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EXPERIMENTAL ARTICLES

Anoxygenic Phototrophic Bacteria of the Kislo-Sladkoe Stratified Lake (White Sea, Kandalaksha Bay)

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Abstract—The community of anoxygenic phototrophic bacteria (APB) in the water column of the Kislo-Sladkoe stratified lake recently isolated from the sea (White Sea, Kandalaksha Bay) was investigated in September 2010. The water of the sulfide-rich zone was greenish-brown due to intense development of green sulfur bacteria (GSB). Nine APB strains were isolated from the water samples: three belonging to GSB, five, to purple sulfur bacteria (PSB), and one, to purple nonsulfur bacteria (PNB). GSB predominated in the phototrophic community of the chemocline. Unexpectedly, two morphologically different green-colored GSB strains were found to be phylogenetically identical and related to the brown-colored *Chlorobium phaeovibrioides* (99% similarity according to the 16S rRNA gene sequencing). Homology to the closest green-colored species (*Chlorobium luteolum*) was 98%. Two morphologically and physiologically similar PSB strains (*Tcr*PS10 and *Am*PS10) had rounded cells containing okenone and gas vesicles. According to the 16S rRNA gene sequencing, these strains were most closely related (99%) to two different *Thiocapsa* species: *Tca. marina* (containing okenonee and no gas vesicles) and *Tca. rosea* (containing spirilloxanthin and gas vesicles). The remaining isolates of purple bacteria were similar to the already described APB species.

Keywords: Arctic ecosystems, White Sea, stratified lakes, meromictic lakes, anoxygenic phototrophic bacteria, green sulfur bacteria, purple sulfur bacteria, purple nonsulfur bacteria, phylogeny of anoxygenic phototrophic bacteria, *Chlorobium phaeovibrioides, Prosthecochloris* sp., *Chlorobaculum chlorovibrioides, Thiocapsa marina, Thiocapsa rosea, Thiorhodococcus kakinadensis, Thiorhodococcus drewsii, Rhodovulum sulfidophilum*

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The small stratified Lake Kislo-Sladkoe (Sour–Sweet) (66°55′ N, 33°14′ E) is located on the Rugozerskaya Gulf shore of the Kandalaksha Bay of the White Sea, 2 km to the east from the Moscow State University White Sea Biological Research Station [1]. The lake is 100 × 60 m in size; its average depth is 1–1.5 m and the maximum depth is 4.5 m. The lake was formed in the mid-20th century as a result of coastal rise. Previously there had been a gully between the continent and a small island with inlet and outlet sills.

The lake is replenished with fresh water mainly in the period of snow thawing, with a minor contribution of rains. The fresh water runoff from the swamp is low (0.01 L s^{-1} in summer); the inflow of salt water percolating under the sill stones is 10-fold greater than that of fresh water (1–1.5 L s^{-1}) [1]. Deep water layers contain hydrogen sulfide. The low depth of the lake and the presence of well-lit hydrogen sulfide zone create favorable conditions for the development of anoxygenic phototrophic bacteria (APB).

The phototrophic community of the recently formed lake is of undoubted interest. APB had not been studied previously in Lake Kislo-Sladkoe, and our work was aimed at studying the conditions of existence of anoxygenic phototrophic bacteria, as well as their isolation and identification by microbiological and molecular methods.

MATERIALS AND METHODS

Water samples were taken in the lake hollow (4.2 m). Water samples were taken with a silicon tube fixed on a calibrated cable and a Whale Premium Submersible Pump GP1352 (Ireland). The total water salinity was measured immediately after sampling with a HANNA HI 98130 salinity-conductivity meter (Germany). Oxygen and sulfide concentrations were measured immediately after sampling using Aquamerck test kits (Merck, Germany).

The rate of photosynthesis was measured by the radioisotope method using ^{14}C-bicarbonate [2]. Labeled bicarbonate solution (0.2 mL, 20 μCu, 50 μg-equiv L^{-1}) was added into 30-mL glass vials filled with lake water. Diuron at a final concentration of 7 mM L^{-1} was used as a selective inhibitor of oxygenic photosynthesis [3]. The vials were suspended on a nylon halyard

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in the layers from which water samples were taken. The time of incubation was 24 h. After incubation, the water samples were fixed with 1 mL of diluted HCl and filtered through membrane filters with a pore size of 0.2 μm. The production of oxygenic photosynthesis was calculated by the difference between the total and anoxygenic (diuron-containing vial) photosynthesis.

The total number of microorganisms was assayed on polycarbonate membrane filters (pore diameter, 0.2 μm) by the fluorescent method using 4',6-diamidino-2-phenylindole hydrochloride (DAPI) [4]. Bacterial cells were counted under an epifluorescence microscope (Zeiss, Germany).

To determine the content of bacteriochlorophylls (BChl), lake water samples (200–400 mL) were filtered through 0.2-μm membranes. Then, under laboratory conditions, the filters were treated with acetone–methanol mixture (7 : 2) for pigment extraction and absorption spectra of the extracts obtained were recorded with a Cary-100 spectrophotometer (Varian, Australia) at 350–900 nm. The content of pigments was calculated by the following formulas [5]:

$$c\ (\mu\text{g Bchl}\ (d+e)) = (1.315 \times \text{E651} - 0.643 \times \text{E663} + 0.005)\ v \times 106/(V \times d \times \varepsilon \times \text{Bchl}\ d),$$

$$c\ (\mu\text{g Chl}\ a) = (1.35 \times \text{E663} - 0.643 \times \text{E651} + 0.005)\ v \times 106/(V \times d \times \varepsilon \times \text{Chl}\ a),$$

where c (μg BChl ($d + e$)) is the concentration of bacteriochlorophylls $d + e$ (mg m^{-3}); E651 and E663 are absorption values of the pigment solutions in acetone at 651 and 663 nm, respectively (minus turbidity measured at E850); v is the volume of acetone extract (mL); V is the volume of filtered lake water sample (mL); d is the width of the cuvette (cm); and ε is the absorption coefficient: εBChl d = 98.0 mg cm^{-1} [6], εChl a = 84.0 mg L g^{-1} cm^{-1} [7]. However, unlike the authors of the article mentioned above, we used an acetone/methanol mixture, rather than acetone. The spectra therefore exhibited a 4-nm shift of light absorption peaks to the long-wave region. Therefore, in the calculations we took the values of E655 and E667 instead of E651 and E663, respectively:

$$c\ (\mu\text{g Bchl}\ (d+e)) = (1.315 \times \text{E655} - 0.643 \times \text{E667} + 0.005)\ v \times 10^6/(V \times d \times \varepsilon \times \text{Bchl}\ d),$$

$$c\ (\mu\text{g Chl}\ a) = (1.35 \times \text{E667} - 0.643 \times \text{E655} + 0.005)\ v \times 10^6/(V \times d \times \varepsilon \times \text{Chl}\ a).$$

APB enrichment cultures were obtained under field conditions by dispensing the freshly collected lake water from the depths of 2, 2.7, 2.9, 3.1, and 4.2 m into sterile penicillin vials, to which 1–2 drops of 10% yeast extract was added. Diuron powder was added into each flask at the tip of a spatula to suppress oxygenic photosynthesis. The vials were hermetically sealed with penicillin stoppers, with a pea-sized air bubble left.

Lake water samples (5 mL) from the depths of 2, 2.7, 2.9, 3.1, and 4.2 m were also inoculated with sterile syringes into hermetically sealed 30-mL glass penicillin vials with the medium. The medium with several variants of salinity (NaCl concentration) contained the following (g/L of distilled water): KH_2PO_4, 0.7; NaCl, 5, 10, 15, 20, or 25; $MgSO_4 \cdot 7H_2O$, 0.5; NH_4Cl, 0.7; KCl, 0.33; $NaHCO_3$, 0.15; $CaCl_2$, 0.1; $Na_2S_2O_3 \cdot 5H_2O$, 1; $Na_2S \cdot 9H_2O$, 0.5; Na acetate, 0.5; Na pyruvate, 0.5; yeast extract, 0.1; trace element solution, 1 mL [8]; vitamin B_{12}, 20 μg; diuron powder; pH 7.5–8.

The relative APB abundance was determined under laboratory conditions by inoculating water samples from the same layers by the method of terminal serial dilutions of the material into semisolid (0.5% agar) nutrient medium (15 g L^{-1} NaCl). Anaerobic cultivation was carried out for a month in a luminostat (at illuminance 2000) at 20–25°C. The cultures were isolated and purified by the terminal dilutions method using liquid and agarized media of different salinity. The vitamin complex [8] was added into the medium for green sulfur bacteria.

The characteristics used for preliminary identification of the microorganisms were as follows: the shape and size of cells and microcolonies, the color of the colonies of phototrophic bacteria, the presence of gas vacuoles, sulfur droplet formation and deposition, and the absorption spectra of the whole-cell suspensions in 50% glycerol and in the acetone–methanol extract (7 : 2).

The pigment composition of APB cultures was studied in whole cell preparations in 50% glycerol and in acetone–methanol (7 : 2) extracts. Absorption spectra were recorded with a Cary 100 spectrophotometer (Varian, United States) at 350–900 nm.

The microphotographs of the cells were taken using an Olympus light microscope (magnification 1200×, 90× oil immersion system) under phase contrast.

Ultrathin sections of the bacteria were obtained as follows: the material was fixed in 2.5% glutaraldehyde solution in 0.05 M cacodylate buffer (pH 7.2) at 4°C for 2 h, washed three times with the same buffer, and additionally fixed in 1% OsO_4 solution in 0.05 M cacodylate buffer (pH 7.2) overnight at 4°C. After dehydration in a series of alcohols, the material was embedded in Epon 812. The sections were mounted on grids and contrasted for 30 min with 3% uranyl acetate solution in 70% alcohol and additionally with lead citrate [9].

Negatively stained preparations were obtained by staining the bacterial suspension with aqueous 0.2% uranyl acetate solution. The preparations were examined in a JEM-100B electron microscope (JEOL, Japan).

DNA was isolated by the technique based on the modified method of alkaline DNA extraction by Birnboim–Doly [10] and the Wizard technology (Promega, United States). Polymerase chain reaction

(PCR) and subsequent sequencing of the fragments of the 16S rRNA gene were performed with a universal primer system [11]. The *fmo* gene fragments were amplified using the primers proposed in [12].

PCR products were analyzed by electrophoresis in 2% agarose gel at 6 V/cm^{-1}. PCR products were isolated and purified from low-melting-point agarose using the Wizard PCR Preps reagent kit (Promega, United States) according to the manufacturer's instructions.

Amplification products were sequenced by the Sanger method [13] using a Big Dye Terminator v. 3.1 reagent kit (Applied Biosystems, Inc., United States) in an ABI PRIZM 3730 genetic analyzer (Applied Biosystems, Inc., United States) according to the manufacturer's instructions. Initial comparison of the newly obtained sequences with the sequences from the GenBank database was performed using the NCBI Blast software (http://www.ncbi.nlm.nih.gov/blast). The sequences were edited using BioEdit (http://jwbrown.mbio.ncsu.edu/BioEdit/bioedit.html). The fragments of the 16S rRNA gene sequences of more than 1400 bp were obtained for all of the isolated strains. The dendrograms of phylogenetic affinity were plotted using MEGA 4.0 [14]. The obtained 16S rRNA gene sequences were deposited in the GenBank International database under accession nos. KC702851–KC702859.

