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EXPERIMENTAL ARTICLES

Application of Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase Genes as Molecular Markers for Assessment of the Diversity of Autotrophic Microbial Communities Inhabiting the Upper Sediment Horizons of the Saline and Soda Lakes of the Kulunda Steppe

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Abstract—The genes encoding the key metabolic reactions are often used as functional markers for phylogenetic analysis and microbial ecology studies. The composition and structure of the genes encoding ribulose-1,5-bisphosphate carboxylase (RuBisCO) of various photoautotrophic bacteria, representatives of the order *Chromatiales*, including collection strains and the strains isolated from saline and soda lakes, were studied in detail. The green-like form I RuBisCO was detected in the majority of the studied strains. In some strains, the genes encoding both form I and form II RuBisCO were present, which has not been previously known for the representatives of this group of bacteria. Moreover, RuBisCO genes were used as functional markers to investigate the autotrophic microbial community inhabiting the upper horizons of bottom sediments of two saline soda lakes and two hypersaline neutral lakes of the Kulunda Steppe. In general, the diversity of autotrophic bacteria in the studied sediment horizons was low. In soda lakes, haloalkaliphilic cyanobacteria and sulfur-oxidizing bacteria (SOB) of the genus *Halorhodospira* were predominant. In saline lakes, halophilic chemoautotrophic SOB *Halothiobacillus* and *Thioalkalivibrio* were found, as well as photoautotrophic bacteria of the genus *Ectothiorhodosinus* and cyanobacteria. Many phylotypes remained unidentified, which indicates the presence of groups of microorganisms with an unknown type of metabolism.

Keywords: Chromatiaceae, RuBisCO, *cbbL/M* genes, saline and soda lakes, autotrophic microbial community.

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The diversity of prokaryotes in hypersaline habitats, including intracontinental saline lakes, has traditionally been considered small, with the predominance of heterotrophic haloarchaea. The main cause of this situation is possibly the lack of attention to sediments. Recent studies of the sediments of hypersaline habitats showed that moderately and extremely halophilic bacteria, including autotrophic microorganisms, are permanent components of prokaryotic communities even in saturated brines [1]. In particular, chemolithoautotrophic sulfur-oxidizing bacteria (SOB) with cultivable forms recently described as several new species- and genus-level taxa of the *Gammaproteobacteria*, including two novel genera of extremely halophilic SOB, play an important role in these communities [2, 3].

Soda lakes represent a unique type of saline lakes characterized by the predominance of sodium carbonates and bicarbonates, which results in stable high pH values (9.0–11.0) in these ecosystems. The resultant unique conditions, while prohibitive for the development of eukaryotes, are optimal for the development of sodaphilic (natronophilic) prokaryotic communities. Autotrophic bacteria, including both primary producers (oxygenic and anoxygenic phototrophs) and chemolithoautotrophs, such as SOB, nitrifiers, and gasotrophic (CH_4/H_2/CO) bacteria, play an important role in these communities. Although the diversity of prokaryotic communities inhabiting soda lakes has received intense scientific attention, the results obtained describe mainly the cultivable representatives of these communities, including new taxa of photo- and chemoautotrophic bacteria [4, 5].

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The study of the phylogenetic diversity of microbial communities inhabiting the soda Mono Lake [6] and Wadi al Natrun [7], which used the most widespread method of the 16S rRNA gene sequencing, made it possible to confirm and expand the results of the previous microbiological studies by adding new data on the high diversity of the bacterial communities of these lakes. This method, however, often fails to reveal the minor constituents of the prokaryotic community, which may still play an important physiological role in the community. These microorganisms include chemolithoautotrophs, whose population density is lower than that of heterotrophs due to a low biomass yield. The use of the genes encoding the key enzymes responsible for various metabolic processes as molecular markers for detection of these ecotypes can be more productive.

