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EXPERIMENTAL ARTICLES

Microbial Community of the Chemocline of the Meromictic Lake Shunet (Khakassia, Russia) during Summer Stratification

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Abstract—The spatio–temporal organization of the bacterial community inhabiting the chemocline of the stratified meromictic Lake Shunet (Khakassia, Russia) was investigated from May to September 2005 by means of microscopy, analysis of photosynthetic pigments, and PCR–DGGE with subsequent 16S rDNA analysis. The samples were collected with a multisyringe stratification sampler, sampling being performed every 5 cm. It was demonstrated that, during the period of investigation, there were no large changes in the bacterial community of the chlemocline, at least among the detected forms. During the whole period of study, purple sulfur bacteria related to *Lamprocystis purpurea* (*Chromatiaceae*) were predominant in the chemocline. Beneath the layer of purple bacteria, green sulfur bacteria were revealed that were phylogenetically distant from strain ShN*Pel*02, which was previously isolated from this lake. Development of phytoflagellates of the genus *Cryptomonas* was observed in the upper zone of the chemocline. In the chemocline of Lake Shunet, the numbers of picoplankton cyanobacteria of the genus *Synechococcus* increased from May to September. It was demonstrated that the application of universal bacterial primers for DGGE resulted in the same qualitative distributional pattern of predominant species as microscopic studies.

Key words: chemocline, meromictic lakes, microstratification, purple sulfur bacteria, green sulfur bacteria, phytoflagellates, cyanobacteria, PCR–DGGE.

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In the water column of meromictic lakes, ecological niches occupied by various groups of planktonic microorganisms, both autotrophic and heterotrophic, displaying different vertical depth distributions are formed due to the stable gradients of physicochemical characteristics. Usually, the most pronounced heterogeneity in the distribution of microbial populations is observed in the chemocline (i.e. at the interface between the aerobic and sulfide-containing horizons of the water column). The microbial communities inhabiting the chemocline zones of various meromictic lakes have received a great deal of attention [1–3]. In Lake Shunet (Khakassia, Russia), meromictic properties are most pronounced, resulting in the occurrence of sharp gradients of all physicochemical characteristics, as well as in the formation of dense stratified populations of anoxygenic phototrophic bacteria in the chemocline [4, 5]. Of all known lakes, Lake Shunet is second only to Lake Mahoney (Canada) in regard to the concentration of purple sulfur bacteria (PSBs) in the chemocline [5].

In some works, the microbial population of Lake Shunet was partially characterized by microscopic techniques, pigment analysis, and cultivation on selective nutrient media [4–6]. The methods applied in these works provide information concerning the numbers of phototrophic microorganisms, their seasonal dynamics, and the total microbial numbers. To determine the structure of microbial communities, methods for identification of the main predominant taxa without cultivation or preliminary identification are most suitable. For instance, analysis of 16S rRNA gene fragments by PCR and subsequent amplicon separation by denaturing gradient gel electrophoresis (DGGE) is used for determination and monitoring of the predominant species in natural communities [7].

The purpose of this work was to study the predominant forms of the microbial community inhabiting the chemocline of the highly stratified Lake Shunet by PCR–DGGE of 16S rDNA fragments, as well as to compare our results with the data on the physicochemical gradients of the water column and the results of microscopic observations.

MATERIALS AND METHODS

Lake Shunet (54.25.10′ N, 90.13.48′ E) is located 19 km from the Shira station (Khakassia, South Siberia, Russia) and 8 km southeast of Lake Shira in the Bei Buluk Valley. The lake (1.2 × 0.4 km) is oval, with a total area of 0.47 km^2; during the period of observations, the maximum depth was 6.2 m. The water is of

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sulfate–chloride–sodium–magnesium type. The lake is a closed basin; a small brook and, possibly, subsurface waters are the sources of fresh water. Mineralization of water in the mixolimnion ranges from 17 to 19 mg l^{-1}; in the monimolimnion, it increases linearly with depth and reaches 66 mg l^{-1} near the bottom. The anaerobic monimolimnion starts at a depth of about 5 m; sulfide content increases sharply with depth and reaches 450 mg l^{-1} in the near-bottom layers.

Sampling and the necessary measurements of the physicochemical water parameters were performed in the deepest (central) part of Lake Shunet (54.25.10 N, 90.13.48 E; GPS GARMIN Olathe, Kansas, United States) on May 25, July 27, and September 10, 2005. In addition, one water sample was collected from the chemocline of the meromictic Lake Shira located 8 km from Lake Shunet on July 29, 2005 [8]. Sampling and all measurements were preformed on a windless day at a wave height of less than 5 cm from a boat fixed in place with two anchors. The water samples from the chemocline were collected using a multisyringe stratification sampler equipped with a hydraulic control system; 15 samples (150 ml each) were collected simultaneously at 5-cm depth intervals [9]. For accurate targeting of the chemocline zone and for precise determination of its depth, a submerged Data-Sonde 4a multichannel probe (Hydrolab, Austin, Texas, United States) was rigidly mounted on the base frame of the sampler. The probe sensors, including the depth sensor, were positioned precisely at the bottom end of the sampler. Drastic changes in the redox potential served as an indicator of the location of the redox zone. The water samples for determination of sulfide content in the near-bottom layers were collected with a standard 0.5-l bathometer.

