ISSN 0026-2617, Microbiology, 2007, Vol. 76, No. 3, pp. 368–379. © Pleiades Publishing, Ltd., 2007.
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EXPERIMENTAL ARTICLES

Seasonal Changes in the Structure of the Anoxygenic Photosynthetic Bacterial Community in Lake Shunet, Khakassia

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Received October 03, 2006

Abstract—Seasonal studies of the anoxygenic phototrophic bacterial community of the water column of the saline eutrophic meromictic Lake Shunet (Khakassia) were performed in 2002 (June) and 2003 (February–March and August). From the redox zone down, the lake water was of dark green color. Green sulfur bacteria predominated in every season. The maximum number of green sulfur bacteria was 10^7 cells/ml in summer and 10^6 cells/ml in winter. A multi-syringe stratification sampler was applied for the study of the fine vertical distribution of phototrophs in August 2003; the sampling was performed every 5 cm. A 5-cm-thick pink-colored water layer inhabited by purple sulfur bacteria was shown to be located above the layer of green bacteria. The species composition and ratio of purple bacterial species depended on the sampling depth and on the season. In summer, the number of purple sulfur bacteria in the layer of pink water was 1.6×10^8 cells/ml. Their number in winter was 3×10^5 cells/ml. In the upper oxygen-containing layer of the chemocline the cells of purple nonsulfur bacteria were detected in summer. The maximum number of nonsulfur purple bacteria, 5×10^2 cells/ml, was recorded in August 2003. According to the results of the phylogenetic analysis of pure cultures of the isolated phototrophic bacteria, which were based on 16S rDNA sequencing, green sulfur bacteria were close to *Prosthecochloris vibrioformis*, purple sulfur bacteria, to *Thiocapsa* and *Halochromatium* species, and purple nonsulfur bacteria, to *Rhodovulum euryhalinum* and *Pinkicyclus mahoneyensis*.

Key words: eutrophic meromictic saline lakes, green sulfur phototrophic bacteria, purple sulfur phototrophic bacteria, purple nonsulfur phototrophic bacteria, anoxygenic photosynthesis.

DOI: 10.1134/S0026261707030149

The eutrophic meromictic saline Lake Shunet in Khakassia is located 19 km from Shira station (Russia, Siberia). This is a small (1.2 × 0.4 km) oval lake, with the total area of 0.47 km^2, with the maximum depth ca. 6 m; the water is of sulfate–chloride–sodium–magnesium type.

The lake is characterized by a sharply pronounced salinity gradient and by significant content of hydrogen sulfide (over 500 mg/l) at the bottom. Preliminary studies of microbial processes in the lake during summer and winter seasons have revealed the high abundance of anoxygenic phototrophic bacteria (APB) [1, 2]. From the redox zone (i.e., the zone where oxygen and hydrogen sulfide are present simultaneously) to the bottom, the water was of intense green color caused by the massive development of these bacteria. However, the species composition and the structure of the community of anoxygenic phototrophic bacteria, as well as its seasonal changes, have not been studied.

The goal of the present work was to perform comparative seasonal studies of the structure of the APB community of Lake Shunet, to isolate pure cultures of phototrophic bacteria, and to identify them by microbiological and molecular methods.

MATERIALS AND METHODS

The water samples were collected in July 2002 and February–March and August 2003 from the central part of the lake with depth 6–6.5 m. A 0.75 l plastic horizontal bathometer, a glass 1 l bathometer, and a multisyringe stratification bathometer [1] were used for sampling. The depth profiles of temperature, salinity, and pH were determined with the aid of a Data-Sonde 4a immersed multichannel probe (Hydrolab, United States).

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The concentrations of oxygen and hydrogen sulfide were measured immediately after sampling with the use the test of collections Aquamerck (Merck, Germany).

The rate of photosynthesis was determined by the radioisotope method with ^{14}C-bicarbonate [3]. For this purpose, 0.1–0.2 ml of labeled bicarbonate was injected into 30 ml glass bottles filled to capacity with lake water (final activity in the bottle, 10 μCi). The flasks, attached to a Nylon halyard, were submerged in the horizons from which water samples were taken. Bottles wrapped in aluminum foil immediately after sampling and submerged at the same depth were used as the dark control. The incubation time was 6–12 h. After the incubation with the labeled substrate, the water and sediment samples were fixed with 0.5–1.0 ml of 10% phosphoric acid. The samples were then filtered through 0.2 μm Nylon filters, washed twice with 5% H_3PO_4 and dried at room temperature; their activity was determined with a RackBeta-1219 scintillation counter (LKB, Sweden).

The total number of microorganisms was determined by epifluorescence microscopy on DAPI (diamidino-4',6-phenyl-2-indol dichlorhydrate)-stained 0.2 μm polycarbonate membrane filters [4]. A Lumam-3 fluorescence microscope (LOMO, St. Petersburg, Russia) was used to count bacterial cells.

The content of bacteriochlorophylls (Bchl) was determined on 0.2 μm Nylon filters after filtration of 10–350 ml of lake water. Then the pigments were extracted with an acetone–methanol mixture (7 : 2) under laboratory conditions. Absorption spectra of the extracts in the wavelength range from 350 to 1000 nm were obtained using a SF 56 spectrophotometer (LOMO, Russia).

The content of the pigments was calculated according to the following formulas [5]:

$$C(\mu g \text{ Bchl } d/l) = 1/kD_{654}(V \text{ extract (ml)}/V \text{ sample (l)})1000,$$

$$C(\mu g \text{ Bchl } a/l) = 1/kD_{770}(V \text{ extract (ml)}/V \text{ sample (l)})1000,$$

where C is the Bchl concentration; k is the absorption coefficient; D is the optical density of the extract as determined in a 1 cm cuvette at 654 or 770 nm; and V is the volume of the extract or sample.

The following absorption coefficients were used: for Bchl *d,* k = 98.0 l g^{-1} cm^{-1} [6], and for Bchl *a,* k = 46.1 l g^{-1} cm^{-1} [7].

For the cultivation of phototrophic sulfur bacteria, a medium was used which approximated Lake Shunet water in composition (g/l of distilled water): KH_2PO_4, 0.5; NaCl, 5.3; $MgSO_4 \cdot 7H_2O$, 0.5; NH_4Cl, 0.7; KCl, 0.33; Na_2SO_4, 21; $MgCl_2 \cdot 7H_2O$, 4.3; $NaHCO_3$, 1; $CaCl_2 \cdot 6H_2O$, 0.1; $Na_2S \cdot 9H_2O$, 0.5; $Na_2S_2O_3 \cdot 5H_2O$, 1; Na acetate $\cdot 3H_2O$, 0.5; Na pyruvate, 0.5; yeast extract, 0.1; vitamin B_{12}, 20 μg; trace elements solution, 1 ml [8].

