake Elmenteita (Kenya), clones affiliated with the genera *Natronococcus*, *Halovivax*, *Halobiforma*, *Halalkalicoccus*, *Halorubrum*, *Natronomonas*, *Natronolimnobius*, and *Natrinema* were detected. However, a considerable percentage of the clones (often 50% or more), although clearly affiliated with the Halobacteriales, did not closely affiliate with any of the organisms in culture, indicating as yet unexplored haloarchaeal diversity.

Hydrolysis products of complex polymers probably derived from the anaerobic decomposition of cyanobacterial mats to acetic acid, hydrogen, and CO₂ (Grant et al. 1998; Zavarzin et al. 1999), in soda lakes in turn may be used by haloalkaliphilic methanogens, (also, strictly speaking, alkaliphilic haloarchaea!) although most of the methanogens isolated to date from hypersaline environments are methylotrophic, utilizing compounds such as methanol and methylamine (Oren 1999). This aspect of archaeal biodiversity in soda lakes is sadly underrepresented in the literature and is largely confined to a few studies on Russian

((Namsaraev et al. 1999a); b) and Indian lakes (Sukarasi et al. 2007). Isolates in culture include *Methanocalculus*, *Methanoculleus*, and *Methanosalsus* spp. from Lake Magadi (Kevbrin et al. 1997). Again, direct molecular analysis indicates other such groups (Sukarasi et al. 2007).