For the majority of autotrophic prokaryotes, ribulose-1,5-bisphosphate carboxylase (RuBisCO), the key enzyme responsible for carbon dioxide fixation via the Calvin cycle, can be used as a molecular marker. Among photoautotrophic bacteria, this pathway of CO_2 fixation is typical of most proteobacteria, cyanobacteria, and some filamentous green nonsulfur bacteria. The bacterial RuBisCO exists in two main forms. Form I is the most widespread and is present in the cells of most autotrophic bacteria, algae, and terrestrial plants. It consists of eight large (L) and eight small (S) subunits, encoded by the *cbbL* and *cbbS* genes, respectively. Only the large subunits have a catalytic function. Form I RuBisCO is subdivided into two types, green-like and red-like, which differ in the amino acid composition of their large subunits. The green-like RuBisCOs are present in the chloroplasts of terrestrial plants, green algae, cyanobacteria, and representatives of the *Alpha-*, *Beta-* and *Gammaproteobacteria*. Among phototrophic bacteria, this form of RuBisCO is present in oxygenic cyanobacteria, anoxygenic purple nonsulfur *Alphaproteobacteria*, and purple sulfur *Gammaproteobacteria*. Thus far, the red-like RuBisCOs have been found in most nongreen algae and some *Gamma-* and *Betaproteobacteria*, including such phototrophs as some purple nonsulfur bacteria, as well as in filamentous green nonsulfur bacteria of the family *Oscillochloridaceae*. Form II RuBisCO (*cbbM* gene) occurs much more rarely and consists of only large subunits (L_n), from two to eight, depending on the organism. Among phototrophic bacteria, this form of RuBisCO can be found in some representatives of purple nonsulfur *Alpha-* and *Betaproteobacteria*. Moreover, the genomes of some studied photoautotrophic bacteria contain several RuBisCO genes in various combinations.

Due to their length and conservatism of structure, RuBisCO genes may be efficiently used in phylogenetic and molecular biological studies. RuBisCO genes have frequently been used as molecular markers in order to assess the microbial diversity of marine habitats; however, the data on the diversity of microorganisms in terrestrial hypersaline habitats are scarce. In particular, analysis of the water column of the saline alkaline Mono Lake [8] and the bottom sediments of the saline and soda lakes of the Kulunda Steppe [9, 10] revealed the predominance of chemoautotrophic halo(alkali)philic *Gammaproteobacteria* in these communities.

The aim of the present work was to study the RuBisCO phylogeny of photoautotrophic bacteria, including the previously unstudied collection strains and the novel strains isolated from soda lakes, as well as to assess the diversity of autotrophic prokaryotes inhabiting the upper horizons of the bottom sediments of soda and saline lakes, favorable for phototrophic bacteria, using RuBisCO genes as molecular markers.

MATERIALS AND METHODS

The subjects of study were microbial strains from the culture collections of the Department of Microbiology, Moscow State University, as well as the recently isolated strains (including those isolated from soda lakes) of purple sulfur bacteria from the culture collection of the Winogradsky Institute of Microbiology, Russian Academy of Sciences (Table 1).

Strains *Thioalkalicoccus limnaeus* A-26 and A-31 were isolated from microbial mats forming a thin (0.2–0.5 cm) layer in the near-shore waters of the soda Lakes Verkhnee Beloe and Tsaidam (Buryatia, Russia), respectively. The pH of the lakes was the same (10.1); the total mineralization of Lakes Verkhnee Beloe and Tsaidam was 7.5 and 15.8 g/l, respectively.

Thioalkalicoccus sp. B7-1 was isolated from a sample of bottom (deep) silt sediments of the stratified weakly saline soda Lake Doroninskoe located 125 km west of Chita (Russia). The total mineralization of the lake water near the bottom was 26–32 g/l; pH of the water was 9.57–9.75.

The novel strain *Thiorhodospira sibirica* B8-1 was isolated in 2009 from a combined sample collected from the soda lake Zun Torei (pH 9.5; mineralization, 7.2 g/l) (Transbaikal Region, Russia).

Strain ShNLb02 was isolated in 2002 from a water sample collected in the water column of the saline meromictic Lake Shunet (Khakassia, Russia) at a depth of 4.5 m. Mineralization of the lake water near the bottom was 15–65 g/l (less than 40 g/l at the sampling depth), pH 7.6. Strain Sivash was isolated from a sample collected in the saline Lake Sivash (Russia) with a water mineralization of 40 g/l and pH 7.8.

Strain Mg3 was isolated in 2010 from a combined sample collected from the soda Lake Davsan-Nur (Mongolia) The lake water has a total mineralization of 150 g/l, pH 9.94.

In addition, samples from the surface horizons of silt sediments of the hypersaline lakes of the Kulunda Steppe collected during the summer season of 2009 were studied as well (Table 2).

