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EXPERIMENTAL ARTICLES

Seasonal Changes in the Structure of the Anoxygenic Phototrophic Bacterial Community in Lake Mogilnoe, a Relict Lake on Kil'din Island in the Barents Sea

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Abstract—An anaerobic phototrophic bacterial community in Lake Mogilnoe, a relict lake on Kil'din Island in the Barents Sea, was studied in June 1999 and September 2001. Irrespective of the season, the upper layer of the anaerobic zone of this lake had a specific species composition of sulfur phototrophic bacteria, which were dominated by the brown-colored green sulfur bacterium *Chlorobium phaeovibrioides.* The maximum number of sulfur phototrophic bacteria was observed in June 1999 at a depth of 9 m, which corresponded to a concentration of bacteriochlorophyll (Bchl) *e* equal to 4.6 mg/l. In September 2001, the maximum concentration of this pigment (3.4 mg/l) was found at a depth of 10 m. In both seasons, the concentration of Bchl *a* did not exceed 3 μg/l. Purple sulfur bacteria were low in number, which can be explained by their poor adaptation to the hydrochemical and optical conditions of the Lake Mogilnoe water. In June 1999, the water contained a considerable number of *Pelodictyon phaeum* microcolonies and *Prosthecochloris phaeoasteroides* cell chains, which was not the case in September 2001. A 16S rDNA–based phylogenetic analysis of pure cultures of phototrophic bacteria isolated from the lake water confirmed that the bacterial community is dominated by *Chl. phaeovibrioides* and showed the presence of three minor species, *Thiocystis gelatinosa, Thiocapsa* sp., and *Thiorhodococcus* sp., the last of which is specific to Lake Mogilnoe.

Key words: meromictic lakes, sulfur phototrophic bacteria, anoxygenic photosynthesis.

Lake Mogilnoe, a relict lake located in the southeastern part of Kil'din Island, is separated from the Barents Sea by a stone–gravel beach. The high gradient of salinity in the lake water is due to seepage of the seawater through the beach and an inflow of surface and underground freshwater from the island side of the lake. The activity of the sulfate-reducing bacteria present in the lake water has led to accumulation of sulfides in its lower layers. These factors (the vertical salinity gradient and the presence of dissolved sulfides in the lower water layers) have given rise to an anaerobic zone in these layers.

The anaerobic phototrophic bacteria living in the chemocline of the deeper part of the lake give a pink color to the surrounding water. At the beginning of the last century, Issatchenko found that the purple sulfur bacteria present in the chemocline are dominated by bacteria of the genus *Chromatium* [1]. In the 1970s, Gorlenko *et al.* showed the presence of only brown-colored green phototrophic bacteria [2, 3]. Recent investigations have confirmed the high activity of anoxygenic phototrophic bacteria in the lake water and the finding of Gorlenko *et al.* [2, 3] that phototrophic bacteria contribute considerably to the production of organic matter in Lake Mogilnoe [4]. The taxonomic position of these phototrophic bacteria has not been established, since they were merely identified on the basis of their morphological properties and pigment composition. Furthermore, relevant microbiological studies have only been carried out in the fall and did not take into account possible seasonal changes in the anaerobic phototrophic bacterial community.

The aim of this work was to study seasonal variations in the structure of the anoxygenic phototrophic bacterial community developing in the upper layer of the hydrogen sulfide zone of Lake Mogilnoe, to isolate phototrophic bacteria in pure cultures, and to identify them using standard microbiological and molecular genetic methods.

MATERIALS AND METHODS

The lake water was sampled in a basin (15.5 m in depth) of the lake using a 0.75-l horizontal plastic bot-

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tle. The water samples were taken at 25-cm depth intervals. The bacterial photosynthesis rate was measured using [^{14}C]bicarbonate [4]. This labeled compound was added, in 0.1- to 0.2-ml aliquots, to 30-ml glass flasks filled with lake water. The flasks were suspended on a nylon halyard at the same depths from which the water samples were taken. In order to measure the dark assimilation of carbon dioxide, the flasks were wrapped in foil. After the flasks had been incubated for 6–12 h, the water and sediment samples were fixed by adding 0.5–1.0 ml of 2 N KOH.

The total bacterial count was estimated using 0.2-μm polycarbonate membrane filters. The bacterial cells adsorbed on the filters were stained with 4',6-diamidino-2-phenylindole (DAPI) [5] and counted under a Lumam-3 luminescence microscope. The content of Cl^- and SO_4^{2-} ions was determined by ion chromatography (Biotronik, Germany). The concentrations of oxygen and hydrogen sulfide and total alkalinity of water were measured immediately after water sampling using Aguamerk kits (Germany).