For the cultivation of nonsulfur bacteria, a medium of the following composition was used (g/ l of distilled water): KH_2PO_4, 0.7; NaCl, 20; $MgSO_4 \cdot 7H_2O$, 0.5; NH_4Cl, 0.7; KCl, 0.33; $NaHCO_3$, 1.5; $CaCl_2 \cdot 6H_2O$, 0.1; $Na_2S \cdot 9H_2O$, 0.05 g; $Na_2S_2O_3 \cdot 5H_2O$, 1; Na acetate $\cdot 3H_2O$, 2; Na pyruvate, 2; yeast extract, 0.2; vitamin B_{12}, 20 μg; trace element solution, 1 ml [8]. For sulfur and nonsulfur phototrophic bacteria, the medium pH was 6.8–7.

In order to obtain enrichment cultures of phototrophic bacteria, lake water samples were injected with sterile syringes into hermetically sealed glass penicillin bottles containing 30 ml of the medium under field conditions.

The numbers of photosynthetic bacteria in July 2002 and February–March 2003 were determined by inoculating the agarized (0.5%) medium for phototrophic sulfur bacteria with serial dilutions of the environmental material (water samples) with the subsequent counting of colonies in each dilution [9]. The test tubes were incubated anaerobically for one month; during the first week of incubation, they were maintained at room temperature out of direct sunlight, and afterwards in a luminostat at 2000 lx illumination and 20–25°C. The quantitative determination of differently colored colonies was performed at the end of the incubation period. Such characteristics as the size and shape of bacteria and microcolonies, cell coloration of bacterial suspension, motility, the presence of gas vacuoles, the manner of elemental sulfur deposition, and the presence and location of intracellular sulfur inclusions were used for bacterial identification.

The numbers of green and purple sulfur bacteria in August 2003 were calculated from the Bchl content on the basis of the previously obtained data on cell numbers and Bchl content in July 2002.

The isolation and purification of the cultures were performed by the MPN (most-probable number) method in the agarized (0.5%) medium of the same composition. The pigment composition of the cultures of anoxygenic phototrophic bacteria was determined in the preparations of whole cells with the glycerol (1: 1) and in acetone–methanol (7: 2) extracts from bacterial biomass.

The microphotographs of the cells were obtained by light microscopy with an immersion system and phase contrast at ×1200. In order to obtain ultrathin sections of bacteria isolated from water samples, the material was prefixed with 2.5% glutaraldehyde and fixed with 1% osmium tetroxide. The dehydration was accomplished in a series of ethanol solutions of increasing concentration and subsequently in absolute acetone. Then the samples were embedded in the mixture of Epon 812 and Araldite M. Ultrathin sections were obtained using an LKB-Nova ultramicrotome. The sections were stained with 2% uranyl acetate in 70% etha-

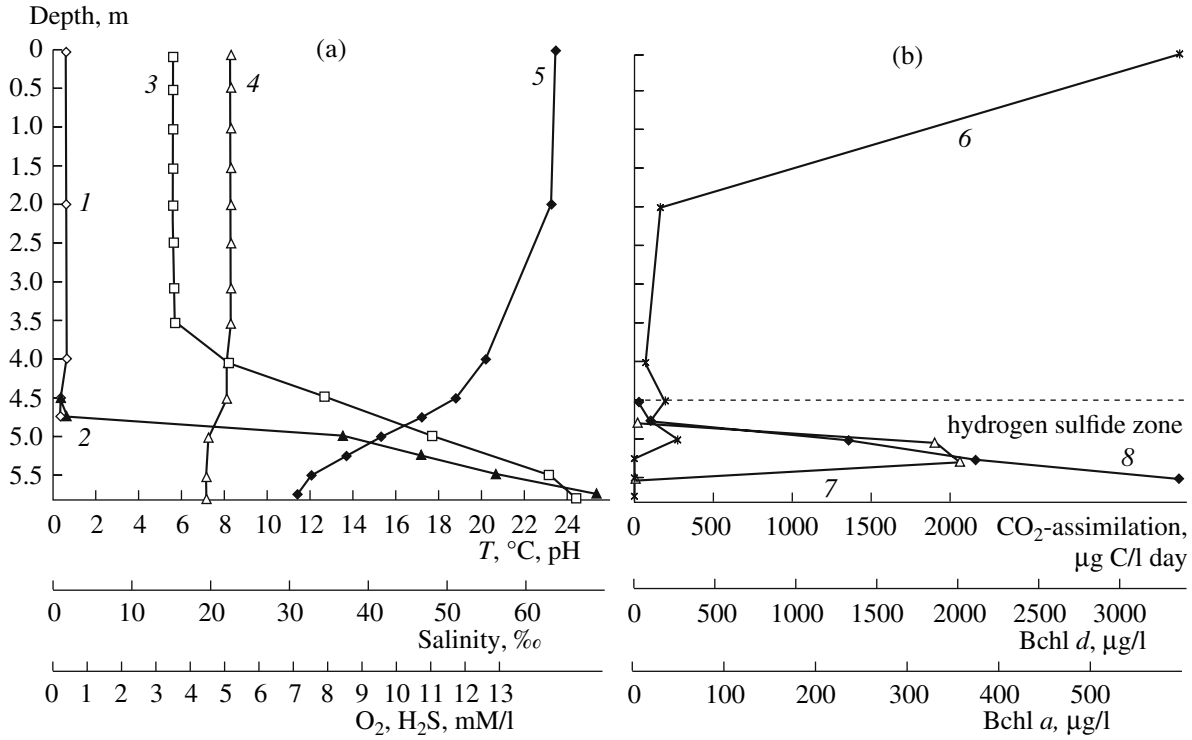


Fig. 1. Hydrochemical characteristics (a); light CO_2 fixation and the distribution of bacteriochlorophylls (b) in the water of Lake Shunet in July 2002: (*1*), O_2 content; (*2*), H_2S content; (*3*), total salinity; (*4*), pH; (*5*), temperature; (*6*), light CO_2 assimilation; (*7*), content of Bchl *a*; (*8*), content of Bchl *d*.

nol additionally with the complex lead contrasting agent [10]. The sections were examined under a JEM-100CXII electron microscope (Japan) at 80 kV accelerating voltage.

DNA isolation was conducted according to the procedure described earlier [11]. The amplification of 16S rRNA genes was achieved with the universal primers 27f and 1492r on a GeneAmp PCR System 2700 (Applied Biosystems). The amplified 16S rDNA fragments were sequenced using a DNA CEQ2000XL automatic sequencer (Beckman Coulter) with the Dye Terminator Cycle Sequencing kit of sequencing reagents (Beckman Coulter) according to the manufacturer's protocol.

For aligning of the nucleotide sequences of 16S rRNA genes, the ClustalX software package was used [12]. The rootless phylogenetic tree was constructed with the aid of the algorithms realized in the TREECON software package [13].