Physicochemical characterization. Prior to sampling, the depth profiles of temperature, turbidity, conductivity, redox potential, and dissolved oxygen were determined with the aid of a submerged multichannel probe Data-Sonde 4a (Hydrolab, Austin, Texas, United States). The dissolved oxygen content in the samples collected with the multisyringe sampler was determined with a titration Aquamerck test kit (Merck, Germany). The sulfide concentrations (up to 5 mg l^{-1}) were measured with a colorimetric Microquant test kit (Merck, Germany). At higher concentrations, the samples were fixed with basic zinc carbonate; then, sulfide concentrations were determined iodometrically [10]. The depth profile of underwater illumination was measured with an LI-193 spherical submerged sensor for photosynthetically active radiation (PAR) attached to an LI-COR 1400 recording device (LI-COR Ltd., Nevada, United States).

Microbial cell numbers. For counting bacterial cells, the water samples were fixed with 4% formalin (final concentration); for counting phytoflagellates, the samples were fixed with 1% glutaraldehyde. The counting of phototrophic anoxygenic bacteria and determination of the total numbers of bacteria were carried out on 0.2-μm black polycarbonate filters (Whatman, United Kingdom). Before filtration, the cells were stained with DAPI. For this purpose, 1 ml of the sample was supplemented with 20 μl of the DAPI solution (100 ng/μl), incubated in the dark for at least 5 min, and filtered; the filters were then examined under a microscope. The cells of green sulfur bacteria (GSB) were counted on the same filters under an MBI-11 microscope (LOMO, Russia) in the reflected light bright field mode at ×1045 magnification; in this case, the GSB cells appeared bluish-green or yellow-green [11].

The counting of PSB and the total numbers of microbial cells was carried out using an Axioskop 40 (Carl Zeiss, Germany) epifluorescence microscope equipped with a Zeiss 02 light filter at ×1000 magnification. The PSB cells were recognized by their shape, size, and pattern of aggregation [12]. During microscopic examination, a pure culture of the PSB strain *Thiocapsa* sp. Shira_1 (AJ633676 in the EMBL/GenBank) isolated from Lake Shira (this study) was used as a control for comparison of the morphological properties.

In all samples, at least 400 cells were enumerated; the results presented are average values ± mean-square error. The number of phytoplankton cells in DAPI-stained preparations was determined microscopically using a Zeiss 15 orange fluorescence filter set (Carl Zeiss, Germany). Picoplanktonic cyanobacteria presumably belonging to the genus Synechococcus were recognized by their size, shape [12], and bright orange autofluorescence.

Phytoflagellates were enumerated in a Fuchs–Rosenthal counting chamber. An MBI-11 microscope (LOMO, Russia) and a Zeiss Axioskop 40 fluorescence microscope (Carl Zeiss, Germany) were used for cell enumeration. Species identification was performed using fixed and living samples according to Kiselev [1].

Pigment analysis. The pigment analysis was performed only in July. Immediately after sampling, the samples were filtered under vacuum through 0.2-μm Nylon filters (BIOKHROM, Russia) to which a 1-mm layer of $BaCO_3$ was applied. The filtered samples were then dried at room temperature in the dark for 6–8 h, placed (together with $BaCO_3$) in penicillin flasks, and stored at −20°C prior to extraction. The extraction was carried out in 5 ml of 90% acetone at 4°C for 24 h [14]. The obtained extracts were then centrifuged at 10000 rpm for 10 min; the supernatant was used for obtaining absorption spectra in the wavelength range from 350 to 900 nm. Bacteriochlorophylls *a* and *d* were identified by absorption peaks at 772 and 654 nm, respectively [15]. Chlorophyll *a* was identified by an absorption peak at 663 nm [15]. The content of the pigments was calculated according to the following extinction values:

$$K_{663}^{\text{Chl } a} = 87.67 \text{ l g}^{-1}\text{cm}^{-1} \text{ [15]},$$

$$K_{772}^{\text{Bchl } a} = 92.3\ \text{l g}^{-1}\text{cm}^{-1}\ [15],$$

$$K_{654.5}^{\text{Bchl } d+e} = 98\ \text{l g}^{-1}\text{cm}^{-1}\ [16].$$

All optical density values were adjusted considering the light diffusion at 850 nm. An absorption peak between 663 and 654 nm was observed when similar concentrations of Chl *a* and Bchl *d* were present simultaneously. In this case, we failed to determine precisely the concentrations of both pigments; the concentrations were therefore calculated using the system of linear equations described in [15]. The extinction coefficient for Chl *a* at 654 nm (56 l g $^{-1}$ cm^{-1}) was calculated from the absorption spectrum of an extract from the green alga *Chlorella vulgaris*. The extinction coefficient for Bchl *d* at 663 nm (62.7 l g $^{-1}$ cm^{-1}) was calculated from the absorption spectrum of an enrichment culture of green sulfur bacteria from Lake Shunet.