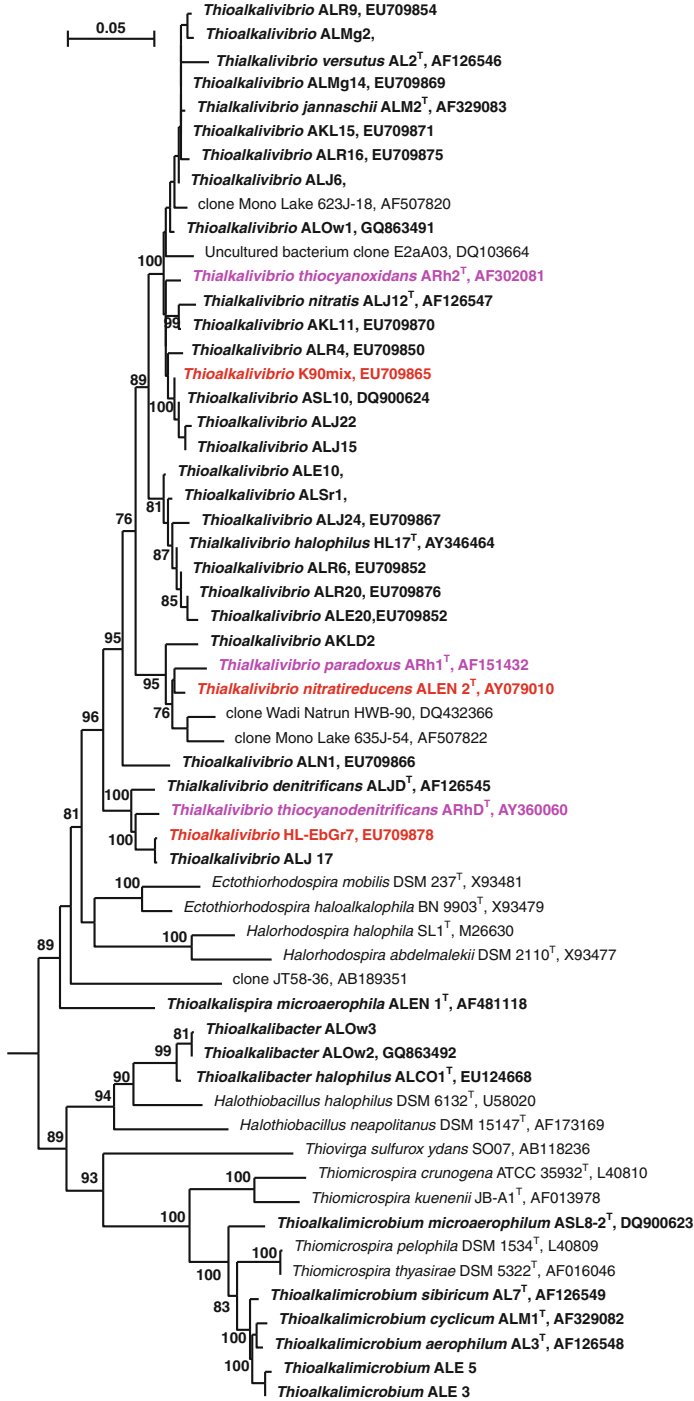
Soda Lake Chemolithotrophs

Aerobic chemolithotrophic bacteria utilizing reduced inorganic compounds as electron donors are important players in the element cycling in natural and technogenic environments. For the last 15 years, the culturable diversity of chemolithotrophs in soda lakes in Asia (southwest Siberia, Transbaikal region, Mongolia), Africa (Kenya), and the USA (Mono Lake, Soap Lake, Searles Lake, Owens Lake) has been studied. Lake pH values vary from 9 to 11, salinity from 5 to 500 g/l (w/v), and alkalinity from 0.005 to 4 M. The work has resulted in the description of novel haloalkaliphilic representatives of all functional groups of chemolithoautotrophs (Sorokin and Kuenen 2005a). One of the important features of these studies was the development of a mineral base medium suitable for the successful enrichment and isolation of a wide range of soda lake chemolithotrophs. The optimized mineral medium is based on the sodium bicarbonate/carbonate buffer with a pH of 10–10.1, variable NaCl concentration, and a total Na⁺ content 0.3–4.0 M. Such media, even at minimal salt content, were able to maintain the pH above 9.2 during active growth of acid-producing chemolithotrophs.

Soda Lake Sulfur-Oxidizing Bacteria (SOB)

With thiosulfate as electron donor, wide distribution of obligately autotrophic haloalkaliphilic SOB in soda lakes of different geographic locations has been demonstrated with abundance ranging from 10³ to 10⁸ viable cells/cm³ in sediments. More than 100 strains of haloalkaliphilic SOB actively growing at pH up to 10.3–10.5 have been obtained in pure culture. They are grouped into four new genera within the Gammaproteobacteria – *Thioalkalimicrobium*, *Thioalkalispira*, *Thioalkalivibrio*, and *Thioalkalibacter* (Fig. 2.2.4) ((Sorokin and Kuenen 2005b; Sorokin et al. 2006b); Banciu et al. 2008). The first three genera include obligate alkaliphiles and the latter contains facultatively alkaliphilic halophiles. Among these, the genus *Thioalkalivibrio* is most diverse phylogenetically and representatives are physiologically the best adapted for life in hypersaline brines. Several large geographical populations each containing about 20 isolates could be distinguished among the extremely salt-tolerant representatives of the genus (Foti et al. 2006): isolates from Kulunda Steppe and Mongolia are clustered together and also resemble the Mono Lake and Soap Lake *Thioalkalivibrio* strains, while most of the Wadi Natrun isolates cluster with *Tv. halophilus*. The basic properties of the soda lake SOB are summarized in Table 2.2.3.

On the basis of their salt tolerance/requirement, *Thioalkalimicrobium*, some of the *Thioalkalivibrio* isolates and *Thioalkalispira* belong to a moderately salt-tolerant type, growing up to 1.2–1.5 M total Na⁺. In contrast, many of the *Thioalkalivibrio* isolates are able to grow in saturated soda brines (4 M Na⁺). Overall, the cultivated forms of SOB from soda lakes cover a complete range of pH/salt concentration typical for their environment. Isolation and study of two facultatively alkaliphilic halophilic SOB species, *Thioalkalivibrio halophilus* and *Thioalkalibacter halophilus*, indicated *natronophily* – a preference of sodium carbonates over sodium chloride by the soda lake organisms. Since sodium carbonates are much weaker electrolytes



■ Fig. 2.2.4 (Continued)