Table 1. Studied strains of photoautotrophic microorganisms (pure cultures)

Order	Species	Strain	Collection/isolation source
Chromatiales	*Thiocapsa* sp. (*Thiocystis violaceae*)	1R	Department of Microbiology, Moscow State University
	Thioalkalicoccus limnaeus	A-26^T	Soda lakes (Southeastern Siberia, Russia)
		A-31	
	Thioalkalicoccus sp.	B7-1	Lake Doroninskoe (Transbaikal region)
	Lamprobacter modestohalophilus	ShNLb02	Lake Shunet (Khakassia)
		Sivash^{nT}	Lake Sivash (Crimea)
	Thiorhodococcus mannitoliphagus	WS^T	Estuary of the White Sea (Poyakonda settlement, Murmansk oblast)
	Thiorhodospira sibirica	B8-1	Lake Zun Torei (Transbaikal region)
	Halorhodospira halophila	Mg3	Lake Davsan-Nur (Mongolia)
Rhodospirillales	*Phaeospirillum fulvum*	5K	Department of Microbiology, Moscow State University
Rhizobiales	*Rhodoblastus acidophilus*	5–6	
	Rhodomicrobium vannielii	K-1	
Rhodobacterales	*Rhodovulum sulfidophilum*	DSM 1374^T Rp-6	
Burkholderiales	*Rubrivivax gelatinosus*	DSM 149	

In 2009, Lake Gorchina-1 (4KL-09) contained saturated soda brine covered with a thick trona. The bottom sediments were represented by black silt with high concentrations of sulfides (up to 3 mM of free sulfide in the pore brine). The sediments were covered with a visible layer consisting of phototrophic microorganisms, presumably cyanobacteria and green algae of the genus *Dunaliella*, as well as of purple sulfur bacteria; however, their numbers were much lower than in the previous years, when the lake was less dry.

Lake Tanatar-5 (6KL-09) is a typical soda lake with moderate water mineralization (usually about 70 g/l). However, its mineralization increased sharply in 2009. The bottom sediments are represented by gray clays containing up to 2.5 mM of free sulfide; the brine is turbid due to the presence of clay particles. On the surface of the sediments, macroaggregates consisting of phototrophic microorganisms (primarily cyanobacteria and algae) can be visualized.

Table 2. Characteristics of the habitats of the studied microbial communities

Sample no.	Lake	pH	Salinity, g/l	Alkalinity, M	
				Na_2CO_3	Total
Hypersaline lakes with high pH					
4 KL-09	Gorchina-1	10.16	300	4.50	4.70
6 KL-09	Tanatar-5	10.1	180	1.40	1.60
Hypersaline lakes with neutral pH					
2 KL-09	Petukhovo	7.7	300	–	–
8 KL-09	Lomovoe	7.8	325	–	–

The hypersaline Lakes Lomovoe (8KL-09) and Petukhovo (2KL-09) are typical saturated chloride–sulfate brines with neutral pH and pink coloration due to the abundance of haloarchaea (*Haloquadratum*, judging from their morphology). The bottom sediments of the lakes are black, clay-sand, and covered with a salt crust containing acid-soluble sulfides in concentrations up to 1 mM (Petukhovo) and 17 mM (Lomovoe). Phototrophs were present in the form of very fine layers and films immediately below the salt crust. They are represented mainly by cyanobacteria and *Dunaliella*.

The samples were stored at 4°C until use.

DNA extraction. DNA was extracted from pure cultures using the Ultra Clean Microbial DNA Isolation Kit (MoBio, United States) according to the manufacturer's protocol.

DNA was extracted from the sediments of the studied soda lakes using the MoBio Power Soil DNA Isolation Kit (MoBio Laboratories). To extract DNA, portions of the sediments (about 10 g) were placed in plastic Falcon tubes and supplemented with 1 M NaCl to a volume of 50 ml. To precipitate sand and other large silt particles, the resultant sediment suspension was centrifuged at low speed for several minutes. The supernatant was then transferred into fresh tubes and recentrifuged. In order to obtain the colloidal fraction, some specimens were centrifuged two or three times, depending of the type of sediments. Portions (2 ml) of the obtained colloidal fraction were used for DNA extraction.

Amplification of the *cbbL/M* genes from the DNAs of pure cultures and sediment samples. Amplification of the *cbbL* genes encoding the green-like RuBisCO of pure cultures was carried out as described in [10]. The green-like RuBisCO genes from the soda lake sediments were amplified using the RubIgF/RubIgR primers [11]. The red-like RuBisCO gene fragments were amplified using the IC537f/IC1212r primers [12]. The *cbbM* genes were amplified using the RuII331f/RuIIR2 primers [16], as well as the primers described by Alfreider et al. in [13].