In order to measure the concentration of Bchl *e*, 10–50 ml of the lake water was passed through 0.2-μm nylon membrane filters. Then, under laboratory conditions, Bchl *e* was extracted from the adsorbed cells with an acetone–methanol (7 : 2) mixture. The absorption spectra of the extracts were recorded at 350–1000 nm in an SF-56 spectrophotometer (LOMO, St. Petersburg). The concentrations of Bchl *e* and Bchl *a* were calculated using the following formula [6]:

$$\text{C (μg Bchl } a\text{/l)} = k\,D_{770}\,(V \text{ extract (ml)}/V \text{ sample (l)}),$$

$$\text{C (μg Bchl } e\text{/l)} = k\,D_{654}\,(V \text{ extract (ml)}/V \text{ sample (l)}).$$

The absorption coefficients *k* for Bchl *e* and Bchl *a* were taken to be 98.0 l g^{-1} cm^{-1} [7] and 46.1 l g^{-1} cm^{-1} [8], respectively.

Phototrophic sulfur bacteria were cultivated in a Pfennig's medium [9] with the following composition (g/l): KH_2PO_4, 0.7; NaCl, 20; KCl, 0.33; $CaCl_2 \cdot 6H_2O$, 0.1; $MgSO_4 \cdot 7H_2O$, 0.5; $NaHCO_3$, 1.5; NH_4Cl, 0.7; $Na_2S \cdot 9H_2O$, 0.7; $Na_2S_2O_3 \cdot 5H_2O$, 1.0; Na acetate · $3H_2O$, 0.5; Na pyruvate, 0.5; yeast extract, 0.1; vitamin B_{12}, 20 μg; and solution of trace elements, 1 ml [9]. The pH of the medium was adjusted to 6.8 and 7.5 for green and purple sulfur bacteria, respectively.

In order to obtain enrichment cultures of phototrophic bacteria, the lake water was placed, under field conditions, in 30-ml sealed serum flasks with the aid of sterile syringes. The flasks were incubated anaerobically for a month. During the first week, the flasks were kept at room temperature without exposure to sunlight or even in the dark (during transportation). Then, the flasks were incubated in a luminostat at 20–25°C and an illuminance intensity of 2000 lx. The cultures were purified by the method of ultimate dilutions using the above cultivation medium solidified with 0.5% agar. The pigment composition of the purified cultures of anaerobic phototrophic bacteria was studied using preparations of intact cells in 50% glycerol as well as acetone–methanol (7 : 2) extracts of the cells.

In order to obtain microphotographs of the bacterial cells, the lake water samples were fixed with 2% glutaraldehyde and analyzed at a magnification of 1200× (objective 90×) using an immersion phase-contrast light microscope.

When carrying out ultramicroscopic studies, bacterial cells from the lake water or pure cultures were fixed with osmium tetroxide [10] and dehydrated in a series of ethanol solutions of increasing concentration and, then, in absolute acetone. The fixed cells were embedded in araldite epoxy resin. After curing the preparation, it was cut on an LKB III ultratome (Sweden) into thin sections, which were contrasted with lead citrate [11] and examined with a JEM-100C electron microscope (JEOL, Japan) operated at an accelerating voltage of 80 kV.

Primary identification of the bacteria was carried out with respect to the shape and size of the cells and microcolonies, the color of the colonies, the presence of gas vacuoles, the presence and pattern of sulfur drops in the cells, and to the absorption spectra of the pure cultures in 50% glycerol.

The optimal growth conditions of the bacteria were studied by measuring their biomass after being grown in a gradient of a particular environmental factor. The cell yield of green and purple sulfur bacteria was calculated from the relative amount of bacteriochlorophylls in the stationary growth phase, when neither the cells nor the medium contained elemental sulfur. Pigments were extracted with an acetone–methanol (7 : 2) mixture. The optical density of the extract was measured at 650 nm with a Specol spectrocolorimeter.

DNA was isolated according to the method described in [12]. Amplification of 16S rRNA genes was carried out with universal 27f and 1525r primers using a GeneAmp PCR System 2700 (Applied Biosystems). The amplified 16S rDNA fragment was sequenced in an automatic CEQ2000XL sequenator (Beckman Coulter) with a Dye Terminator Cycle Sequencing kit (Beckman Coulter) according to the manufacturer's instruction.

The nucleotide sequences of the 16S rDNA genes were aligned with the aid of the CLUSTAL X program [13]. An unrooted phylogenetic tree was generated using the neighbor-joining algorithm of the TREECON software package [14].

RESULTS

During both survey periods (June 19–25, 1999, and September 15–20, 2001), oxygen was detected in the Lake Mogilnoe water to a depth of 9–10 m (Figs. 1a, 1b); the chemocline was located at a depth of 8–10 m; and

