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**EXPERIMENTAL ARTICLES**

# *Microcoleus* Mats from Alkaliphilic and Halophilic Communities

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**Abstract—**A detailed description of the macrostructure, the ultrastructure, and the species diversity of alkaliphilic mat from Lake Khilganta (Buryatiya) is presented. The structure of this mat was found to be similar to that of halophilic mats from hypersaline lagoons of Lake Sivash (Crimea) that we studied earlier. *Microcoleus chthonoplastes* was the dominant form of cyanobacteria in both mats (in the alkaliphilic mat, *Phormidium molle* was another dominant form). Both mats had a pronounced laminated structure. However, unlike halophilic mats with calcium carbonate and gypsum laminas, the alkaliphilic mat contained calcium phosphate laminas instead of gypsum ones. The species diversity of microorganisms in the alkaliphilic mat was at least as rich as that in the halophilic mat; however, in the halophilic mat, the distribution of organisms between layers was more clear-cut. In the alkaliphilic mat, the highest species diversity was observed in the upper mat layers, at the boundary between zones of oxygenic and anoxygenic photosynthesis. This fact can be explained by the ephemeral nature of soda lakes.

*Key words*: alkaliphilic, halophilic cyanobacterial community, mat, cyanobacteria, mineralization, ultrastructure, biodiversity.

In the last two decades, cyanobacterial communities have been studied in many laboratories worldwide in connection with the claimed analogy between modern mats and ancient stromatolites. Although thermophilic and halophilic mats have been described in great detail [1–10], very few papers have been devoted to alkaliphilic communities [11–13]. In recent years, there has been increasing interest in these communities in connection with the hypothesis about the role they presumably played in the emergence of species diversity [14].

To find experimental verification of the contended hypothesis, several expeditions were undertaken to study soda lakes situated in central Africa, Tuva, Chita oblast, and the southeastern Transbaikal region. As a result of these investigations, the functional diversity of microorganisms in alkaliphilic microbial communities was demonstrated and several new microorganisms were discovered and described [15]. In 1995, a complex expedition organized by the Institute of Microbiology, Russian Academy of Sciences (the Laboratory of Relict Microbial Communities, headed by Academician G.A. Zavarzin, and the Laboratory of the Ecology and Geochemical Activity of Microorganisms, headed by Dr. V.M. Gorlenko), and the Institute of General and Experimental Biology (Dr. B.B. Namsaraev) surveyed numerous steppe lakes of the Transbaikal region and, in a soda lake, Lake Khilganta, found a laminated cyanobacterial mat, which was very similar to halophilic mats in its appearance.

The goal of this work was to compare alkaliphilic and halophilic mats with respect to their macrostructure and ultrastructure and the species diversity of microorganisms.

## MATERIALS AND METHODS

Lake Khilganta is situated in a small hollow and is 600 m in diameter and 50 cm in depth. The fact that the discharge of underground waters fails to affect water salinity in the lake indicates their high mineralization. According to the classification scheme of M.G. Valyashko, Lake Khilganta belongs to the sodium sulfate type and is characterized by a high concentration of carbonate ions (≈1.5 g/l); it has a total mineralization of 46.63 g/l and pH 9.5 [16]. Cyanobacterial mat develops on the lake bottom.

Samples were collected by T.N. Zhilina in the summer of 1995. Samples of mat for electron microscopy were enclosed in gelatin capsules and stored until arrival in Moscow in 2.5% glutaraldehyde. In the laboratory, bits of mat were divided into individual layers, additionally fixed in $OsO_4$, and subjected to conventional treatment for electron microscopy studies [17].

The macrostructure of the mat was examined under an Amplival light microscope and an SEM-300 scanning microscope. The ultrastructure of microorganisms was studied using a JEM-100 electron microscope.