RESULTS

Physicochemical characteristics of the lake. The work at Lake Kislo-Sladkoe was carried out on September 6–13, 2010 [15]. In the period of our studies, in the deepest part of the lake (4.2 m) oxygen was present to the depth of 2.7 m, sulfide was detected in the water at a depth of 2.6 m (Fig. 1), and the redox zone was 2.6–2.7 m deep. Sulfide concentration in the bottom layer was 32 mg L^{-1}. The total water salinity in the lake varied from 18.0 g L^{-1} in the surface layers to 22.5 g L^{-1} near the bottom. The thermocline was observed at a depth of 2.5–3 m and practically coincided with the redox zone. The water temperature decreased from 12.8°C on the surface to 8.8°C near the bottom.

Primary production and optical characteristics of the water. The water in the upper layers was transparent. The water was light-lettuce green in the redox zone (2.6–2.7 m) and light green at a depth of 2.8 m due to the development of green-colored anoxygenic phototrophic bacteria.

Immediately below the redox zone, at a depth of 2.9 m, there was a layer of pink-colored water. However, contrary to our expectations, pink water color was imparted not by the purple sulfur bacteria but by unicellular eukaryotic algae containing pink carotenoids (Fig. 2). At a depth of 3 m and lower, the water

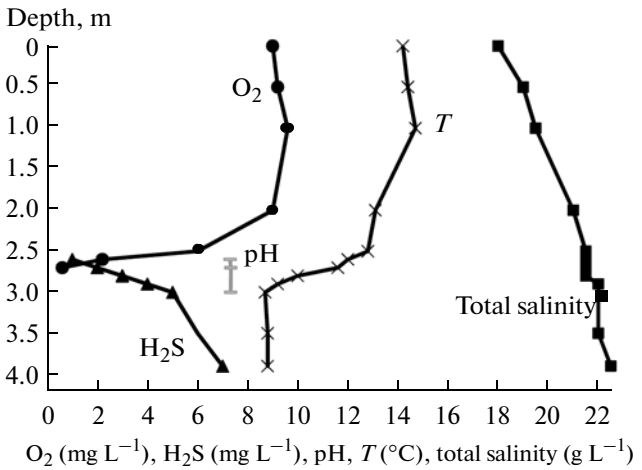

Fig. 1. Hydrochemical characteristics of the water in Lake Kislo-Sladkoe, September 2010.

was brown-green due to the presence of green- and brown-colored green sulfur bacteria.

The spectra of the pigments extracted with acetone–methanol from water samples collected at different depths of the lake are shown in Figure 3. Overlapping of the peaks of bacteriochlorophyll *d* (654–659 nm) from green-colored GSB and BChl *e* (654 nm) of brown-colored GSB made it impossible to determine the content of each Bchl in the water. The content of photosynthetic pigments in lake water is given in Table 1. The most active APB development was recorded at a depth of 2.9 m. Here, the peaks of biomass content and production of anoxygenic photosynthesis were recorded (Fig. 4). During the period of our studies, production of anoxygenic photosynthesis in the lake was more than twice higher than the maximum production of oxygenic photosynthesis (411 and 182 μg C L^{-1} day^{-1}, respectively).

Zonality of APB development in lake water. APBs developed in the lake water at depths of 2.7 m and more. Analysis of absorption spectra of the pigments extracted from the water samples collected at different depths (Fig. 3, Table 2) and the quantitative account of APB colonies indicated that green-colored GSB were predominant in the upper part of the anoxic water column, while purple nonsulfur bacteria were present as a

Table 1. The content of photosynthetic pigments in the water of Lake Kislo-Sladkoe, September 2010

Depth	Chl *a* (mg m^{-3})	BChl (*d* + *e*) (mg m^{-3})
2	8.2	–
2.7	0	118.6
2.9	688.9	65.6
3.1	0	132.5
4.2	0	161.3

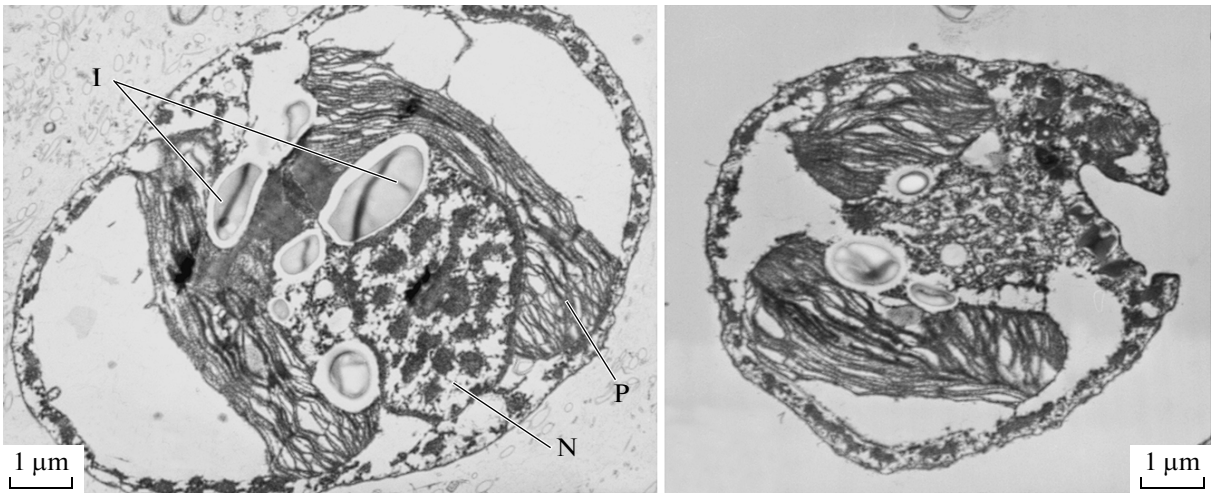

Fig. 2. Ultrathin cell sections of algal cells from Lake Kislo-Sladkoe water, September 2010, 2.9-m deep (pink layer): the nucleus (N) with different chromatin condensation, a cup-shaped pyrenoid (P), and amyloid inclusions (I) are in view.

minor component. Brown-colored GSB were predominant in the lower part of the anoxic zone. Minor amounts of PSB occurred in all of the studied layers and showed no strict zonation (Table 2).

The pattern of depth-related APB distribution in the lake water was almost fully confirmed by the analysis of enrichment cultures from water samples of different layers.

Patterns of enrichment culture growth. APB enrichment cultures in liquid media were obtained from the depths of 2, 2.7, 2.9, 3.1, and 4.2 m, although the chemocline in the lake was located at 2.6 m. Growth was observed in 2–4 weeks after inoculation, generally with prevalence of one of the discovered GSB species.

The distinctive feature of isolated GSB was their very small cell size: 0.1 × 0.2–0.5 μm. Mainly green-colored GSB grew in the medium with salinity of 5–20 g L^{-1}. During the growth of enrichment cultures in the vials at salinity of 25 g L^{-1}, green-colored forms were competitively displaced by brown forms. Brown-colored GSB grew in the medium with salinity of 25 g L^{-1}, as well as in the medium with low salinity inoculated with water samples taken from the lower layers of the lake (5 g L^{-1} from 3.1 m and 15 g L^{-1} from 2.9–4 m). This pattern was in agreement with the prevalence of brown-colored GSB in more mineralized, poorly illuminated deep layers of the lake.

PSB enrichment cultures phylogenetically close to the genus *Thiocapsa* grew from the water samples taken at the depths of 2 and 2.7 m, while PSB phylogenetically close to the genus *Thiorhodococcus* grew from the samples of water above the sediment and of the sediment. It was also in good agreement with the physiological characteristics of the isolated strains. *Thiocapsa* prefer slightly saline water and are able to grow in the presence of oxygen, whereas all of the known *Thiorhodococcus* species prefer the salinity similar to that of sea water.

PNB did not form enrichment cultures in vials of liquid medium inoculated with the water samples. They were isolated from single colonies emerging on solid media together with the purple sulfur bacteria.

Characteristics of the APB isolates. We have isolated from the water samples of Lake Kislo-Sladkoe one brown-colored GSB strain (*Phv*PS10); two morphologically different green-colored GSB strains (*Pr*PS10 and *Chlv*PS10); five PSB strains (*Tca*PS10, *Tcyf*PS10, *Tcyr*PS10, *Am*PS10, and *Trcc*PS10); and

Table 2. Results of quantification of APB colonies grown on agar medium for APB inoculated with lake water

Depth, m	Viable cell number		
	green-colored GSB (cells mL^{-1})	brown-colored GSB (cells mL^{-1})	PSB (cells mL^{-1})
2.7	1.12×10^4	$0.5–0.8 \times 10^3$	3.4×10^3
2.9	1.13×10^4	6.75×10^3	3.4×10^3
3.1	3.5×10^3	$3–9 \times 10^3$	4.5×10^3
4.2	9.9×10^4	5.06×10^4	3.4×10^3

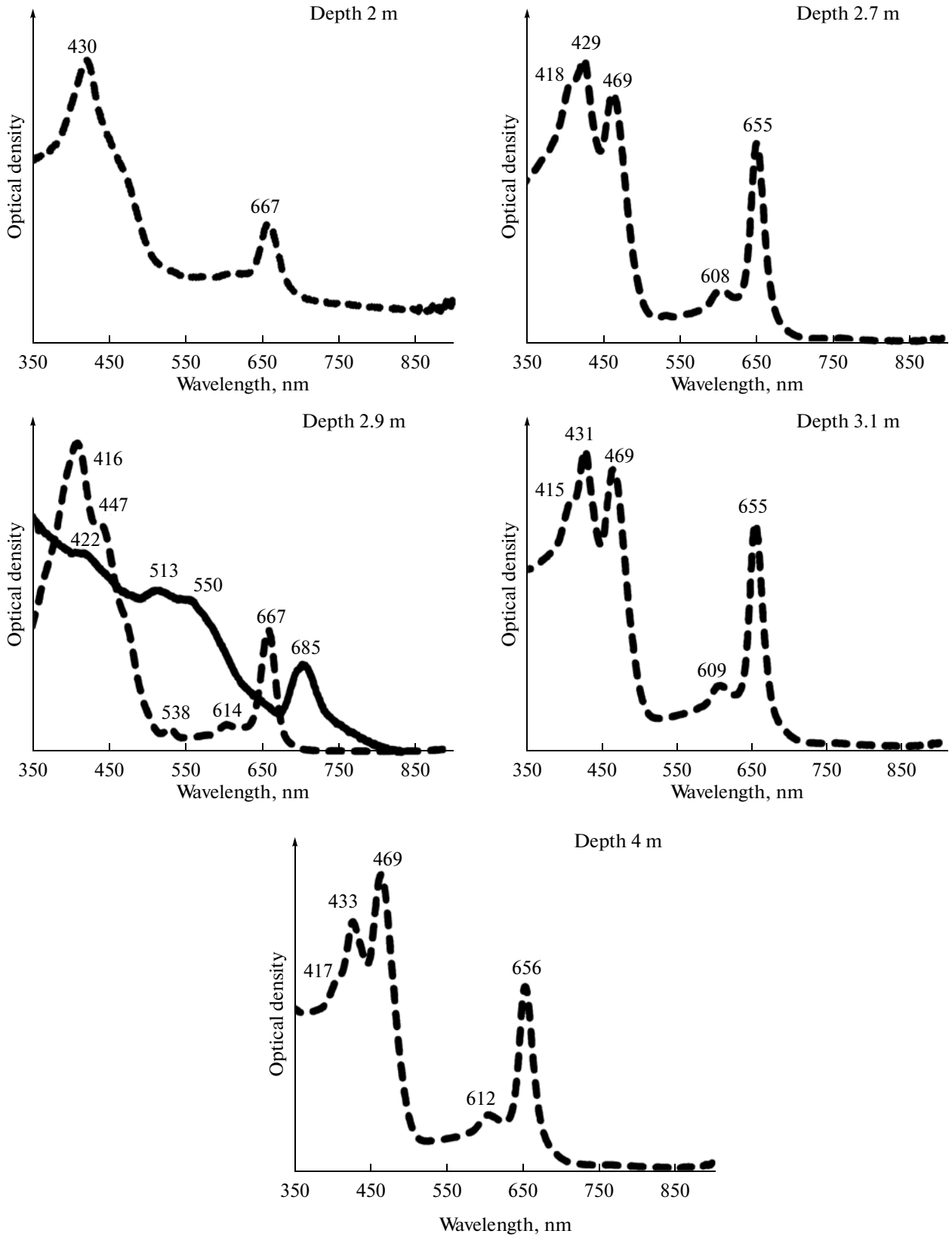

Fig. 3. Lake water spectra (Lake Kislo-Sladkoe, September 2010). The solid line is the spectrum of unfixed lake water, water/glycerol (1 : 1). The dotted line is the spectrum of the pigments extracted from lake suspended matter with acetone/methanol (7 : 2).