RESULTS

June 2002

Physicochemical characterization. During this season, the sampling was performed with 0.75 and 1 l bathometers; it was therefore not possible to reveal the fine structure of the phototrophic microbial community near the redox zone (i.e., the zone where oxygen and hydrogen sulfide are present simultaneously). According to our data, the redox zone was located at the depth of 4.5–4.75 m (Fig. 1a). Salinity increased sharply from 15 g/l at 3.5 m to 65 g/l at the bottom. In summer the upper water layers (3–4 m) were heated to 18–22°C; the thermocline was located at 3 m depth, and the water temperature at the bottom of the lake was close to 10°C. In the upper layers, the pH of the water was 8.3–8.6; in the layers directly above the redox zone, it decreased sharply to 7.6–7.3. From the redox zone down, the water had dark green coloration caused by the massive development of green sulfur phototrophic bacteria. Microbial activity in the water layer directly below the redox zone was confirmed by the peak of CO_2 assimilation at 273 μg C/(l day) (Fig. 1b).

Anoxygenic phototrophic bacterial community. During July 2002 the APB community of Lake Shunet was represented by green sulfur bacteria, purple sulfur bacteria, and purple nonsulfur bacteria.

The highest number of phototrophic bacteria was recorded at the depth of 5 m, 0.5 m below the redox zone. The water at this depth was of a dark green color; its absorption spectrum is shown in Fig. 2a.

The quantitative analysis of the APB colonies grown in the agarized medium revealed 9.65×10^6 and 4.8×10^6 cells/ml of green sulfur bacteria and purple sulfur bacteria, respectively at the depth of 5 m; the respective

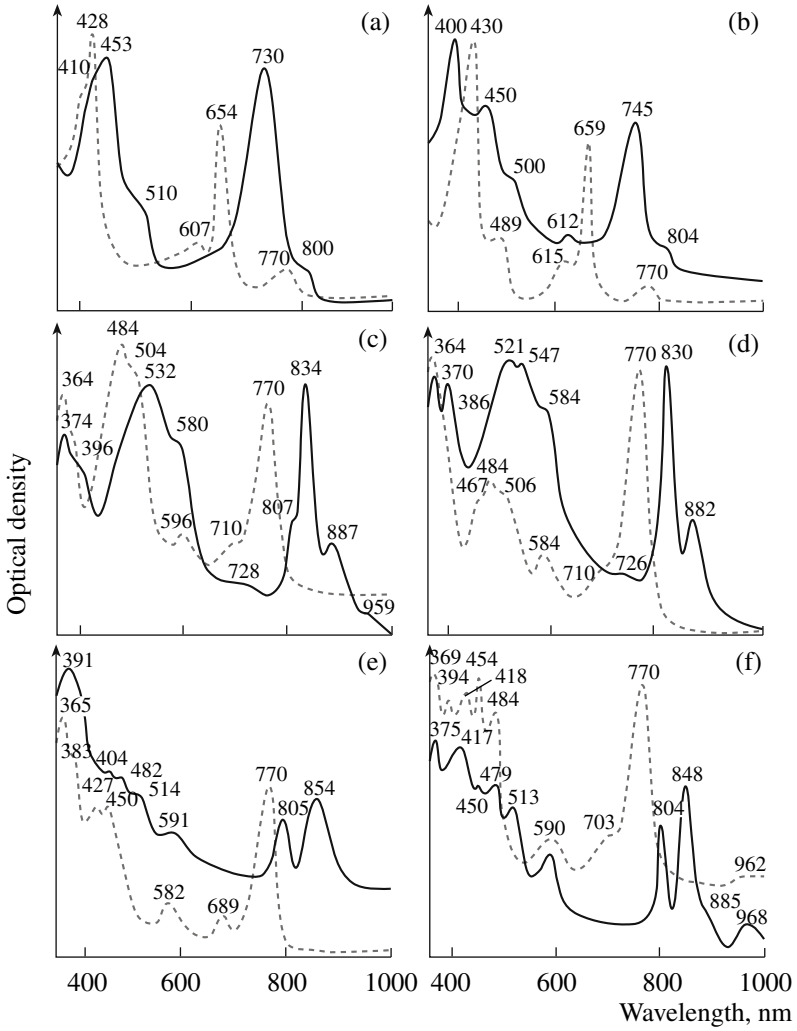


Fig. 2. Absorption spectra of the pigments of Lake Shunet anoxygenic phototrophic bacteria: (a), the spectrum of Lake Shunet water from the depth of 5 m, July 2002; (b), green sulfur bacteria, strain ShN*Pel*02; (c), purple sulfur bacteria, strain ShN*Am*02; (d), purple sulfur bacteria, strain ShN*Lb*02; (e), purple nonsulfur bacteria, strain ShN*Rb*02; and (f), purple nonsulfur bacteria, strain ShN*Br*03. Solid line, spectrum of the live culture in glycerol; dotted line, spectrum of the acetone-methanol extract.

contents of Bchl *d* and Bchl *a* in the lake water were 1303.5 and 324.2 μg/l.

The pure culture of green sulfur bacteria isolated from the 5 m water sample was designated as strain ShN*Pel*02. The absorption spectrum of this strain is shown in Fig. 2b; it was practically identical to the absorption spectrum of lake water. A strain of purple sulfur bacteria isolated from the same sample was designated ShN*Am*02.

In the deeper water layers at 5.25 m, the numbers of green sulfur bacteria remained the same as at 5 m depth (9.65×10^6 cells/ml), while the number of purple sulfur bacteria decreased to 7.4×10^5 cells/ml.

Purple sulfur bacteria predominated in the layers above the peak of APB development, at the depth of 4.5 m (redox zone). According to the data of quantitative analysis, the number of bacteria morphologically similar to strain ShN*Am* was 10^4–10^5 cells/ml.

In the water samples from the redox zone (4.5 m), isolated cells of another purple sulfur bacterium and of a motile nonsulfur purple bacterium were present; these organisms were not revealed in other water layers. We have obtained pure cultures of these microorganisms, designated as strains ShN*Lb*02 and ShN*Rb*02, respectively.

August 2003

Physicochemical characterization. A multisyringe stratification bathometer was used for sampling during this season; it enabled water sampling with 5 cm intervals without disruption of the layer structure [1]. Detailed examination of the redox zone and adjacent water layers of Lake Shunet water has been performed.