■ **Table 2.2.3**

Basic properties of haloalkaliphilic SOB from soda lakes

Property	<i>Thioalkali</i> <i>microbium</i> spp.	<i>Thioalkalispira</i> <i>microaerophila</i>	<i>Thioalkalivibrio</i> spp.	<i>Thioalkalibacter</i> <i>halophilus</i>
Number of species	3	1	9	1
Closest relative	<i>Thiomicrospira</i> <i>pelophila</i>	No	<i>Ectothiorhodospira</i>	<i>Halothiobacillus</i>
Cell morphology	Rods and spirilla	Spirilla	Rods, vibrios, spirilla, and cocci	Rods
Sulfur compounds oxidized	HS^- , S_n^{2-} , $\text{S}_2\text{O}_3^{2-}$	HS^- , S_n^{2-} , $\text{S}_2\text{O}_3^{2-}$	HS^- , S_n^{2-} , $\text{S}_2\text{O}_3^{2-}$, S_8 , $\text{S}_4\text{O}_6^{2-}$, SCN^-	HS^- , S_n^{2-} , $\text{S}_2\text{O}_3^{2-}$
Electron acceptors	O_2	O_2 microaerophile	O_2 , NO_3^- , NO_2^- , N_2O	O_2
pH optimum	9.5–10.0	10.0	10.0–10.2	8.5
Upper salt limit	1.5 M Na^+	1.4 M Na^+	4.3 M Na^+	3.5 M Na^+
Maximal specific growth rate	0.33 h^{-1}	0.08 h^{-1}	0.25 h^{-1}	0.22 h^{-1}
Maximal growth yield with $\text{S}_2\text{O}_3^{2-}$	3.5 mg protein mmol^{-1}	5.8 mg protein mmol^{-1}	6.5 mg protein mmol^{-1}	3.5 mg protein mmol^{-1}
Rate of HS^- oxidation	Extremely high	Low	Low	High
Dominant compatible solute	Ectoine	nd	Glycine betaine	Ectoine
Yellow membrane pigment	–	+	+	–
Distribution	Asia, Africa, North America	Egypt	Asia, Africa, North America	Asia, North America

■ **Fig. 2.2.4**

Phylogenetic position of haloalkaliphilic sulfur-oxidizing bacteria (SOB) from soda lakes (in **bold**) within the Gammaproteobacteria based on 16S rRNA sequence analysis. In the cluster of the genus *Thioalkalivibrio* the unclassified strain abbreviation is as follows: ALMg, strains from Mongolia; ALJ, strains from Kenya; AKL, strains from Kulunda Steppe; ALE, strains from Wadi Natrun in Egypt; ASL, strain from Soap Lake (USA); ASLr, strain from Searles Lake (USA); ALN1, extremely haloalkaliphilic nitrate-reducing strain from Wadi Natrun; ALR, strains from a lab-scale bioreactor; HL-Ebgr7, strain from a full-scale bioreactor. In red are the genome sequenced strains and in pink are strains for which genome sequence is in progress. Numbers at the nodes indicate the percentage of bootstrap values for the clade of this group in 1,000 replications (the values for maximum-likelihood method are given in parentheses). Only values above 70% are shown. *Bar*, 5 substitution per 100 nt

than NaCl, their osmotic burden and water stress on cells must be significantly less than that of NaCl. Therefore, organisms living in soda must have an advantage in synthesizing less compatible solutes as compared to halophiles living in NaCl brines of the same sodium molarity. Analysis of compatible solute content in *Tv. halophilus* (Banciu et al. 2004) and *Thioalkalibacter* (Banciu et al. 2008) grown either in NaCl or in soda brines demonstrated that, indeed, the cells grown in NaCl brines contained roughly twice more osmolytes than the soda-grown cells, exactly corresponding to the difference in electrochemical and ionic properties of the two sodium salts (► [Table 2.2.4](#)).

Furthermore, the reaction of extremely salt-tolerant SOB on sodium salts with different electrolytic properties correlated with the anionic composition of their habitats. Strains isolated from sodium carbonate-dominated lakes, such as *Thioalkalivibrio versutus* ALJ15 (Kenya), respired more actively in sodium carbonate and sodium sulfate brines (weak electrolytes), while *Thioalkalivibrio halophilus* ALE 20 from a NaCl-dominated alkaline lake in Wadi Natrun functioned better in NaCl brines (► [Fig. 2.2.5](#)). Therefore, the preference of soda lake organisms for sodium carbonates, despite the high pH stress, has a rational explanation and must be taken into consideration as a specific biological phenomenon.

