

Microbial diversity and ecology of the Soda Lakes of East Africa

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ABSTRACT

Soda lakes are highly alkaline aquatic environments where evaporative concentration results in carbonate as a major dissolved anion. In these very productive environments prokaryotic photo-synthetic primary production is probably the driving force behind all nutrient recycling in these lakes. The major trophic groups responsible for cycling of carbon and sulphur have in general now been identified. Although there are many parallels with athalassohaline salt lake systems, systematic studies have shown that the microbes are obligately alkaliphilic or alkali-tolerant and many appear to represent separate alkaliphilic lineages within recognized taxa, indicating they may have evolved separately within the alkaline environment. As evaporative concentration continues, chloride ions also dominate in solution. As a consequence, a quite different population of prokaryotes is present in the trona (sodium sesquicarbonate) beds and concentrated lagoon brines of hypersaline lakes (Magadi-Natron basin) compared with more dilute lakes elsewhere in the East African Rift Valley.

Introduction

Soda Lakes

Soda lakes are highly alkaline aquatic environments. Often impermanent in nature, their terrestrial equivalents, soda deserts, represent their desiccated remains. Although soda lakes have a worldwide distribution, they are mainly confined to (sub)tropical latitudes in continental interiors or rain-shadow zones. Owing to their hostile nature they are often remote from the main centers of human activity and perhaps for this reason they have been little studied. The best studied regions are the lakes and solonchaks of the Central Asian (Siberia) steppes and the Rift Valley of Eastern Africa.

The Great Rift Valley running through East Africa is an arid tropical zone where tectonic activity has created a series of shallow depressions. These are often closed basins with no obvious outflow where ground water and seasonal streams flowing from the surrounding highlands collect to form (semi)permanent standing bodies of water. Surface evaporation rates exceed the rate of inflow of water allowing the dissolved minerals to concentrate into a caustic alkaline brine with CO_3^{2-} and Cl^- as major anions, creating a pH of 8.5 to >12 [7,8]. Total salts vary from about 5% w/v in the northern lakes (Bogoria, Nakuru, Elmenteita, Sonachi) to saturation in parts of the Magadi-Natron basin in the south. These apparently hostile extreme pH environments are in fact extremely productive because of

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high ambient temperatures, high light intensities and unlimited supplies of CO₂. Primary production rates of >10 gC m⁻²day⁻¹ [13] have been recorded making these among the most productive aquatic environments in terms of biomass anywhere in the world. This remarkable photoautotrophic primary production stands at the top of the food chain and is presumably the driving force behind all biological processes in what is essentially a closed environment.

Diversity and Salinity

A systematic examination of chosen examples of the many strains brought into laboratory culture reveals a remarkable diversity of prokaryotes with alkaliphiles represented in most of the major taxonomic groups. This has permitted the identification of many of the major trophic groups responsible for the recycling of carbon, sulphur and nitrogen in the lakes using the obvious parallels with better characterized aquatic ecosystems. Many of the microorganisms so far characterized from soda lakes have relatives in salt lakes except that they are all alkaliphilic or at least highly alkali-tolerant [8]. However, salinity is also an important defining factor in the alkaline lakes. There is a distinct difference in microbial community composition between the hypersaline, alkaline lakes such as parts of Lake Magadi with salinity approaching saturation or higher, compared with the more dilute lakes like Nakuru.

Several hundred strains of non-photo-trophic aerobic organotrophs have been isolated from the environs of Rift Valley soda lakes on a variety of media. About 100 of these have been examined phenotypically and chemotaxonomically in some considerable detail for the purpose of numerical taxonomy [8]. Phylogenetic analysis has been performed on around 30 isolates [2]. Many of the strains isolated in pure culture can be assigned to existing taxa as new species or novel genera, but some isolates have no close phylogenetic ties with known microbes and appear to be separate lines of evolutionary descent perhaps peculiar to the soda lake environment.