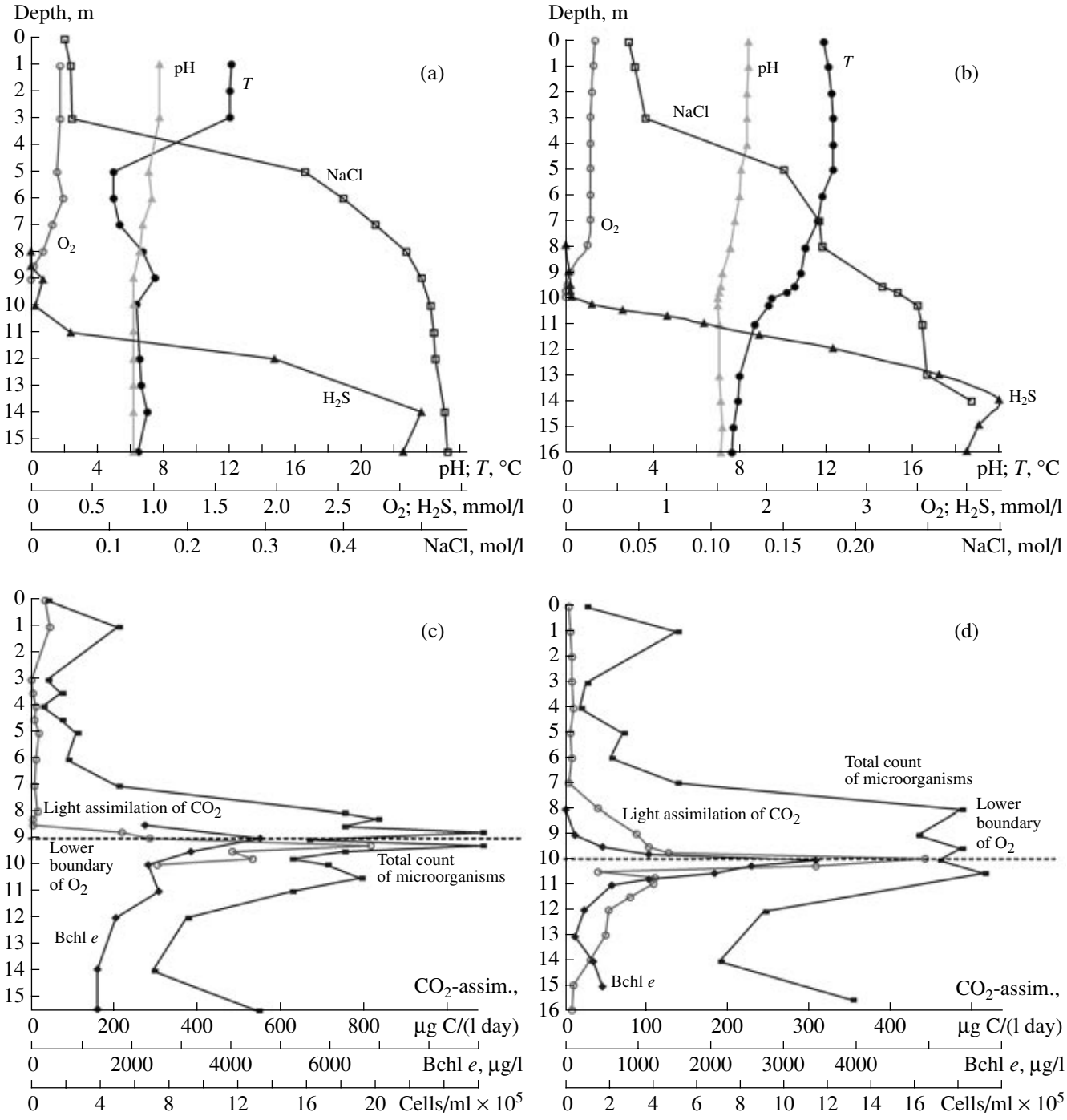

Fig. 1. Hydrochemical parameters of the water, the total number of microorganisms, and their activity in Lake Mogilnoe in (a, c) June 1999 and (b, d) September 2001. Curves in panels a and b: ○ shows oxygen concentration; ▲, hydrogen sulfide concentration; ▲, pH; ●, temperature; and □, sodium chloride concentration. Curves in panels c and d: ■ indicates the total count of microorganisms; ○, light assimilation of CO_2; ◆, concentration of Bchl *e* in microbial cells occurring in 1 l of the lake water; and –, the lower boundary of the oxic zone in the lake.

the concentration of hydrogen sulfide showed a sharp increase from below the chemocline downward to the bottom. The concentration of NaCl in the lake water began to increase from a depth of 3 m. The salinity gradient was less distinct in September 2001 than in June 1999, which can be attributed to the substantial inflow of freshwater in the summer period. In September 2001, the summer-warmed water of the lake had a temperature of 10–12°C to a depth of 10 m, whereas, in June 1999, we observed a distinct thermocline at a

Table 1. Anoxygenic phototrophic bacteria in the chemocline water samples from Lake Mogilnoe

Date	Isolated sulfur bacteria		Photosynthetic pigments	Optimal growth conditions
June 1999	Dominant species	**Chlorobium phaeovibrioides*	Bchl *e*, carotenoid isorenieratin	30 g/l NaCl, pH 6.8–7, 18–32°C
	Subdominant species	*Pelodictyon phaeum*		30 g/l NaCl, pH 7, 25°C
		Prosthecochloris phaeoasteroidea		30–35 g/l NaCl, pH 7, 25°C
	Minor species isolated from single colonies	**Thiocapsa* sp.	Bchl *a*, spirilloxanthin carotenoids	10 g/l NaCl, pH 6.5–8.5, 23–25°C
		**Thiorhodococcus* sp.	Bchl *a*, spheroidene carotenoids	5 g/l NaCl, pH 7.5, 23–25°C
September 2001	Dominant species	**Chlorobium phaeovibrioides*	Bchl *e*, carotenoid isorenieratin	30 g/l NaCl, pH 6.8–7, 18–32°C
	Subdominant species	–	–	–
	Minor species isolated from single colonies	**Thiocystis gelatinosa*	Bchl *a*, okenone carotenoids	Up to 20 g/l NaCl, pH 6.5–7.6, 23–25°C

* The isolates were identified taking into account the 16S rDNA gene sequencing data.

depth of 3–5 m, which was characterized by a temperature drop from 12 to 6°C. The pH of the lake water varied from 6.5 to 7.5 in June 1999 and from 7 to 8.5 in September 2001.

