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MiniReview

Chemolithotrophic haloalkaliphiles from soda lakes

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Abstract

This paper summarizes recent data on the occurrence and properties of lithotrophic prokaryotes found in extremely alkaline, saline (soda) lakes. Among the chemolithotrophs found in these lakes the obligately autotrophic sulfur-oxidizing bacteria were the dominant, most diverse group, best adapted to haloalkaline conditions. The culturable forms are represented by three new genera, *Thioalkalimicrobium*, *Thioalkalivibrio* and *Thioalkalispira* in the Gammaproteobacteria. Among them, the genus *Thioalkalivibrio* was most metabolically diverse, including denitrifying, thiocyanate-oxidizing and facultatively alkaliphilic species. Culturable methane-oxidizing populations in the soda lakes belong to the type I methanotroph group in the *Gammaproteobacteria*, mostly in the genus *Methylomicrobium*. The nitrifying bacteria in hyposaline soda lakes were represented by a new species *Nitrobacter alkalicus* (*Alphaproteobacteria*), and by an alkaliphilic subspecies of *Nitrosomonas halophila* (*Betaproteobacteria*). Both belonged to the low salt-tolerant alkaliphiles. The facultatively autotrophic haloalkaliphilic isolates able to grow with hydrogen as electron donor were identified as representatives of the α-3 subclass of the Proteobacteria (aerobic) and of the *Natronolimnicola – Alkalispirillum* group in the gammaproteobacteria (nitrate-reducing). While all chemolithotrophic isolates from soda lakes belong to the alkaliphiles with a pH optimum for growth around 10, only the sulfur-oxidizing group included species able to grow under hypersaline conditions. This indicates that carbon and nitrogen cycles in the hypersaline alkaline lakes might not be closed.

Keywords: Soda lakes; Haloalkaliphilic; Sulfur-oxidizing bacteria; Nitrifying bacteria; Methanotrophic bacteria; Hydrogenotrophs

1. Introduction

Chemolithotrophic bacteria, which utilize reduced inorganic compounds as electron donors, are important players in the element cycles of natural and industrial environments. They are widely distributed in various habitats, associating mostly with the interface zones where opposed fluxes of reduced (substrates) and oxidized (acceptors) inorganic compounds meet. The chemolithotrophs include aerobic, facultatively anaerobic and obligate anaerobic bacteria. This review is focused on the aerobic and denitrifying chemolithotrophs including sulfur-oxidizing, nitrifying, hydrogenotrophic and methanotrophic bacteria active at extremely high pH, found recently in saline alkaline (soda) lakes.

2. Soda lake habitat

Soda lakes represent a specific type of salt lakes, which contain an alkaline sodium carbonate/bicarbonate fraction among the dominant salts. They are mostly confined to dry areas with high evaporation rates that facilitate salt accumulation in local depressions. The main geochemical conditions of their formation include leaching of rock material, rich in sodium but low in Ca

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and Mg, by CO_2-saturated waters in an area with a dry and warm climate, facilitating evaporative concentration of the brines in natural depressions [1,2]. Under such conditions, sodium becomes dominant among the cations and HCO_3^-/CO_3^{2-}, Cl^- and SO_4^{2-} – are the dominant anions in the solution. The presence of sodium carbonate in variable combinations with sodium chloride and sodium sulfate creates a unique, buffered haloalkaline habitat appropriate for a stable development of obligately (halo)alkaliphilic microorganisms growing optimally at pH around 10. Apart from the high salt/high pH effects, which demand adaptations well known for haloalkaliphiles (osmolytes, pH homeostasis, elements of sodium cycle), there are other extreme factors associated with high alkalinity, which might be important specifically for the chemolithotrophs (Fig. 1). In particular, the availability of metal cofactors could be limited at highly alkaline conditions. Fortunately, the carbonate ion forms alkaline complexes with the metal ions, which are much more soluble than the respective hydroxides [3], as in case of the moderately soluble basic magnesium carbonate [$Mg_2(OH)_2CO_3$]. At pH 10 and higher, NH_4^+ is mostly converted to toxic and volatile NH_3. On the other hand, nitrite becomes less toxic at high pH, to the benefit of the nitrite-metabolizing bacteria. A high carbonate/bicarbonate ratio at pH > 10 may result in limitation of autotrophic bacteria by their carbon source (HCO_3^-) as has been suggested, for example, for alkaliphilic cyanobacteria [4,5]. On the other hand, the high alkaline buffering capacity might be advantageous for the growth of chemolithotrophic bacteria that neutralize acids produced during oxidation of reduced inorganic compounds. With respect to the sulfur cycle, the nucleophilic attack of HS^- and SO_3^{2-} on elemental sulfur at high pH results in the spontaneous formation of polysulfides ($^-S–S_x–S^-$) and thiosulfate, respectively. While the latter is also common for neutral conditions, the polysulfides are specific for highly alkaline conditions.