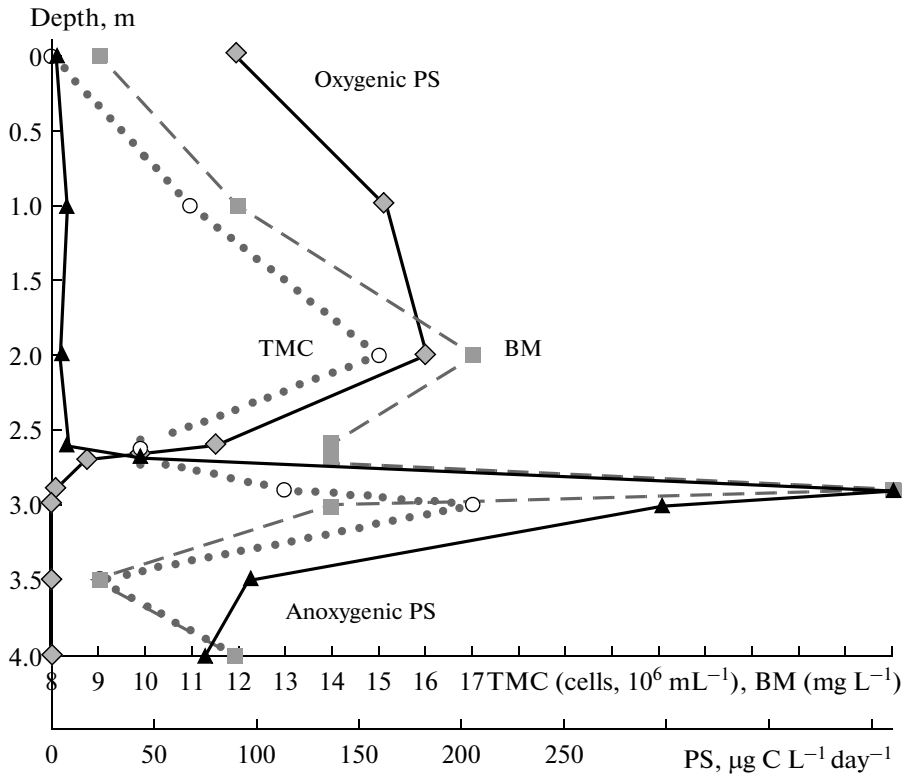

Fig. 4. The profiles of photosynthesis, distribution of microorganisms and biomass in Lake Kislo-Sladkoe, September 2010. Total number of microorganisms (TNM); biomass (BM); photosynthesis (PS).

one PNB strain (*Rv*PS10). The main characteristics of the isolates are given in Table 3.

The brown-colored GSB strain *Phv*PS10 was isolated from the enrichment culture obtained from a water sample collected at the depth of 4 m in the medium containing 25 g L^{-1} NaCl. Bacterial cells were nonmotile, round or oval, 0.2–0.5 μm in size (Figs. 5j–5l). The major photosynthetic pigments in the cells were BChl *e* and the carotenoid isorenieratene, which was responsible for the chocolate brown color of the bacteria (Fig. 6). The optimal salinity for growth was 15 g L^{-1}, pH 8 (Fig. 7). Good growth occurred at NaCl concentrations from 10 to 30 g L^{-1}, pH 7.5–8.5. The morphological, physiological, and phylogenetic characteristics of the strain *Phv*PS10 (GenBank KC702853) indicated it to be most closely related to *Chlorobium phaeovibrioides*, with 100 and 99% similarity to *Chl. phaeovibrioides* strains DSM 265 and DSM 269 (type strain), respectively. Surprisingly, the isolated brown-colored strain *Chl. phaeo-vibrioides* showed 100% 16S rRNA gene similarity to two green-colored GSB strains, *Pr*PS10 and *Chlv*PS10, which were also isolated from the anaerobic water zone of Lake Kislo-Sladkoe (Fig. 8).

The green-colored GSB strain *Pr*PS10 was isolated from the depth of 4 m from an enrichment culture with 15 g L^{-1} NaCl. On agar medium, the bacteria formed dense mucous colonies. Microscopy showed short filaments falling into separate oval cells (0.15–0.2 × 0.2–1 μm) covered with a thin mucous sheath and forming irregularly shaped aggregates in liquid medium (Figs. 5b–5d). Ultrathin sections clearly showed the chlorosomes arranged along the cell periphery and gas vesicles in the center of the cytoplasm (Figs. 5e, 5f). The major pigments of these bacteria were BChl *d* with the maximum at 730 nm and the carotenoid chlorobactene (Fig. 6). Good growth was observed at NaCl concentrations of 10–20 g L^{-1} (the optimum of 15 g L^{-1}) and pH 7.5–8 (pH optimum, 8.0) (Fig. 7). The strain *Pr*PS10 was morphologically similar to *Prosthecochloris vibrioformis* isolated from Lake Shunet (strain Sh*Pel*02), which also contained gas vacuoles [16]. However, the 16S rRNA gene sequencing showed that the strain *Pr*PS10 (GenBank KC702854) exhibited 99% similarity to the *Chl. phaeovibrioides* strains DSM 265 and DSM 269 (type strain) and only 93–94% similarity to *Prosthecochloris* species (Fig. 8) [17].

Another strain of green-colored GSB, *Chlv*PS10, was isolated from the enrichment culture (5 g L^{-1} NaCl) inoculated with a water sample from the depth of 2.9 m. Bacterial cells were nonmotile rods (0.1–0.2 × 0.2–0.5 μm) surrounded by a mucous sheath separating the cells from each other (Figs. 5g, 5h). The cells of the strain *Chlv*PS10 had no gas vacuoles. Chlorosomes and the deposits of dense polyphosphate

Table 3. Basic properties of the APB strains isolated from Lake Kislo-Sladkoe water samples in September 2010

Isolated APB strain, the ranges of salinity and pH values for its growth	Description of the isolated strain	The species most similar to the isolated strain in morphophysiological characteristics	The species phylogenetically most similar to the isolated strain
		Green sulfur bacteria	
Pr*PS10 10–20 g L^{-1} NaCl (opt. 15 g L^{-1}), pH 7.5–8 (opt. 8.0)	Nonmotile oval cells, 0.15–0.2 × 0.2–1 μm, covered with a thin mucous sheath and forming irregularly shaped aggregates. Contain gas vacuoles, BChl *d*, and the green carotenoid chlorobactene	*Prosthecochloris* sp. ShN*Pel*02	*Chl. phaeovibrioides* (DSM 269 and DSM 265): 99.3 and 100%, respectively
***Chlv*PS10** 10–15 g L^{-1} NaCl (opt. 10 g L^{-1}), pH 7.5–8 (opt. 7.5)	Nonmotile rods (0.1–0.2 × 0.2–0.5 μm) with a large mucous sheath; contain no gas vacuoles; possess BChl *d* deposits and the green carotenoid chlorobactene	*Chlorobaculum chlorovibrioides* (previously *Chlorobium chlorovibrioides*)	Strain *Pr*PS10 with 100% similarity
***Phv*PS10** 10–30 g L^{-1} NaCl (opt. 15 g L^{-1}), pH 7.5–8.5 (opt. 8)	Nonmotile round or oval, 0.2–0.5 μm in size, BChl *e* and the brown carotenoid isorenieratene	*Chl. phaeovibrioides*	Green-colored strains *Pr*PS10 and ChlvPS10—100% similarity *Chl. phaeovibrioide*s (DSM 265)—100% similarity
		Purple sulfur bacteria	
*Tca*PS10 20–40 g L^{-1} NaCl (opt. 30 g L^{-1}), pH 6.5–7 (opt. 6.5)	Rounded nonmotile cells (1–1.2 μm), with a mucous sheath; contain sulfur droplets, BChl *a*, and the carotenoid spirilloxanthin	Bacteria of the genus *Thiocapsa*	*Tca. pendens*. *Tca. roseopersicina*—98.1%, strain Mog1 *Tca*. sp.—98.6% similarity
***Tcyr*PS10** 10–30 g L^{-1} NaCl (opt. 15 g L^{-1}), pH 7.5–8.5 (opt. 7.5)	Rounded cells of 1.5–2 or 1–3 μm (respectively) containing sulfur droplets, either motile due to the tuft of 3 flagella or nonmotile with gas vacuoles	–	*Thiocapsa rosea* and *Thiocapsa marina*—99% similarity each
***Am*PS10** 20–35 g L^{-1} NaCl (opt. 30 g L^{-1}), pH 7.5–8.5 (opt. 8)			
*Tcyf*PS10 25–45 g L^{-1} NaCl (opt. 30–35 g L^{-1}), pH 6.5–7.5 (opt. 7)	Rounded or oval cells (1–2 × 3–4 μm); motile due a single flagellum; contain sulfur droplets, BChl *a*, and the carotenoid rhodopinal (violet-colored)	Bacteria of the genus *Thiorhodococcus*	*Thiorhodococcus kakinadensis*—99.7% similarity
***Trcc*PS10** NaCl 25–40 g L^{-1} (opt. 35 g L^{-1}) and pH 7.5–8 (opt. 8)	Rounded cells, 1–1.5 μm, motile due to a single polar flagellum. Contain sulfur droplets, BChl *a*, and the carotenoid rhodopin (brown-colored)	*Thiorhodococcus drewsii*	Strain *Trcf*PS10—99% similarity
		Purple nonsulfur bacteria	
*Rv*PS10 15–50 g L^{-1} NaCl, pH 7–8 (opt. 7.5)	Motile cells, vibrioid- or oval-shaped with sharp edges (0.3 × 0.5 μm), with a tuft of flagella at one end. Contain BChl *a* and carotenoids of the spheroidene series	Bacteria of the *Rhodobacter–Rhodovulum* morphotype	*Rhodovulum sulfidophilum*—100% similarity

* The strains claiming to be the novel APB species are in boldface.