Isolation and cultivation. To obtain enrichment cultures of purple sulfur bacteria, we used a water sample collected from Lake Shira in July 2000 from a depth of 13 m. Isolation, purification, and cultivation of the PSB culture were carried out according to Pfennig [17].

Isolation of the total bacterial DNA. Samples for DNA analysis were collected into sterile flasks and fixed with sterile formalin (at the final concentration of 4%). Genomic DNA was isolated from the bacterial communities of the chemocline using the technique described in [18].

PCR amplification of the 16S rRNA gene fragments (586 bp) from the total DNA of bacterioplankton was carried out using the universal GC341F and 907R primes [19]. A negative control using sterile water as a template was used to monitor contamination. Analysis of the PCR products was carried out by electrophoresis in 1.2% agarose gel. The PCR products were extracted with chloroform and precipitated. The obtained DNA (800 ng) was analyzed by denaturing gradient gel electrophoresis (DGGE).

Denaturing gradient gel electrophoresis was carried out in 6% polyacrylamide gel with a denaturing gradient from 30% (40%) to 70% (100% denaturing gradient is a mixture of a 7 M urea solution and 40% deionized formamide) using a DCode Universal Mutation Detection System (BioRad, United States). Electrophoresis was carried out at 60°C in TAE buffer at 100 V for 17 h. The obtained gel slabs were stained with ethidium bromide and recorded with an AlphaImager Workstation for Gel Documentation and Fluorescent Imaging (Alpha Innotech Corp., United States) in UV light (302 nm). DNA bands were excised from the gel slabs, and the obtained DNA was eluted and amplified.

DNA sequencing and analysis. Analysis of the obtained nucleotide sequences of DGGE fragments was performed in the Interinstitute Center for DNA Sequencing (Siberian Branch, Russian Academy of Sciences, Novosibirsk, http://sequest.niboch.nsc.ru). The obtained nucleotide sequences of the 16S rDNA fragments were aligned using the ClustalX software package. [20]. The rootless phylogenetic tree was constructed using the algorithms implemented in the TREECON software package [21]. The significance of the branching order was determined by bootstrap analysis of 100 alternative trees.

RESULTS

Physicochemical characterization. Throughout the whole period of sampling, Lake Shunet was pronouncedly stratified. The chemocline zone, which is understood here as the interface between aerobic anaerobic conditions, was located at the same depth (4.91–5.1 m) during the entire period of study (Fig. 1). In the water column of the lake, in the chemocline, a suspended horizontal high-turbidity layer of bright purple color was detected (Fig. 1). Above the "purple horizon," sulfide was not detected, whereas it was present in trace amounts in the purple horizon. Below this layer, the sulfide concentration gradually increased downward in the water column, reaching 450 mg/l near the bottom.

Phototrophic anoxygenic bacteria. On all sampling dates, the vertical distribution profile of purple sulfur bacteria (PSB) exhibited a well-pronounced peak in the chemocline (Fig. 1); its depth was the same as that of the purple horizon. In the chemocline, the predominant PSB morphotype was similar in shape, size, and type of aggregation to the species previously described by Lunina et al. [5] and related to *Lamprocystis purpurea*, as well as to our isolate, strain *Thiocapsa* sp. Shira_1 (AJ633676 in EMBL/GenBank).

The highest number of PSB visible in reflected light, approximately $(1.8 \pm 0.4) \times 10^8$ cells/ml, were observed in July. During this time, the color of the purple horizon was the most intense. In May and September, the number of these microorganisms in the purple horizon was about 4×10^7 cells/ml.

The vertical distribution profile of green sulfur bacteria (GSB) also exhibited a peak in the chemocline (Fig. 1). In May and September, the highest number of GSB was detected 5 cm deeper than the highest number of PSB (in the neighboring syringe of the sampler); in July, is was observed in the purple horizon. The highest PSB concentration in May was approximately 4.6×10^6 cells/ml; in July and August it was about 8×10^6 cells/ml.

Total bacterial counts. During the whole period of sampling, the number of DAPI-stained cells (not including PSB) varied from 2×10^8 to 4×10^8 cells/ml. In the depth ranges under study, the total number of bacterial cells was higher above the purple horizon; it decreased within the purple horizon, formed a local minimum below this layer, and then increased again with depth (Fig. 1).