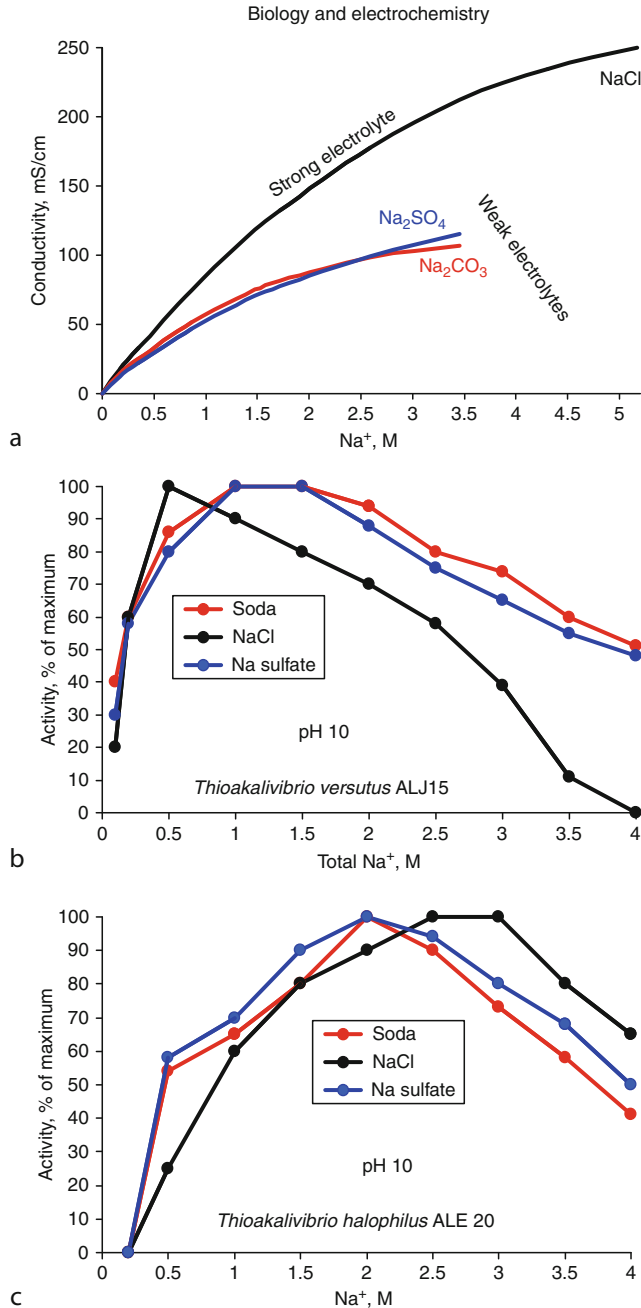
Use of thiocyanate ($N\equiv C-S^-$) as electron donor instead of thiosulfate at pH 10 has resulted in the isolation in pure culture of two different phenotypes of haloalkaliphilic SOB from various soda lakes described as *Thioalkalivibrio thiocyanoxidans* and *Thioalkalivibrio paradoxus* (Sorokin et al. 2001a; Sorokin et al. 2002). These bacteria degraded thiocyanate via cyanate ($N=C=O^-$) with final production of ammonia, CO_2 , and sulfate. But, in contrast to anaerobic hydrolysis, suggested previously as a mechanism of the primary thiocyanate degradation in neutrophilic thiobacilli, in alkaliphilic SOB this reaction is obligately aerobic and the enzyme responsible for it appears to act as an thiocyanate-cytochrome *c* oxidoreductase with elemental sulfur as an immediate product. The novel enzyme is a soluble periplasmic ~60-kDa monomer with a unique primary peptide structure representing a novel class of proteins and a novel type of oxidoreductases (our unpublished results).

Both very active sulfide oxidation and the ability to utilize thiocyanate at highly alkaline pH make natronophilic SOB attractive for bio-based environmental technology (Janssen et al. 2009). For example, cyanide waste is only safe at high pH and is usually

■ **Table 2.2.4**

Synthesis of compatible solutes (% from dry cells) by facultatively alkaliphilic SOB during growth in NaCl or in soda brines

Compatible solute	<i>Thioalkalivibrio halophilus</i> HL17		<i>Thioalkalibacter halophilus</i> ALCO1	
	4 M Na ⁺	4 M Na ⁺	3 M Na ⁺	3 M Na ⁺
	NaCl	soda	NaCl	soda
	pH 7.5	pH 10	pH 7.5	pH 10
Glycine betaine	19.8	12.4	13.5	5.2
Ectoine	0	0	0.9	1.3
Total	19.8	12.4	14.4	6.5



■ Fig. 2.2.5

Illustration of the presence of different “salt ecotypes” among the SOB isolates from alkaline lakes. (a) electrochemical difference in sodium salts indicated by conductivity dependence from concentration. (b and c) influence of sodium salts with different ionic properties on respiratory activity at pH 10 in two strains of extremely salt-tolerant strains of the genus *Thioalkalivibrio*

converted into less harmful, but still toxic, thiocyanate waste. The latter could be treated with alkaliphilic SOB.