The redox zone in August 2003 was located 0.5 m lower than in July 2002. The oxic zone ended at the

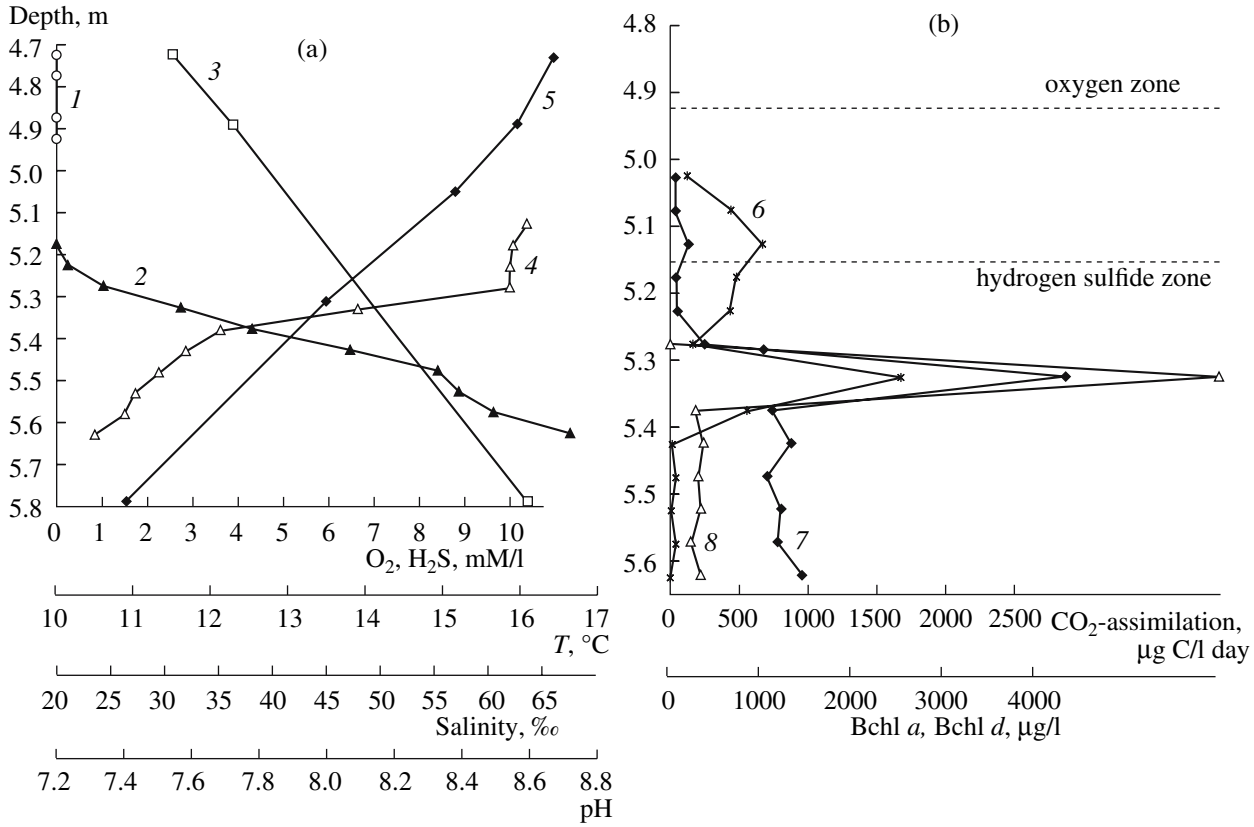


Fig. 3. The chemocline zone of Lake Shunet, August of 2003: (a), the hydrochemical indices; (b), light CO_2 fixation and the distribution of bacteriochlorophylls. Designations (*1–8*) are as in Fig. 1.

depth of 4.93 m, while the traces of hydrogen sulfide appeared only at the depth of 5.18 m (Fig. 3a). Thus, the size of the redox zone was 0.25 m. The profiles of salinity, temperature, and pH practically coincided with those of July 2002.

The new sampling method made it possible to reveal a significant peak of photosynthesis, 1679 μg C/(l day), at the depth of 5.33 m; this coincided with the peaks of bacteriochlorophyll content in the lake (Fig. 3b).

The water in the upper 5-cm layer of the zone of mass development of anoxygenic phototrophic bacteria was of pink color due to the presence of high numbers of purple sulfur bacteria; the deeper layers were dark green in color.

Anoxygenic phototrophic bacterial community. The peak of the numbers of phototrophic bacteria was located on the depth of 5.33 m (i.e., 15 cm below the redox zone), in the layer of pink-colored water. Green sulfur bacteria were morphologically similar to the bacteria of strain ShN*Pel*02. Cells of two morphotypes, ShN*Am*02 and ShN*Lb*02, were present among the purple bacteria; cells of the first morphotype were predominant. The content of Bchl *a* in the zone of maximal development of phototrophic bacteria was 5984 μg/l, of Bchl *d*, 4295 μg/l, which corresponded to approximately 1.6×10^8 and 3.2×10^7 cells/ml of purple and green sulfur bacteria, respectively.

The lower water layers were of green color due to the decrease in the numbers of purple sulfur bacteria. At the depth of 5.43 m, green sulfur bacteria of the ShN*Pel*02 morphotype prevailed. Both morphotypes of purple sulfur bacteria detected in the upper layers were encountered; however, the ShN*Lb*02 morphotype predominated. The content of Bchl *a* decreased to 346 μg/l and that of Bchl *d* to 1303 μg/l; these values correspond to ca 9.5×10^6 and 9.7×10^6 cells/ml of purple and green bacteria, respectively.

Of sulfur bacteria, only the cells of ShN*Lb*02 morphotype were detected in small numbers (tens of cells/ml) in the water of the redox zone at the depth of 4.97 m. Unlike in all earlier studies, small nonmotile cells, resembling nonsulfur purple bacteria, were revealed in the water of the redox zone (5×10^2 cells/ml). The culture of this microorganism was designated as strain ShN*Br*03.

February–March 2003

Physicochemical characterization. During the winter season, when the lake was covered with ice, the

