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EXPERIMENTAL ARTICLES

Anoxygenic Phototrophic Bacterial Community of Lake Shira (Khakassia)

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Abstract—The anoxygenic phototrophic bacterial community of the brackish meromictic Lake Shira (Khakassia) was investigated in August 2001, July 2002, and February–March 2003. In all the periods of investigation, the prevailing microorganisms were purple sulfur bacteria similar to *Lamprocystis purpurea* in morphology and pigment composition. Their highest number (3×10^5 cells/ml) was recorded in July 2002 at the depth of 15 m. According to 16S rRNA gene analysis, the strain of purple sulfur bacteria isolated in 2001 and designated Sh*Am*01 exhibited 98.6% similarity to the type strain of *Thiocapsa roseopersicina* and 97.1–94.4% similarity to the type strains of *Tca. pendens, Tca. litoralis*, and *Tca. rosea*. The minor microorganisms of the anoxygenic phototrophic bacterial community within the period of investigation were nonsulfur purple bacteria phylogenetically close to *Rhodovulum strictum* (98.3% similarity, strain Sh*Rb*01), *Ahrensia kielensis* (of 93.9% similarity, strain Sh*Rb*02), *Rhodomicrobium vannieli* (of 99.7% similarity, strain Sh*Rmc*01), and green sulfur bacteria, phylogenetically close to *Chlorobium limicola* (of 98.7% similarity, strain Sh*Cl*03).

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

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Lake Shira is located ten km northeast of the Shira regional center (Khakassia, Siberia, Russia). It is a brackish meromictic lake with a pronounced sulfide zone in the water column. The lake is 35.9 km^2 in area and its maximum depth is 22–24 m; the water mineral composition is of the sulfate–chloride–sodium–magnesium type.

Although Lake Shira is well-investigated in a number of respects [1], its community of anoxygenic phototrophic bacteria (APB) had not been studied before August 2001.

We investigated the APB community in August 2001, July 2002, and February–March 2003; these results have been partially published [2, 3]. The present work provides generalized results of the microbiological study of the APB community of Lake Shira; the isolated APB cultures (five strains) are characterized, and the results of their genetic study presented.

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MATERIALS AND METHODS

The field research of the brackish meromictic Lake Shira was performed on August 23–29 2001, July 12–17 2002, and February 24–March 3 2003. The water was sampled in the deep depression of the lake (22–23 m). The water samples were taken at 25-cm intervals with a 0.75-l plastic horizontal bathometer. The rate of photosynthesis was determined by the radioisotope method with ^{14}C bicarbonate [2]. Labeled bicarbonate (0.1–0.2 ml) was injected into 30-ml glass vials with water; the vials were tied to a nylon halyard and incubated at the depth of sampling for 6–12 h. Control vials were immediately wrapped with aluminum foil and incubated under the same conditions. After incubation, the samples were fixed with 0.5–1.0 ml of 10% phosphoric acid, filtered through 0.2-μm nylon membranes, washed twice with 5% H_3PO_4, and dried. The filters were analyzed on a Rackbeta-1219 scintillation counter (LKB, Sweden).

The vertical profiles of temperature, salinity, and pH were determined with a submerged multichannel device Data-Sonde 4a (Hydrolab, United States).

The concentrations of oxygen and sulfide were determined immediately after sampling with Aquamerk test kits (Merck, Germany).

In order to determine the concentration of bacteriochlorophylls (Bchl) in the lake water, the samples (10–350 ml) were filtered through 0.2-μm nylon membranes. The pigments were extracted with an acetone–methanol mixture (7 : 2) under laboratory conditions; the spectra of the extracts in the 350–1000 nm range were analyzed on a LOMO SF 56 spectrophotometer (LOMO, Russia).

The pigment content was calculated according to [4]:

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

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

The absorption factor for Bchl *a* was $k = 46.1\ l\ g^{-1}\ cm^{-1}$ [5].

The medium of the following composition (per 1 l of distilled water) was used for the isolation of all phototrophic bacteria and for cultivation of sulfur phototrophic bacteria: KH_2PO_4, 0.7 g; NaCl, 20 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; NH_4Cl, 0.7 g; KCl, 0.33 g; $NaHCO_3$, 1.5 g; $CaCl_2 \cdot 6H_2O$, 0.1 g; $Na_2S \cdot 9H_2O$, 0.5 g; $Na_2S_2O_3 \cdot 5H_2O$, 1.0 g; Na acetate $\cdot 3H_2O$, 0.5g; Na pyruvate, 0.5 g; yeast extract, 0.1 g; vitamin B_{12}, 20 μg; trace element solution [6], 1 ml; pH, 7.0.

The medium of the following composition (per 1 l of distilled water) was used for the cultivation of nonsulfur purple bacteria: KH_2PO_4, 0.7 g; NaCl, 20 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; NH_4Cl, 0.7 g; KCl, 0.33 g; $NaHCO_3$, 1.5 g; $CaCl_2 \cdot 6H_2O$, 0.1 g; $Na_2S \cdot 9H_2O$, 0.05 g; $Na_2S_2O_3 \cdot 5H_2O$, 1.0 g; Na acetate $\cdot 3H_2O$, 2 g; Na pyruvate, 2 g; yeast extract, 0.2 g; vitamin B_{12}, 20 μg; trace elements solution, 1 ml; pH, 7.0.

To obtain enrichment cultures of phototrophic bacteria, lake water samples were injected into hermetically sealed penicillin vials with 30 ml of the medium.

The numbers of photosynthetic bacteria were determined by inoculating the serial dilutions of the natural material (water samples) into test tubes with the agarized (0.5%) medium for sulfur phototrophic bacteria. The colonies in each dilution were enumerated [7]. The dilution series were incubated anaerobically for one month: for the first week, at room temperature away from direct sunlight and afterwards, in a luminostat at 2000 lx and temperature of 20–25°C. The colored colonies grown in each dilution were enumerated after the incubation. The characteristics used for microbial identification included the size and shape of cells and microcolonies, cell color in bacterial populations, motility, the presence of gas vacuoles, the mode of elemental sulfur deposition, and the presence and location in intracellular sulfur, as well as the absorption spectra of whole cell suspensions in 50% glycerol and spectra of the acetone–methanol (7 : 2) pigment extract.

The isolation and purification of microbial cultures was achieved by serial dilutions of environmental material on agarized (0.5%) medium of the same composition. The pigment composition of the cultures of anoxygenic phototrophic bacteria was studied in whole cell samples with glycerol (1 : 1) and in acetone–methanol (7 : 2) extracts from bacterial biomass.

Microphotographs at ×1200 were obtained using a light microscope with phase contrast and immersion system. For ultrathin sections, the cell pellet was fixed with 1.5% glutaric aldehyde in 0.05 M cacodylate buffer (pH 7.2) at 4°C for an hour, washed three times in the same buffer, and postfixed in 1% OsO_4 solution in 0.05 M cacodylate buffer (pH 7.2) for three h at 20°C. The material was dehydrated in a series of alcohols and embedded in Epon 812 epoxy resin. Ultrathin sections were mounted on grids, contrasted for 30 min with 3% uranyl acetate solution in 70% ethanol, and with Reynolds lead citrate [8]. Ultrathin sections were examined in a JEM-100CXII electron microscope (Japan) at 80 kV accelerating voltage.