Primary Production

In the moderately saline lakes cyanobacteria (notably *Spirulina* spp.) are the main contributors to primary production. *Spirulina* spp. not only support large numbers of organotrophic bacteria (10⁶ cfu ml⁻¹) but are also the only food source for the vast flocks (>10⁶ individuals) of Lesser Flamingo (*Phoeniconaias minor*) that graze on these lakes. The essential role of *Spirulina* as primary producer is the fixation of N₂ and production of O₂. Other species of cyanobacteria have been recorded, e.g. *Cyanospira*, *Chroococcus*, *Synechococcus*, but their significance is probably minor in comparison with *Spirulina* spp. There is a probable contribution to primary production by anoxygenic phototrophic bacteria of the genus *Ectothiorhodospira* that is likely to be highly significant but is as yet unquantified [6]. Their role in the re-cycling of sulphur is also important.

In hypersaline lakes like Magadi it is rather uncertain what organisms are responsible for primary production, especially in the trona beds that are usually dominated by organotrophic archaea. Cyanobacterial blooms do occur occasionally in the lagoon waters, but only after an unusually wet rainy season has caused substantial dilution of the brine [1].

Carbon Cycle

Aerobes

Even though these data are clearly incomplete it is possible to make some predictions as to the roles played by the different organisms in the recycling of nutrients in this highly alkaline environment. Especially abundant in Kenyan soda lakes are Gram-negative bacteria related to, but clearly different from members of the *Halomonadaceae* family of moderately halophilic bacteria found in a range of terrestrial and marine saline environments. We have characterized 17 isolates by phenotypic, chemotaxonomic and phylogenetic analysis indicating that they fall into 3 groups along with known halomonad species, probably representing at least 5 or 6 novel species [2]. Given the dynamic nature of *Halomonas* systematics at present we feel confident only to propose one new species, *Halomonas magadii* at this time (in preparation). While it is increasingly clear that halomonads often constitute a major group in moderately saline environments, it is now obvious that they have counterparts in the alkaline, saline environment. They are also likely to be widespread since similar strains have been isolated from an alkaline, saline lake in the Pacific Northwest of the USA (M. Mormile, personal communication).

As might be expected considering the nutrient rich habitat that they inhabit, the soda lake isolates are biochemically reactive. Besides hydrolyzing proteins and polymeric carbohydrates, they utilize a very wide range of sugars, organic and amino acids, and are nutritionally far less demanding than previously characterized halomonads. However, what clearly sets the soda lake halomonads apart from *Halomonas sensu strictu* is their ability to reduce nitrate to nitrite. One group of soda lake isolates is further capable of reducing nitrite and of anaerobic growth with and without nitrate. Since these organisms form a major part of the soda lake microbial community they presumably play an important and substantial role in the nitrogen cycle. Other strains with an uncertain affiliation within the $\gamma 3$ subdivision of the Proteobacteria [2] appear strongly proteolytic or have a strong preference for lipids.

Gram-positive isolates are found in both the high G+C and low G+C divisions. Especially abundant are bacteria associated with the diverse *Bacillus* spectrum. Some of these can be included in *Bacillus* rRNA group 6 [14]. However, other strains form a distinct but related group (“rRNA group7”) of alkaliphilic bacilli [2]. These *Bacillus* spp. probably play an important role in the breakdown of bio-polymers since they have an arsenal of extracellular hydrolytic enzymes: proteinases, cellulases, xylanases and other enzymes capable of degrading complex carbohydrates. Some evidence suggests that the two groups of bacilli may be segregated within the soda lake environment. The rRNA group 6 strains seem to predominate in shore line muds and dried foreshore soils that are subject to fluctuating conditions of alkalinity and salinity as water levels change with the seasons. The other group of strains have a high absolute Na^+ requirement for growth and are seemingly more abundant in lake waters and sediments, where owing to the high pH buffering, conditions are less variable. These spore-forming organisms enjoy a worldwide distribution and are likely to be dispersed widely by wind blown dust and migratory birds.