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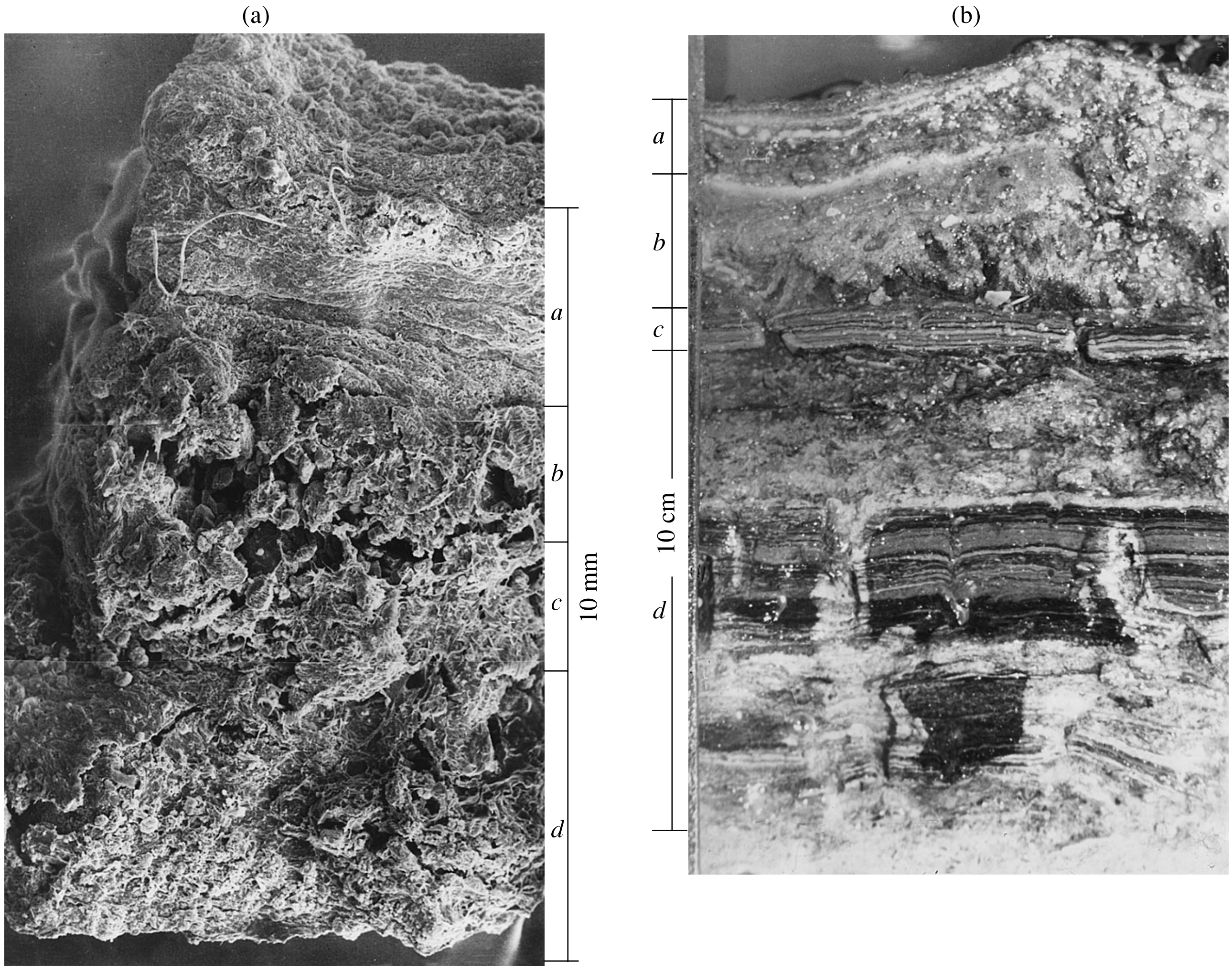


**Fig. 1.** Cross sections of (a) alkaliphilic and (b) halophilic mats displaying (*a*) the zone of oxygenic photosynthesis with mineral laminas; (*b*) the zone of anoxygenic photosynthesis; (*c*) the burial zone of cyanobacteria; and (*d*) the zone of sulfate reduction.

The elemental composition of mineral mat laminas was determined on a Link analyzer via sample scanning.

## RESULTS AND DISCUSSION

The mat under study had a thickness of 10 mm and a stratified structure with manifest layers that differed in color. It was relatively loose because of the presence in all layers of undecomposed filaments of cyanobacteria (Fig. 1a). Meanwhile, halophilic mats from Lake Sivash were quite dense and more than 30 mm in thickness (Fig. 1b). In the alkaliphilic mat, four zones separated by mineral laminas could be readily distinguished. Zone 1 is the zone of oxygenic photosynthesis, green in color and populated by filamentous cyanobacteria. Zone 2 is the zone of anoxygenic photosynthesis, brownish-crimson in color. In this zone, layers of purple bacteria are separated by mineral laminas. Zone 3 is the burial zone for cyanobacteria, brown-green in color. It has several mineral laminas. Zone 4 is a gray-to-black sulfate reduction zone (Table 1).