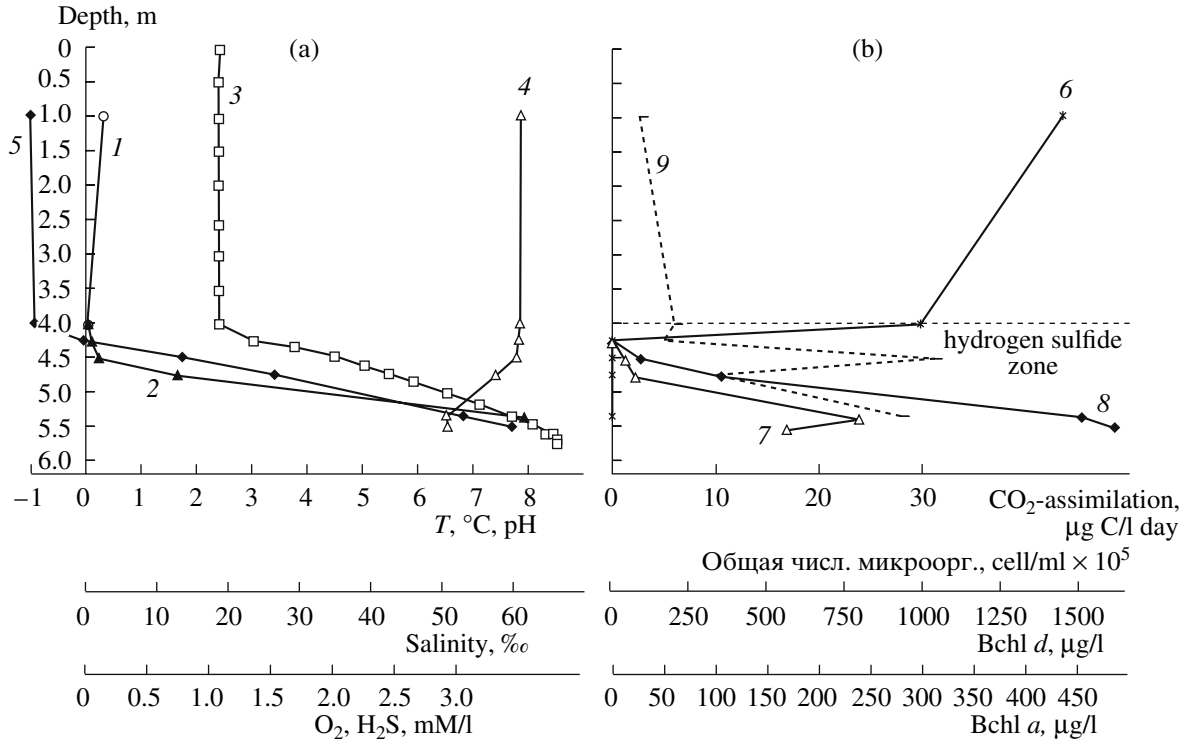


Fig. 4. Hydrochemical indices (a); light CO_2 fixation and the distribution of bacteriochlorophylls (b) in the water of Lake Shunet in February–March 2003. Designations *1–8* are as in Fig. 1; *9*, total number of microorganisms.

redox zone was located half a meter higher than in summer, i.e., at the depth of 4 m (Fig. 4a) [2].

The winter profile of salinity completely coincided with the profile of August 2003. The halocline and thermocline began at the depth of 4 m. The temperature in the upper layers of water decreased to –1°C; at the bottom it was close to 10°C, as in summer; pH of the upper water layers was about 8. The change in pH was located 0.5 m below the chemocline (i.e., at the depth of 4.5 m) and did not coincide with the area of development of anoxygenic phototrophs.

In spite of the use of a multisyringe stratification bathometer, no peak of photosynthesis was revealed in winter in the zone where anoxygenic phototrophs were present (Fig. 4b). However, the water of the hydrogen sulfide zone of the lake was, as in summer, of a greenish-marshy color due to the presence of green sulfur bacteria.

Anoxygenic phototrophic bacterial community. As in the summer period, one morphotype of green sulfur bacteria (ShN*Pel*02) and two morphotypes of purple sulfur bacteria (ShN*Am*02 and ShN*Lb*02) were revealed. Nonsulfur purple bacteria were not revealed during this season.

The peak of APB numbers was located at the depth of 4.75 m (i.e., 75 cm below the redox zone). The colony counts revealed ca. 2×10^6 cells/ml of green sulfur bacteria of the ShN*Pel*02 morphotype (0.36 μg/l of Bchl *d*) and more than 6.7×10^3 cells/ml of purple sulfur bacteria (0.06 μg/l of Bchl *a*). Among the purple sulfur bacteria, the cells of the ShN*Lb*02 morphotype predominated.

In the deeper water layers, at 5.35 m, green sulfur bacteria of the ShN*Pel*02 morphotype prevailed (9.1×10^5 cells/ml); among the purple sulfur bacteria, cells of the ShN*Am*02 morphotype predominated (3×10^5 cells/ml).

At the depth of 4.5 m, in the water layer above the peak of APB development, the numbers of purple bacteria of the ShN*Am*02 morphotype and green sulfur bacteria of the ShN*Pel*02 morphotype were identical, equaling 7.4×10^4 cells/ml.

Morphophysiological characteristics and taxonomic position of the isolated APB cultures

The green sulfur bacterial strain ShN*Pel*02 isolated in 2002 from the water sample obtained from the depth of 5 m consists of mostly ovoid nonmotile cells (0.5–1×2 μm) assembled into mucous aggregates. Some cells of green bacteria form twisted filaments. Usually the cells contain gas vacuoles; elemental sulfur is not present (Fig. 5a, 5b). The photosynthetic apparatus is represented by chlorosomes located along the cytoplasmic membrane (CPM); Bchl *d* and a carotenoid (chlorobactin) are the photosynthetic pigments of this bacterium (Fig. 2b). The morphological characteristics and the pigment composition of the strain resemble most

closely those of *Chlorobium luteolum* (previously *Pelodictyon luteolum*) [14, 15].

Strain ShN*Pel*02 has highest level of 16S rRNA similarity (99.6%) with the type strain of *Prosthecochloris vibrioformis* [14]. It should be mentioned that gas vesicles have not been previously observed in the cells of *Prs. vibrioformis*. The phylogenetic tree (Fig. 6) demonstrates the position of isolate ShN*Pel*02 (registration number GenBank EF149016) among the type strains of the closely related genera and species of the family *Chlorobiaceae*.

The purple sulfur bacterial strain ShN*Am*02 isolated in 2002 from the water sample obtained from the depth of 5 m has nonmotile rounded cells (1.5–2.5 μm) with gas vacuoles and inclusions of elemental sulfur. The cells are covered with a thick polysaccharide layer; when grown in liquid media, they formed large mucous aggregates. Microscopy revealed the cells singly, in pairs, and more seldom in tetrads or bigger aggregates (Figs. 5c–5e). The photosynthetic apparatus is represented by vesicular outgrowths of the cytoplasmic membrane (CPM); Bchl *a* and carotenoids of the okenone series are the photosynthetic pigments (Fig. 2c). The morphological characteristics and the pigment composition of the strain resemble most closely those of *Lamprocystis purpurea*. According to our data, the DNA G+C content of strain ShN*Am*02 was 63.5 mol %, while that of *Lamprocystis purpurea* was 63.5–63.6 mol % [16]. The phylogenetic tree (Fig. 7) demonstrates the position of isolate ShN*Am*02 (registration number GenBank EF153289) among the type strains of the closely related genera and species of the family *Chromatiaceae*. Strain ShN*Am*02 proved to be most closely related (97.3–94.8% similarity) to the type strains of *Thiocapsa pendens*, *Tca. litoralis*, and *Tca. rosea*, that are located in the same cluster.

The purple sulfur bacterial strain ShN*Lb*02 isolated in 2002 from the water sample from the depth of 4.5 m has large oval cells (2.0–2.5 × 4–5 μm). Cells of two types are present in the culture. Young cells are motile; they do not contain gas vacuoles and granules of elemental sulfur. Nonmotile cells of this culture have gas vacuoles located on the cell periphery and contain granules of elemental sulfur. The bacterium possesses a well-developed vesicular photosynthetic apparatus; Bchl *a* and carotenoids of the okenone series are the photosynthetic pigments (Fig. 2d, Figs. 5g, 5h). The morphological characteristics and the pigment composition of the strain resemble most closely those of *Lamprobacter modestohalophilus* [17]. According to our data, the DNA G+C content of strain ShN*Lb*02 was 62.4 mol %, while that of *L modestohalophilus* was 62.5 mol %. The phylogenetic tree (Fig. 7) demonstrates the position of isolate ShN*Lb*02 (registration number GenBank EF153292) among the type strains of the closely related genera and species of the family *Chromatiaceae.* Strain ShN*Lb*02 falls into a common cluster with the strains of the genus *Halochromatium*, with 96.4–96.8% similarity of 16S rRNA with those of the type strains of this genus.