During both survey periods, the concentration of microorganisms attained its maximum in the chemocline (Figs. 1c, 1d), where it reached (18–26) × 10^5 cells/ml in June 1999 and (16–18) × 10^5 cells/ml in September 2000, and the carbon dioxide fixation rates reached maximum at the lower boundary of the chemocline. The intensity of photosynthesis was two times higher in June 1999 than in September 2001, indicating an outbreak of anoxygenic phototrophic bacteria after ice thawing.

The chemocline water was pink in color due to the growth of anoxygenic phototrophic bacteria. The color was more intense in June 1999 than in September 2001. This water contained a considerable amount of Bchl *e* but no Bchl *a*. This circumstance implies that, as in the case of earlier studies [2, 3], the pink color of the chemocline water is due to the growth of brown-colored green sulfur bacteria, which contain Bchl *e* and the brown carotenoid isorenieratin. The absence of Bchl *a* indicated that the number of purple bacteria in the chemocline water was low or nonexistent. The maximum concentration of Bchl *e* was detected in June 1999 (4.6 mg/l), whereas its concentration in September 2001 was equal to 3.4 mg/l.

The chemocline water samples taken in June 1999 were found to contain several morphotypes of green sulfur bacteria, which were also observed by Gorlenko *et al.* [2, 3]. The thin sections of cells taken from the water samples contained chlorosomes (Figs. 2a–2d), which are typical photosynthetic structures of green sulfur bacteria. The dominant bacterial morphotype corresponded to *Chl. phaeovibrioides*, which appear as small slightly curved cells forming large aggregates of an irregular shape (Figs. 2a, 2d). In some cases, we observed cell chains enclosed in a common sheath. This structure probably represents a living form of the same species (Fig. 2c). A minor morphotype consisted of large round bacteria resembling *Pld. phaeum* (Fig. 2b). The gas vesicles visible in these bacteria had an irregular shape, which could be due to incorrect prefixation of the samples. It should be noted that *Prs. phaeoasteroides* cells were not detected in the natural water samples, as they were probably scarce in the lake water during the survey periods.

An analysis of the enrichment cultures of anaerobic phototrophic bacteria showed that the chemocline water contained, in addition to green sulfur bacteria, purple sulfur bacteria.

As is evident from Table 1, the community of phototrophic bacteria in the chemocline water was different in June and September.

During both seasons, the chemocline water was dominated by one species, the green sulfur bacterium *Chl. phaeovibrioides* (Figs. 3a, 3b), whereas the minor purple sulfur bacteria were different. In June 1999, minor species were represented by purple sulfur bacteria phenotypically close to *Thiocapsa* sp. (Figs. 3d, 3e) and *Thiorhodococcus* sp. (Figs. 3f, 3g). In September 2001, instead of these species, we detected purple sulfur bacteria phenotypically close to *Chromatium–Thio-*