Zone 1 is composed of two layers. The topmost layer is feltlike, yellowish-green in color, and formed by thin entangled filaments of *Phormidium molle* (Fig. 2a). The underlying bright green layer is thicker and consists of bundles of the filamentous cyanobacterium *Microcoleus* (Figs. 2b, 2c). Zone 2 is mostly populated by photosynthesizing bacteria *Chromatium* and *Ectothiorhodospira* (as determined by Gorlenko and Kompantseva). Mineral laminas consist of aragonite, calcite, and calcium phosphate (Fig. 2d). Buried valves of diatoms were noted in this layer (Fig. 2e). In zone 3, the detrital mass contains occasional inclusions of *Microcoleus* bundles (Fig. 3f), very thin filaments of *Phormidium*, and unicellular cyanobacteria of the genus *Aphanothece*. Microorganisms in zone 4 could not be identified under a light microscope because of their small size, making them hardly distinguishable within the detrital mass.

**Table 1.** Mat macrostructure

| Zone | mm | Color | Microorganisms |
|---|---|---|---|
| I | 0–0.5 | Yellow-green | Cyanobacteria (*Phormidium molle*) |
| | 0.5–4.0 | Green | Cyanobacteria (*Microcoleus chthonoplastes* and others) |
| | 4.0–4.25 | Brownish-crimson | Purple bacteria (*Chromatium* and *Ectothiorhodospira*) |
| | 4.25–4.75 | White to gray | Minerals |
| II | 4.75–5.0 | Brownish-crimson | Purple bacteria (*Chromatium*) |
| | 5.0–5.5 | White to gray | Minerals |
| III | 5.5–5.75 | Brownish-green | Cyanobacteria (*Microcoleus* and *Aphanothece*) |
| | 5.75–6.25 | White to gray | Minerals |
| IV | 6.25–6.5 | Brownish-green | Cyanobacteria |
| | 6.5–10.0 | Gray to black | Sulfate reducers |

Electron microscopic analysis of individual mat layers (Figs. 3–5) provided more accurate evidence on the species diversity of the alkaliphilic system. Two forms of cyanobacteria prevailed in the oxygenic zone (this is not the case with halophilic mats): *Phormidium molle* in the top layer (Figs. 3a, 3b) and *Microcoleus chthonoplastes* (Figs. 3c, 3d) in the bottom layer. In the oxygenic zone, we also occasionally observed *Aphanothece salina* (Fig. 3e) and a green alga, which, based on its ultrastructure, was attributed to *Chlorella minutissima*. As in halophilic mats, filaments of *Microcoleus* were arranged in bundles enclosed in a common slimy sheath (Fig. 3c), whereas filaments of *Phormidium* were singular (Fig. 3a).

The internal structure of cells of alkaliphilic cyanobacteria had several distinctive features. They contained one or more electron-dense inclusions resembling cyanophycin granules (Figs. 3a–3d). In some cells, these inclusions occupied the entire central part of the cell and were as large as 1 μm (Fig. 3f) (note that, under unfavorable conditions, cyanophycin granules in the alkaliphilic cyanobacterium *Spirulina platensis* can be as large as 2.4 μm [18]). Such inclusions were never encountered in cyanobacteria located in any other mat layer. This is additional evidence in favor of the cyanophycin nature of the inclusions, since cyanophycin granules are synthesized only in the light and are quickly spent in the dark [19]. The lamellar system is well developed, and one can clearly see phycobilisomes located among the lamellae (Figs. 3b, 3d, 3f). Polyribosomes are not concentrated in the central part of the cell, as is usually the case, but occur in small zones spread all over the cell (Figs. 3e, 3g).

The lower part of the oxygenic zone was characterized by a very wide diversity of bacteria from various taxonomic groups. Many of these bacteria had inclusions of elemental sulfur and poly-β-hydroxybutyrate (Figs. 4a, 4b, 4d, 4g). Some cells had a peculiar cell wall typical of archaebacteria (Fig. 4b). The occurrence of anaerobic organisms in the photic zone was astonishing. However, methanogens were also found in the top layers of mat from the Canadian Goodenough Lake [13]. In Fig. 4a, one can see a filamentous sulfur bacterium of the *Thiothrix* type. A nitrifying bacterium with a well-developed lamellar structure is displayed in Fig. 4c. Many bacteria had a peeling-off S-layer (Fig. 4b). Some bacteria exhibited an ordered structured layer looking dark in photographs, and this, apparently, points to the presence of a carbohydrate component (Fig. 4g). Cyanobacteria were represented exclusively by *Microcoleus*, their filaments enclosed in a slimy sheath. The entire layer was very slimy, and the bacteria were submerged in glycocalyx, particularly developed in this layer.