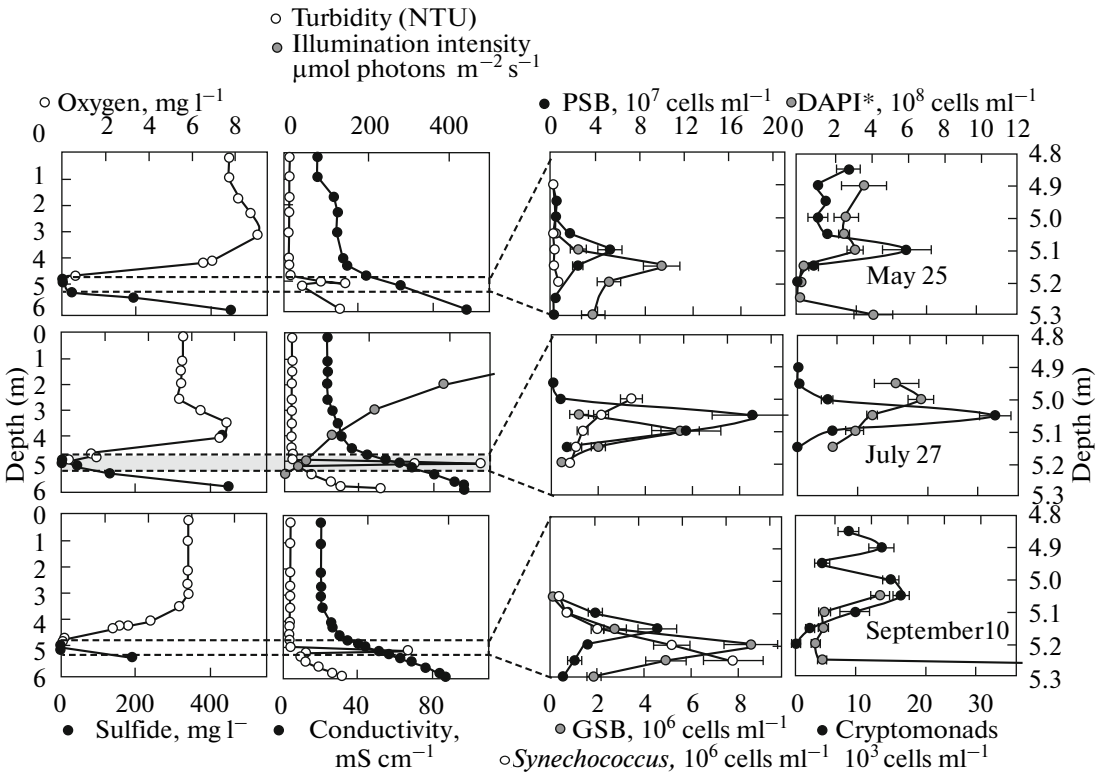

Fig. 1. Vertical distribution of the physicochemical characteristics in the water column of Lake Shunet and of the number of microorganisms in the Lake Shunet chemocline in 2005. The total number of bacterial cells (DAPI) does not include the number of PSB cells.

Cyanobacteria. In all samples, the picoplankton form of cyanobacteria were detected. These microorganisms presumably belonged to the genus *Synechococcus* and were represented by small (less than 0.5 μm in diameter) spherical cells appearing in pairs or separately and brightly fluorescing in the orange spectral region under green illumination (see Materials and Methods). In May, the number of cyanobacteria was relatively low and the distribution of these microorganisms in the chemocline was almost uniform (Fig. 1). In July, the total number of bacterial cells increased and a tendency toward a decrease in the cell number was observed with depth. In September, the total number of cells increased even more and the cell concentration increased with depth (Fig. 1).

Cryptomonads. During the whole period of study, a dense population of cryptophytic phytoflagellates (*Cryptophyta*; *Cryptomonadaceae*) represented by the species *Chroomonas* sp., *Rhodomonas salina, Proteomonas sulcata, Pyrenomonas helgolandii*, and unidentified cryptomonads) was observed in the chemocline of Lake Shunet.

In July, the highest numbers of cryptophytic phytoflagellates were observed in the tenth layer in the upper part of the chemocline. In spring and autumn, the distribution curves of cryptomonads did not exhibit any statistically significant maximum, and uniform distribution of the population was observed in a 30-cm horizon above the chemocline. In July and May, the localization of the highest population density of cryptomonads coincided with that of purple sulfur bacteria (purple horizon), while in September cryptomonads were localized above the purple horizon (Fig. 1).

Pigments of phototrophic microorganisms. In the absorption spectra of the acetone extracts of all samples, a specific absorption peak at 488 nm with a shoulder at 504 nm was detected (Fig. 2). A similar peak was detected in the spectrum of the pure culture of *Thiocapsa* sp. Shira_1 (Fig. 2). The main absorption peak of Bchl *a* at 772 nm was detected only as a trace; thus, we failed to determine the Bchl *a* concentration.

In July, absorption peaks at 430 and 654 nm, typical of the spectra of green sulfur bacteria from Lake Shunet [5], were detected below the purple horizon (Fig. 2). In July, the concentration of Bchl *d* at the highest numbers of GSB, i.e., 5 cm below the purple horizon, was 1440 μg/l, which corresponds to 5 × 10^6 cells/ml [5]. In May, the absorption peak of Bchl *d* in the purple horizon (depth 5.1 m) shifted to the long-wavelength spectral region (659 nm), which indicated the presence of Chl *a* (see Discussion). The concentrations of Chl *a* and Bchl *d* were 89 and 81 μg/l, respectively.