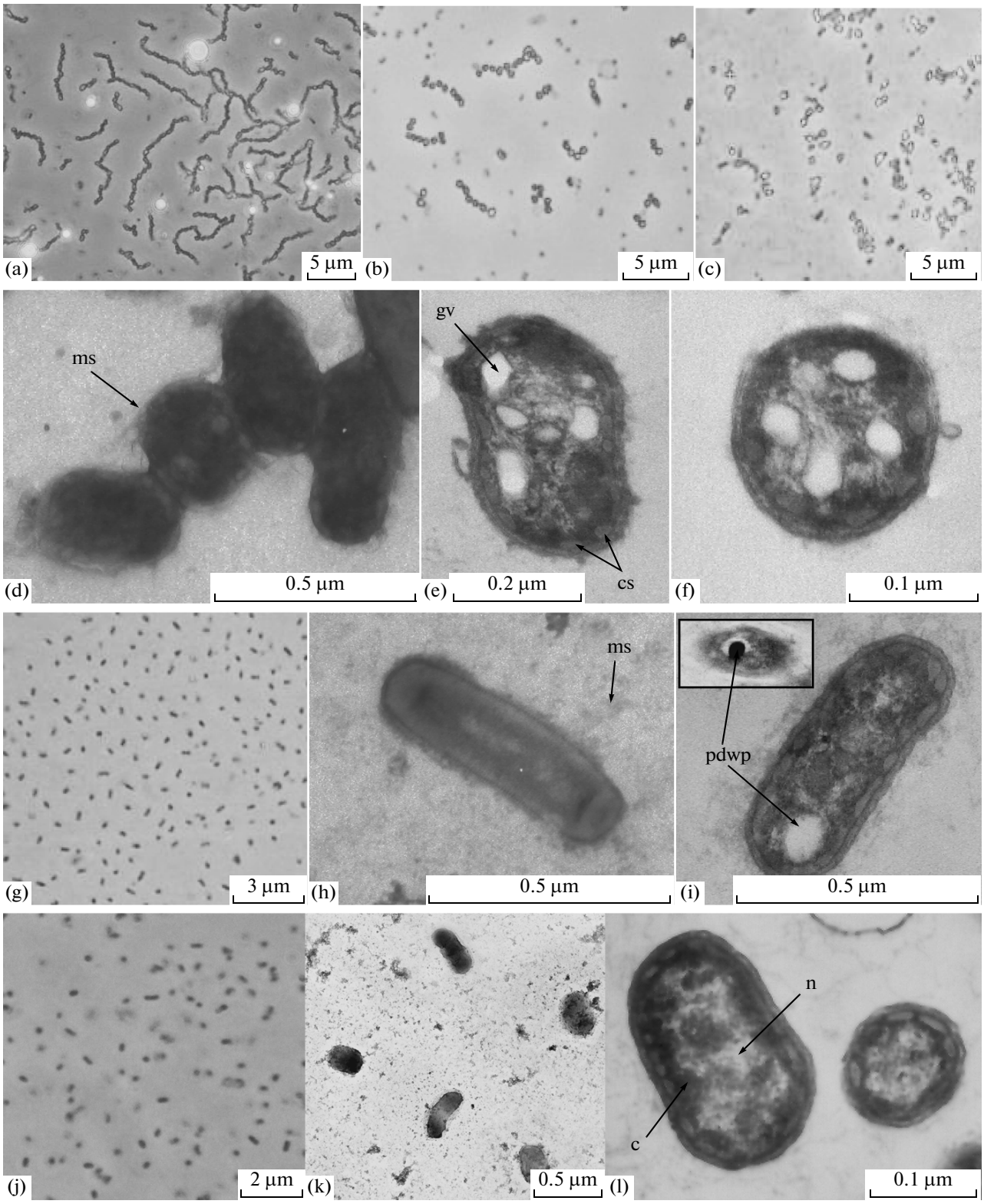

Fig. 5. Morphology and ultrathin structure of the cells of green sulfur bacteria isolated from Lake Kislo-Sladkoe in September 2010: strain *Pr*PS10 (a–f), strain *Chlv*PS10 (g–i), strain *Phv*PS10 (j–l); light microscope, phase contrast (a–c, g, j); electron microphotographs (d, h, k) of total preparations and (e, f, i, l) of bacterial cell ultrathin sections. Strain *Pr*PS10: the pattern of cell division and growth during purification and cultivation changes from long filaments to separate cells (a–c); a thin dense mucous sheath (ms) surrounds the cells (d); gas vacuoles (gv) can be seen (e, f). Strain *Chlv*PS10: the cells are surrounded with a thick mucous sheath (h); polyphosphate deposit washout places (pdwp) are visible (i). In the center of the cells on ultrathin sections (e, f, i, l), one can clearly see the light zone of the nucleoid (n), the dark zone of the cytoplasm (c) with ribosomes, and the chlorosomes (cs) adjacent to the plasma membrane.

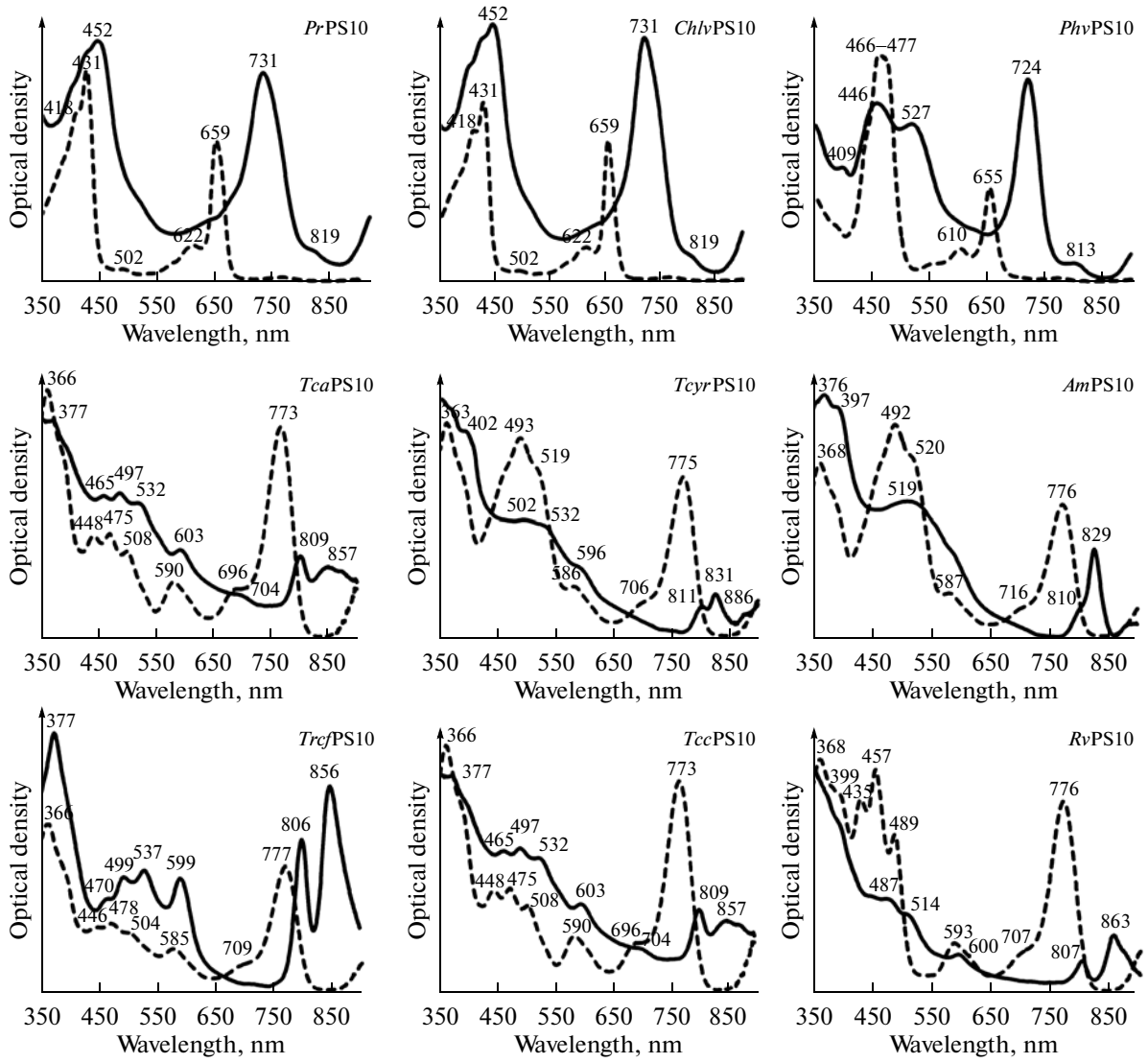

Fig. 6. Spectra of anoxygenic phototrophic bacteria isolated from Lake Kislo-Sladkoe, September 2010.

granules, as well as the empty spaces from which these granules have been washed out during the treatment of the preparations, can be seen in the ultrathin sections of the cells (Fig. 5i). Absorption spectrum of the pigments of strain *Chlv*PS10 completely coincided with that of the strain *Pr*PS10. The major photosynthetic pigments were BChl *d* and the carotenoid chlorobactene (Fig. 6). The optimal salinity for growth *Chlv*PS10 was 10 g L^{-1}, pH 7.5 (Fig. 7). Good growth was observed at a NaCl concentrations from 10 to 15 g L^{-1}, pH 7.5–8. The strain *Chlv*PS10 (GenBank KC702852) was morphologically similar to the bacterium *Chlorobaculum chlorovibrioides* (formerly, *Chlorobium chlorovibrioides*); however, its 16S rRNA gene sequence had only 92.1% similarity to the sequence of the type strain of this species (UdG 6026 = DSM 1377, GenBank accession number Y10649) and 92–95% similarity to other *Chlorobaculum* species [11]. It is interesting that the nucleotide sequences of the 16S rRNA gene of green-colored strains *Chlv*PS10 and *Pr*PS10 proved to be identical. Comparison of the nucleotide sequences of the strains *Chlv*PS10 and *Pr*PS10 with that of the brown-colored strain *Chl. phaeovibrioides Phv*PS10 also showed their 100% similarity, in spite of the differences in morphology and composition of bacteriochlorophylls and carotenoid pigments (Fig. 8, Table 3). The dendrogram based on the results of phylogenetic analysis of the 16S rRNA gene sequences showed that the strains *Phv*PS10, *Chlv*PS10, and *Pr*PS10 formed a cluster together with the sequences of the type strain *Chlorobium phaeovibrioides* DSM 269^T (Y08105) and *Chlorobium phaeovibrioides* DSM 265 (NC_009337), with the 99.3 and 100% similarity levels, respectively.

Since these results were unexpected, we additionally analyzed the *fmo* genes encoding the FMO protein