Zone 2 was dominated by photosynthesizing purple bacteria (Figs. 5b–5d) with photosynthesizing membranes of a vesicular type. The zone was divided into several layers by mineral laminas (Figs. 1b, 2d). In these laminas, mineralized cells of cyanobacteria occurred among mineral particles made up of calcium carbonate and calcium phosphate (Figs. 2d, 2f, 2g). At the same time, they also contained undamaged cells of cyanobacteria with well-preserved structure. It should be noted that, unlike the halophilic mat, the alkaliphilic mat was characterized by high integrity of cyanobacteria even in the bottom mat layers. Cyanobacteria in zones 3 and 4 did not differ from organisms in the top layers and had well-preserved cell envelopes and undamaged lamellae with phycobilisomes (Figs. 5e, 5f).

It is difficult to make any conclusions concerning the species diversity in the sulfate reduction zone (zone 4) because, due to the high mineralization of this layer, cells tended to crumble out from microscopic sections and were rarely visible in micrographs.

Unlike the halophilic community, characterized by clear-cut localization of microorganisms with respect to mat layers [17], the entire diversity of species in the alkaliphilic mat is encountered in the three top layers (the photic zone). The most striking feature is the variety of species in the oxygenic zone,

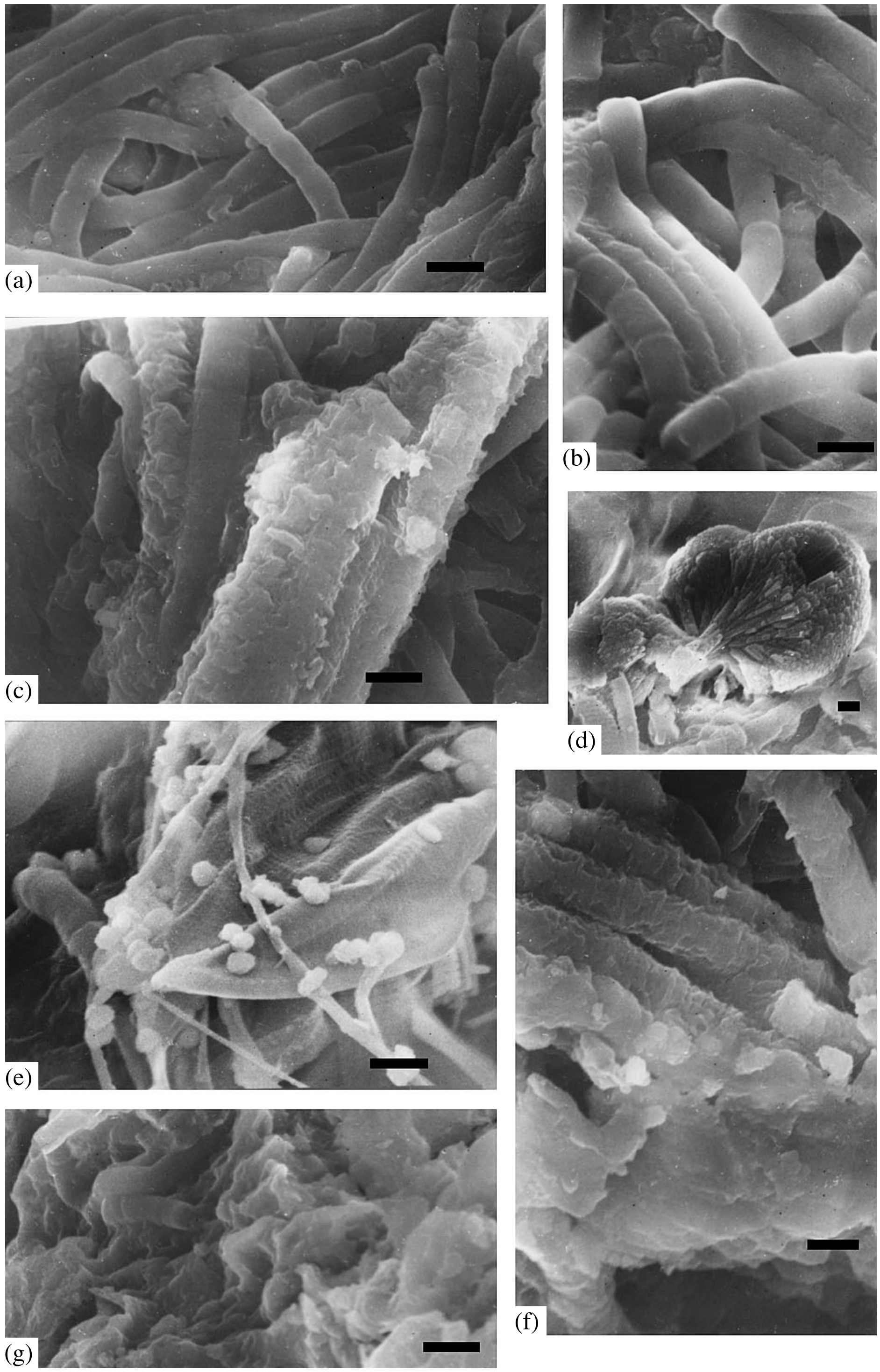

**Fig. 2.** Macrostructure of alkaliphilic mat as seen under a scanning microscope: (a) *Phormidium molle*; (b) *Microcoleus chthonoplastes*; (c) *M. chthonoplastes* (viable and mineralized trichomes in the second layer); (d) mineral calcite laminas; (e) purple bacteria among valves of diatoms; (f) mineralized filaments of cyanobacteria in lower mat layers; and (g) zone of sulfate reduction (zone 4) with remnants of filaments of cyanobacteria. Bar represents 3 μm.

where microorganisms from various taxonomic groups with different relation to oxygen occur side by side. Their coexistence can be explained either by the presence of micron-size redox zones [6] or by the occurrence in anaerobes of a mechanism protecting them from oxygen produced by cyanobacteria during daytime [5]. The authors of the latter paper argue that all microorganisms develop only in upper mat layers, with bottom layers containing only nonviable organisms.