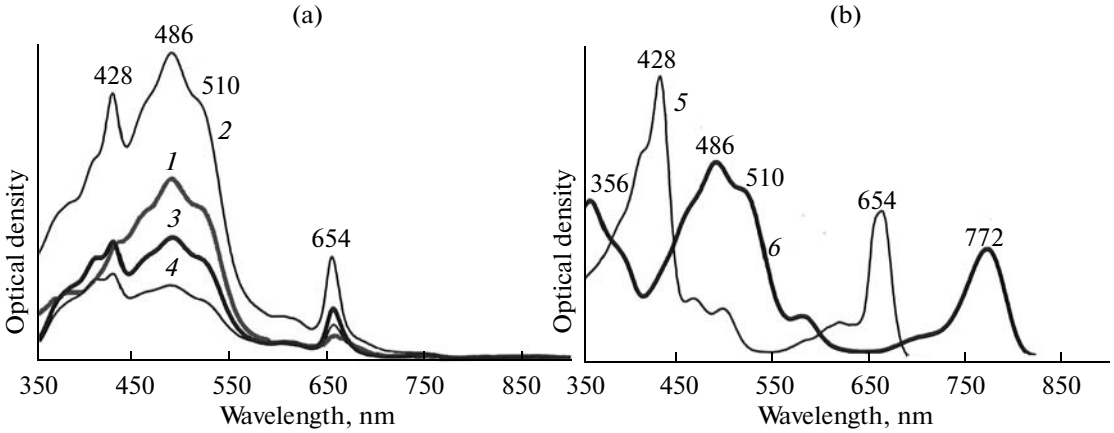

Fig. 2. Absorption spectra of the acetone extracts of the pigments of photosynthetic microorganisms from (a) the water samples collected from the chemocline of Lake Shunet in July 2005 and (b) the cultures of anoxygenic phototrophic bacteria: 5.05 m depth (*1*), 5.1 m depth (*2*), 5.15 m depth (*3*), and 5.2 m depth (*4*); enrichment culture of unidentified green sulfur bacteria from Lake Shunet (*5*); purple sulfur bacterium *Thiocapsa* sp. Shira_1 (*6*).

The component structure of the bacterial community determined using the DGGE profiles. All the described groups of microorganisms, including PSB, GSB, cryptomonads, and cyanobacteria, were detected by DGGE analysis (Fig. 3). The bands corresponding to the chloroplasts of cryptophytic algae (Shunet 2005-A in Figs. 3–5) were present in the samples collected from the microaerophilic and aerobic zones. In May, these bands were detected in the upper part of the anaerobic zone as well; however, during the whole period of study, they were not detected in the deep anaerobic zone (Fig. 3). Green sulfur bacteria were detected in DGGE profiles during the entire period of sampling and usually occurred in the anaerobic zone (Shunet 2005-C; Figs. 3 and 5). These bacteria were found to be phylogenetically close to *Prosthecochloris* sp. (Fig. 5). Pronounced bands of cyanobacterial species phylogenetically close to the genus *Synechococcus* (Shunet 2005-F; Figs. 3–5) were obtained only from the samples collected in September; they were detected in the chemocline at all depths (Figs. 3 and 4). Very faint bands of these organisms were obtained from the samples collected from the upper part of the chemocline in July. Every lane contained bands corresponding to *Gammaproteobacteria* phylogenetically related to *Halomonas* sp. (Shunet 2005-G) and *Pseudoalteromonas* sp. (Shunet 2005-E) (Figs. 3–5). The samples collected from the chemocline of the meromictic Lake Shira yielded bands of the same organisms (Fig. 2). The samples collected from the anaerobic zones of both lakes during the entire period of sampling yielded faint lanes of *Deltaproteobacteria* (Shunet 2005-B) related to uncultured bacteria from the active sludge of waste treatment plants (Figs. 3 and 5).

In a gel slab obtained using the denaturing gradient from 30 to 70%, no PSB bands were detected; however, these bands were revealed with the denaturing gradient from 40 to 70% (Fig. 4). Figure 4 shows that all samples, except for one, yielded a band coinciding with that of the pure culture of the purple sulfur bacterium *Thiocapsa* sp. Shira_1. The morphology and pigment composition of this strain are most similar to those of *Lamprocystis purpurea* [5]; phylogenetically, it is most closely related to the species of the genus *Thiocapsa* (approximately 97% similarity; Fig. 4).

DISCUSSION

Both microscopic examination and genetic analysis demonstrated that no significant changes occurred in the bacterial community of the chemocline from May to September, at least among the forms identified in the present work. This conclusion is true for both the numbers and the vertical distribution profiles. The only group of microorganisms for which a spatial dynamics was demonstrated during the entire period of study were cyanobacteria of the genus *Synechococcus* (Figs. 1 and 3), which were detected in the DGGE profile only in September, when their numbers in the chemocline increased significantly, probably due to precipitation from the upper horizons (Fig. 1). The development of cyanobacteria of the genus *Synechococcus* near the chemocline is typical for meromictic lakes [22].