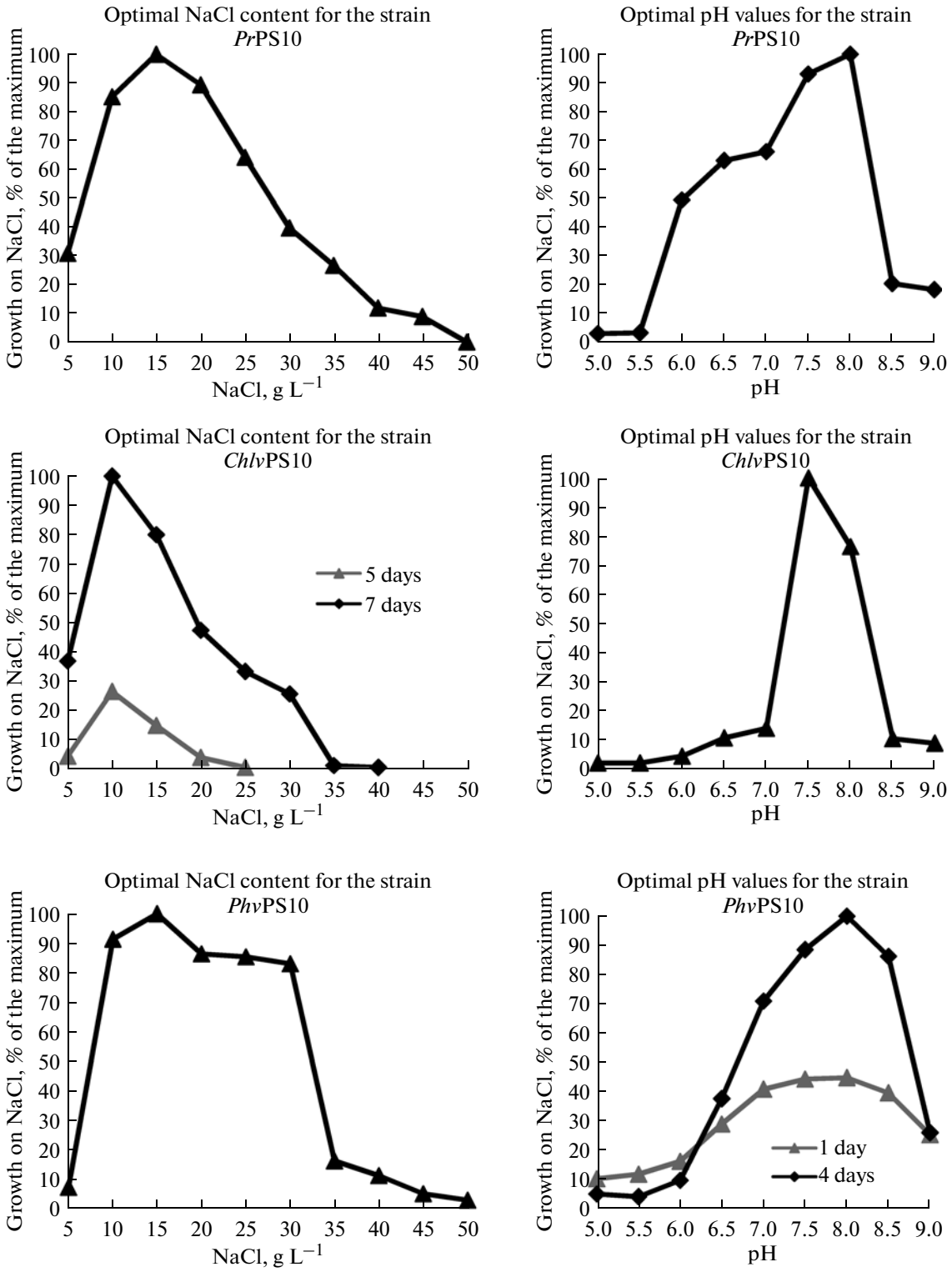

Fig. 7. Optimal salinity and pH values of the medium for the growth of green sulfur bacteria isolated from Lake Kislo-Sladkoe in September 2010.

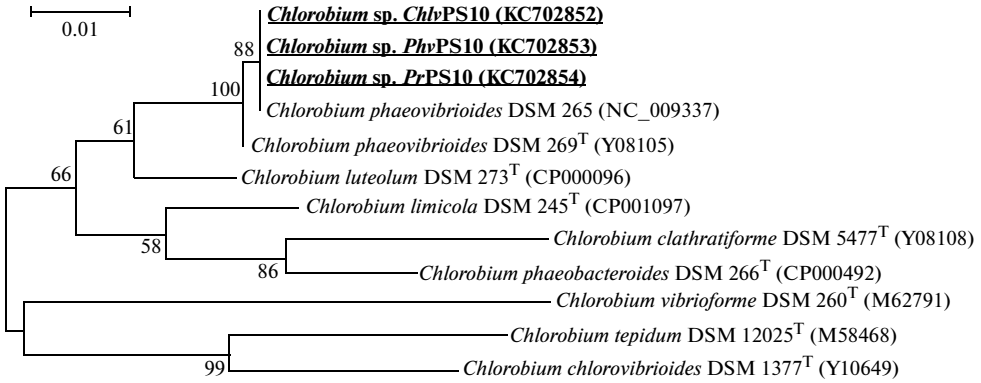

Fig. 8. Phylogenetic positions of the isolates *Chlv*PS10, *Phv*PS10, and *Pr*PS10 among representatives of the species of the genus *Chlorobium*. The sequences of the type strains of the species are marked with superscript T. The rootless dendrogram was plotted as a result of comparison of the 1306-nucleotide 16S rRNA gene sequences using the maximum-likelihood algorithm. The accuracy of branching obtained from the analysis of 1000 alternative replicas is given in percentage. The scale corresponding to 1 substitution per 100 nucleotides is given at the upper right.

of the photosynthetic reaction center (the Fenna–Matthews–Olson protein) specific for representatives of the phylum *Chlorobi* [12]. Successful application of this analysis as an alternative molecular marker resulted in substantial changes in the taxonomy of green sulfur bacteria [18]. Comparison of the nucleotide and amino acid sequences of the *fmo* genes in all three isolated strains of green sulfur bacteria (*Phv*PS10, *Chlv*PS10, and *Pr*PS10) demonstrated their complete identity (100% similarity), confirming the data of the 16S rRNA gene analysis.

The PSB strain *Tca*PS10 was isolated from the enrichment culture (15 g L^{-1} NaCl) obtained from a water sample from the depth of 2 m. The bacterial suspension had the typical whitish-pink color due to high content of intracellular elemental sulfur. Bacterial cells were rounded (1–1.2 μm), nonmotile, and had a mucous sheath. The cells contained sulfur droplets. Ultrathin sections showed a vesicular-type photosynthetic apparatus (Figs. 9a–9c). The major photosynthetic pigments were BChl *a* and the carotenoids of the spirilloxanthin series (Fig. 6). Good photolithoautotrophic growth was observed at NaCl concentrations of 20–40 g L^{-1} (the optimum of 30 g L^{-1}) and pH 6.5–7 (the optimum of 6.5) (Fig. 10). The strain *Tca*PS10 was related to bacteria of the genus *Thiocapsa* based on its morphological, physiological, and genetic properties. According to the data of phylogenetic analysis of the 16S rRNA gene sequences (Fig. 11), the strain *Tca*PS10 (GenBank KC702857), together with the strain *Thiocapsa* sp. Mog1 (EF149012) isolated previously from Lake Mogilnoye [19], formed an individual cluster (with a sequence similarity level of 98.6%). The sequences of the type strains *Tca. pendens* DSM 236 (AJ002797) and *Tca. roseopersicina* DSM 217 (AF113000) were most similar to the sequences of this cluster: the similarity with *Tca*PS10 was 98.1% [20].

Two phylogenetically identical PSB strains, *Tcr*PS10 and *Am*PS10, were isolated from a water sample taken from the depth of 2 m. The bacterial suspensions of both strains were pink-crimson. Rounded bacterial cells (1.5–2 and 1–3 μm in diameter, respectively) contained chromatophore vesicles and sulfur droplets (Figs. 9d–9f). The cells of both strains could be either motile due to a tuft of flagella or nonmotile and containing gas vacuoles (Figs. 9g, 9i). The main photosynthetic pigments were BChl *a* and the okenone carotenoids (Fig. 6). The optimal salinity values for the growth of *Tcr*PS10 and *Am*PS10 were 15 and 30 g L^{-1} (pH 7.5 and 8), respectively (Fig. 10). The sequences of the strains *Am*PS10 (GenBank KC702856) and *Tcyr*PS10 (GenBank KC702858) formed a cluster on the dendrogram together with the spirilloxanthin-containing type strain *Thiocapsa rosea* DSM 235T (AJ006062) (99.1% similarity) (Fig. 11) and, in accordance with the modern concepts [21], were at the very least novel strains of this species. The okenone-containing species closest to the isolated strains was *Thiocapsa marina*; their phylogenetic similarity was 99%.

The PSB strain *Trcf*PS10 was isolated from the enrichment culture (15 g L^{-1} NaCl) obtained from a sample of water collected above the sediment (4.2 m). Bacterial cells were rounded (1.5–2 μm in diameter) or oval (1–2 × 3–4 μm), motile, contained chromatophores and sulfur droplets (Figs. 12a, 12b). The major photosynthetic pigments were BChl *a* and the carotenoid rhodopinal (Fig. 6). The optimal salinity was 30–35 g L^{-1}, pH 7. Good growth was observed at a NaCl concentrations of 25–45 g L^{-1}, pH 6.5–7.5 (Fig. 13). The morphological and physiological properties, as well as the 16S rRNA gene sequence analysis, showed that the strain *Trcf*PS10 (GenBank KC702851) was most closely related (99.7% similarity) to the type strain *Thiorhodococcus kakinadensis*

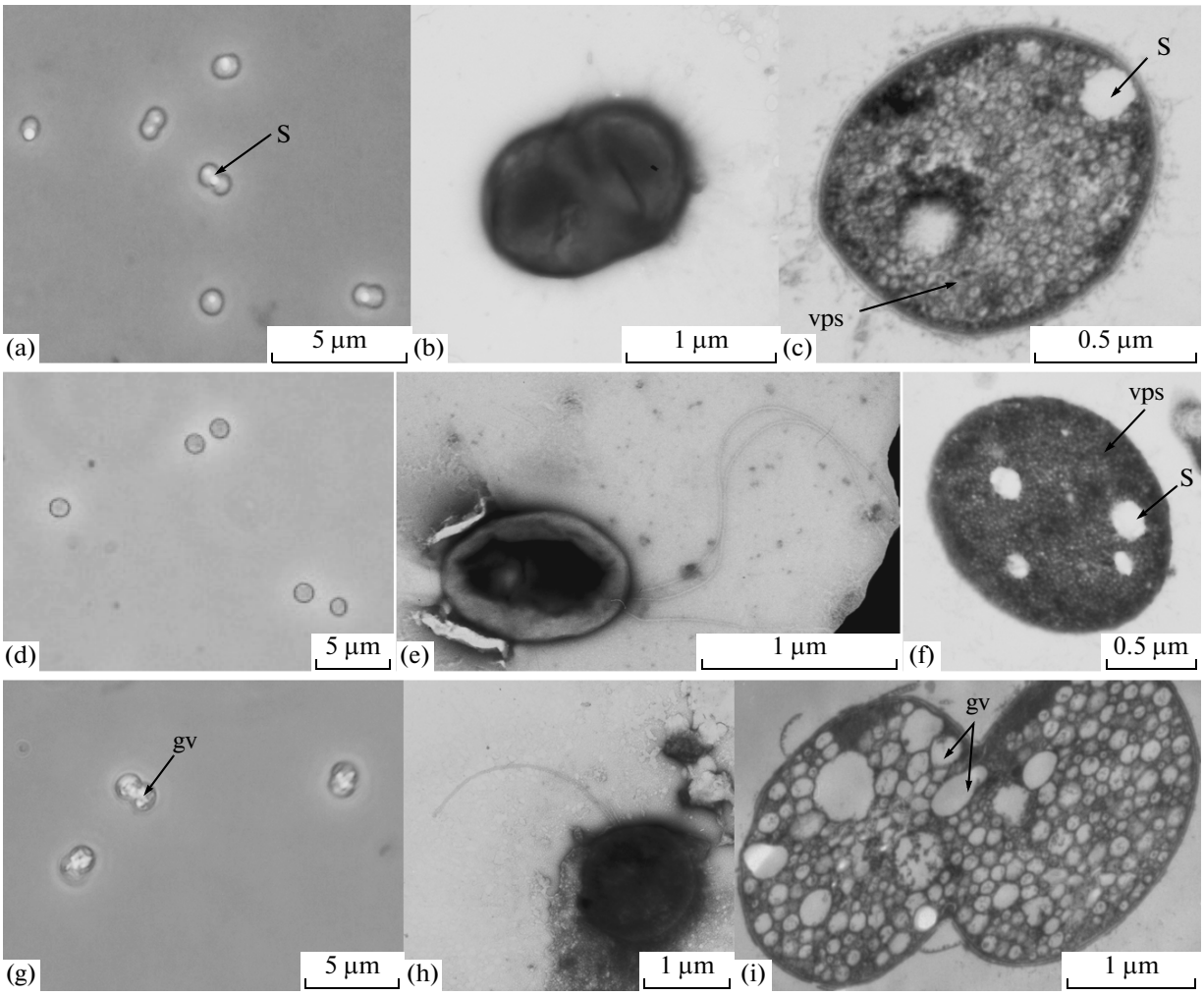

Fig. 9. Morphology and ultrathin structure of the cells of purple sulfur bacteria, phylogenetically close to the genus *Thiocapsa*, isolated from Lake Kislo-Sladkoe in September 2010: strain *Tca*PS10 (a–c), strain *Tcr*PS10 (d–f), strain *Am*PS10 (g–i); light microscope, phase contrast (a, d, g); electron microphotographs of total preparations (b, e, h) and ultrathin section of bacterial cells (c, f, i); elemental sulfur deposits (S); vesicular photosynthetic apparatus (vps); gas vacuoles (gv); a tuft of 3 flagella can be seen, which have been broken off during fixation and treatment of the preparations (e, h).