DNA isolation was performed as described previously [9]. Amplification of 16S rRNA was performed with 27f and 1492r universal primers on GeneAmp PCR System 2700 (Applied Biosystems). The amplified 16S rDNA was sequenced in a CEQ2000XL automatic DNA sequencer (Beckman Coulter) with the Dye Terminator Cycle Sequencing sequencing kit (Beckman Coulter) according to the manufacturer's recommendations.

The 16S rDNA nucleotide sequences were aligned using the ClustalX software package [10]. The rootless phylogenetic tree was constructed by means of the algorithms embedded in TREECON software package [11].

RESULTS

Physicochemical characterization. During the summer period, in the central deepest part of Lake Shira (22–23 m), oxygen was absent below 13 m; substantial concentrations of sulfide (>0.5 mg/l) were observed at the depth of 12.5 m (Fig. 1a). In July 2002, the redox zone (zone where oxygen and sulfide are present simultaneously) was located within the 11–13-m interval of depths; it was then somewhat wider and closer to the surface than in the other seasons of observation: in August 2001 it was at 13–13.5 m [2], and in winter 2003, it was at 15–15.5 m (Fig. 1b).

The absolute maximum of dissolved oxygen concentration was observed in summer at a depth of 8 m (10.2 mg/l in August 2001, 7.7 mg/l in July 2002); in winter, the oxygen concentration decreased gradually with depth. The sulfide concentration in the near-bottom horizons was 12–15 mg/l; it did not change significantly in the summer and winter periods [2, 3].

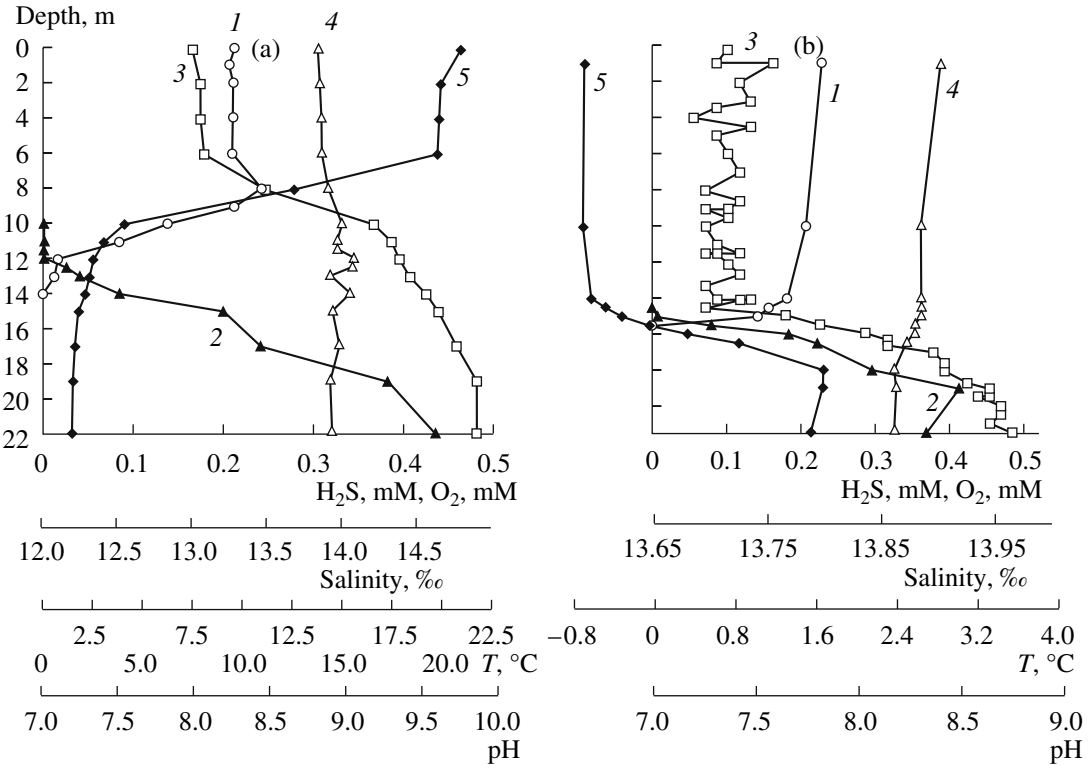

Fig. 1. Hydrochemical characteristics of Lake Shira water: (a), July 2002; (b), February–March 2003, from [3]. *1*, O_2 concentration; *2*, H_2S concentration; *3*, total salinity; *4*, pH; *5*, temperature.

According to the data from the submerged probe, in summer the thermocline with a temperature drop from 19.7 to 2.95°C was located at 6–11 m depths; in winter, a less pronounced thermocline with a temperature increase from –0.65 to +1.64°C was located at 14–18 m [3].

In summer, the water pH in the redox zone was 8.5–9; in winter, it was lower (8.2–8).

Anoxygenic phototrophic bacterial community. A weakly pronounced water layer where purple sulfur APB predominately developed was present in the upper part of the sulfide zone in all the periods of investigation. The highest content of purple sulfur bacteria was detected at 12–14 m in the summer and at 15–16.5 m in the winter.

In summer, the highest Bchl *a* content in bacterial cells was 30.6 μg/l (August 2001, depth 14 m); in winter, the maximal pigment concentration decreased to 8.9 μg/l (February–March 2003, depth 15 m) (Fig 2a) [2].

In summer, the rate of light CO_2 assimilation in the zone of maximal APB growth was 56.7–81.5 μg C/(l day); in winter, this value was 15.2 μg C/(l day) (Fig. 2b, 3).

In all the periods of investigation, purple sulfur bacteria similar to *Lamprocystis purpurea* (formerly *Pfennigia purpurea, Amoebobacter purpureus*) [2, 3] in morphology and pigment composition dominated in the APB community. The numbers of purple sulfur bacteria determined by colony counts were 2×10^5–6×10^5 cells/ml in summer at 12–15 m depth; in winter, this value decreased by 1–2 orders of magnitude (1.65×10^4 cells/ml at 15 m and 5.5×10^3–6.7×10^3 at 15.5–16.5 m).

In summer, the minor components of the community at the depth of 13.5–15 m were nonsulfur exospore-forming budding bacteria similar to *Rhodomicrobium vannieli* and two varieties of nonsulfur purple bacteria, which presumably belong to spheroidene-containing bacteria of the *Rhodobacter-Rhodovulum* morphotype. The numbers of bacteria of each minor morphotype determined by colony counts did not exceed 10^3 cells/ml [2]. In winter, small quantities of green sulfur bacteria were found at 16 m depth; they were classified as *Chlorobium limicola* [3] on the basis of their morphology and pigment composition.