Analysis of the PCR products was carried out by electrophoresis in 1.0% agarose gel stained with ethidium bromide (0.5 μg/ml). PCR fragments of the expected sizes were isolated and purified using the Wizard PCR Preps Kit (Promega, United States). The purified fragments were sequenced directly (pure cultures) or cloned (soda lake sediments).

Cloning. Cloning was carried out using the CloneJet PCR Cloning kit (Fermentas) and the plasmid pJET1.2/blunt according to the manufacturer's protocol. From each clone library, 50 clones were randomly chosen for analysis. To determine the clones containing an insert, direct PCR using specific primers was carried out. The inserts were detected by electrophoresis in 1.0% agarose gel stained with ethidium bromide (0.5 μg/ml). The clones containing the target insert were sequenced and used for further analyses.

Phylogenetic analysis. The nucleotide sequences of the studied genes were edited using the BioEdit software [http://jwbrown.mbio.ncsu.edu/BioEdit/bioediTca.html]. Primary comparison of the de novo-obtained sequences with the sequences within the GenBank database was performed using the BLAST NCBI software package [http://www.ncbi.nlm.nih.gov/blast]. The 16S rRNA and *cbbL–cbbM* gene sequences from the GenBank database were used for subsequent comparative analysis. The nucleotide sequences and the deduced amino acid sequences of the studied genes were aligned with the appropriate sequences from the closest relatives using CLUSTALX 2.0 software package [http://bips.u-strasbg.fr/fr/Documentation/ClustalX/]. The phylogenetic trees were constructed by the methods implemented in the TREECONW software package [http://bioc-www.uia.ac.be/u/yvdp/treeconw.html]. The significance of the branching order (%) was determined by bootstrap analysis of 1000 alternative trees.

Deposition of the nucleotide sequences. The 16S rRNA and *cbbL–cbbM* gene fragments obtained in this work were deposited in the GenBank under accession numbers HQ877032–HQ877096.