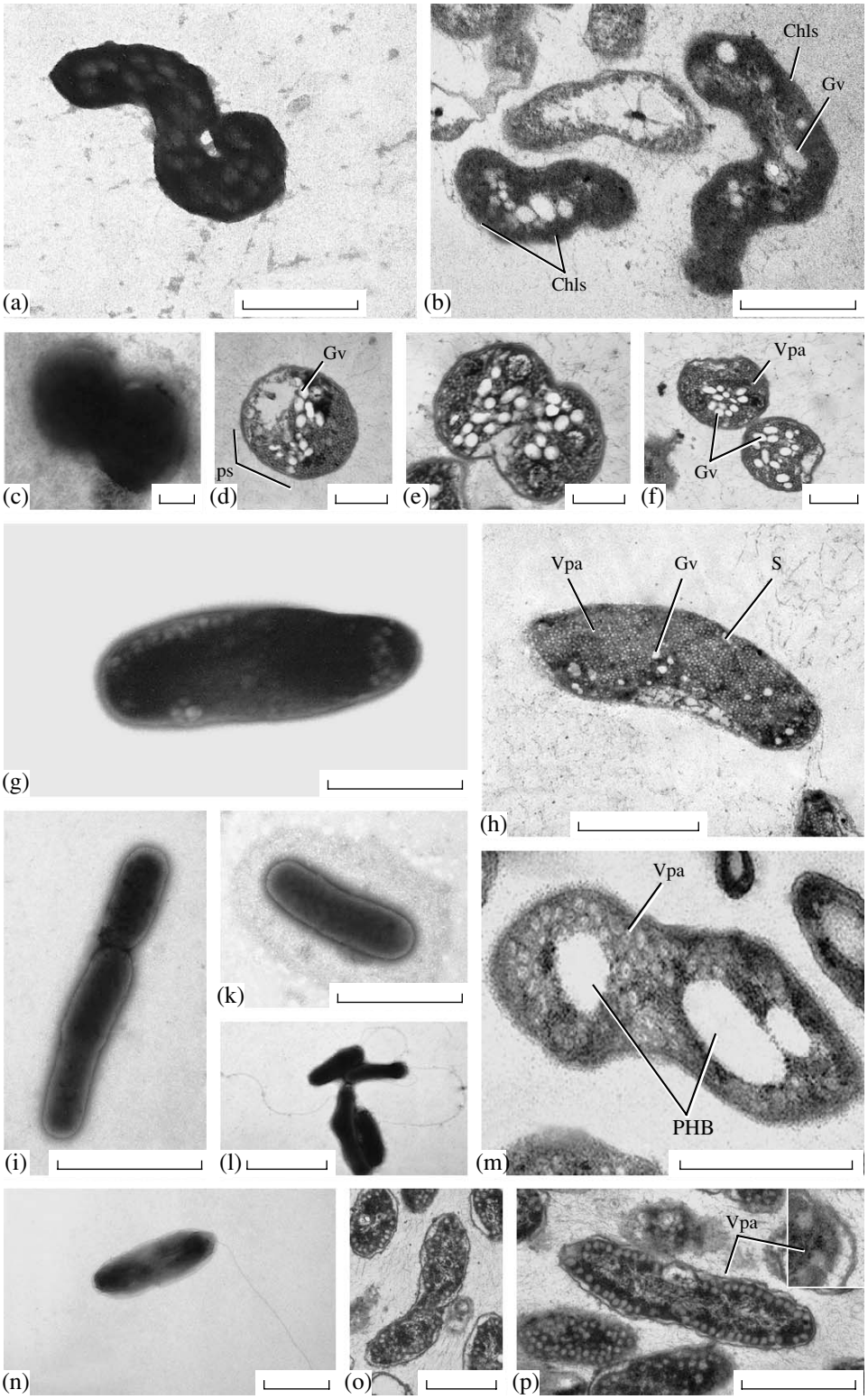
The nonsulfur purple bacterial strain ShN*Rb*02 isolated in 2002 from the water sample from the depth of 4.5 m contains single rod-shaped (0.3 × 3–4 μm) motile cells without inclusions. Under laboratory conditions of cultivation, the cells accumulate poly-β-hydroxybutyrate (PHB) and can synthesize an external mucous capsule (Fig. 2d, Figs. 5i–5l). The cells have a vesicular photosynthetic apparatus; Bchl *a* and carotenoids of the spheroidene series are the photosynthetic pigments. The morphological characteristics and the pigment composition of the strain resemble most closely those of the purple nonsulfur bacteria of the *Rhodobacter–Rhodovulum* morphotype.

The phototrophic bacteria ShN*Br*03 isolated in 2003 from the water sample from the depth of 4.97 m has single nonmotile, although flagellated, rod-shaped cells (0.5–0.8 × 1.5–1.7 μm). The cells contain neither gas vacuoles nor globules of elemental sulfur. The culture can grow both aerobically and anaerobically. The photosynthetic apparatus consists of individual vesicles, located strictly in a row along the CPM (Figs. 5n, 5p); Bchl *a* and carotenoids of the spheroidene series are the photosynthetic pigments (Fig. 2f). Under oxygen-free conditions, the color of the cell suspension is yellowish brown. In spite of the presence of carotenoids of the spheroidene series, the color of the cell suspension did not change after prolonged (more than a week) incubation under oxic conditions. The morphological characteristics and the pigment composition of the strain resemble most closely those of the purple nonsulfur bacteria of the *Rhodobacter–Rhodovulum* morphotype; it, however, differs strongly in some of its morphophysiological features.

The phylogenetic tree (Fig. 8) demonstrates the position of isolates ShN*Rb*02 (registration number GenBank EF153290) and ShN*Br*03 (registration number GenBank EF154519) among the closely related genera and species of the family *"Rhodobacteraceae"*. Strain ShN*Rb*02 exhibits 99.4% similarity to the type strain *Rhodovulum euryhalinum* and strain ShN*Br*03 to *Roseicyclus mahoneyensis* (99.5% similarity) [18]. It should be noted that the species *Roseicyclus mahoneyensis* is not a purple nonsulfur bacterium; it belongs to the group of aerobic bacteriochlorophyll-containing bacteria.