Soda Lake Methanotrophs

Methanotrophic enrichments at pH 9–10 from soda lake sediments, even from the hypersaline ones, were positive only at a salt concentration below 1.5 M total Na⁺, despite in situ experiments with ¹⁴CH₄ demonstrating measurable rates of methane oxidation in hypersaline soda lakes. In the hyposaline and moderately saline lakes, as was in the case of the northeastern Mongolia, the rates of methane oxidation were sufficient to balance the rates of methane formation, while at higher salinity the methane cycle was unbalanced (Sorokin et al. 2004). Molecular probing of the methanotrophic population in soda lakes of Central Asia demonstrated ubiquitous domination of type I methanotrophy belonging to the genus *Methylomicrobium* in the Gammaproteobacteria (Li et al. 2004).

A pure culture of an obligate methanotroph was obtained from a Kenyan soda lake after enrichment at 0.6 M total Na⁺ and pH 10. Strain AMO 1 was identified as a member of the genus *Methylomicrobium* in the Gammaproteobacteria (Sorokin et al. 2000) and later classified as *M. kenyense* (Kalyuzhnaya et al. 2008). Other moderately alkaliphilic methanotrophic isolates from hyposaline alkaline lakes (Trotsenko and Khmelenina 2002) were all classified within the same genus (Kalyuzhnaya et al. 2008). On ultrastructure and biochemical properties, it is a typical representative of the type I methanotrophs with lamellar intracellular membrane structures and RuMP pathway of carbon assimilation, utilizing methane and methanol as carbon and energy source. Strain AMO 1 had a very narrow pH range for growth between 9 and 10.2 with an optimum at pH 9.9–10, although washed cells respired methane and methanol within much broader pH range from 6.0 to 11.0 with the same pH optimum. The total salt concentration in the form of sodium carbonate/bicarbonate and NaCl suitable for growth ranged from 0.2 to 1.2 M total Na⁺, thus belonging to the low salt-tolerant alkaliphiles. The ecological role of the AMO-like alkaliphiles might not be limited only by methane oxidation, since the strain was found to possess an additional potential to oxidize ammonia and carbon disulfide (CS₂). Ammonia was converted to nitrite in presence of moderate concentrations of methane or methanol and only at very alkaline pH between 10 and 11. CS₂ was oxidized with relatively high rates of polysulfide formation, probably resulting from abiotic reaction of the true products sulfur and sulfide at high pH. Addition of low numbers of alkaliphilic SOB (a *Thioalkalimicrobium* strain) to the cells of AMO 1 increased the rate of CS₂ oxidation and changed the product to elemental sulfur, mimicking a possible variant of cooperation between these two groups of haloalkaliphiles.

Soda Lake Nitrifiers

The in situ activity of ammonia oxidation and the presence of 16S rRNA, *amoA* (ammonium monooxygenase), and *cbbL* (RuBisCO) gene sequences related to the *Nitrosomonas europaea* lineage have been demonstrated in the chemocline of saline alkaline Mono Lake and Big Soda Lake (Cloern et al. 1983; Giri et al. 2004; Joye et al. 1999; Ward et al. 2000). Five stable enrichment cultures of the ammonia-oxidizing bacteria (AOB) growing at pH 10 have been obtained from sediments of the Mongolian soda lakes and one from the Wadi Natrun lake

using ammonium concentration <4 mM and salt concentration 0.6 M total Na^+ . Nitrite production ceased at higher ammonium concentrations and salt content >1 M total Na^+ (Sorokin 1998; Sorokin et al. 2001b). Five strains obtained from the enrichments were very close genetically to each other and closely related to the known marine species *Nitrosomonas halophila*. In continuous culture with pH control, one of the strains was able to grow up to pH 11.4 – an absolute maximum not only among chemolithotrophs but also close to a proven maximum (11.5) for alkaliphilic heterotrophs (Sturr et al. 1994). It seems that the soda lake AOB have somehow managed to overcome a major problem of autotrophic metabolism at extremely high carbonate/bicarbonate ratio (e.g., NaHCO_3 limitation). Growth was possible only at low salt from 0.1 to 0.9 M Na^+ in purely sodium carbonate media without any Cl^- . Apart from high salt, ammonia toxicity was evident at pH above 10, isolates tolerating no higher than 8 mM of NH_4Cl and growing without lag phase only when ammonium concentration was <4 mM.