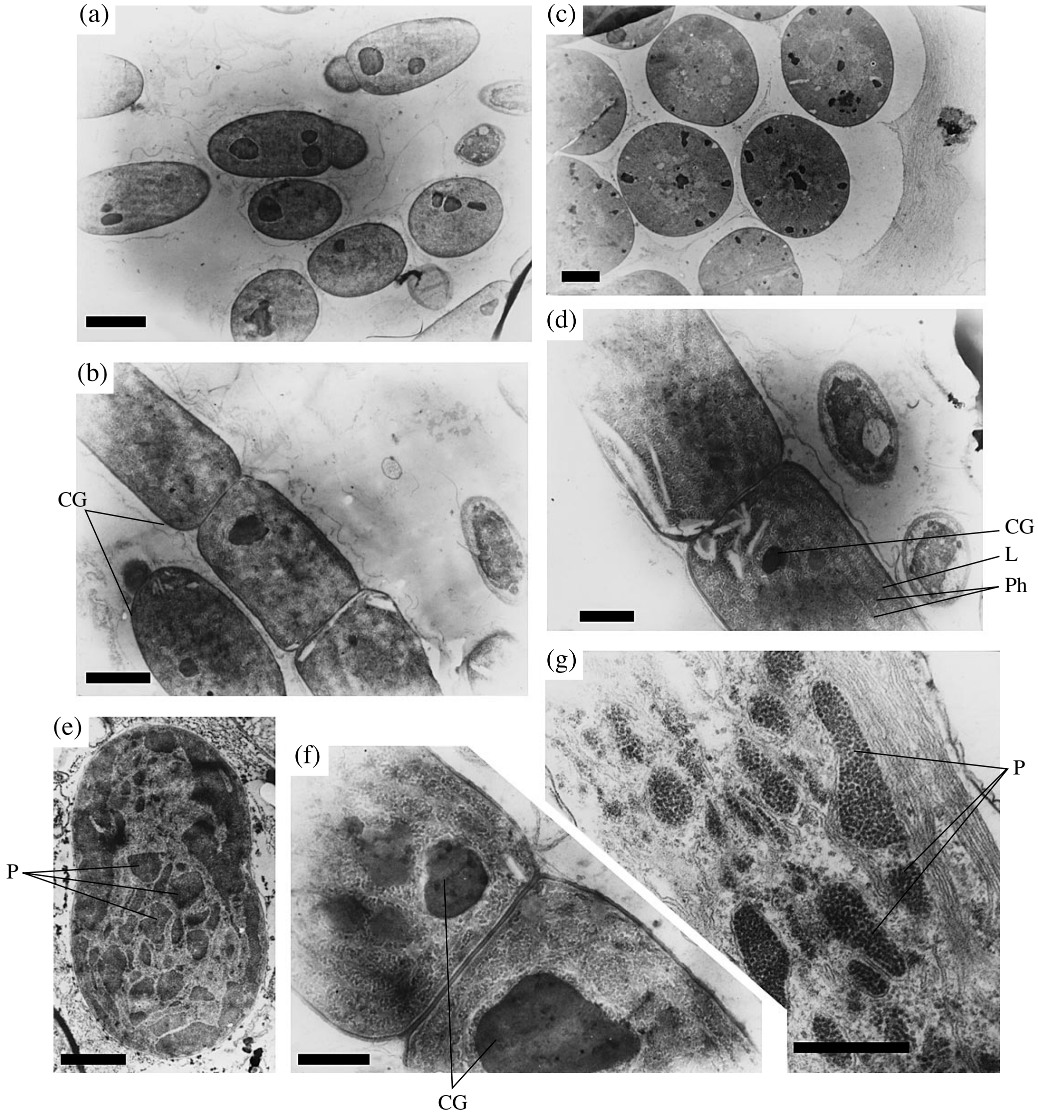


**Fig. 3.** Ultrastructure of cyanobacteria: (a, b) *Phormidium molle*; (c, d) *Microcoleus chthonoplastes*; (e) *Aphanothece salina* with islets of polyribosomes; (f) *P. molle* with granules of cyanophycin; and (g) a filament of *P. molle* with islets of polyribosomes. Bar represents 1 μm. CG, cyanophycin granules; P, polyribosomes; L, lamellae of thylakoids; Ph, phycobilisomes.

As previously noted, the alkaliphilic mat had a looser structure than halophilic mats and was similar to a 1-year-old halophilic mat from Lake Sivash [7]. In winter, alkaliphilic mats are likely to freeze solid and be destroyed, and this explains why they are so thin. Typical halophilic mats have a large number of interlaced layers of cyanobacteria and photosynthesizing bacteria.

The emergence of a thick layer with the development of *P. molle* in the upper part of the mat can be explained by the desalinization of lake water during the summer of 1995. This is confirmed by the ecophysiology of *P. molle*, which, unlike the moderately alkaliphilic *M. chthonoplastes*, is haloalkalitolerant. It is of interest that *M. chthonoplastes* is the predominant organism both in halophilic and in alkaliphilic communities. Its development is usually connected with the formation of cyanobacterial mats, because the capacity of this organism to form thick slimy sheaths enclosing several trichomes allows it to withstand large variations in the concentrations of NaCl and $Na_2CO_3$. The organism remains viable at concentrations of NaCl + $Na_2CO_3$ ranging from 2 to 16%.

Cyanobacterial mats were found only in two salt lakes out of eleven inspected in Tuva and Buryatiya. These were Lake Kak-Khol' (the Ubsu Nur depression, Tuva) [20] and Lake Khilganta (Buryatiya). The other nine lakes had a lower mineralization level and different cation–anion compositions [15]. Another factor