Only one PSB species prevailing in the chemocline of Lake Shunet was detected by PCR–DGGE. Judging from the coinciding bands in the DGGE profile, the same species was isolated from the chemocline of Lake Shira on selective nutrient media. Judging from its morphological properties, this was also the pre-

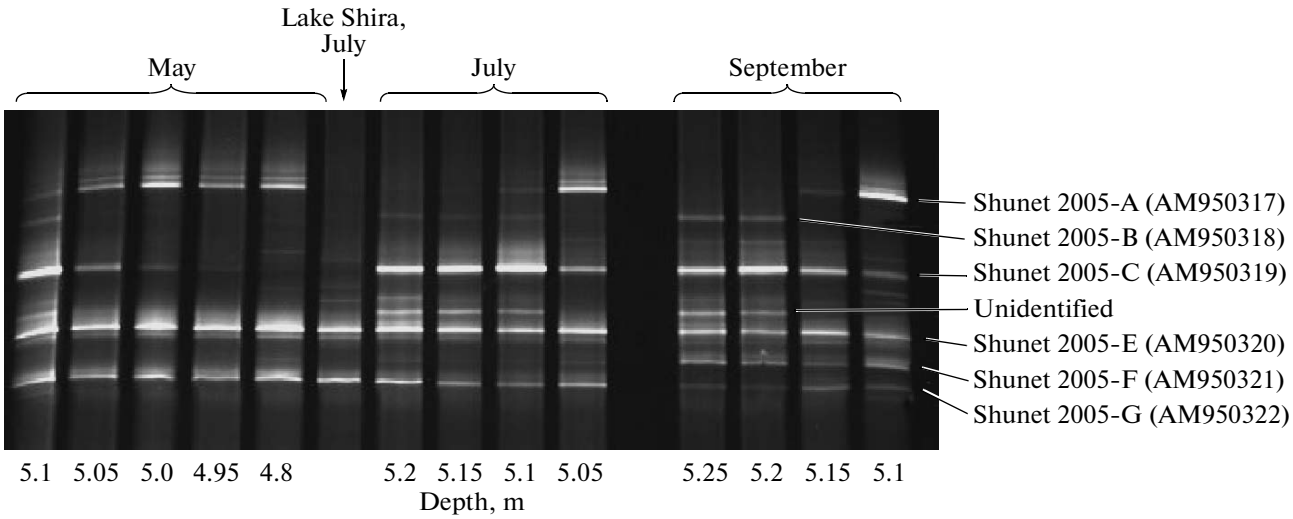

Fig. 3. PCR-DGGE profile of the bacterial community inhabiting the chemocline of Lakes Shira and Shunet obtained with the use of the 30–70% gradient.

dominant species in the water samples collected from the purple horizon of Lake Shunet. PSB strains almost identical to this one were previously isolated by Lunina et al. [5] from Lakes Shira and Shunet (Fig. 5). Hence, comparison of the results of PCR–DGGE with the results of direct microscopic observations, pigment analysis, and cultivation indicates that the predominant PSB species isolated from Lake Shunet was adequately identified by PCR–DGGE.

The only GSB species (phylotype) identified in this work by PCR–DGGE differed from the strain previously isolated in pure culture by Lunina (97% similarity; Fig. 5). A similar incongruity was described by Casamayor et al. for two Spanish stratified lakes [3]; in that case, the GSB sequences obtained from DGGE profiles differed from those of the species previously isolated in pure culture. Nevertheless, absorption peaks (Fig. 2) similar to those of the pure culture isolated by Lunina et al. (strain ShN*Pel*02) and to those of the cultures isolated from Lake Shunet in 2003 [5] and typical of green sulfur bacteria were observed in the absorption spectra of the water below the purple layer. Therefore, the predominant GSB populations detected in both years were similar in their pigment compositions; they were similar to strain ShN*Pel*02 as well. The vertical distribution of the GSB numbers, exhibiting a pronounced peak below the purple horizon, was confirmed by the results of DGGE analysis (Fig. 3).

According to the published data, the number of purple sulfur bacteria exceeding 10^8 cells/ml^{-1} is extremely high and, apart from Lake Shunet, was detected only in Lake Mahoney (Canada) [2]. In the chemocline of this lake, the illumination intensity and the sulfide flux are as favorable as in Lake Shunet; this obviously promotes PSB development, and the sharp density gradient ensures a hydrophysical stability that promotes accumulation of the PSB biomass in a narrow layer. However, mass development of green sulfur bacteria was not detected in Lake Mahoney [2], which probably results from the weakly alkaline reaction of its water, which is not optimal for this group of microorganisms [7].

The presence of a large population of cryptophytic algae of the genus *Cryptomonas* (*Eukaryota, Cryptophyta, Cryptomonadaceae*) near the chemocline is a characteristic trait of many meromictic lakes; the highest numbers of these microorganisms are usually detected in the zones where purple sulfur bacteria develop [23]. In meromictic lakes, the chloroplasts of cryptomonads are often detected in the DGGE profiles obtained with bacterial primers [3].