High G+C Gram-positive bacteria in the soda lake environment include novel *Streptomyces* spp. that grow at pH 10 [9] and hydrolyze proteins and carbohydrates, a

novel genus *Bogoriella* [4], a new species of *Dietzia*, [3] and uncharacterized relatives of *Nesterenkonia* [2].

A quite different population of prokaryotes is present in the trona beds and concentrated alkaline brines of hypersaline lakes such as those found in the Magadi-Natron basin (Kenya/Tanzania). Lake Magadi, where salt concentrations achieve saturation levels, is the center of a commercial enterprise. The salt making ponds provide the most extreme alkaline environment at >pH12 and are dominated by bright red blooms of haloalkaliphilic archaea. These organisms are organotrophs. Since the inorganic components of the environment have accumulated by evaporative concentration it seems reasonable to suppose that organic material also accumulates and this alone may be sufficient to support a substantial but perhaps less diverse community of organotrophs where photosynthetic primary production plays a lesser role. The organisms most commonly cultured from the hypersaline, alkaline lakes have been assigned to a distinct physiological group of the archaeal halophile lineage. These organisms not only require high concentrations of NaCl but also high pH (8.5 - 11) and low Mg^{2+} (<10mM). Originally assigned to two genera, *Natronobacterium* and *Natronococcus*, they have recently been recognized as representing considerable diversity and accordingly proposals for 3 additional genera have been made [10]. 16S rDNA sequencing of selected isolates indicates a greater diversity [2]. The only other organisms that have been cultivated from this hypersaline environment are haloalkaliphilic *Bacillus* spp. Although these isolates are related phylogenetically to the Na^+ requiring species ("rRNA group 7") [2] they are phenotypically quite distinct, being able to grow well in 25-30% w/v NaCl with a minimum requirement for at least 15% w/v NaCl. The diversity of this group of bacilli is also evident from signature sequences in the 16S rRNA gene [9].

Anaerobes

Hydrolysis products of complex polymers presumably form the substrates for not only alkaliphilic *Halomonas* spp. but also for anaerobic fermentative bacteria. Viable counts on the anoxic soda lake sediments indicate they contain $>10^6$ cfu ml^{-1} (37°C) chemoorganotrophic alkaliphilic anaerobes. 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) [18]. These organisms utilize a wide variety of pentoses, hexoses and disaccharides producing acetate, lactate, ethanol and H_2 . Our own work shows that anaerobic enrichment cultures performed on complex polymers yields almost exclusively facultative anaerobes, while obligate anaerobes could be isolated on glucose supplemented with appropriate amounts of NaCl at the correct pH. This would seem to suggest that in the soda lake environment obligate anaerobes are 'secondary organotrophs' utilizing the products of primary hydrolysis provided by aerobes and facultative anaerobes. Obligately anaerobic isolates from the moderately saline lakes were associated with the *Clostridium* group XI [9]. 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.[9]. These strains have a high tolerance for NaCl (25% w/v) and a minimum requirement of at least 12-16% w/v at pH 9.5. These too

ferment a range of sugars producing mainly iso-valeric acid with smaller amounts of iso-butyric and acetic acid as end products. Fatty acids can be consumed by homoacetogens [17].

There is plenty of evidence for biogenic methane production in alkaline lakes although at Lake Magadi it is mainly anecdotal. Methanogenic bacteria isolated to date from soda lakes are mainly obligately methylotrophic utilizing a variety of C1 compounds and not H₂/CO₂ as energy yielding substrates. Compounds such as methanol, methylamine and dimethyl sulphide are probably abundant in the alkaline environment being derived from the anaerobic digestion of algal mats and from compatible solutes such as betaine and ectoine which have been detected in some alkaliphilic organotrophs [unpublished]. This suggests that in general methanogens are not functioning as a H₂ sink and are not competing with sulphate-reducing bacteria for resources in soda lakes, although there may be exceptions. The methanogens characteristic of soda lakes have all been assigned to *Methanohalophilus* (*Methanosalsus*) as a separate genus within the Methanomicrobiales [11]. The fate of methane in the soda lake system has recently also become clear. Aerobic methane-oxidizing bacteria have been isolated from Central Asian soda lakes [12], and more recently from the Rift Valley soda lakes [Sorokin *et al.* unpublished].