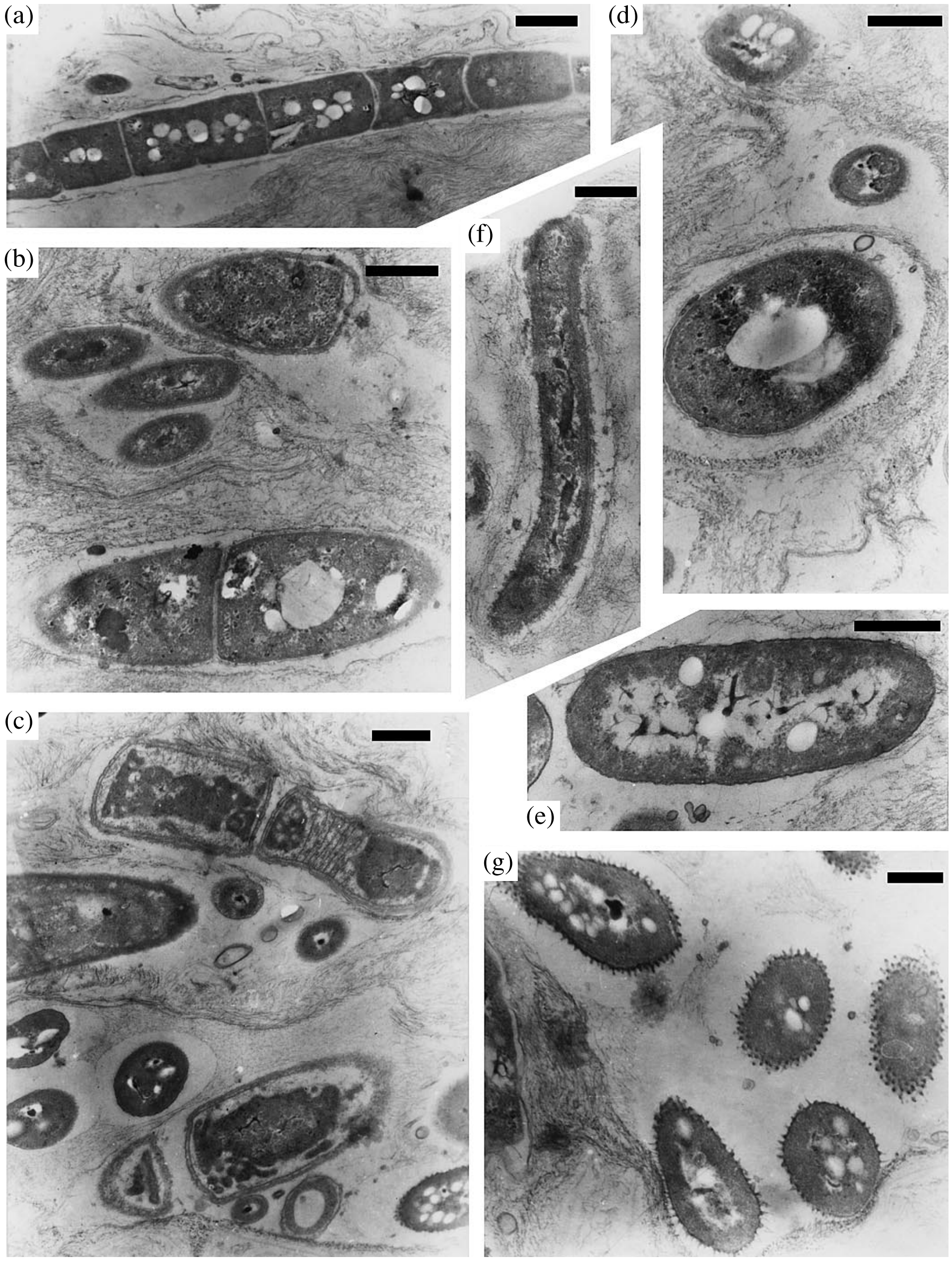


**Fig. 4.** Ultrastructure of microorganisms in the lower layer of the oxygenic zone: (a) a filament of *Thiothrix* with inclusions of sulfur and poly-β-hydroxybutyric acid; (b) archaebacteria and bacteria with a peeling-off outer layer; (c) a nitrifying bacterium with a well-developed lamellar system; (d) bacteria with sulfur inclusions; (e, f) unidentified bacteria; and (g) unidentified microorganism with an ordered structured layer. Bar represents 1 μm.

contributing to survival of the mat is a constant inflow of underground waters, saving the mat from complete destruction (according to local residents, these lakes never dry up in summer).

Alkaliphilic and halophilic *Microcoleus* mats are compared in Table 2.

It can be concluded that alkaliphilic and halophilic mats are similar in their structures, in the presence of several mineral laminas, in the diversity of species, and in the role of *Microcoleus chthonoplastes* as a predominant cyanobacterium [8]. However, alkaliphilic mats differ from halophilic ones by having two dominant cyanobacteria strictly confined to different mat layers, by the abundance of different groups of bacteria in the upper layers, and by good preservation of cyanobacteria in lower mat layers.