The fact that the bands of *Synechococcus* were obtained only from the September profiles and were absent from the profiles obtained in May and July (Fig. 3) allows us to assess the DGGE sensitivity threshold. In September, the proportion of *Synechococcus* cells exceeded 3% of the total bacterial counts; however, in May and July, it was about 0.01 and 0.3%, respectively. Hence, in our case, the sensitivity threshold for *Synechococcus* was several percent, which corresponds to the data obtained by other authors on the 1% sensitivity of DGGE [7]. It should be noted that, in our work, this was not the case for green sulfur bacteria. The GSB bands could be clearly seen in the DGGE profiles of the relevant samples, despite the fact that the ratio of GSB never exceeded 1% (Fig. 1). The most plausible explanation of this phenomenon is that the numbers of green sulfur bacteria determined microscopically under reflected light were underestimated. It should be noted that the numbers of cryptomonads in all samples did not exceed tenths of a per-

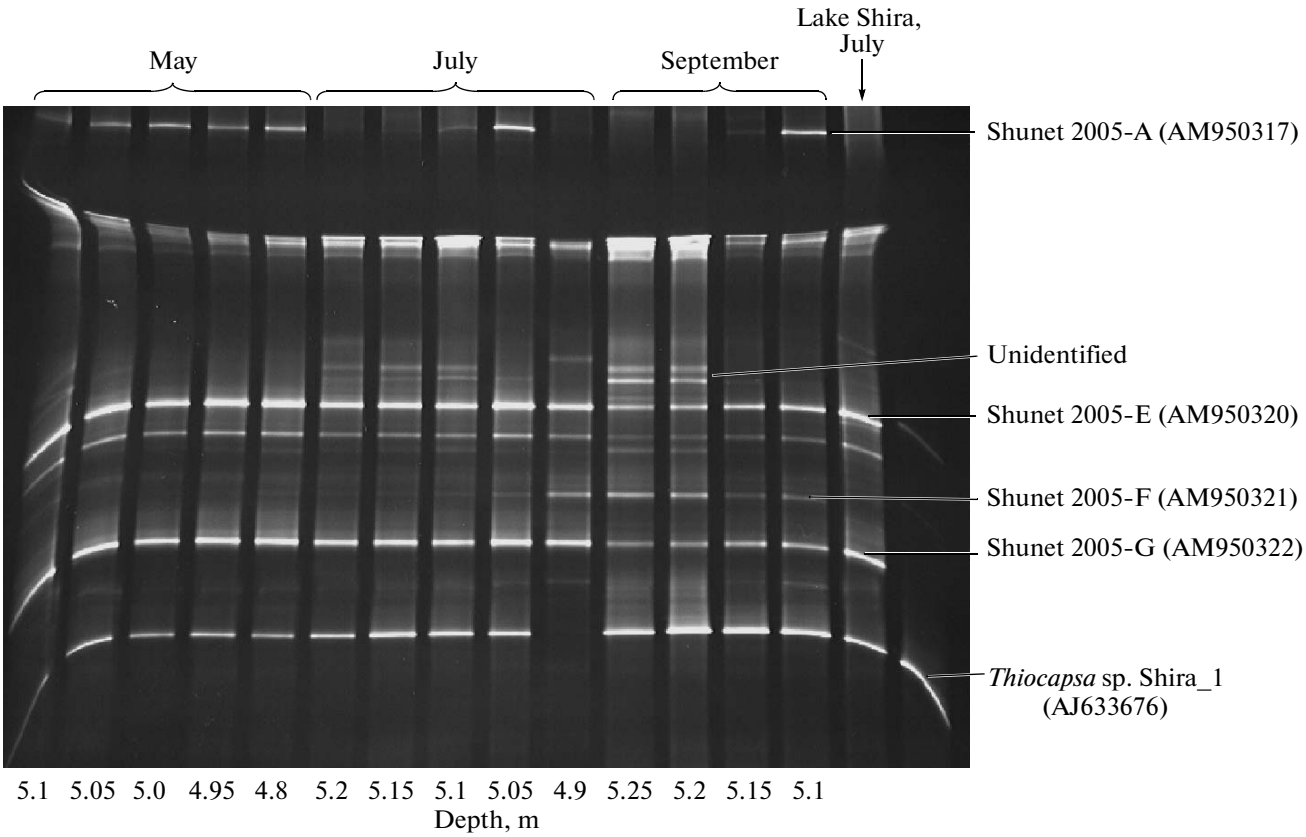

Fig. 4. PCR-DGGE profile of the bacterial community inhabiting the chemocline of Lakes Shira and Shunet obtained with the use of the 40–70% gradient .

cent and, in most cases, thousandths of a percent of the total bacterial counts (Fig. 1). Since one cell of cryptomonads contains two chloroplasts, the fact that the bands corresponding to *Cryptomonas* chloroplasts were clearly seen in the DGGE profile (Fig. 3) contradicts the general principle of the sensitivity threshold of this technique. However, similar results were obtained in other studies, for instance, in Lake Ciso (Spain) [3].

The absence of characteristic bands (Fig. 3) indicates that, in the chemocline of neighboring Lake Shira, green sulfur bacteria, cryptomonads, and cyanobacteria did not develop in large numbers, which correlates with the results obtained by direct methods (the figures do not show the data obtained). The PSB band was present in the sample from the chemocline of Lake Shira (Fig. 4), which corresponds to the result of microscopic observations; the number of PSB in this sample was 2.5×10^6 cells ml^{-1}.