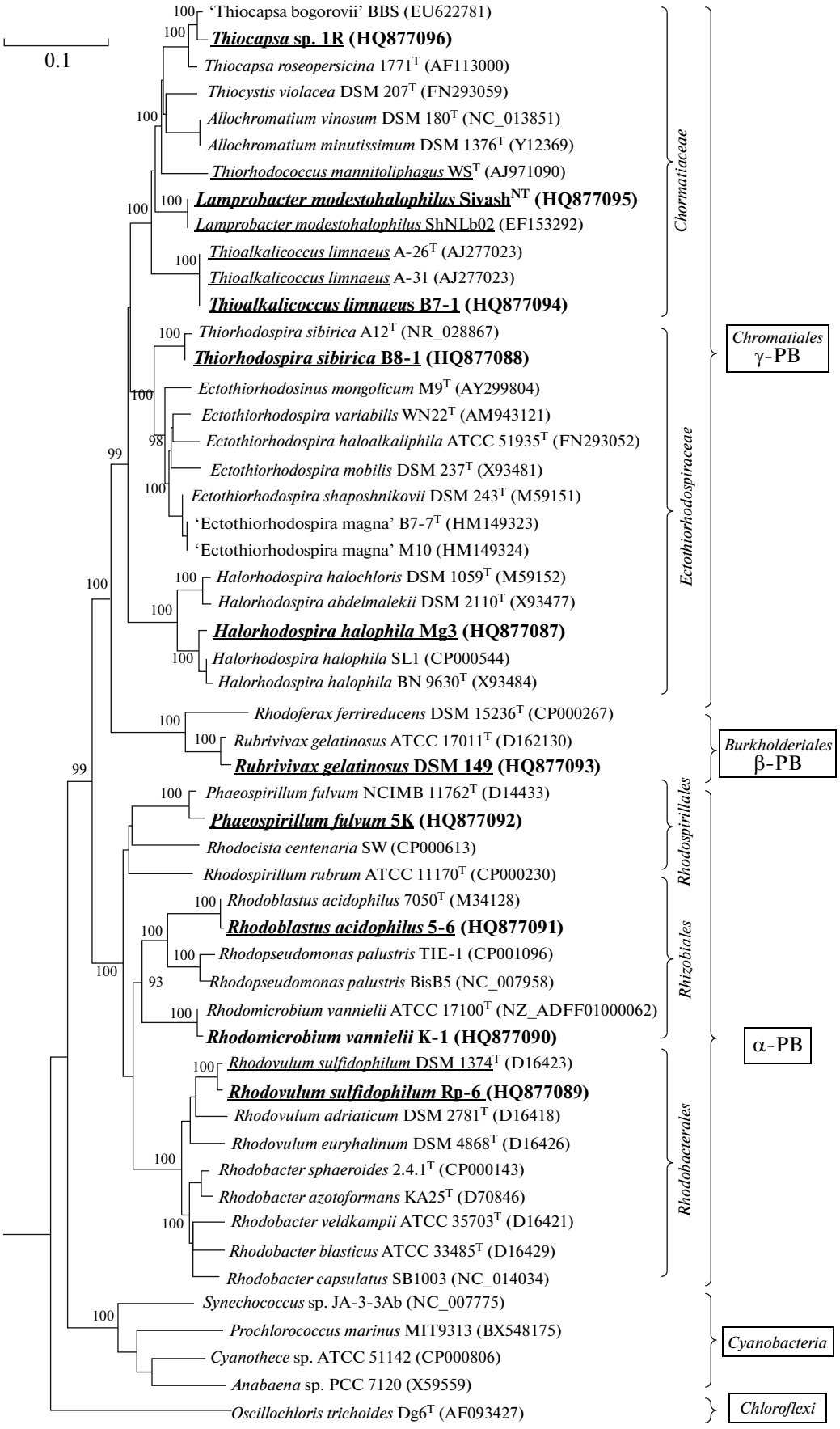
RESULTS AND DISCUSSION

Phylogeny of photoautotrophic bacteria according to the results of comparative analyses of the 16S rRNA and RuBisCO genes. Nearly complete 16S rRNA gene sequences (more than 1400 bp approximately corresponding to *E. coli* positions between 50 and 1500) were determined for ten of the studied collection and novel strains of photoautotrophic bacteria and compared with the gene sequences of phototrophic bacteria from the GenBank database. As was expected, in the obtained phylogenetic tree (Fig. 1), photoautotrophic bacteria involved in the process of CO_2 assimilation via the Calvin cycle fell into three main evolutionary lineages corresponding to proteobacteria, cyanobacteria, and green nonsulfur bacteria. The strains of photoautotrophic bacteria studied in this work, including those isolated from soda lakes, belonged to the phyla *Alpha-*, *Beta-*, and *Gammaproteobacrteria*. According to existing concepts [14], the level of 16S rRNA similarity required for identification of novel strains as representatives of previously described species or, alternatively, for their classification as new species, is 98–100%. Identification of the strains of photoautotrophic proteobacteria studied in this work was carried out according to this criterion, taking into account the phenotypic properties of these strains.

Analysis of the 16S rRNA gene sequences of the majority of the studied collection strains confirmed the results of their initial phenotypic identification. In the obtained phylogenetic tree, the strains of purple nonsulfur bacteria *Phaeospirillum fulvum* 5K, *Rhodoblastus acidophilus* 5-6, *Rhodomicrobium vannielii* K-1, *Rhodovulum sulfidophilum* Rp-6, and *Rubrivivax gelatinosus* DSM 149 occupied the same taxonomic positions as the type strains of these species. The similarity level between their 16S rRNA gene sequences and those of the type strains was 98.0–99.8%, which corresponded to the above-mentioned intraspecific criterion. However, the strain of purple nonsulfur bacteria 1R, which was initially identified as a representative of *Thiocystis violaceae*, was found to be a close relative of *Thiocapsa* species (98.7–99.1% similarity), according to the results of the16S rRNA gene analysis, and was therefore identified as *Thiocapsa* sp.

The results of the 16S rRNA gene analysis of the newly isolated strains of alkali(halo)phlic purple nonsulfur bacteria also confirmed the results of their initial identification (based on their phenotypic properties). The 16S rRNA gene sequences of strains ShNLb02 and Sivash (proposed as the neotype strain for *Lamprobacter modestohalophilus*) were almost identical (99.8% similarity), which confirmed their classification as members of this species. The results of identification of strains B8-1 B7-1, and Mg3 as representatives of *Thiorhodospira sibirica* (99.6% similarity with the type strain), *Thioalkalicoccus limnaeus* (99.8% similarity with the type strain), and *Halorhodospira halophila* (98.6% similarity with the type strain), respectively, were confirmed as well.