In contrast to ammonia, nitrite toxicity decreases with increasing pH, which made it possible to use 20 mM nitrite for the isolation of alkaliphilic NOB. Five pure cultures obtained from soda lakes and soda soils at 0.6 M Na^+ and pH 10 with nitrite represented a typical *Nitrobacter* phenotype, which was confirmed by phylogenetic analysis. However, despite very high sequence similarity to a type species *Nb. winogradskii*, there was very little DNA homology between the soda lake isolates and the type strain, which, together with distinct alkalitolerance justified the description of a new species *Nb. alkalicus* (Sorokin et al. 1998). In contrast to the soda lake AOB isolates, the NOB strains were facultatively alkaliphilic, only moderately alkalitolerant (pH maximum 10.2) and even less salt tolerant (up to 0.5 M Na^+). This is most probably due to a very low energy yield of the nitrite oxidation (the lowest among lithotrophs). Accordingly, it may be concluded that the nitrogen cycle in hypersaline soda lakes may be devoid of its oxidative part.

Soda Lake Hydrogenotrophs

Recent work with sulfidogenic populations in soda lakes (our unpublished results) demonstrated that, in contrast to neutral habitats, hydrogen is not a favorable substrate for sulfate-reducing haloalkaliphiles. Therefore, part of the hydrogen might escape from the anoxic sediments to the more oxidized water-sediment interface and thus become available for aerobic and denitrifying hydrogenotrophs. One such bacterium, strain AHO 1, was obtained from an aerobic enrichment inoculated with mixed sediment samples from Kenyan soda lakes at pH 10 and 0.6 M total Na^+ (Sorokin et al. 2000). The isolate AHO 1 was identified as a novel member of the alpha-3 subdivision of the Proteobacteria and was given a tentative name “*Natronohydrogenobacter thiooxidans*.” It is a facultative autotroph growing chemolithoautotrophically with hydrogen, heterotrophically with various organic acids and sugars, and mixotrophically with hydrogen or sulfide as energy source and acetate as carbon source. Hydrogenotrophic growth was optimal at pH 9.5–9.8 and possible up to pH 10.25 at a salt concentration from 0.2 to 1 M total Na^+ . An anaerobic enrichment under denitrifying conditions yielded a pure culture, strain AHN 1, which grew autotrophically with hydrogen and nitrate at pH 10 and salt concentration up to 1.5 M total Na^+ . Strain AHN 1 is a partial denitrifier with a gap in the middle of its denitrification pathway, for example, it can reduce nitrate to nitrite (with hydrogen or acetate) and N_2O to dinitrogen (with acetate), but cannot grow anaerobically with nitrite. The strain was identified as a member of the genus *Alcalilimnicola* in the

Gammaproteobacteria (Sorokin et al. 2006a). Another haloalkaliphilic strain belonging to this cluster and described as *Alkalilimnicola ehrlichii* (Hoeft et al. 2007) was obtained from Mono Lake using somewhat “exotic” combination of substrates, previously unknown to sustain lithoautotrophic growth. Strain MLHE-1 was able to grow autotrophically with arsenite, hydrogen, sulfide, and thiosulfate coupled to nitrate reduction to nitrite (Oremland et al. 2002).