Characterization of the isolated anoxygenic phototrophic bacteria. The purple sulfur bacterial isolate obtained in August 2001 from 14 m depth and tentatively assigned to *Lamprocystis purpurea* was designated strain Sh*Am*01 [2, 12]. The cells were spherical or ovoid, their size ranging from 1.8 to 3.5 μm. Their cell wall was of the typical gram-negative structure with a well-pronounced external membrane; a regular S layer was present on its outer surface. Both gas vesicles (visible on ultrathin sections as regular, slightly elongated polygons with low electron density) and carboxysomes as electron-dense regular polyhedrons with hexagonal symmetry were present in the cells. Electron-transparent sulfur inclusions were revealed in the cells, mainly

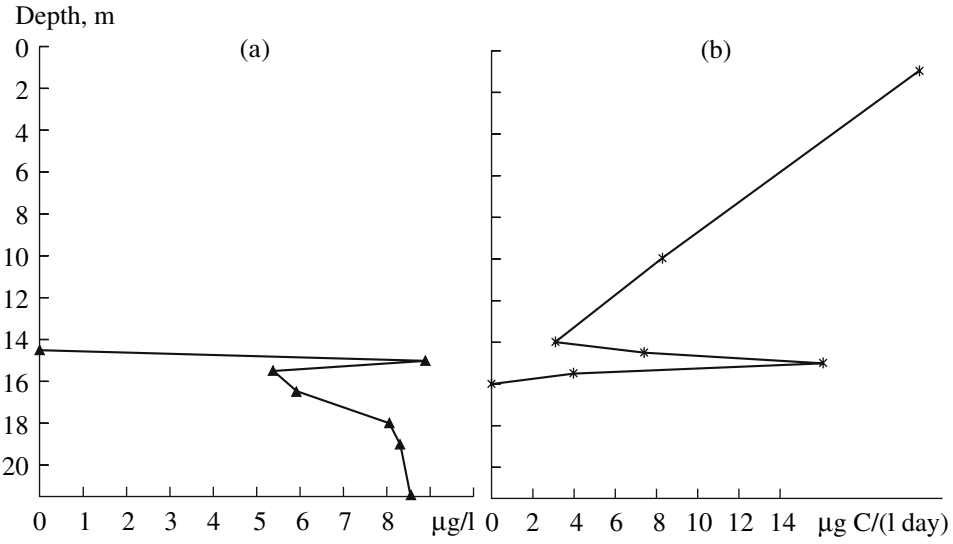

Fig. 2. Bchl *a* content in bacterial cells (a) and rate of light CO_2 assimilation (b) in Lake Shira, February–March 2003.

at the periphery of the cytoplasm. The photosynthetic apparatus consisted of numerous small spherical vesicles (chromatophores) densely distributed in the cytoplasm. Bchl *a* and carotenoids of the okenone series were the photosynthetic pigments (Fig. 4a, Fig. 5a–5g). In the variants of the enrichment culture from which strain Sh*Am*01 was obtained, a gram-negative bacterium was detected, which in the course of its life cycle behaved as an intracellular parasite of bacteria of the Sh*Am*01 morphotype. In a free state, the parasitic bacterium had short rod-shaped cells ~300 nm in diameter. In the intracellular stage, within the cytoplasm of Sh*Am*01 morphotype bacteria, the diameter of the parasitic cells varied from 200 to 280 nm (Fig. 5i–k).

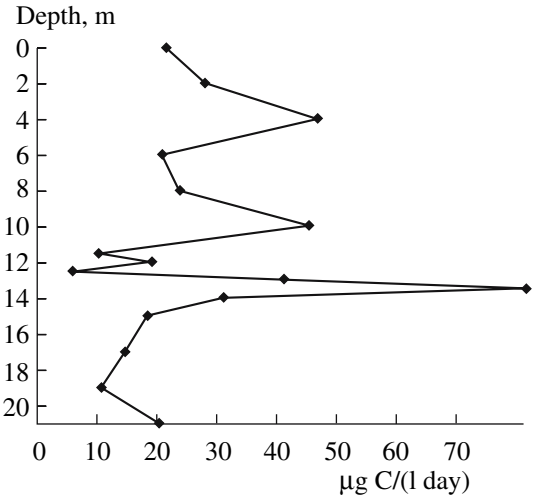

Fig. 3. Rate of light CO_2 assimilation in Lake Shira, July 2002.

The morphological, pigment, and phylogenetic characteristics of strain Sh*Am*01 closely resembled those of strain ShN*Am*02, isolated by us from Lake Shunet in 2002. However, Sh*Am*01 were bigger; unlike strain ShN*Am*02, strain Sh*Am*01 never formed mucous aggregates in liquid medium [13]. We determined the DNA G + C content of strain Sh*Am*01 as 64.2 mol %, that of *Lamprocystis purpurea* as 63.5–63.6 mol %, and that of strain ShN*Am*02 as 63.5 mol %. Our determination of the DNA–DNA hybridization level between strains Sh*Am*01 and ShN*Am*02 gave the value of 78%.

Analysis of 16S rRNA sequences of strain Sh*Am*01 (GenBank accession no. EF153293) revealed that it was closest to the type strain of *Thiocapsa roseopersicina* (98.6%). The similarity between strain Sh*Am*01 and the type strains of *Tca. pendens, Tca. litoralis*, and *Tca. rosea*, which belong to the same cluster, is 97.1–94.4%. Strain Sh*Am*01 from Lake Shira and strain ShN*Am*02 from Lake Shunet (GenBank accession no. EF153289) exhibit 100% similarity in 16S rRNA (Fig. 6).

Only one okenone-containing *Thiocapsa* species is presently known, *Tca. marina*; it does not contain gas vacuoles and is phylogenetically close to the type strains of *Tca. pendens* and *Tca. litoralis* [14]. The DNA G + C content of strain *Tca. marina* BM-3 is 63.2 mol %; our analysis of DNA–DNA hybridization between strains Sh*Am*01 and *Tca. marina* BM-3 revealed 40% values.

Nonsulfur purple bacteria, which have been previously assigned to spheroidene-containing bacteria of the *Rhodobacter-Rhodovulum* morphotype [2], were isolated from the 14-m water layer in August 2001. The bacteria were designated as strain Sh*Rb*01. The cells were oval (0.3–0.4 × 0.5–0.7 µm) and motile, with a polar flagellum. Cell division occurred by strangula-