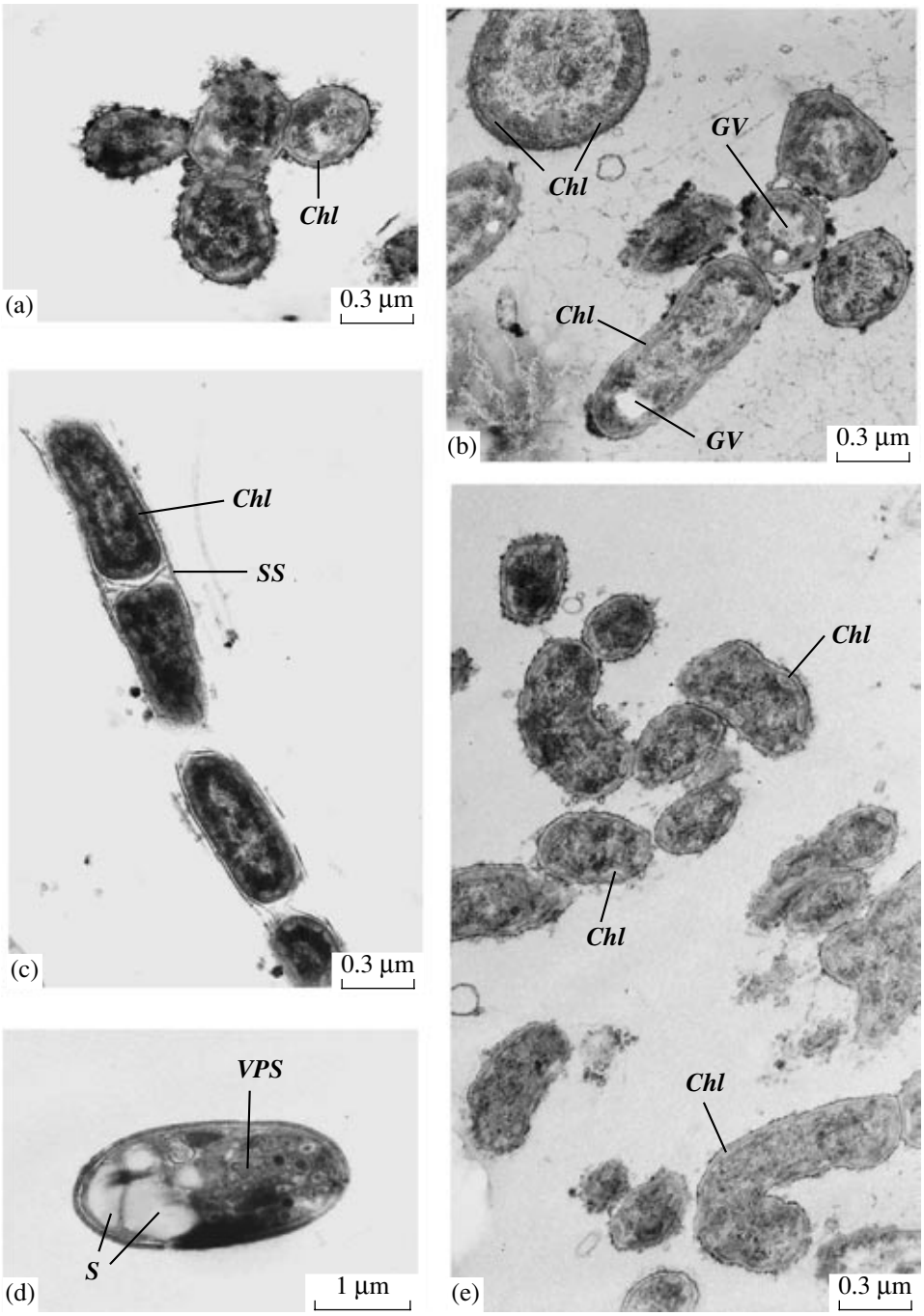

Fig. 2. Ultrastructure of the sulfur bacteria found in the chemocline water of Lake Mogilnoe: (a–d) the green sulfur bacterium *Chl. phaeovibrioides*, the cell periphery contains chlorosomes (photosynthetic antenna structures); (b) presumably, the microaggregates of *Pld. phaeum* cells, which contain gas vesicles; (c) chains of green sulfur bacteria in a common sheath (this structure was not observed in the pure cultures); and (e) presumably, the purple sulfur bacterium *Tcs. gelatinosa.* Abbreviations: *Chl,* stands for chlorosomes; *VPS*, vesicular photosynthetic structures (chromatophores) of purple bacteria; *GV*, gas vesicles; *S*, sulfur depositions; and *SS*, slimy sheath.

cystis spp. (Fig. 3c). It should also be noted that we failed to detect the subdominant species of green sulfur bacteria that were described in earlier studies.

Figure 4 shows the absorption spectra of the sulfur bacteria, which characterize their pigment composition.

In order to identify the taxonomic position of the four isolated bacterial species, we analyzed the nucleotide sequences of their 16S rDNA genes with the aid of the BLAST program (Table 2). This analysis made it possible to attribute strains Mog1, Mog2, and Mog3 to the family *Chromatiaceae* and strain Mog4, to the fam-