Soda Lake Carboxydrotrophs

Early attempts to enrich for alkaliphilic aerobic carboxydrotrophs from soda lakes were not successful. The most probable reason for this result was too high CO and O₂ concentrations (50% and 20% in the gas phase, respectively). When those concentrations were reduced below 20% for CO and 5% for O₂, four positive enrichments were obtained at pH 10 and salt content from 0.2 to 2.0 M Na⁺ from sediments of hypersaline soda lakes in Kulunda Steppe and a single culture from the Wadi Natrun hypersaline alkaline lakes. The enrichments resulted in isolation of five pure cultures of bacteria with a potential to grow chemolithoautotrophically with CO. Four of them were members of the genus *Alkalispirillum* and one belongs to the “sister” genus *Alkalilimnicola*, once again indicating the importance of this group of facultatively autotrophic haloalkaliphiles for the “aerobic filter” in soda lakes (Sorokin et al. 2010). Earlier, the potential to consume CO, but not to grow with it, and the presence of a typical aerobic CO-dehydrogenase (CODH) have been demonstrated in *Alkalilimnicola ehrlichii* (Hoeft et al. 2007). The ACO isolates were able to grow with CO within a relatively narrow alkaline pH range from 8.5 to 10.6 and up to a very high salt concentration (3.5 M total Na⁺) possible only in the SOB group. Such a salt tolerance is probably possible due to a high energy yield of CO oxidation. Apart from CO, the strains utilized formate and oxidized sulfide also within the highly alkaline pH range but did not grow autotrophically with hydrogen, in contrast to most of the known aerobic carboxydrotrophs. The CODH activity was detectable in the membranes and in the periplasm but conventional primers for the *coxL* forms I and II did not amplify the gene.

Overview of Lithotrophs in Soda Lakes

All known groups of aerobic chemolithoautotrophic bacteria that can be cultivated at least at pH 10 and sometimes higher have been found in soda lakes and cultivated using mineral medium highly buffered with sodium carbonate. The generalized scheme of their role in the soda lake element cycling is presented in [Fig. 2.2.6](#). All of these use the Calvin–Benson cycle for inorganic carbon fixation in the form of HCO₃⁻. The preference of sodium carbonate environment with its extremely high pH and alkalinity, that is, *natronophily* rather than haloalkaliphily, is actually the main property that discriminates these bacteria from their neutrophilic counterparts. Based on comparison of the pH profiles, three different pH-iono-philes can be discriminated among the salt-tolerant chemolithoautotrophs: NaCl-, NaHCO₃-, and Na₂CO₃-preferring types. The NaCl-type is usual for the marine environment, chloride-sulfate salt lakes, and solar salterns (e.g., *Nitrococcus halophilus* and *Halothiobacillus halophilus*); the NaHCO₃-type is probably adapted to increasing NaHCO₃ alkalinity in photosynthetic microbial mats of the tidal flats, with diurnal fluctuation of pH/HCO₃⁻ (e.g., *Nitrosomonas halophilus* Nm1 and *Thiomicrospira pelophila*, both from the North Sea). Neither of them, though, can tolerate Na₂CO₃-dominating environment with a pH ≥ 10, which