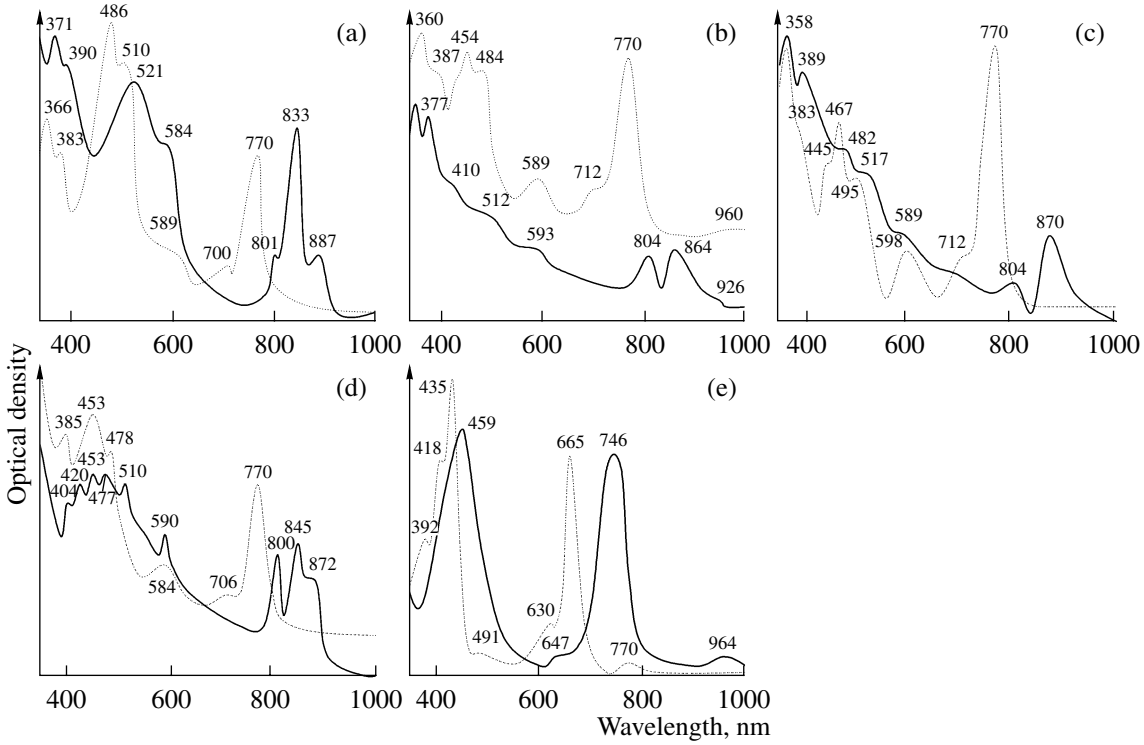

Fig. 4. Absorption spectra of the pigments of anoxygenic phototrophic bacteria from Lake Shira: (a) purple sulfur bacteria, strain Sh*Am*01; (b) nonsulfur purple bacteria, strain Sh*Rb*01; (c) nonsulfur purple bacteria, strain Sh*Rmc*01; (d) nonsulfur purple bacteria, strain Sh*Rb*02; (e) green sulfur bacteria, strain Sh*Cl*03. Solid line, spectrum of a live culture in glycerol; dotted line, spectrum of an acetone–methanol extract.

tion, not always at the cell center. The photosynthetic apparatus consisted of some rounded vesicles localized both in the periphery and in the central zone of the cytoplasm. Bchl *a* and carotenoids of the spheroidene series were the photosynthetic pigments (Fig. 4b, 7). The DNA G + C content of strain Sh*Rb*01 was 65 mol %. Analysis of 16S rRNA sequences revealed 98.3% similarity between strain Sh*Rb*01 (GenBank accession no. EF153294) and the type strain of *Rhodovulum strictum* (Fig. 8).

In August 2001, from 14 m depth, other nonsulfur purple bacteria were isolated; on the basis of their characteristic morphology and pigment compositions, they were previously identified as an exospore-forming budding bacterium *Rhodomicrobium vannieli* [2, 15]. The bacterial strain was designated Sh*Rmc*01. The cells were of oval or irregular shape (1–1.2 μm × 2–2.8 μm); long hyphae were formed, with daughter cells or exospores at their ends. The cells had a lamellar photosynthetic apparatus, which was localized around the cytoplasmic membrane in dense layers. Bchl *a* and carotenoids of the spirilloxanthin series were the photosynthetic pigments; the bacterial suspension had a dirty red color (Fig. 4c, 9). Analysis of 16S rRNA sequences revealed 99.7% similarity between strain Sh*Rmc*01 (GenBank accession no. EF153296) and the type strain of *R. vannielii*, the only species of the genus *Rhodomicrobium*.

From the water samples from 13.5 and 15 m depth in June 2002, nonsulfur purple bacteria were isolated, which were previously tentatively classified as spheroidene-containing bacteria of the *Rhodobacter-Rhodovulum* morphotype. This strain was designated Sh*Rb*02. Bacterial cells were rod-shaped (0.4–0.5 × 1–4 μm) and motile. The cells of a young culture were short regular rods. In the old culture, bacteria had a shape of elongated rods with pronounced polymorphism (long curved cells with asymmetrically located stretches); in some cases, bud-type extrusions were formed, possibly as the result of cultivation on acetate. The cell wall structure was typical for gram-negative bacteria. Numerous electron-transparent inclusions were revealed on electron micrographs, possibly of poly-β-roxybutyrate. Small electron-dense inclusions of polyphosphates were also present; gas vacuoles and elemental sulfur inclusions were absent. The photosynthetic apparatus consisted of isolated flat extended vesicles in the peripheral zone of the cytoplasm. Bchl *a* and carotenoids of the spheroidene series were the photosynthetic pigments; the cell suspension, however, was of light pink color under anaerobic conditions (Fig. 4d, 10). The G + C content in the DNA of strain Sh*Rb*02 was 65.1 mol %. The morphological and spectral characteristics of strain Sh*Rb*02 were different from those of the nonsulfur purple bacterial strain Sh*Rb*01 obtained by us in August 2001. The DNA–DNA hybridization