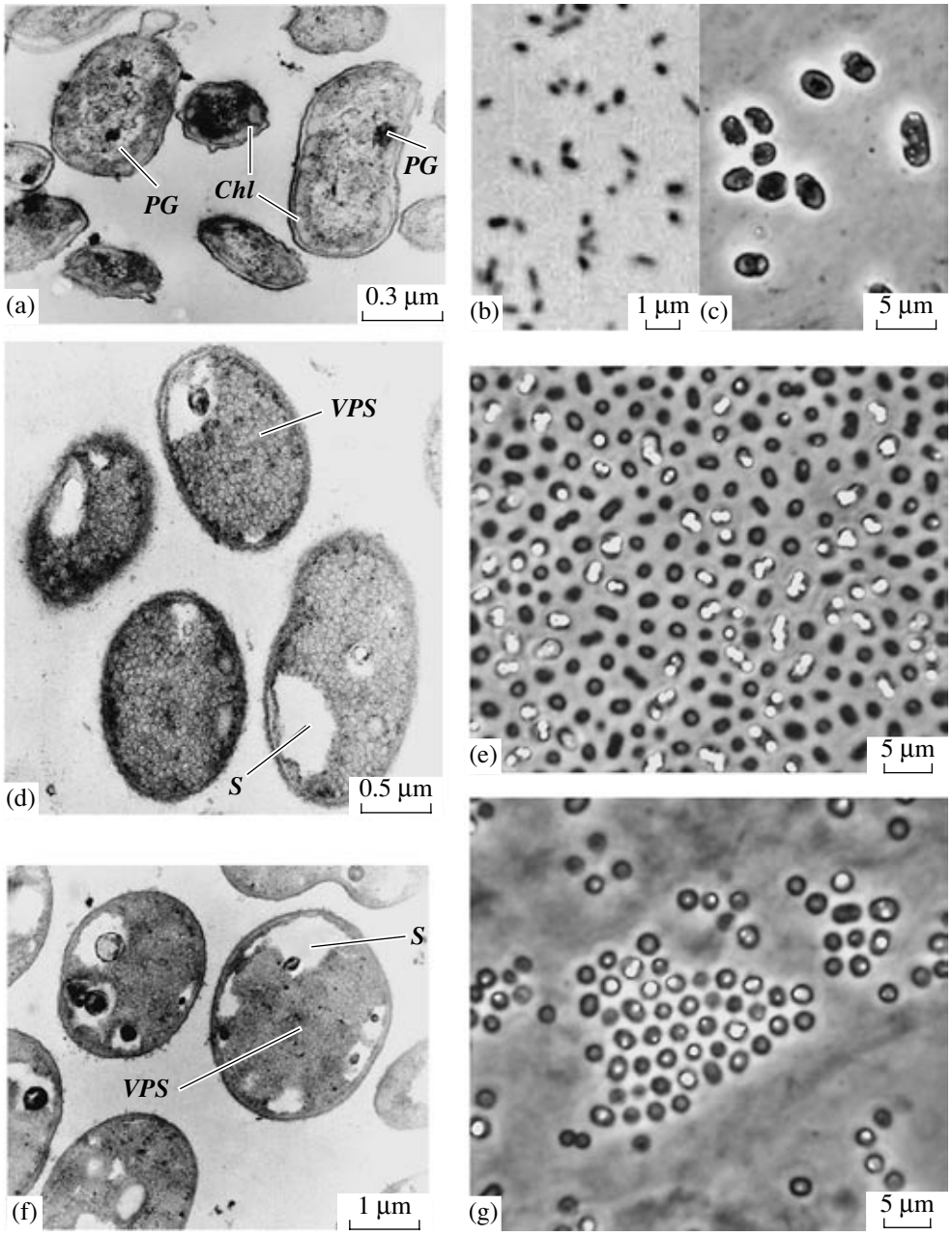

Fig. 3. Morphology and structure of the green and purple sulfur bacteria isolated from the Lake Mogilnoe water: (a, b) *Chl. phaeovibrioides*; (c) *Tcs. gelatinosa*; (d, e) *Thiocapsa* sp.; (f, g) *Thiorhodococcus* sp.; (a, d, f) electron microscopy; and (b, c, e, g) phase-contrast light microscopy. The cells on photographs c, e, and g contain luminescent sulfur deposits. *Chl*, chlorosomes (antenna structures of green bacteria); *VPS*, vesicular photosynthetic structures of purple bacteria (chromatophores); *S*, sulfur depositions; *PG*, polyphosphate granules.

ily *Chlorobiaceae*. Figure 5 shows the position of strains Mog1, Mog2, and Mog3 among the closest genera and species of the family *Chromatiaceae*. Strain Mog3 showed a 99.9% similarity to the type strain of *Tcs. gelatinosa*. Strain Mog1 comprised a cluster with bacteria of the genus *Thiocapsa*, being closest to the species *Tca. roseopersicina* (98.5% similarity). This strain may be a new species of the genus *Thiocapsa*. Strain Mog2 comprised a cluster with *Thiorhodococcus minor* (95.9% similarity of nucleotide sequences and 88% bootstrap value), suggesting its affiliation to the genus *Thiorhodococcus* as a new species.

Figure 6 shows the position of Mog4 among the type strains of the closest genera and species of the family *Chlorobiaceae*. This isolate exhibited the highest degree of similarity (99%) to the type strain of *Chl. phaeovibrioides*.

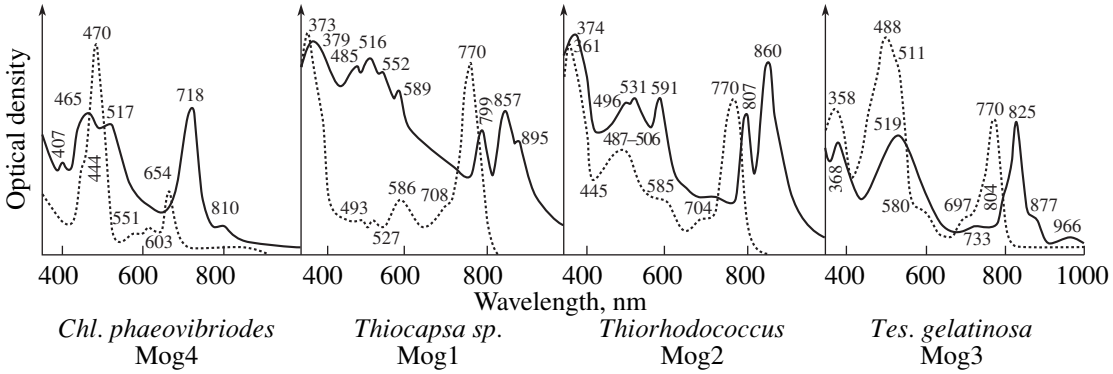

Fig. 4. Absorption spectra of the anoxygenic phototrophic bacteria isolated from Lake Mogilnoe. The solid lines represent the spectra of intact cells in 50% glycerol. The dashed lines represent the spectra of acetone–methanol (7 : 2) extracts of pigments.