In general, the RuBisCO phylogeny differs considerably from the "ribosomal" one [15]. This is true, in particular, for the RuBisCO phylogeny of phototrophic bacteria, for which the distribution of the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'Thiocapsa bogorovii' BBS (EU622781)
Thiocapsa sp. 1R (HQ877096)
Thiocapsa roseopersicina 1771T (AF113000)
Thiocystis violacea DSM 207T (FN293059)
Allochromatium vinosum DSM 180T (NC_013851)
Allochromatium minutissimum DSM 1376T (Y12369)
Thiorhodococcus mannitoliphagus WST (AJ971090)
Lamprobacter modestohalophilus SivashNT (HQ877095)
Lamprobacter modestohalophilus ShNLb02 (EF153292)
Thioalkalicoccus limnaeus A-26T (AJ277023)
Thioalkalicoccus limnaeus A-31 (AJ277023)
Thioalkalicoccus limnaeus B7-1 (HQ877094)
Thiorhodospira sibirica A12T (NR_028867)
Thiorhodospira sibirica B8-1 (HQ877088)
Ectothiorhodosinus mongolicum M9T (AY299804)
Ectothiorhodospira variabilis WN22T (AM943121)
Ectothiorhodospira haloalkaliphila ATCC 51935T (FN293052)
Ectothiorhodospira mobilis DSM 237T (X93481)
Ectothiorhodospira shaposhnikovii DSM 243T (M59151)
'Ectothiorhodospira magna' B7-7T (HM149323)
'Ectothiorhodospira magna' M10 (HM149324)
Halorhodospira halochloris DSM 1059T (M59152)
Halorhodospira abdelmalekii DSM 2110T (X93477)
Halorhodospira halophila Mg3 (HQ877087)
Halorhodospira halophila SL1 (CP000544)
Halorhodospira halophila BN 9630T (X93484)
Rhodoferax ferrireducens DSM 15236T (CP000267)
Rubrivivax gelatinosus ATCC 17011T (D162130)
Rubrivivax gelatinosus DSM 149 (HQ877093)
Phaeospirillum fulvum NCIMB 11762T (D14433)
Phaeospirillum fulvum 5K (HQ877092)
Rhodocista centenaria SW (CP000613)
Rhodospirillum rubrum ATCC 11170T (CP000230)
Rhodoblastus acidophilus 7050T (M34128)
Rhodoblastus acidophilus 5-6 (HQ877091)
Rhodopseudomonas palustris TIE-1 (CP001096)
Rhodopseudomonas palustris BisB5 (NC_007958)
Rhodomicrobium vannielii ATCC 17100T (NZ_ADFF01000062)
Rhodomicrobium vannielii K-1 (HQ877090)
Rhodovulum sulfidophilum DSM 1374T (D16423)
Rhodovulum sulfidophilum Rp-6 (HQ877089)
Rhodovulum adriaticum DSM 2781T (D16418)
Rhodovulum euryhalinum DSM 4868T (D16426)
Rhodobacter sphaeroides 2.4.1T (CP000143)
Rhodobacter azotoformans KA25T (D70846)
Rhodobacter veldkampii ATCC 35703T (D16421)
Rhodobacter blasticus ATCC 33485T (D16429)
Rhodobacter capsulatus SB1003 (NC_014034)
Synechococcus sp. JA-3-3Ab (NC_007775)
Prochlorococcus marinus MIT9313 (BX548175)
Cyanothece sp. ATCC 51142 (CP000806)
Anabaena sp. PCC 7120 (X59559)
Oscillochloris trichoides Dg6T (AF093427)
Chromatiaceae
Ectothiorhodospiraceae
Rhodospirillales
Rhizobiales
Rhodobacterales
Chromatiales γ-PB
Burkholderiales β-PB
α-PB
Cyanobacteria
Chloroflexi

Fig. 1. Phylogenetic tree showing the taxonomic position of the studied strains on the phylogenetic tree constructed for photoautotrophic bacteria on the basis of their 16S rRNA gene sequences. The strains, for which the 16S rRNA gene sequences were determined in this work are in boldface; the strains, for which the RuBisCO gene sequences were determined in this work are underlined. The tree was constructed by the neighbor-joining method. The bar shows the evolutionary distance corresponding to 10 replacements per 100 nucleotides. The numerals show the significance of the branching order as determined by bootstrap analysis of 1000 alternative trees (only bootstrap values above 90% were considered significant).

forms and types of the enzyme, and even within some forms and types, has only a degree of correspondence to their taxonomic position determined on the basis of the results of phenotypic analysis and the "ribosomal" phylogeny (Fig. 2). Therefore, prior to analysis of the natural ecosystems, a detailed database on pure cultures was required. Otherwise, identification of the microorganisms carrying RuBisCO genes can be quite difficult.