In the DGGE profiles from lakes Shunet and Shira, the same heterotrophic bacteria prevailed; however, there are not enough data to make a conclusion regarding the similarity between the predominant species. The representatives of both genera, *Halomonas* and *Pseudoalteromonas* (bands E and G; Fig. 3), are, for the most part, halotolerant aerobic microorganisms capable of nitrate respiration [12].

The application of denaturing gradient gel electrophoresis for detection of objects that are visible to an unaided eye, such as PSB, *Synechococcus*, and cryptomonads, revealed that this method, coupled with the use of universal bacterial primers, yields the same results regarding the distribution patterns of the predominant species as microscopic observations and, therefore, can be used for monitoring of the dynamics of the microbial communities of brackish meromictic lakes, including Lakes Shunet and Shira (Khakassia).

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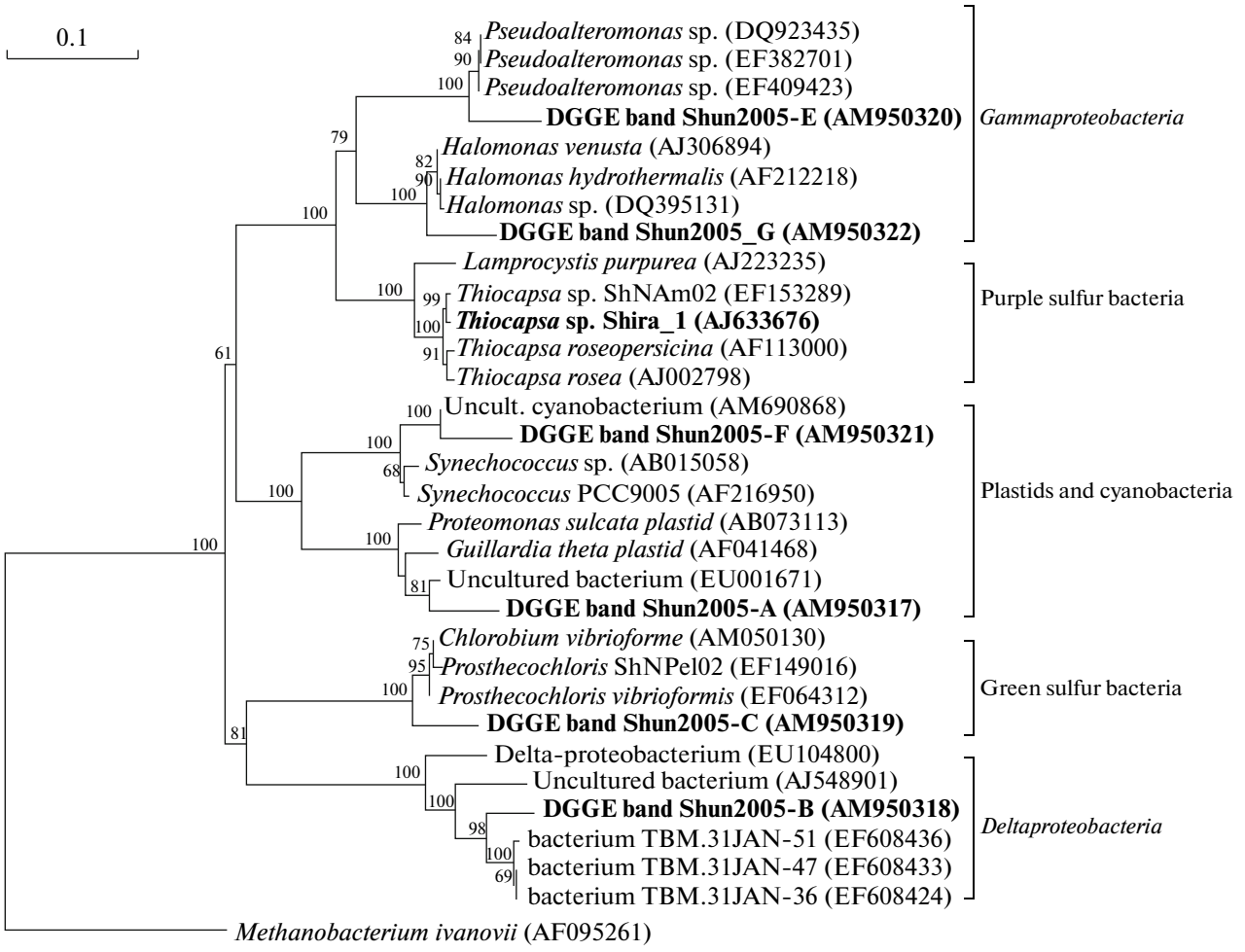

Fig. 5. Unrooted phylogenetic tree of strain *Thiocapsa* Shira_1 and of the nucleotide sequences of the chemocline of Lake Shunet obtained by PCR-DGGE (set out in bold). The numerals show the bootstrap values above 65%.

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