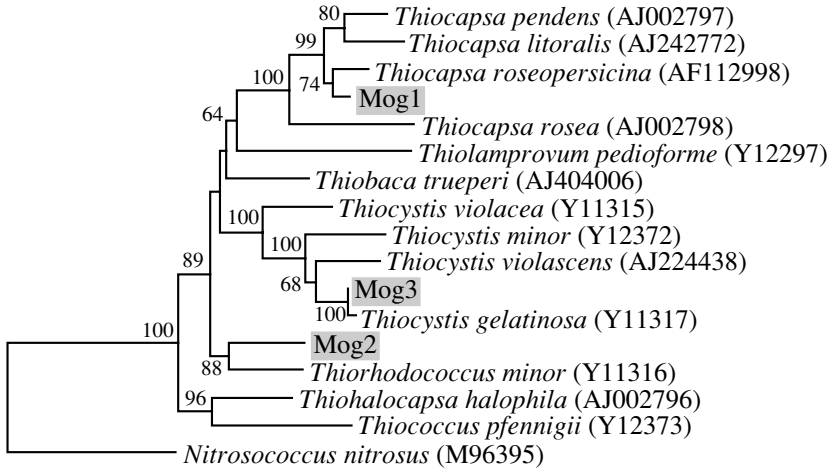

Fig. 5. Unrooted phylogenetic tree showing the position of strains Mog1, Mog2, and Mog3 among representatives of the family *Chromatiaceae*. The numerals indicate bootstrap values.

DISCUSSION

As compared to freshwater lakes, stratified saline lakes show a poorer diversity of phototrophic bacterial species, with one species completely dominating [15]. This study confirmed the earlier observations of Gorlenko *et al.* [3] that the species composition of the sulfur phototrophic bacterial community of Lake Mogilnoe is poor and completely dominated by *Chl. phaeovibrioides.*

The domination of this species is due to its very good adaptation to the hydrochemical conditions of the lake (primarily, water salinity and pH) and to the spectral composition of the light penetrating down to the chemocline. Indeed, *Chl. phaeovibrioides* prefers pH 6.8–7.0 and 1% water salinity, which corresponds to the parameters of Lake Mogilnoe. The presence of Bchl *e*, with an absorption maximum at 710–725 nm (Fig. 4), and

Table 2. Phylogenetic analysis of microorganisms isolated from the chemocline water of Lake Mogilnoe in June 1999 and September 2001

Isolate phenotypically close to	Source and time of isolation	Strain	Number of nucleotides analyzed	Similarity according to sequence data
Thiocapsa sp.	Chemocline water, June 1999	Mog1	1387	98.5% similarity to *Tca. roseopersicina*
Thiorhodococcus sp.		Mog2	1257	95–93% similarity to representatives of the genera *Thiorhodococcus* and *Thiocystis*
Chromatium-Thiocystis sp.	Chemocline water, September 2001	Mog3	1346	99.9% similarity to *Tcs. gelatinosa*
Chlorobium phaeovibrioides		Mog4	1414	99% similarity to *Chl. phaeovibrioides*

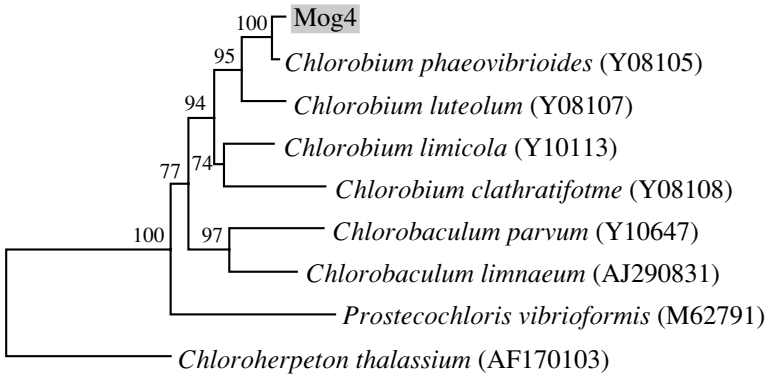

Fig. 6. Unrooted phylogenetic tree showing the position of strain Mog4 among representatives of the family *Chlorobiaceae.* The numerals indicate bootstrap values.

the brown carotenoid isorenieratin, which widens the absorption range of pigments in the blue–green spectral region between 480 and 550 nm, allows *Chl. phaeovibrioides* to grow at a considerable depth even in the high-turbidity water of Lake Mogilnoe [16–18]. The small number of purple sulfur bacteria and even the lack of them during some unfavorable periods can be explained by their poor adaptation to the hydrochemical and optical conditions of the Lake Mogilnoe water.