DSM 18858 (AM282561) and, thus, was one of the strains of this species (Fig. 14).

The PSB strain *Trcc*PS10 was isolated from the enrichment culture (15 g L^{-1} NaCl) obtained from a sediment sample. Bacterial suspension could be greenish, yellow, or beige-yellow at early growth stages and reddish-brown at late growth stages. Bacterial cells were rounded (1–1.5 μm in diameter), motile due to a single polar flagellum, contained chromatophores and sulfur droplets (Figs. 12c–12e). The major photosynthetic pigments were BChl *a* and carotenoids of the spirilloxanthin series with rhodopin as the primary pigment (Fig. 6). The optimal salinity was 35 g L^{-1}, pH 8. Good culture growth was observed at a NaCl concentrations from 25 to 40 g L^{-1}, pH 7.5–8 (Fig. 13). The strain *Trcc*PS10 was morphologically similar to bacteria of the genus *Thiorhodococcus*. According to the 16S rRNA gene sequence analysis, the strain *Trcc*PS10 (GenBank KC702859) was close to the strain *Trcf*PS10 (98.5% similarity) (Fig. 14). The sequence of the strain *Trcc*PS10 formed a separate branch on the dendrogram between the cluster of *Thiorhodococcus kakinadensis* DSM 18858T/ *Trcf*PS10 (AM282561, 98.3% similarity) and *Thiorhodococcus drewsii* DSM 15006 (FM178273, 97.5% similarity) [22].

The PNB strain *Rv*PS10 was isolated on agarized medium with 15 g L^{-1} NaCl from a water sample taken from the depth of 2.7 m. Bacterial suspension was light yellowish-orange. Motile bacterial cells were vibrioid- or oval-shaped with tapered edges (0.3 × 0.5 μm) and a tuft of flagella at one end (Fig. 15). The major photosynthetic pigments were BChl *a* and the carotenoids of the spheroidene series; this was confirmed by bacte-

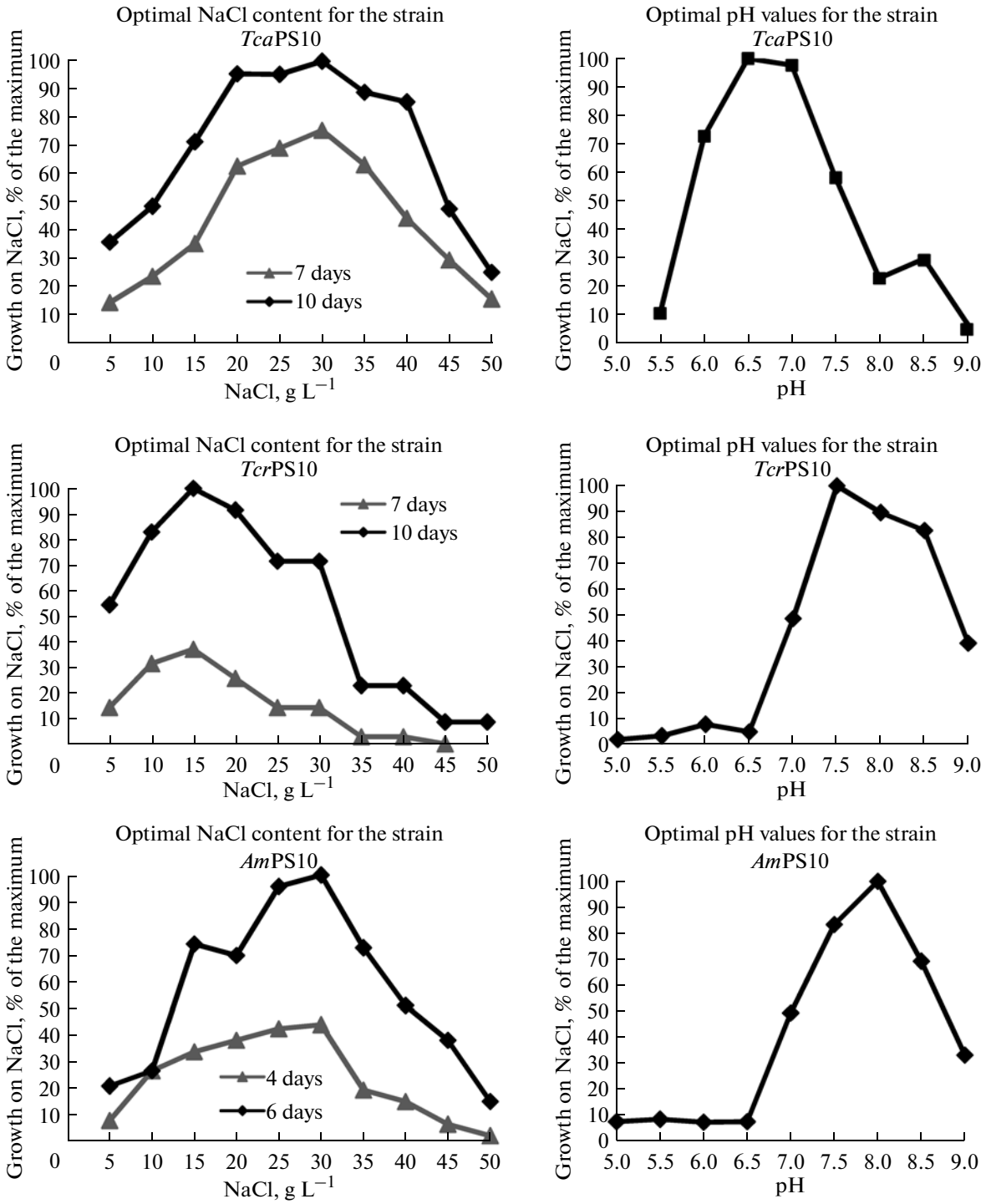

Fig. 10. Optimal salinity and pH values of the medium for growth of the purple sulfur bacteria phylogenetically close to the genus *Thiocapsa* isolated from Lake Kislo-Sladkoe in September 2010.

rial suspension changing its color to pink-red in the presence of oxygen (Fig. 6). Cell sections showed a thick laminated cell wall, vesicular-type photosynthetic membranes, and the places of deposition of poly-β-hydroxybutyric acid granules (Fig. 15c). The optimal pH value for growth was 7.5. The bacteria grew well at pH 7–8 and in a broad range of salinity: from 15 to 50 g L^{-1} NaCl (Fig. 16). According to the 16S rRNA gene sequence analysis, the strain *Rv*PS10 (GenBank KC702855) had 100% similarity with the strain *Rhodovulum sulfidophilum* JA198, 98% similarity with the type strain *Rh. sulfidophilum*, and 98–99% similarity with other strains of this species.

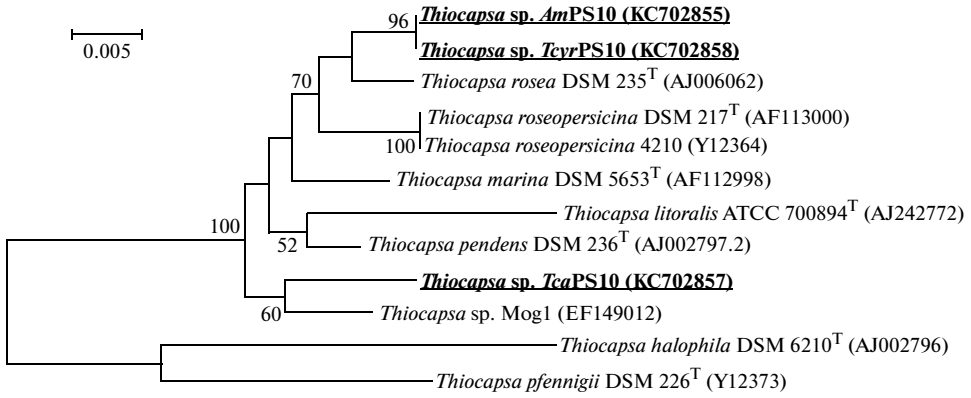

Fig. 11. Phylogenetic positions of the isolates *Am*PS10, *Tcyr*PS10, and *Tca*PS10 among representatives of the species of the genus *Thiocapsa*. The sequences of the type strains of the species are marked with superscript T. The rootless dendrogram was plotted as a result of comparison of the 1369-nucleotide 16S rRNA gene sequences using the maximum-likelihood algorithm. The accuracy of branching obtained from the analysis of 1000 alternative replicas is in percentage; the values above 50% are presented. The scale corresponding to 5 substitutions per 1000 nucleotides is given at the upper right.

DISCUSSION

The shallow stratified basin that we have examined is of undoubted interest, first of all, because it became separated from the water area of the Kandalaksha Bay (the White Sea) not long ago, in the mid-20th century. It should be emphasized that a unique APB community developed in the lake in spite of its recent formation. According to the results of the 16S rRNA gene analysis, the APB isolates belong to four known genera (*Chlorobium, Thiocapsa, Thiorhodococcus*, and *Rhodovulum*); however, nearly all isolated strains differ from the previously known species in some respects.

Stable water stratification at the moment of sampling was maintained due to the thermocline. APB depth distribution profile was in accordance with pigmentation, the presence of gas vacuoles in the cells, and their attitude to salinity and oxygen. Among the dominating GSB, green-colored species developed above the brown-colored ones. Minor PSB were under great pressure from the dominating green sulfur bacteria and, hence, their development in the zone with the maximum APB quantity was hindered. Oxygen-resistant bacteria of the genus *Thiocapsa*, containing gas vacuoles, were isolated from some water samples taken at a depth of 2 m. This fact indicates that they develop in minor quantities in the upper layers of the sulfide zone and get into the oxygen zone as single cells. The minor strictly anaerobic bacteria of the genus *Thiorhodococcus* were isolated from the samples of the near-bottom water layer and from the sediment, where they had sunk from the upper layers of the sulfide zone after losing mobility.

Our data show that salinity in Lake Kislo-Sladkoe water at the time of sampling varied from 18.0 g L^{-1} in the surface layers to 22.5 g L^{-1} near the bottom. These are boundary salinity values allowing the development of both salt-tolerant (GSB: *Pr*PS10, *Chlv*PS10, *Phv*PS10; PSB: *Tca*PS10, *Tcyr*PS10) and marine microorganisms (PSB: *Am*PS10, *Trcf*PS10, *Trcc*PS10) in the lake water, as was demonstrated by the diversity of behavior of the APB isolates in relation to salinity.