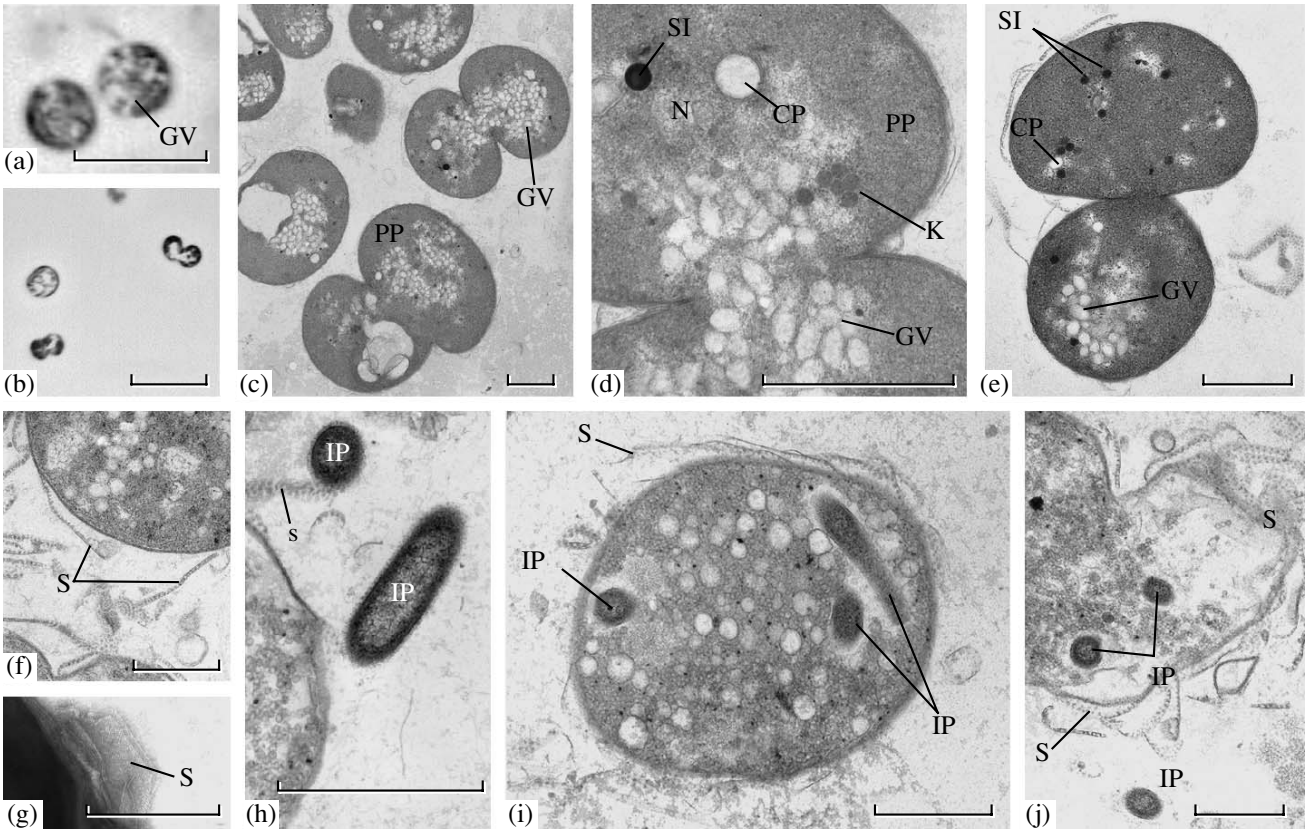

Fig. 5. Morphology and ultrastructure of strain Sh*Am*01. Phase contrast microscopy (a, b), electron microscopy of ultrathin sections (c–f, i–k) and of the negatively stained preparation of the S layer (g); (d, f) fragments of ultrathin sections with characteristic intracytoplasmic organelles, intercellular inclusions, and the external S layer of the cell wall; (i–k) interaction of the parasitic bacterium with Sh*Am*01 cells: (i) attachment to the surface; (j) growth in the cytoplasm of the host cell; (k) destruction of the host cell. CP, chromatophores; GV, gas vesicles; C, carboxysomes; N, nucleoid; PP, polyphosphate inclusions; SI, sulfur inclusions; S, cell wall S layer; IP, Intrecellular parasite. Scale bar: (a, b) 10 μm; (c–g) 0.2 μm; (i–k) 1 μm.

level between strains Sh*Rb*01 and Sh*Rb*02 was 86%. Analysis of 16S rRNA nucleotide sequences revealed 93.9% similarity between strain Sh*Rb*02 (GenBank accession no. EF153295) and the type strain of *Ahrensia kielensis* (previously *Agrobacterium kielensis*) (Fig. 8).

Green sulfur bacteria previously identified by us as *Chlorobium limicola* [3, 16] were isolated in February–March 2003 from 16 m depth. The strain was designated Sh*Cl*03. The cells were nonmotile rods (0.7–1.1 × 0.9–1.5 μm) without gas vacuoles and sulfur inclusions. The cells were mostly in filaments of 2–20 or more (on average, ten cells). Apart from the cytoplasmic membrane and the outer membrane, a proteinaceous S layer was present; it detached periodically from the surface together with the fragments of an internal membrane. The photosynthetic apparatus consisted of chlorosomes, located along the cytoplasmic membrane. Bchl *c* and the chlorobactin carotenoid were the photosynthetic membrane (Fig. 4e, 11). Analysis of 16S rDNA nucleotide sequences revealed the highest similarity (98.7%) between strain Sh*Cl*03 (GenBank accession no. EF153291) and the type strain of *Chlorobium limicola* (Fig. 12).

DISCUSSION

The results obtained clearly demonstrate that in summer the conditions were more favorable for the development of the APB community than in winter. In spite of the higher pH values (8.5–9) in the redox zone, higher illumination and water temperature in summer provided for APB growth in a 6.5-m thick layer (Fig. 3). In winter, pH decreased to 8.3 and became more favorable for APB. However, the water temperature in the zone of the community localization became negative; illumination was practically absent as a result of a dense ice layer (over one meter). A weak peak of photosynthesis indicated that in spite of such conditions, APB development did not stop completely; the zone of APB presence decreased to 1 m (Fig. 2a).

In all the seasons of investigation, purple sulfur bacteria similar to *Lamprocystis purpurea* in morphology and pigment composition were the dominant organisms in the Lake Shira APB community.

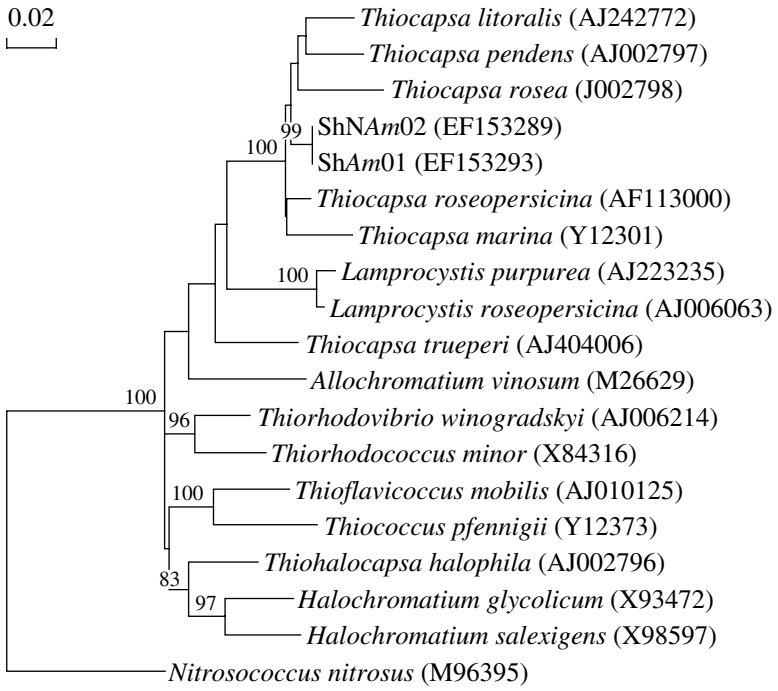

Fig. 6. Rootless phylogenetic tree of the representatives of the family *Chromatiaceae*, showing the position of strains Sh*Am*01 and ShN*Am*02. The numbers show the values of bootstrap analysis higher than 70%.