DISCUSSION

In spite of the seasonal changes in activity and position of the APB layer, the basic APB composition remained constant throughout a year. According to our data, Lake Shunet can be classified as a shallow eutrophic meromictic reservoir with water of the sulfate–chloride–sodium–magnesium type. Lakes with a similar type of water are known, in which the species composition of anoxygenic phototrophic bacterial communities


(a)
(b)
Chls
Gv
Chls
(c)
(d)
Gv
ps
(e)
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S
(i)
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Vpa
PHB
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(o)
(p)
Vpa

Fig. 5. Electron microphotographs of APB: (a), the total preparation of the dividing cells of strain ShN*Pel*02; (b), ultrathin section of the cells of strain ShN*Pel*02: the photosynthetic apparatus in the form by chlorosomes (Chls), gas vacuoles (Gv); scale bar, 0.5 μm; (c), the total preparation of the dividing cells of strain ShN*Am*02; (d–f), ultrathin sections of the cells of strain ShN*Am*02: gas vacuoles (Gv), vesicular photosynthetic apparatus (Vpa), threads of exopolysaccharides (ps); scale bar, 0.5 μm; (g), total preparation of the cells of strain ShN*Lb*02; (h), ultrathin section of the cells of strain ShN*Lb*02: gas vacuoles (Gv), vesicular photosynthetic apparatus (Vpa), deposition of sulfur (S); scale bar, 1 μm; (i–l), total preparation of the cells of strain ShN*Rb*02: the process of cell division, with formation of short chains (i), the cells of this strain are capable of synthesizing the mucous capsule (j), at one of the stages of the life cycle, the cells have a polar flagellum (l); scale bar, 1 μm; (m), ultrathin shear of the cells of strain ShN*Rb*02: vesicular photosynthetic apparatus (Vpa), poly-β-hydroxybutyrate granules (PHB); scale bar, 0.5 μm; (n), the total preparation of a motile cell of strain ShN*Br*03; (o, p), ultrathin sections of the cells of strain ShN*Br*03: photosynthetic apparatus in the form of isolated vesicles (Vpa) located along the cytoplasmic membrane; scale bar, 0.5 μm.

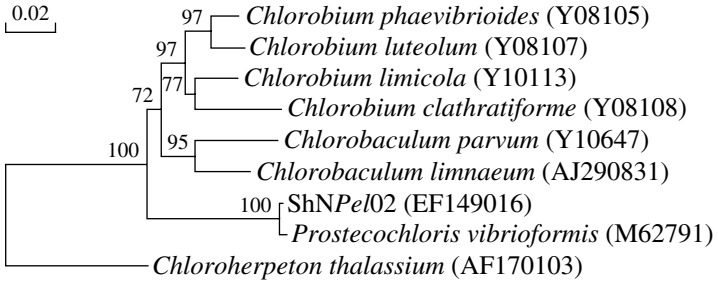

Fig. 6. Rootless phylogenetic tree of the representatives of the family *Chlorobiaceae*, showing the position of strain ShN*Pel*02. The numbers show the values of bootstrap analysis.

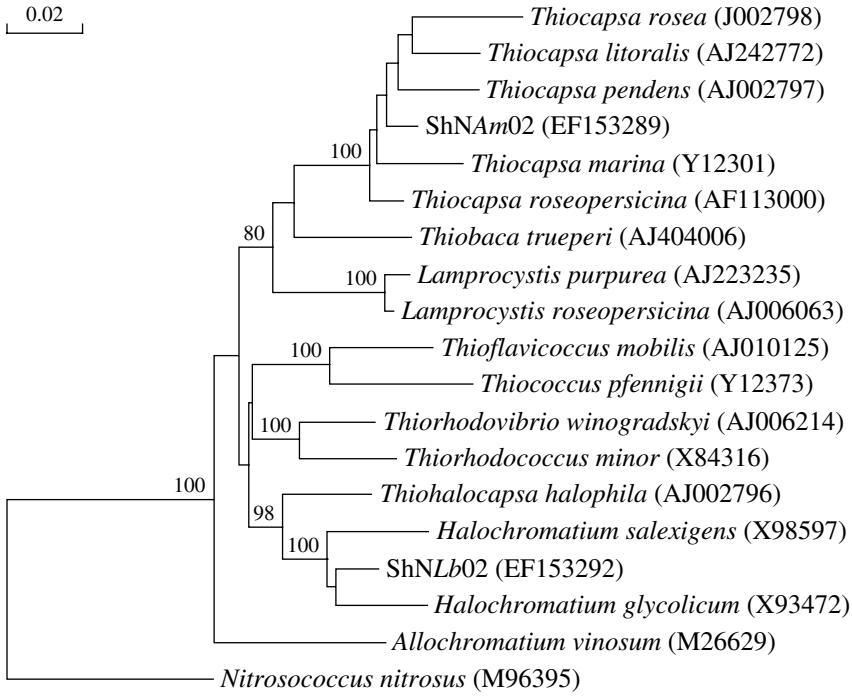

Fig. 7. Rootless phylogenetic tree of the representatives of the family *Chromatiaceae*, showing the position of strains ShN*Am*02 and ShN*Lb*02. The numbers show the values of bootstrap analysis higher than 70%.

resembles that of Lake Shunet. The shallow eutrophic meromictic Chiprana Lake (Monegros, Spain) has the maximum depth of ca. 5.6 m, water salinity 30–73 g/l, and hydrogen sulfide concentration at the bottom 7 mM. The redox zone in this lake was located at the depth of 3.5–3.6 m, and a layer of green-colored water, inhabited by bacteria *Chlorobium vibrioforme* and *Prosthecochloris aestuarii*, developed below the redox zone [19].

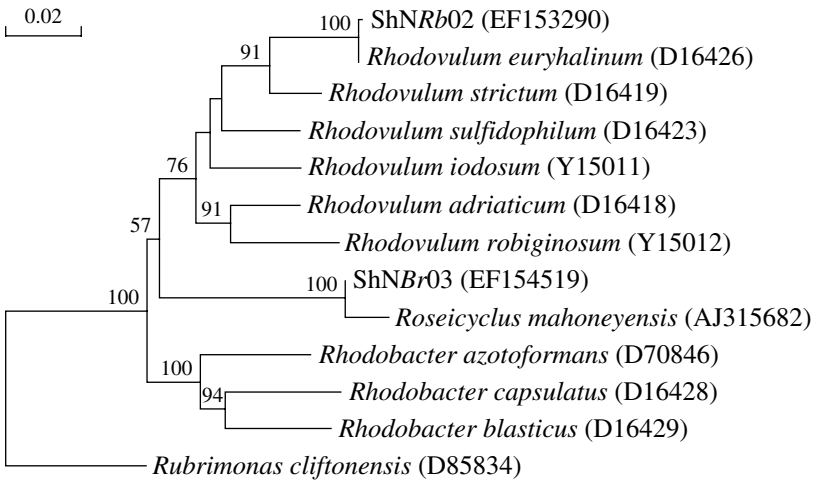

Fig. 8. Rootless phylogenetic tree of the representatives of the family *"Rhodobacteraceae"*, showing the position of the strains ShN*Rb*02 and ShN*Br*03. The numbers show the values of bootstrap analysis.