Marine species included the brown-colored GSB *Chlorobium phaeovibrioides*, PSB *Thiorhodococcus* sp., and PNB *Rhodovulum sulfidophilum*. Other isolates (both GSB and PSB) developed preferentially under a freshwater or brackish conditions, which is in good agreement with their natural habitats. We should put special emphasis on the development of brown-colored typically marine GSB *Chlorobium phaeovibrioides* in Lake Kislo-Sladkoe. These organisms were previously discovered only in deep-water meromictic basins such as the Black Sea and Lake Mogilnoye. This GSB species contains bacteriochlorophyll *e* and is enriched with the carotenoid isorenieratene, allowing it to win the competition with green-colored GSB species at great depths, where mainly low-intensity, short-wave visible light penetrates. Coastal microbial mats do not include brown-colored GSB, and their appearance in the young stratified basin cannot be explained by secondary adaptation of the benthic forms of anoxygenic phototrophs to planktonic existence. Most of the other species identified could migrate from the shallow-water benthic algal–bacterial communities of the White Sea [20].

Three bacterial strains of the genus *Thiocapsa* isolated from the lake seem to be novel microorganisms.

The strain *Tca*PS10 shows only 98% similarity to the phylogenetically closest microorganisms (*Tca. pendens, Tca. roseopersicina*, and *Tca.* sp. strain Mog1). At the same time, attention should be drawn to the fact that strain *Tca.* sp. Mog1 isolated from Lake Mogilnoye in 1999 is among the closest relatives. It is known that Lake Mogilnoye, like Lake Kislo-Sladkoe, is connected with the sea through a bridge of stones and gravel and is located at similar geographical lati-

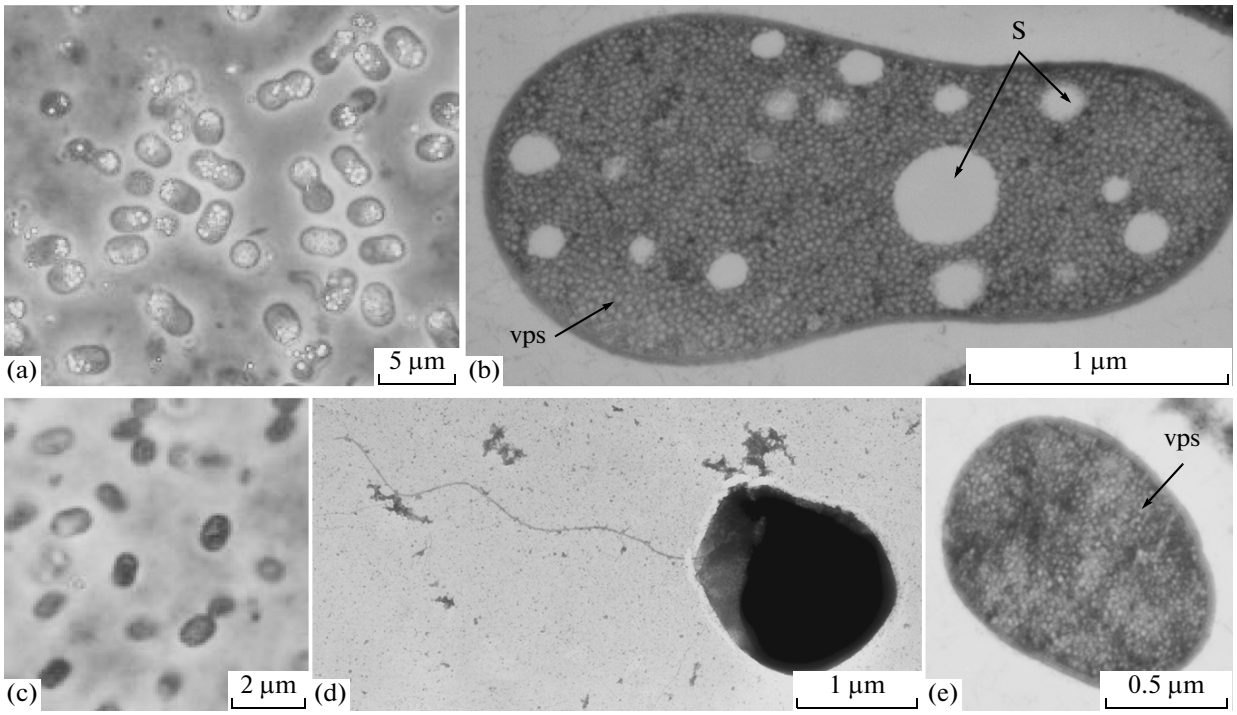

Fig. 12. Morphology and ultrathin structure of the cells of purple sulfur bacteria of the genus *Thiorhodococcus* isolated from Lake Kislo-Sladkoe in September 2010: strain *Trcf*PS10 (a, b) strain *Trcc*PS10; (c–e); light microscope, phase contrast (a, c); electron microphotograph; of total preparation (b) and ultrathin section of bacterial cells (b, e); elemental sulfur deposits (S); vesicular photosynthetic apparatus (vps); a flagellum is in view (d).

tudes (within the Arctic Circle and at the Arctic Circle boundary, respectively).

Other two strains (*Tcr*PS10 and *Am*PS10) containing gas vacuoles and the carotenoid okenone demonstrate a close relationship (99%) with the okenone-containing species *Thiocapsa marina*, which does not form gas vacuoles, and with the type strain *Thiocapsa rosea*, which forms gas vacuoles but contains the carotenoid spirilloxanthin.

Among the isolated bacteria of the genus *Thiorhodococcus*, the strain *Trcc*PS10 was the most similar to the rhodopin-containing species *Thiorhodococcus drewsii* in its morphophysiological characteristics. As regards its phylogenetic properties, it takes an intermediate position between rhodopinal-containing *Thiorhodococcus kakinadensis* and rhodopin-containing *Thiorhodococcus drewsii* and is probably a novel species of this genus.

One more surprise was the 100% phylogenetic similarity (based on the 16S rRNA gene analysis) of three isolated GSB morphotypes, one brown and two green-colored due to the presence of bacteriochlorophyll *d* and only a minor amount of the carotenoid chlorobactene. Importantly, these isolates did not differ significantly from each other according to the results of the FMO protein sequencing (unpublished data of T.P. Tourova not presented in this article).

As is clear from the results of molecular genetic research, the isolated strain of brown-colored GSB *Phv*PS10 had 99 and 100% similarity to *Chl. phaeovibrioides* strains DSM 269 (type strain) and DSM 265, respectively. It is known that DSM 269 (type strain) contains also BChl *e* and the carotenoid isorenieratene and is brown-colored. At the same time, strain DSM 265, which is still closer genetically, contains BChl *d* and BChl *c* and the carotenoid chlorobactene and is green-colored [18]. The strain DSM 265 was initially described as *Chl. vibrioforme* forma *thiosulfatophilum*; however, based on the data of the 16S rRNA gene and FMO protein sequencing, it was later attributed to the species *Chlorobium phaeovibrioides*, in spite of its different pigmentation [18].

A similar situation, when a brown-colored strain is found to be closer to a green-colored strain of absolutely different morphology than to brown-colored strains of the same morphology, was observed for the unique strain BS1 isolated from the zone of the deep-water chemocline of the Black Sea and described previously as *Chlorobium phaeobacteroides*. Based on the data of the 16S rRNA gene and FMO protein sequencing, the Black Sea brown-colored strain BS1 was attributed to the species *Prosthecochloris*; the green-colored *Ptc. aestuarii* DSM 271 (type strain) containing BChl *c* and the carotenoids chlorobactene and rhodopin, with absolutely different cell morphology, proved to be most close relative of the above strain [17, 23, 24].

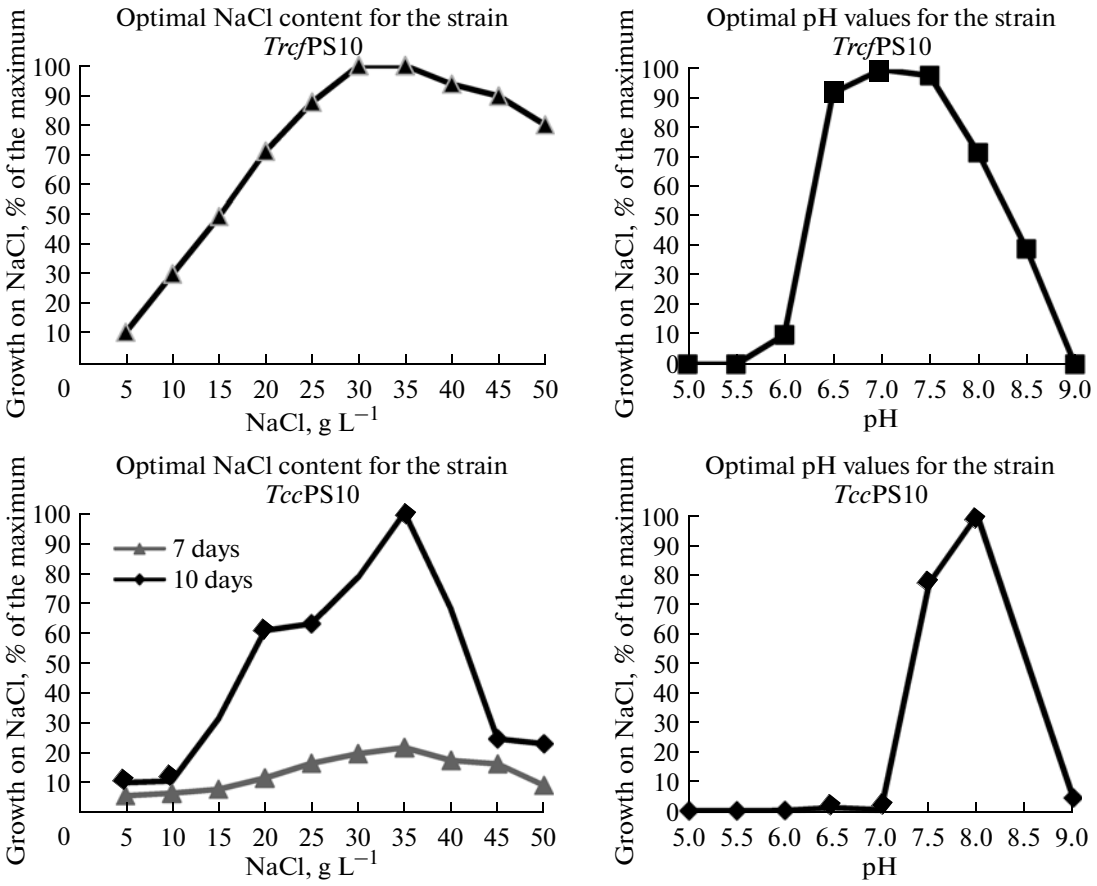

Fig. 13. The optimal salinity and pH values of the medium for the growth of purple sulfur bacteria of the genus *Thiorhodococcus* isolated from Lake Kislo-Sladkoe in September 2010.

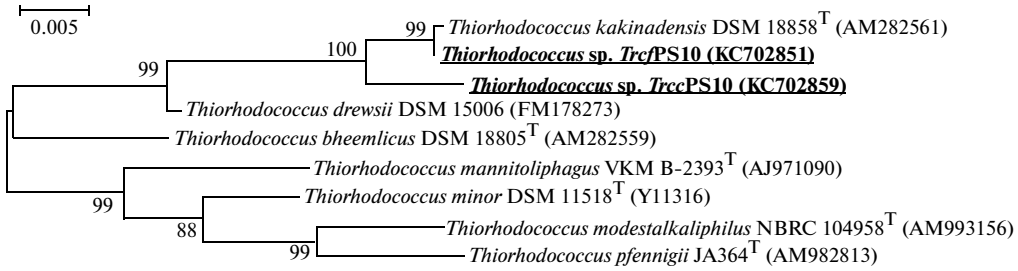

Fig. 14. Phylogenetic positions of the isolates *Trcf*PS10 and *Trcc*PS10 among representatives of the species of the genus *Thiorhodococcus*. The sequences of the type strains of the species are marked with superscript T. The rootless dendrogram was plotted as a result of comparison of the 1388-nucleotide 16S rRNA gene sequences using the maximum-likelihood algorithm. The accuracy of branching is given in percentage and obtained from the analysis of 1000 alternative replicas; the values above 50% are presented. The scale corresponding to 5 substitutions per 1000 nucleotides is given at the upper right.