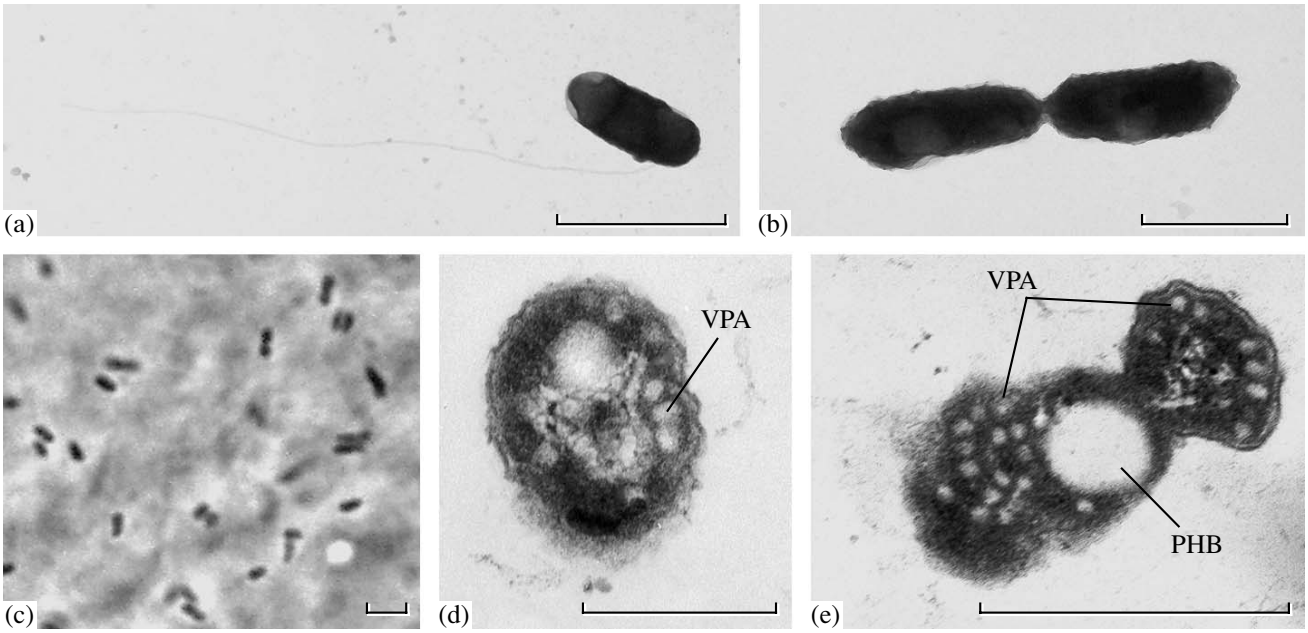

Fig. 7. Morphology and ultrastructural organization of strain Sh*Rb*01. Electron microscopy of negatively stained samples (a, b) and ultrathin sections (d, e), phase contrast microscopy (c); (a, c) overall cell morphology; (b) cell division. PHB, poly-β-hydroxybutyrate inclusions; VPA, vesicular photosynthetic apparatus. Scale bar: (a, c), μm; (b, e) 0.5 μm; (d) 0.25 μm.

However, strain Sh*Am*01 was phylogenetically most closely related to the type strains of *Thiocapsa pendens*, *Tca. litoralis*, and *Tca. rosea*, which contain carotenoids of the spirilloxanthin series. Moreover, according to the results of DNA–DNA hybridization, strain Sh*Am*01 was remote from *Tca. marina*, the only okenone-containing *Thiocapsa* species. Strain Sh*Am*01 therefore belongs to the genus *Thiocapsa*, although its species status requires further investigation.

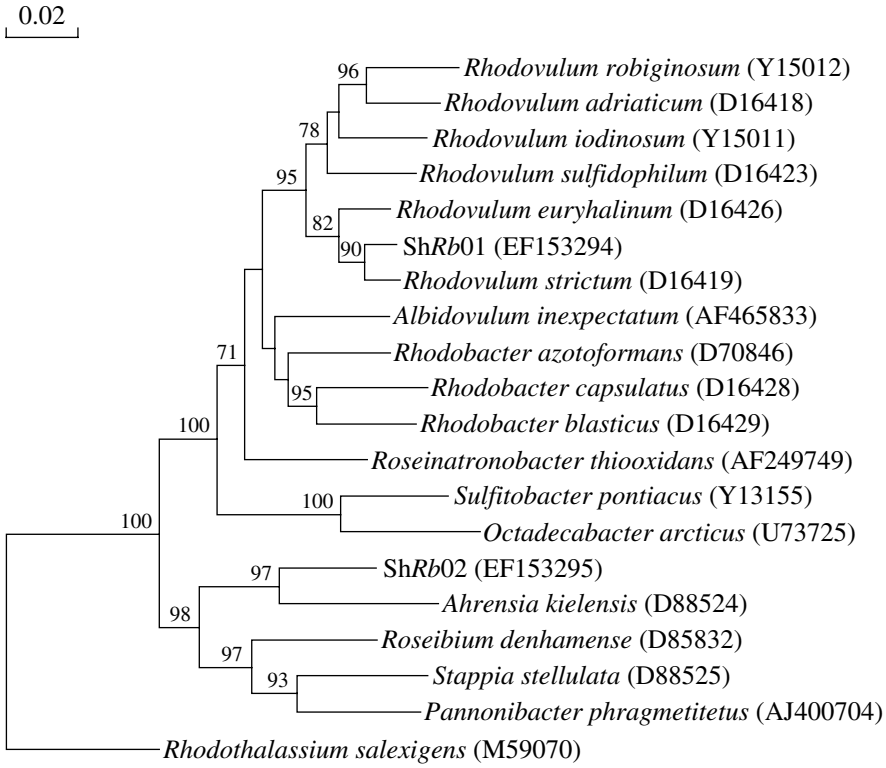

Fig. 8. Phylogenetic tree of the representatives of the family *Rhodobacteraceae*, showing the position of strains Sh*Rb*01 and Sh*Rb*02. The numbers show the values of bootstrap analysis.