Sulphur Cycle

The probable role of *Ectothiorhodospira* spp. in primary production has already been noted. These bacteria require reduced sulphur species as photosynthetic electron donors which are oxidized to sulphur and then sulphate. Growth may be photoautotrophic by the fixation of CO₂ via the ribulose-bisphosphate pathway or photoheterotrophically on volatile fatty acids. *Ectothiorhodospira* spp. are also a major source of the nitrogenous compounds glycine betaine and ectoine, which function as intracellular compatible solutes in these extreme halophiles. These observations point to an active sulphur cycle that is confirmed by recent data. *Desulfonatronovibrio hydrogenovorans* is a H₂-utilising sulfate reducing bacterium (SRB) isolated from the trona beds of Lake Magadi which functions optimally at pH 9.5 and 3% w/v NaCl [19]. Although this is the only fully characterized example of an alkaliphilic SRB at present, our observations would suggest that it is unlikely to be a sole representative because their role in the soda lake ecosystem is crucial. As a H₂ sink SRB's form an important link between the carbon and sulphur cycles providing sulphide for anaerobic photoautotrophic primary production. The sulphur cycle also has an aerobic component and aerobic sulphur-oxidizing bacteria occupy a narrow zone where H₂S and O₂ coexist. At least 2 new genera of alkaliphilic obligately chemo-lithoautotrophic sulphur-oxidizing bacteria have been isolated from Siberian and Kenyan soda lakes. While some strains appear to be slightly affiliated with the genus *Thiomicrospira*, others appear to be a new lineage within the γ -Proteobacteria [15].

Nitrogen Cycle

The nitrogen fixing and denitrification activities of *Spirulina* spp. and some *Halomonas* spp. respectively, have already been noted. Recent data point to further novel alkaliphiles that are involved in the cycling of nitrogen compounds. Several strains of an autotrophic, CO₃²⁻-dependent nitrite-oxidizing bacterium have been isolated from Siberian and Kenyan soda lakes. Although isolated from diverse locations the strains form a compact species group

related to *Nitrobacter* but distinct from known species [16]. At alkaline pH ammonia is volatile and leads to loss of nitrogen from the environment. The methane-oxidizing bacterium from the Kenyan soda lakes mentioned above is also able to oxidize ammonia to nitrite optimally at pH 10-10.5 [16]. These data suggest that the nitrogen flux remains in balance and that losses due to an “unfavourable” pH are adequately compensated.

The Soda Lake Explained?

Clearly the ability to culture organisms is a major hurdle to our complete understanding of soda lake microbial communities and this is further biased by our manner of sampling, culture conditions employed and the choice of isolates for study. An examination of the sequences of PCR amplified 16S rRNA genes from DNA extracted on site revealed an unexpected diversity even in the salterns at Magadi – the most extreme environment in terms of alkalinity and salinity. Sequence signatures were found from micro-organisms clearly phylogenetically related to, but distinct from the cultivated haloalkaliphilic archaea. Two of the 16S rRNA clones exhibited little relatedness to reported species with only 76% identity with known archaea, placing them as members of a deeply branching group of the Euryarchaeota. Signature sequence analysis revealed a number of features in common with the extreme halophilic archaea and also a close relationship to the methanogens, but without a definite tie to any of the 3 major methanogen families [5]. It is a distinct possibility that this phylotype represents a new type of methanogen, which would need to be taken into account during any attempt to recover the organism in pure culture.

It is evident from these preliminary studies that the full extent of the soda lake microbial diversity, community structure and the roles played by individual organisms has yet to be wholly revealed. It is an enterprise that is not aided by the remoteness of these extraordinary extreme environments.

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