A picture of the distribution of the anoxygenic phototrophic bacteria in the form of separate layers, similar to that observed in Lake Shunet, has also been observed in the meromictic El Tobar (Cuenca, Spain), in the zone located below the halocline. This lake, just like Shunet, has the sulfate–chloride–sodium–magnesium type of water, but the hydrogen-sulfide zone begins above the halocline. Depending on the water salinity, four layers of anoxygenic phototrophic bacteria were formed in Lake El Tobar, with different dominant species in each layer. In the upper layer of the anaerobic zone, where the water was still fresh, a layer was formed where the purple sulfur bacteria *Chromatium minus* prevailed; below it, down to the halocline, there was a layer with the predominance of brown-colored green sulfur bacteria *Chlorobium phaeobacteroides*. Below the halocline, in the zone where the water salinity reached 20 g/l, a layer of purple sulfur bacteria was formed, where *Lamprobacter modestohalophilus* prevailed; still lower, there was a layer with the predominance of green sulfur bacteria *Chlorobium vibrioforme* [20].

It is noteworthy that anoxygenic phototrophic bacterial communities of a similar species composition also occur in lakes with the chloride type of water. Thus, in the eutrophic meromictic lake Veisovo (Slavyanskie lakes in the vicinity of Slavyansk, Donetsk oblast) at the depth of 1.5 m, photosynthetic sulfur bacteria *Pelodictyon phaeum* and, to a lesser degree, *Pld. luteolum* and purple sulfur bacteria *Lamprocystis* sp. developed. The authors noted that the purple bacteria, being more tolerant to oxygen, developed to a higher degree under microaerophilic conditions than green sulfur bacteria. The depth of the larger part of the lake does not exceed 3 m, although a 17-m-deep funnel has also been recorded. Beginning from the depth of 1.25 m, hydrogen sulfide was present; its concentration at the bottom reached 736 mg/l. In Veisovo Lake, the halocline with salinity increase from 18.4 to 26 g/l was located at the depth of 1–1.25 m [21].

Although the anoxygenic phototrophic bacterial community of Lake Shunet is similar to the above-mentioned associations of other lakes, it should be noted that three isolates out of the five obtained by us from Lake Shunet proved to be quite different from the known species of anoxygenic phototrophic bacteria.

Strain ShN*Pel*02 has the highest level of 16s rRNA similarity (99.6%) to the type strain *Prostecochloris vibrioformis*; however, unlike the latter it contains intracellular gas vacuoles; the morphological properties and the pigment composition of strain ShN*Pel*02 most closely resemble *Chlorobium luteolum* (previously *Pelodictyon luteolum*) [14, 15].

Strain ShN*Am*02 contains carotenoids of the okenone series and most closely resembles *Lamprocystis purpurea*; however, it is different from the latter in its phylogenetic characteristics. It is interesting that the type strains of *Thiocapsa pendens*, *Tca. litoralis,* and *Tca. rosea*, which contain carotenoids of the spirilloxanthin series, were closest to strain ShN*Am*O2 (97.3–94.8% similarity). Thus, strain ShN*Am*O2 certainly belongs to the genus *Thiocapsa*; however, its species status requires further investigation.

Strain ShN*Lb*02 is similar to *Lamprobacter modestohalophilus* in its morphology and pigment composition; they have similar values of DNA G+C content. However, according to 16S rRNA analysis of strain ShN*Lb*02, it was most close to the type strains of the genus *Halochromatium* (96.4–96.8% similarity). It is therefore possible to conclude that not only the species, but also the generic position of strain ShN*Lb*02 require further refinement.

The study of Lake Shunet in 2003 demonstrated that in shallow lakes with sharp gradients of salinity, alkalinity, etc., it is expedient to perform sampling only

with the use of a multisyringe stratification bathometer, which enables sampling from close depths without disrupting the structure of the layer water. Thus, we failed to reveal the thin-layered structure of the anoxygenic phototrophic bacterial community of Lake Shunet in July 2002, when a horizontal bathometer was used. During August 2003, it was shown for the first time that mass development of purple bacteria occurred above the layer of green water and that a layer of pink water about 5 cm thick was formed. Such sharp differentiation of the layers indicates that oxygen in minute quantities penetrates into the area where phototrophs develop; purple bacteria therefore gain an advantage over green bacteria.

It should be emphasized that in summer, in spite of the presence of differently colored layers of sulfur bacteria, the peak numbers of both green and purple sulfur bacteria were located at one and the same depth somewhat below the redox zone.

The fact that in July 2002 strain ShN*Am*02 predominated among the purple sulfur bacteria in the layer of the maximum concentration of phototrops, while in August 2003, strain ShN*Lb*02 prevailed, is possibly due primarily to the changes of salinity and pH in the area of phototroph development.

It can be seen from Figs. 1 and 3 that the water salinity in the area of phototroph development was 33 g/l in July 2002 and 52 g/l in August 2003. The conditions of August of 2003 were more suitable for strain ShN*Lb*02 than for strain ShN*Am*02, since the optimal salinity ranges for the species closest to ShN*Lb*02 in morphology and genetics are 10–90 and 20–200 g/l, while the species closest to ShN*Am*02 require the salinity of 10 g/l.

In July 2002, pH in this zone was 8.1, while in August 2003 it was 8.5–8.1. Taking into consideration that the optima for growth of the species closest to ShN*Am*02 are 6.5 and 6.7–7.5, while for the species closest to ShN*Lb*02 the values are 7.2–7.4 and 7.4-7.6, it is evident that in August 2003 the conditions were less favorable for ShN*Am*02.

During the winter season, the activity of anoxygenic photosynthesis and the numbers of all species of phototrophic bacteria decreased sharply, mostly as the result of the small amount of light which penetrated through the ice cover. The illumination in the development area of phototrophs in winter was two orders of magnitude less than in summer; the temperature decreased by 10–12 degrees and the salinity by approximately 8 g/l [2]. In winter, pH in the development area of phototrophs decreased to 7.3, i.e., 1.5 units below the summer value. These changes affected, first of all, the development of purple sulfur bacteria, including ShN*Lb*02, which prefer high salinity and pH. The green sulfur bacteria, which prefer pH 6.5–7, were less affected by the changes in the hydrochemical parameters. Thus, in the winter season the numbers of green sulfur bacteria decreased by an order of magnitude, while the numbers of purple sulfur bacteria decreased by three orders of magnitude in comparison with July 2002.

ACKNOWLEDGMENTS

The authors express deep gratitude to V.M. Gorlenko for invaluable help with the interpretation of obtained data, constant support, and great interest in our work and in the isolated microbial cultures, to Z.B. Namsaraev, and also to A.S. Savvichev, E.E. Zakharova, and to other participants in the expeditions to Khakassia.

The work was performed with the partial financial support of the Russian Foundation for Basic Research, projects nos. 05-04-48710, 04-04-48602, and 05-04-97708 "Enisei"; the grant for support of the leading scientific schools within the framework of the Federal Task Program "Studies and Development for Priority Trends in Development of Science and Technology" for 2002–2006 (State Committee and Rosnauka, no. 02.445.11.7409); the Presidium of the Russian Academy of Sciences Programs for Basic Research "Molecular and Cellular Biology" and "Origin and Evolution of Biosphere".

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