In this work, with the application of the oligonucleotide primers targeting different forms and types of RuBisCO genes, these genes were detected in all 14 collection and novel strains of photoautotrophic proteobacteria, including those isolated from soda lakes (Table 1). The PCR products of expected length (about 800 bp) corresponding to the green-like and red-like form I RuBisCO, as well as to form II RuBisCO, were used in the phylogenetic analysis, together with the gene sequences of other phototrophic bacteria from the GenBank database.

The majority of the studied photoautotrophs have green-like form I RuBisCO (Fig. 2). On the whole, cyanobacteria form a separate cluster (1B) in the CbbL phylogenetic tree. However, some cyanobacteria, probably due to gene transfer, fall into another, larger CbbL cluster, 1A [19], which, in addition to various chemoautotrophs, contains all purple bacteria with green-like form I RuBisCO.

According to the results of phenotypic and "ribosomal" phylogenetic analysis, purple sulfur *Gammaproteobacteria* of the order *Chromatiales* may be divided into two families, *Ectothiorhodospiraceae* and *Chromatiaceae*. The RuBisCO phylogeny of bacteria belonging to the family *Ectothiorhodospiraceae* was described in detail in our previous work [16], according to which all autotrophic bacteria of this family carry one *cbbL* gene in their genomes. The representatives of the genera belonging to this family do not form a single phylogenetic group, as in the "ribosomal" phylogenetic tree. Each of these genera, including those comprising phototrophic bacteria (*Ectothiorhodospira, Halorhodospira, Thiorhodospira*, and *Ectothiorhodosinus*), form separate branches/clusters in the CbbL tree consisting of easily distinguishable species. In the genome of the novel strain Mg3 studied in this work and identified as a representative of the species *Halorhodospira halophila*, the only green-like form I RuBisCO gene was found to be identical to that of the type strain of this species (100% similarity of nucleotides and amino acids).

The most noticeable discrepancy between the "ribosomal" and CbbL phylogenies of phototrophic bacteria of the family *Ectothiorhodospiraceae* was the similarity between the *cbbL* gene sequences of the type strain of the alkaliphile *T. sibirica* A12 and those of the *Alphaproteobacteria*, rather than *Gammaproteobacteria*. However, the nucleotide composition of DNA and codon usage frequency of *T. sibirica* A12 were the same for the *cbbL* genes, as well as for the total genome, but differed significantly from those of *Alphaproteobacteria*. Therefore, this phenomenon was tentatively explained by an increase in the rate of nonsynonymous substitutions in the *cbbL* sequence of this microorganism rather than horizontal gene transfer [16]. In this work, a novel alkaliphilic strain, B8-1, was studied. On the basis of its phenotypic and phylogenetic characteristics, the strain was identified as a representative of *T. sibirica*. The sequence of the only *cbbL* gene detected in the genome of this microorganism differed insignificantly from that of the type strain A12 (98.6 and 96.2% similarity of nucleotides and amino acids, respectively), which confirmed the unusual phylogenetic position of this photoautotroph in the CbbL tree.

Unlike the family *Ectothiorhodospiraceae*, data on the structure of RuBisCO of representatives of the family *Chromatiaceae* are scarce. The type strain of *Allochromatium vinosum*, whose genome contains two different green-like form I *cbbL* genes [17] has long been the only microorganism studied in this regard. Later, very similar *cbbL* genes were detected in another representative of this genus, the type strain of *A. minutissimum* [18]. In this work, we demonstrated that the genomes of two species of purple sulfur bacteria, *Thiocapsa roseopersicina* DSM 211 and '*Tc. bogorovii*' BBS, contain one *cbbL* gene similar to one of *A. vinosum* genes (designated as *rbcL* [17]) each.