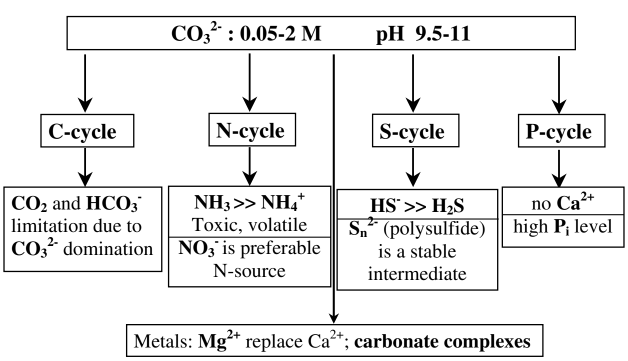

Fig. 1. Schematic representation of the possible impact of carbonate alkalinity on microbial element cycling in soda lakes.

The soda lakes are populated almost exclusively by prokaryotes, which can form dense communities (possibly due to the absence of grazing pressure) even in saturated alkaline brines [6–11]. Alkaliphilic cyanobacteria (*Spirulina*, *Arthrospira*, *Anabaenopsis*, *Cyanospira*) are responsible for the generally high level of primary production and nitrogen fixation in soda lakes [12–14]. The polymers produced by the primary producers are degraded by the aerobic and anaerobic hydrolytics, such as haloalkaliphilic *Bacillus* spp. and *Clostridia*, respectively [8,10]. The main groups of primary and secondary anaerobes using monomers and oligomers, such as fermentative (*Spirochaeta alkalica*, *Spirochaeta asiatica*, *Spirochaeta africana*, *Tindalia magadii*, *Alkalibacter*, *Alkaliflexus*), acetogenic (*Natroniella acetigena*, *Natronoincola histidinovorans*), methanogenic (*Methanohalophilus zhilinae*, *Methanosalsus zhilinaeae*) and sulfate-reducing bacteria (*Desulfonatronovibrio*, *Desufonatronum*), well adapted to haloalkaline conditions, have also been identified [10,15]. The microbial sulfur cycle appears to be very active in the soda lakes. It is driven by the extremely haloalkaliphilic anaerobic purple sulfur bacteria (*Ectothiorhodospira* and *Halorhodospira*) and hydrogenotrophic sulfate-reducing bacteria [6,10]. Haloalkaliphilic sulfur/polysulfide-reducing hydrogenotrophs however, remained to be identified.

Until recently, the bacterial ''filter'' responsible for the reoxidation of reduced inorganic compounds, such as methane, hydrogen, sulfide and ammonia, produced by the haloalkaliphilic anaerobes in the soda lake sediments, remained unknown despite some evidence of their activity [16,17], and of functional genes for these organisms [18] being found in different soda lakes. Our investigation of the soda lakes in various geographic areas (Table 1) revealed culturable representatives of all missing groups of aerobic and denitrifying chemo-

Table 1
Soda lake samples used for study of haloalkaliphilic chemolithotrophs

Area	Number of investigated lakes	Total salts ($g\,l^{-1}$)	pH	Total carbonate alkalinity (M)
Kenya (sampled by B. Jones and W. Grant)	6	20–220	9.5–11.0	0.12–1.16
Wadi Natrun (Egypt)	8	200–380	9.5–10.3	0.11–0.75
Mono Lake, California (sampled by V. Gorlenko)	1	90	9.7	0.4
Tuva, Russia (sampled by T.N. Zhilina)	1	20	10.0	nd
Kunkur steppe, Russia	10	5–40	9.5–10.2	0.02–0.11
North-eastern Mongolia	16	5–360	9.2–10.5	0.02–1.20
Kulunda steppe, Altai (Russia)	20	20–380	9.3–10.6	0.02–5.20