As an illustration, the species *Tcs. gelatinosa*, which requires a pH of 6.5–7.6 [19] and water salinity of 10–20 g/l for optimal growth, was not detected in the lake in June 1999, when the chemocline water had a pH of 6.2–6.5 and salinity of 28–30 g/l. Nor was this species detected in the chemocline water from August to September 1973, when its hydrochemical parameters were close to optimal [3]. This fact can be attributed to the unfavorable spectral composition of the light reaching the chemocline. This suggestion is confirmed by the higher turbidity of the lake water and deeper location of the chemocline over that period as compared to September 2001. A similar phenomenon was observed in the mesotrophic meromictic Lake Belovod (the Vladimir region), where, after ice thawing in May and early June, the water layer containing brown-colored *Pelochromatium roseum* symbionts was first observed at a depth of 7 m. As the water became more transparent, other species of phototrophic sulfur bacteria were found at a depth of 6.5 m. These species became dominant from June to August, although the brown-colored water layer remained at the same depth of 7 m [15].

Isolation of the bacteria *Thiocapsa* sp. and *Thiorhodococcus* sp. from the chemocline water in June 1999 indicates that the hydrochemical parameters of the chemocline water in Lake Mogilnoe may vary considerably. Indeed, these species grow optimally at a pH of 7–7.5 and salinity of 5 and 10 g/l, respectively. Consequently, the hydrochemical conditions of the lake chemocline in the spring are very unfavorable to *Thiocapsa* sp. and *Thiorhodococcus* sp. (Fig. 1). It is known that the water of Lake Mogilnoe is stratified from the fall onwards [20], which makes the development of brackish-water sulfur bacteria impossible in winter. The freshening of the upper water layers in Lake Mogilnoe recorded in August 1973 [3] and September 2001 was probably due to high precipitates over those periods. We can suggest that, during the summer and fall periods, the chemocline water can favor the growth of brackish-water sulfur bacteria provided that the lake water is sufficiently freshened. It is likely that the *Thiocapsa* sp. and *Thiorhodococcus* sp. bacteria detected in the chemocline water in June 1999 had survived there from the fall of 1998. This suggestion is confirmed by the fact of their isolation from single colonies. The absence of these species in August 1973 and September 2001 can be attributed to the high salinity of the chemocline water in those years (18–19 and 10–14 g NaCl/l, respectively), a different depth of the chemocline, a different intensity of the light reaching the chemocline, etc.

The presence of the sessile *Thiocapsa* sp. in the deep parts of meromictic bodies of water is uncommon. We were the first to show the development of this species at the boundary of the anaerobic zone of Lake Mogilnoe. This species is typical of shallow meromictic bodies of water and cyanobacterial mats of marine lagoons, where it develops in the upper part of the layer containing phototrophic bacteria due to its ability to tolerate oxygen or even utilize it in the dark [15, 21]. The lower layers of saline meromictic bodies of water often contain anaerobic purple sulfur bacteria such as *Chromatium* (*Halochromatium*) and *Thiocystis*, as well as green sulfur bacteria. A good example is the 6.5-m deep eutrophic meromictic Lake Repnoe in the environs of Slavyansk in the Donetsk region described by Gorlenko *et al.* [15]. This lake showed a mass development of *Chl. phaeovibrioides* beginning from the upper layer of the chemocline (4.5 m), with a maximum concentration of this bacterium at a depth of 5.75 m. The ecological niche of *Tca. roseopersicina* was found to be at a depth of 5.5 m. Purple bacteria resembling *Chr. vinosum* lived in the same zone as *Chl. phaeovibrioides.* Among the minor species of Lake Repnoe, Gorlenko *et al.* detected the purple nonsulfur bacterium *Rhodopseudomonas sulfidophila* and the green sulfur bacterium *Chl. chlorovibrioides.*

Changes in the hydrochemical parameters of the chemocline water in Lake Mogilnoe influence the growth of green phototrophic bacteria. The low salinity of this water (10–14 g/l) in September 2001 is probably responsible for the absence of the brown-colored sulfur bacteria *Pld. phaeum* and *Prs. phaeoasteroides* over that period. For comparison, these bacteria require 20–30 g/l NaCl and pH 6.5–7.0 [3, 19]. Both of these species were found in August 1973, when the chemocline water had a pH of 6.5–7 and salinity of 18–19 g/l [3], and in June 1999, when this water had a pH of 6.2–6.5 and salinity of 28–30 g/l [3]. The isolation of these two species is difficult because of their small concentration as compared to that of *Chl. phaeovibrioides.*

To conclude, the low salinity and favorable optical properties of the Lake Mogilnoe water over the summer and fall periods may provide for the development of some species of halotolerant purple sulfur bacteria, in addition to the deep-water, halophilic bacteria *Chl. phaeovibrioides, Pld. phaeum*, and *Prs. phaeoasteroides.*

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