Thus, currently available data show that, in spite of the morphophysiological similarity, individual GSB strains may be genetically distant from each other, while morphophysiologically different strains may be genetically close. It is one more piece of evidence that GSB are a genetically conservative group of APBs [23, 25]. Individual species have insignificant differences in the genome, which reflects the adaptive tendencies induced by their existence in a particular ecosystem.

In the case of adaptation of our three isolates to existence in the stratified Lake Kislo-Sladkoe, there are two obviously dominant ecological factors: the salinity and the spectral composition of light that vary

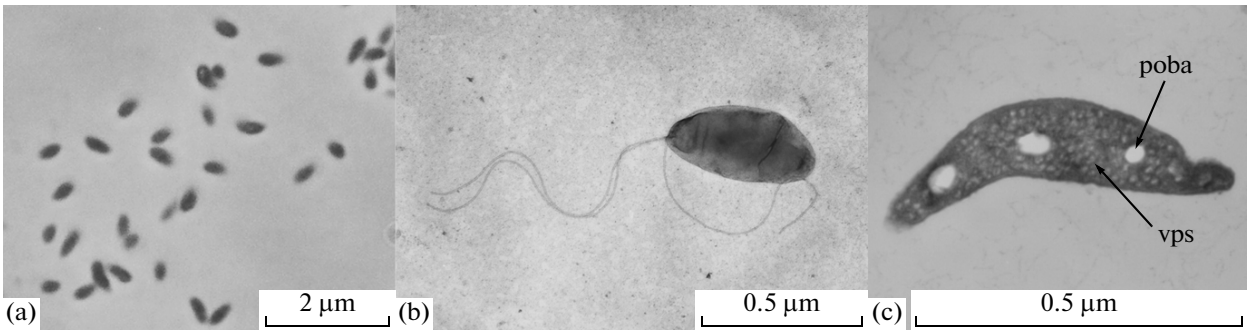

Fig. 15. Morphology and ultrathin structure of the cells of purple nonsulfur bacteria *Rhodovulum* sp. strain *Rv*PS10 isolated from Lake Kislo-Sladkoe in September 2010: light microscope, phase contrast (a); electron microphotographs of total preparations (b); and ultrathin sections (c) of bacterial cells; poly-β-oxybutyric acid granules (poba); vesicular photosynthetic apparatus (vps).

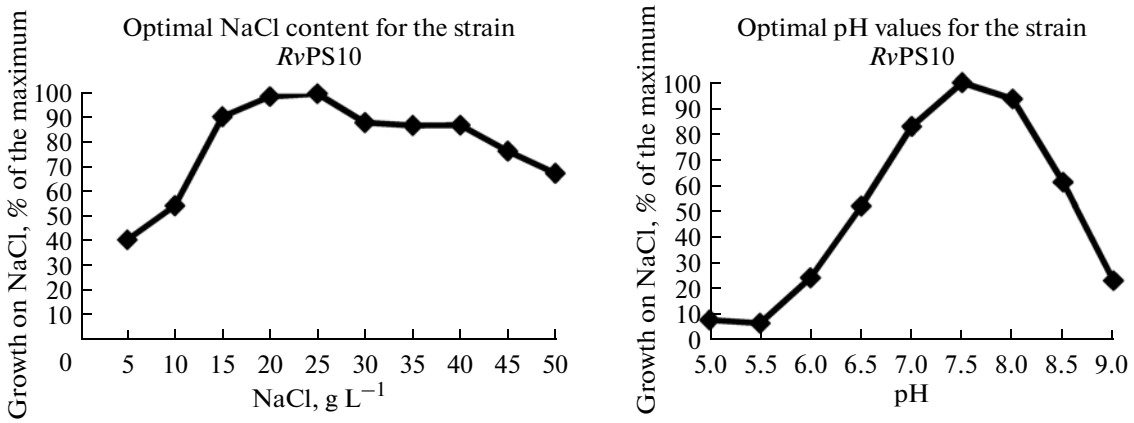

Fig. 16. The optimal salinity and pH values of the medium for the growth of purple nonsulfur bacteria strain *Rv*PS10 isolated from Lake Kislo-Sladkoe in September 2010.

with the lake depth. It is evident that the degree of GSB tolerance to oxygen also plays an important role in the competition. Genetic similarity of co-existing GSB populations of different quality in a basin that has recently become isolated from an open gulf suggests the high tempo of emergence and genetic consolidation of essential adaptive characteristics, such as the types of antenna chlorophylls and carotenoids. It is also obvious that the presence of gas vesicles in GSB, similar to PSB, is not caused by substantial changes in their genome.

ACKNOWLEDGMENTS

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REFERENCES

1. Krasnova, E.D., *Lake Kislo-Sladkoe*, Tula: Grif i Ko, 2008.

2. Pimenov, N.V., Rusanov, I.I., Karnachuk, O.V., Rogozin, D.Yu., Bryantseva, I.A., Lunina, O.N., Yusupov, S.K., Parnachev, V.P., and Ivanov, M.V., Microbial processes of the carbon and sulfur cycles in Lake Shira (Khakasia), *Microbiology* (Moscow), 2003, vol. 72, no. 2, pp. 221–229.

3. Dawson, R.M., Elliott, D.C., Elliott, W.H., and Jones, K.M., *Data for Biochemical Research*, 1986, Oxford: Clarendon, 1991, pp. 399–415.

4. Hobbie, J.T., Daley, R.J., and Jasper, S., Use of Nucleopore filters for counting bacteria by fluorescence microscopy, *Appl. Environ. Microbiol.*, 1977, vol. 33, pp. 1225–1228.

5. Overmann, J. and Tilzer, M.M., Control of primary productivity and the significance of photosynthetic bacteria in a meromictic kettle lake Mittlerer Buchensee, West-Germany, *Aquat. Sci.*, 1989, vol. 51, pp. 261–278.

6. Montesinos, E., Guerrero, R., Abella, C., and Esteve, I., Ecology and physiology of the competition for light between *Chlorobium limicola* and *Chlorobium paeobacteroides* in natural habitans, *Appl. Environ. Microbiol.*, 1983, vol. 46, pp. 1007–1016.

7. Smith, J.H.C. and Benitez, A., Chlorophylls: analysis in plant materials, in *Moderne methoden der Pflanzenanalyse*, Peach, K. and Tracey, M.V., Eds., Berlin: Springer, 1955, vol. 4, pp. 142–196.
8. Pfennig, N. and Lippert, K.D., Über das Vitamin B_{12}-Bedüfnis phototropher Schwefelbakterien, *Arch. Mikrobiol.*, 1966, vol. 55, pp. 245–256.
9. Reynolds, E.S., The use of lead citrate at high pH as an electron-opaque stain in electron microscopy, *J. Cell Biol.*, 1963, vol. 17, pp. 208–213.
10. Birnboim, H.C. and Doly, J., A rapid alkaline extraction procedure for screening recombinant plasmid DNA, *Nucleic Acid Res.*, 1979, vol. 7, no. 6, pp. 1513–1523.
11. Lane, D.J., 16S/23S sequencing, in *Nucleic Acid Techniques in Bacterial Systematics*, Stackebrandt, E. and Goodfellow, M., Eds., Chichester: Wiley, 1991, pp. 115–175.
12. Alexander, B., Andersen, J.H., Cox, R.P., and Imhoff, J.F., Phylogeny of green sulfur bacteria on the basis of gene sequences of 16S rRNA and of the Fenna–Matthews–Olson protein, *Arch. Microbiol.*, 2002, vol. 178, pp. 131–140.
13. Sanger, F., Nicklen, S., and Coulson, A.R., DNA sequencing with chain-terminating inhibitors, *Proc. Natl. Acad. Sci. USA*, 1977, vol. 84, pp. 5463–5467.
14. Tamura, K., Dudley, J., Nei, M., and Kumar, S., MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0, *Mol. Biol. Evol.*, 2007, vol. 24, pp. 1596–1599.
15. Savvichev, A.S., Lunina, O.N., Rusanov, I.I., Zakharova, E.E., Veslopolova, E.F., and Ivanov, M.V., Microbiological and isotopic geochemical investigation of Lake Kislo-Sladkoe, a meromictic water body at the Kandalaksha Bay shore (White Sea), *Microbiology* (Moscow), 2014, vol. 83, no. 2 (in press).
16. Lunina, O.N., Bryantseva, I.A., Akimov, V.N., Rusanov, I.I., Rogozin, D.Yu., Barinova, E.S., Lysenko, A.M., and Pimenov, N.V., Seasonal changes in the structure of the anoxygenic photosynthetic bacterial community in Lake Shunet, Khakassia, *Microbiology* (Moscow), 2007, vol. 76, no. 3, pp. 368–379.
17. Imhoff, J.F. and Thiel, V., Phylogeny and taxonomy of *Chlorobiaceae, Photosynth. Res.*, 2010, vol. 104, pp. 123–136.
18. Imhoff, J.F., Phylogenetic taxonomy of the family *Chlorobiaceae* on the basis of 16S rRNA and *fmo* (Fenna–Matthews–Olson protein) gene sequences, *Int. J. Syst. Evol. Microbiol.*, 2003, vol. 53, pp. 941–951.
19. Lunina, O.N., Gorlenko, V.M., Solov'eva, O.A., Akimov, V.N., Rusanov, I.I., and Pimenov, N.V., 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, *Microbiology* (Moscow), 2005, vol. 74, no. 5, pp. 588–596.
20. Gorlenko, V.M., Puchkova, N.N., and Demchev, V.V., Photosynthetic microorganisms of the White Sea supralittoral, *Dokl. Akad. Nauk*, 1985, vol. 5, pp. 66–72.
21. Tindall, B.J., Rossello-Mora, R., Busse, H.-J., Ludwig, W., and Kampfer, P., Notes on the characterization of prokaryote strains for taxonomic purposes, *Int. J. Syst. Evol. Microbiol.*, 2010, vol. 60, pp. 249–266.
22. Tank, M., Thiel, V., and Imhoff, J.F., Phylogenetic relationship of phototrophic purple sulfur bacteria according to *pufL* and *pufM* genes, *Int. Microbiol.*, 2009, vol. 12, pp. 175–185.
23. Davenport, C. and Ussery, D.W., Comparative genomics of green sulfur bacteria, *Photosynth. Res.*, 2010, vol. 104, pp. 137–152.
24. Gorlenko, V.M., Genus *Prosthecochloris*, in *Bergey's Manual of Systematic Bacteriology*, 2nd ed., Boone, D.R. and Castenholz, R.W., Eds., Berlin: Springer, 2001, vol. 1.
25. Bryant, D.A., Liu, Z., Li, T., Zhao, F., Garcia Costas, A.M., Klatt, C.G., Ward, D.M., Frigaard, N.U., and Overmann, J., Comparative and functional genomics of anoxygenic green bacteria from the taxa *Chlorobi, Chloroflexi* and *Acidobacteria*, in *Advances in Photosynthesis and Respiration. Functional Genomics and Evolution of Photosynthetic Systems*, Burnap, R.L. and Vermaas, W., Eds., Dordrecht: Springer, 2012, vol. 35, pp. 47–102.

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