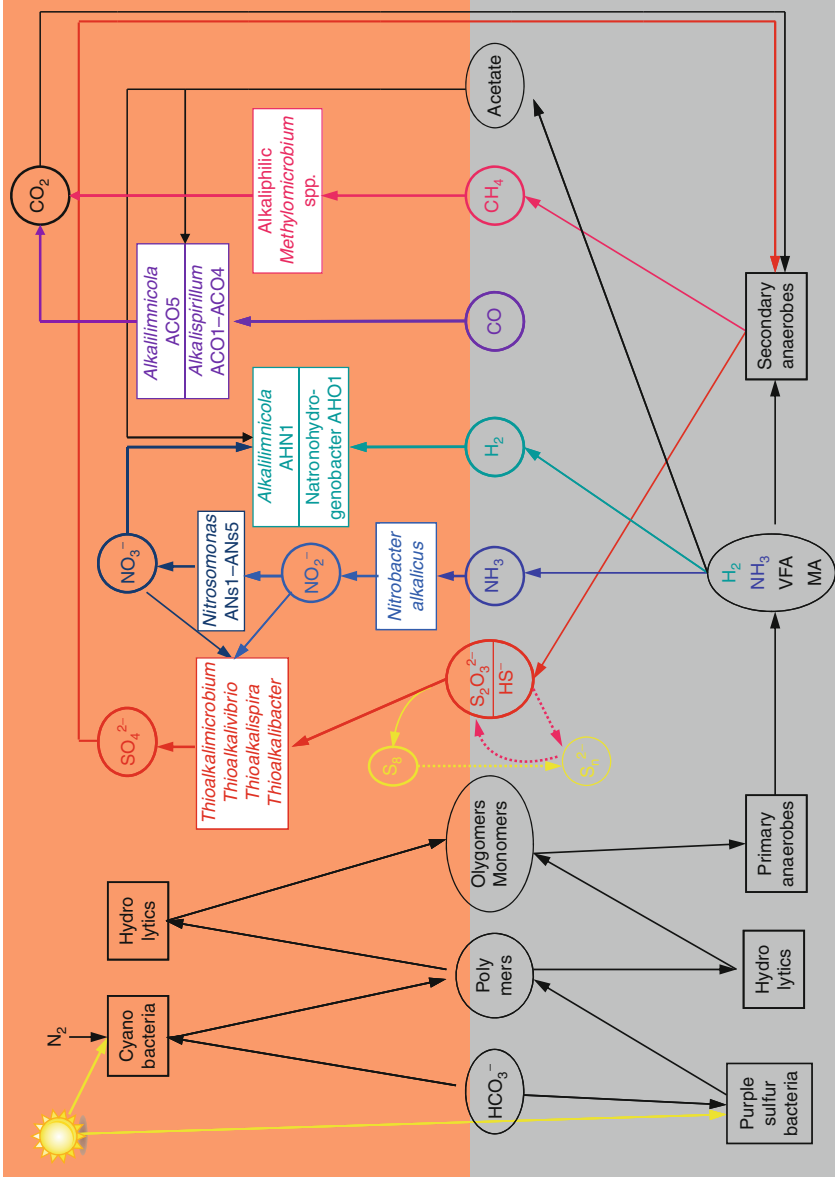
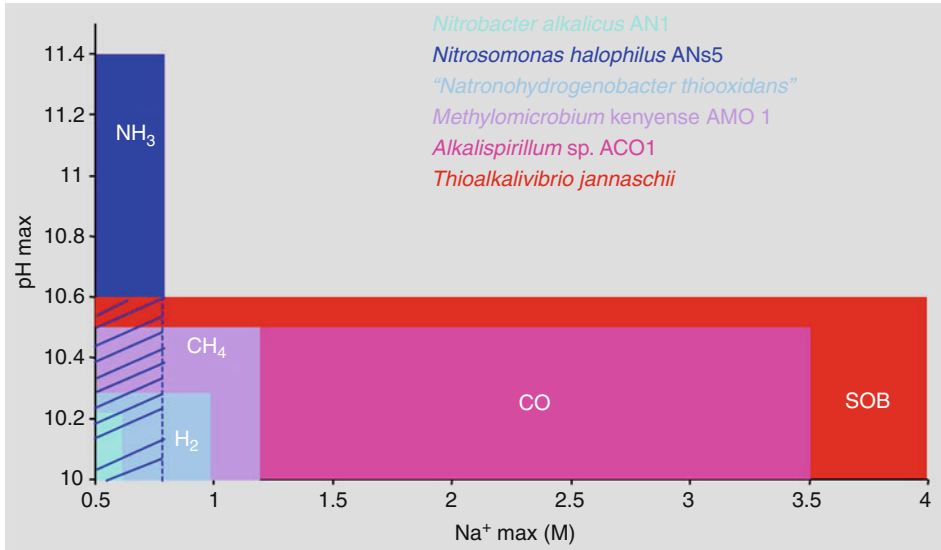


Fig. 2.2.6 Generalized scheme of the soda lake microbial system showing the place of chemolithoautotrophic natronophiles



■ Fig. 2.2.7

Range of pH-salt values for growth of chemolithotrophic natronophiles from soda lakes

is a real domain of the soda lake natronophiles (e.g., *Nitrosomonas halophila* ANs1 and *Thioalkalimicrobium aerophilum*).

The second important characteristic is salt tolerance. From five groups of chemolithoautotrophs discovered in soda lakes, only some of the SOB and carboxydrotrophs are fully capable to function in hypersaline soda lakes, while the activity of others either partially or completely restricted to moderately saline lakes (► Fig. 2.2.7). Their presence in hypersaline lakes might be explained by either existence of less saline microniches or by the salinity fluctuation.

Cross-References

- 2.3 Environmental Distribution and Taxonomic Diversity of Alkaliphiles
- 2.4 Anaerobic Alkaliphiles and Alkaliphilic Poly-Extremophiles
- 2.5 General Physiology of Alkaliphiles
- 2.6 Adaptive Mechanisms of Extreme Alkaliphiles
- 2.8 Enzymes Isolated from Alkaliphiles
- 2.11 Alkaline Enzymes in Current Detergency

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