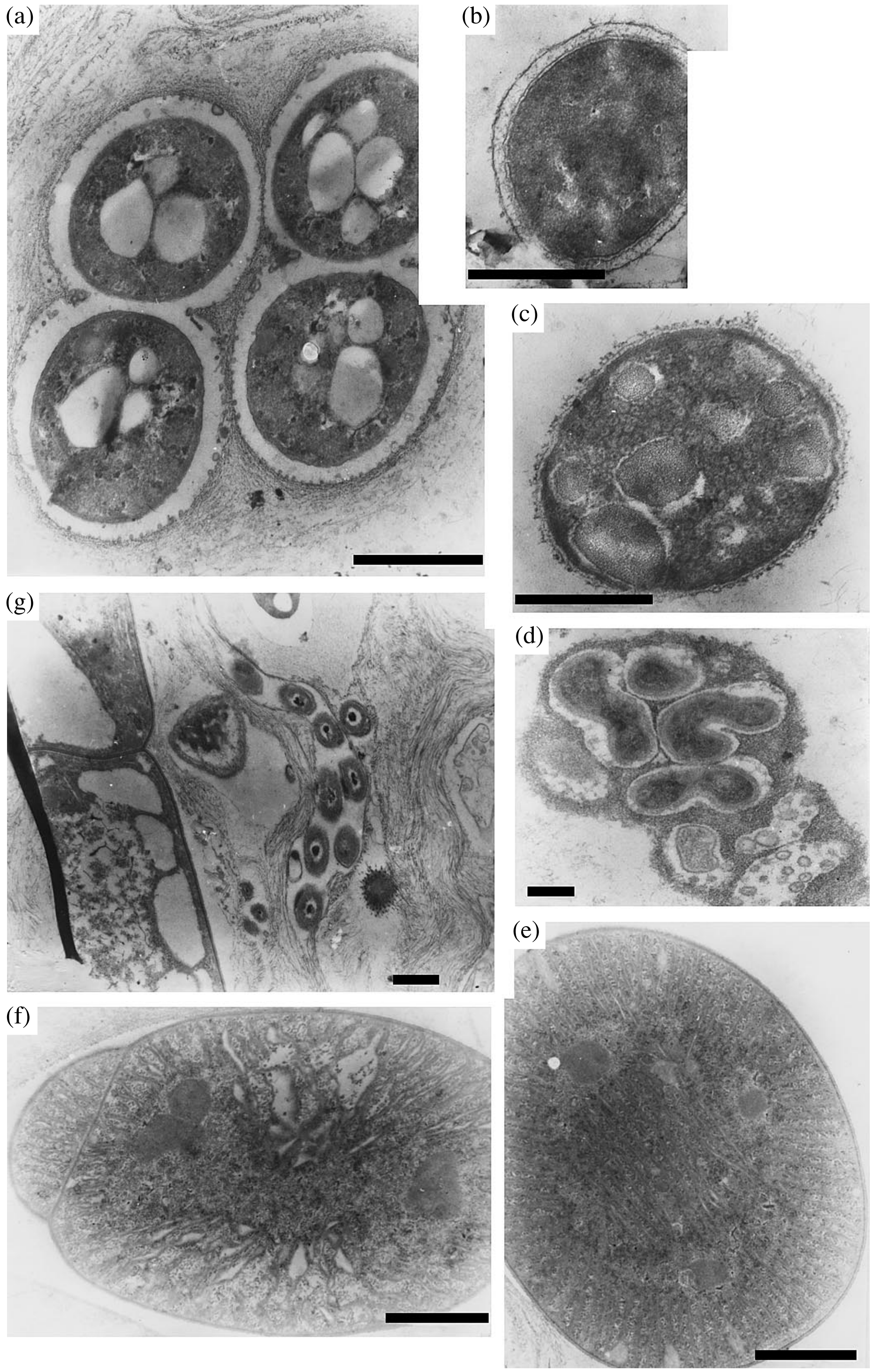
(a) (b) (c) (d) (e) (f) (g)

**Fig. 5.** Ultrastructure of microorganisms in the zone of anoxygenic photosynthesis and sulfate reduction: (a) cells of a colonial microorganism with binary cell fission and sulfur inclusions; (b–d) different photosynthesizing bacteria; (e, f) *Microcoleus chthonoplastes* from lower mat layers; and (g) a degraded filament of a cyanobacterium and unidentified microorganisms from the sulfate reduction zone. Bar represents 1 μm.

Based on the obtained evidence, it can be claimed that the structure of the mat is determined mostly by the biology of *Microcoleus* as the edifying organism and to a lesser extent by the physicochemical characteristics of the environment. Mats in continental bodies of water turn out to be very similar to mats in marine bodies of water.

The alkaliphilic mat that we studied develops in the mixing zone of saline upper waters of Lake Khilganta

**Table 2.** Characteristic features of alkaliphilic and halophilic mats

| Characteristics of mats | Alkaliphilic | Halophilic |
|---|---|---|
| Manifest laminated structure | + | + |
| *Microcoleus* as the dominant form of cyanobacteria | + | + |
| Presence of other dominant cyanobacteria | *Ph. molle* | |
| Mat thickness | up to 10 mm | up to 10 cm |
| Presence of photosynthesizing bacteria | + | + |
| Strict confinement of organisms to certain layers | – | + |
| Presence of mineral laminas of | | |
| calcium carbonate | + | + |
| gypsum | – | + |
| calcium phosphate | + | – |
| Preservation of cyanobacteria in lower layers | + | – |

with carbonate-rich underground waters, causing mat mineralization. This mat, therefore, can be considered as a modern analogue of ancient stromatolites.

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