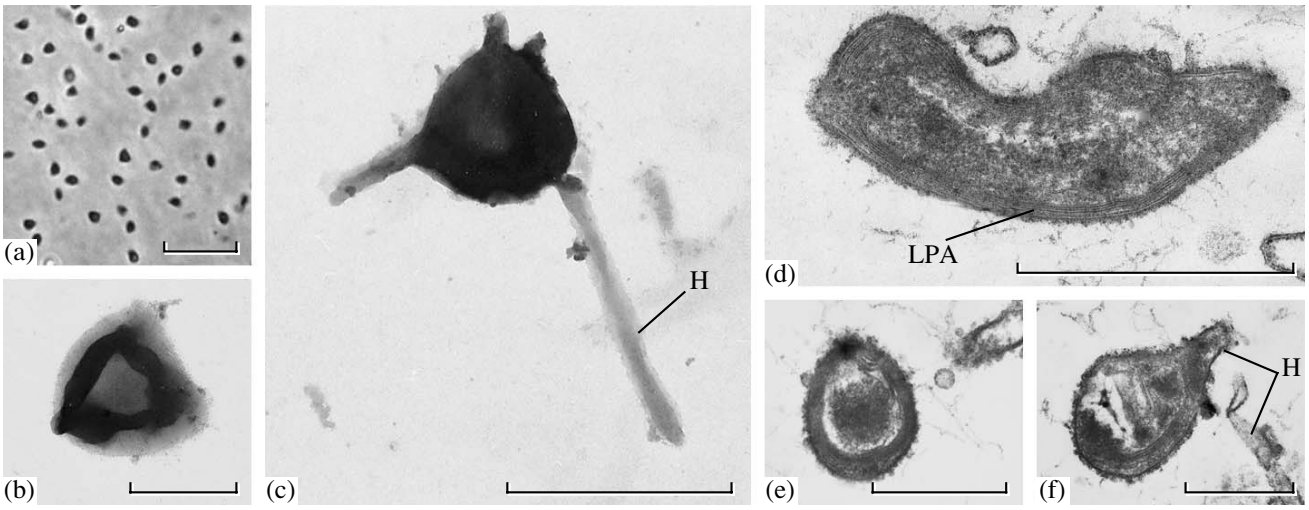

Fig. 9. Morphology and ultrastructural organization of strain Sh*Rmc*01. Phase contrast microscopy (a), electron microscopy of negative stained samples (b, c) and ultrathin sections (d—f); (a, b) overall morphology of the exospores; (c, d) vegetative cell with hyphae; (e) exospore formation of the hyphal tip; (f) exospore germination. H, hyphae; LPA, lamellar photosynthetic apparatus. Scale bar: (a) 5 μm; (b, d–f) 0.5 μm; (c) 1 μm.

We believe that the dominance of this morphotype of purple sulfur bacteria in Lake Shira is not accidental. Lakes exist with the water type similar to that of Lake Shira, where *Lamprocystis purpurea* (previously *Amoebobacter purpureus*) is known to dominate in the APB community. The meromictic Lake Mahoney near Penticton in the Ocanagan Valley (British Columbia, Canada) can serve as an example. This lake has an area of 11.5 ha, a depth of 14.5 m, and water of the sulfate–carbonate–chloride type. The total water salinity in the lake was 7.5–16 g/l in the surface layers and 39 at the bottom. The sulfide content in the monimolimnion was

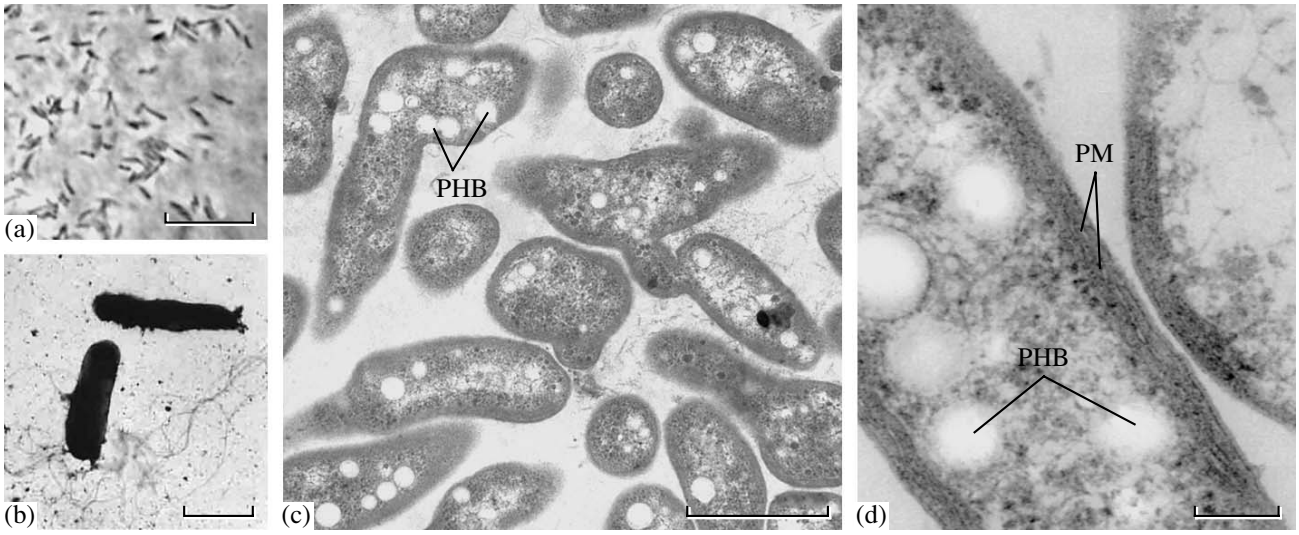

Fig. 10. Morphology and ultrastructural organization of strain Sh*Rb*02. Phase contrast microscopy (a), electron microscopy of negative stained samples (b) and ultrathin sections (c, d); (d) fragment of ultrathin section with photosynthetic membranes in the peripheral zone of the cytoplasm. PHB, poly-β-hydroxybutyrate inclusions; PM, photosynthetic membranes. Scale bar: (a) 10 μm; (b, c) 1 μm; (d) 0.1 μm.

as high as 60 mM. The quantitative ratio of phototrophic bacterial species in the water of the Lake Mahohey redox zone was as follows: *Lamprocystis purpurea* – 97.9%, *Thiocapsa roseopersicina* up to 2%; *Rhodobacter capsulatus*, up to 0.02%; green sulfur bacteria *Chloroherpeton thalassium* and *Prosthecochloris aestuarii*, up to 0.002% [17].

The meromictic Lake Cadagno in the southern Swiss Alps is another example. This is a lake with 800 × 400 m dimensions, 24 ha area, and 21 m maximal depth. The water is enriched with sulfate; water conductivity in the zone of APB development is 250–350 μS/cm. The sulfide concentration at the bottom is 1 mM. Purple sulfur bacteria *Lamprocystis purpurea* were shown to dominate in the Lake Cadagno APB community in autumn and winter, while *C. okenii* dominates in summer [18].

In the APB community of the meromictic Lake Shunet, bacteria develop which resemble *Lamprocystis purpurea*, although they do not belong to this species. Lake Shunet is located 20 km to the southwest of Lake Shira; its dimensions are 1.2 × 0.4 km, its area is 0.47 km^2, and its depth is about 6 m. The water of Lake Shunet is of the sulfate–chloride–sodium–magnesium type; the total salinity is 15 g/l in the upper layers and 66 g/l near the bottom. The sulfide content near the bottom exceeds 500 mg/l. Green sulfur bacteria phylogenetically similar to *Prosthecochloris vibrioformis* dominate in the APB community of this lake. The upper five cm of the zone of APB development, however, was formed by purple sulfur bacteria of the *Lamprocystis purpurea* and *Lamprobacter modestohalophilus* morphotypes; the first morphotype was usually dominant. Nonsulfur purple bacteria *Rhodovulum euryhalinum* and nonsulfur purple bacteria phylogenetically close to *Roseicyclus mahoneyensis* were also found in the APB community of Lake Shunet [13].

Unlike other lakes, in Lake Shira only one morphotype of purple sulfur bacteria was revealed, morphotype Sh*Am*01.

Bacteria of the Sh*Am*01 morphotype in Lake Shira are never as numerous as in other lakes. This is certainly the result of the rate of sulfide flow from the lower horizons of the water column into the zone of APB development. It has already been mentioned that the sulfide concentration near the bottom was 60 mM in Lake Mahoney, over 500 mg/l in Lake Shunet, and less than 16 mM in Lake Shira.

We believe that high pH values in the zone of APB development play an important role as a limiting factor for the growth of purple sulfur bacteria in Lake Shira. For *Lamprocystis purpurea*, a species phylogenetically close to strain Sh*Am*01, the pH is 6.5; 6.7–7.5 are the optimal values [12]. In the redox zone of Lake Mahoney, the pH was 8.0, and *Lamprocystis purpurea* numbers were as high as 4×10^8 cells/ml; in Lake Cadagno, pH was close to 7 and *Lamprocystis purpurea* numbers exceeded 2×10^6 [19]; in Lake Shunet at pH 8.1–8.5, the total number of purple sulfur bacteria (obtained from colony counts on agarized media inoculated with water samples) was 1.6×10^8 cells/ml [13]. In Lake Shira, pH in summer in the zone of APB development was 8.7–9.0; the numbers of bacteria of the Sh*Am*01 morphotype (obtained from colony counts on agarized media inoculated with water samples) did not exceed 3×10^5 cells/ml.

The winter pH values in Lake Shire in the zone of APB development decreased to 8.3; however, the negative temperature and low illumination did not promote

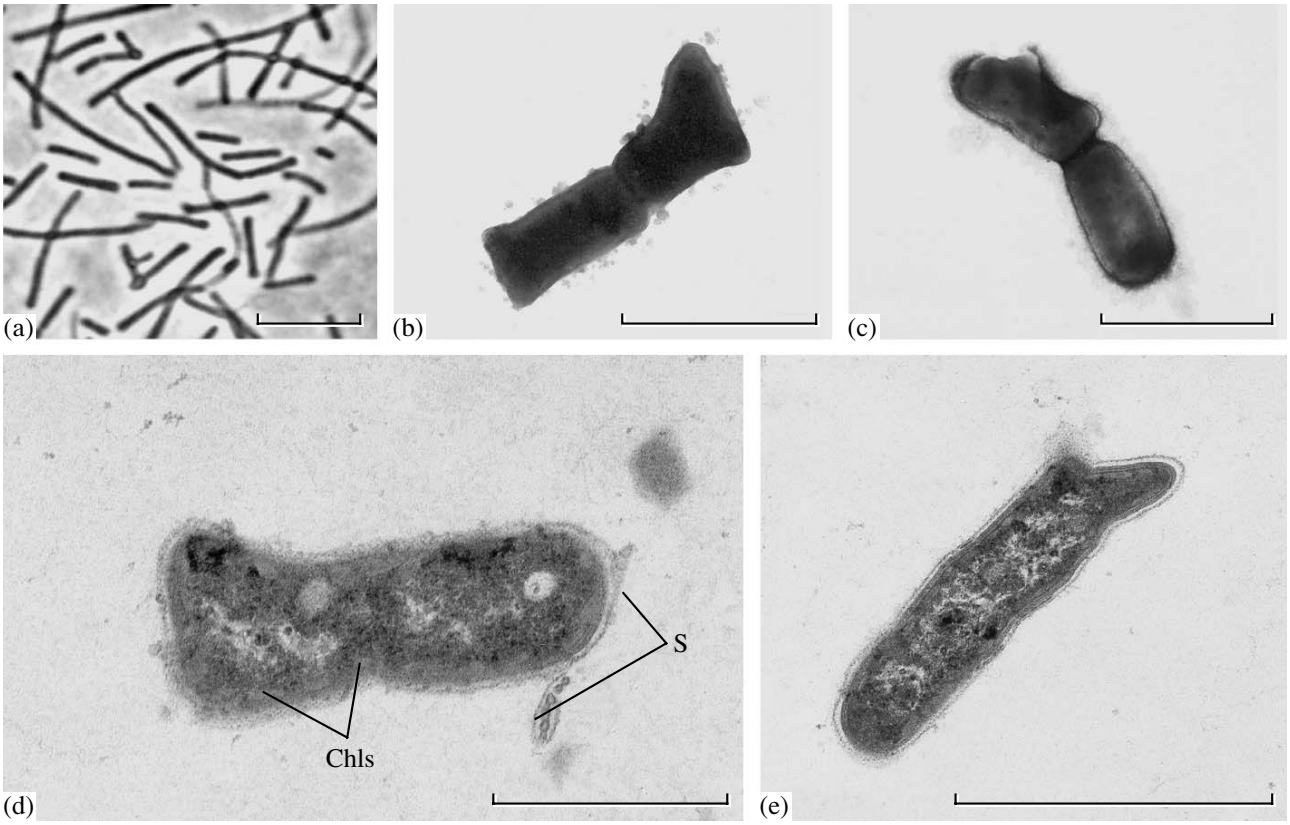

Fig. 11. Morphology and ultrastructural organization of strain Sh*Cl*03. Phase contrast microscopy (a), electron microscopy of negative stained samples (b, c) and ultrathin sections (d, e); (a) cells in filaments, (b, c) cell division with a septum formed between daughter cells. Chls, chlorosomes; S, S layers. Scale bar: (a) 5 μm; (b, c, e) 1 μm; (d) 0.5 μm.

abundant growth of the purple sulfur bacteria of the Sh*Am*01 morphotype.

This combination of pH values and low illumination enabled, however, the development of the only minor sulfur bacterium species detected in Lake Shira—green sulfur bacteria of the Sh*Cl*03 morphotype. The pigment analysis of lake water in winter revealed no noticeable Bchl *c* peak in the zone of APB development; this pigment is present in Sh*Cl*03 cells. The green sulfur bacteria most closely related to strain Sh*Cl*03 require pH 6.5–7 for growth. The winter pH values in Lake Shira were probably too high for the Sh*Cl*03 morphotype bacteria; these bacteria were therefore detected in small amounts only in the lower part of the zone of APB development [16]. The previously published data on the presence of high numbers of green sulfur bacteria within the APB community were therefore not confirmed for any of the seasons investigated [20].

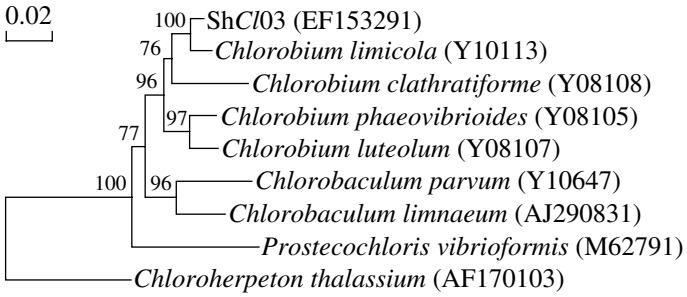

Fig. 12. Rootless phylogenetic tree of the representatives of the family *Chlorobiaceae*, showing the position of strain Sh*Cl*03. The numbers show the values of bootstrap analysis.

Strain Sh*Rb*02, which was isolated in July 2002, is of great interest. Its morphology, physiological characteristics, and pigment composition are close to those of the *Rhodobacter-Rhodovulum* morphotype of nonsulfur purple bacteria; phylogenetically, it is related to nonphototrophic bacteria *Ahrensia kielensis* (formerly *Agrobacterium kielensis*). Thus, both the generic and the species position of strain Sh*Rb*02 require further specification.

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