Several other representatives of the family *Chromatiaceae* were also studied: collection strain 1R identified as *Thiocapsa* sp., 2 *Lamprobacter modestohalophilus* strains (including the neotype strain), 3 *Thioalcalicoccus limnaeus* strains (including the type strain), and the type strain of *Thiorhodococcus mannitoliphagus*. The genomes of *Thiocapsa* sp. 1R and the type strain of *Thc. mannitoliphagus* were found to contain one green-like form I *cbbL* gene each, whereas the genomes of *Thc. limnaeus* contained three RuBisCO genes, two genes encoding the green-like form I enzyme and one gene encoding form II. A double set of genes encoding form I and form II was detected in the genome of the neotype strain *L. modestohalophilus*

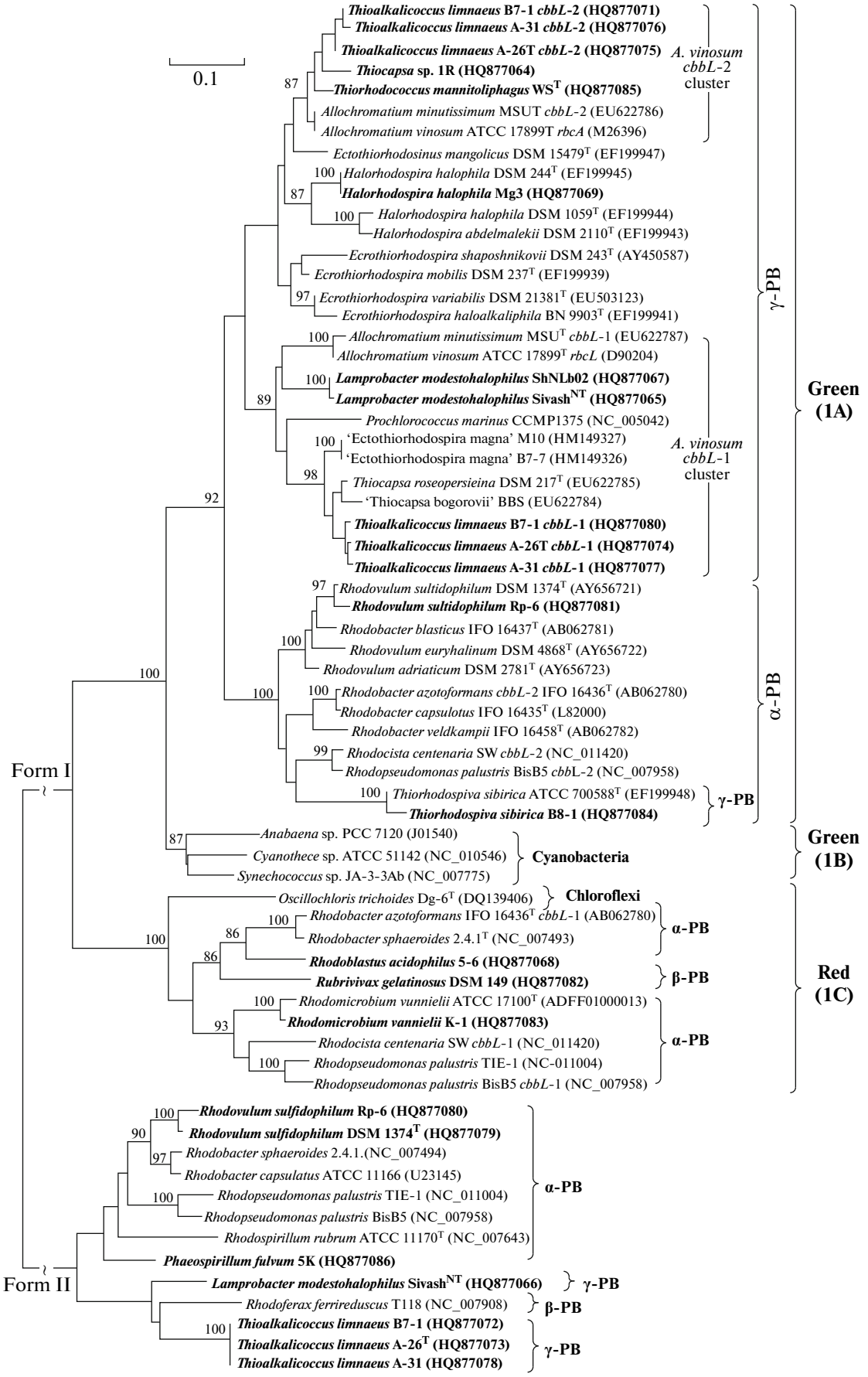

0.1
Thioalkalicoccus limnaeus B7-1 cbbL-2 (HQ877071)
Thioalkalicoccus limnaeus A-31 cbbL-2 (HQ877076)
Thioalkalicoccus limnaeus A-26T cbbL-2 (HQ877075)
Thiocapsa sp. 1R (HQ877064)
Thiorhodococcus mannitoliphagus WST (HQ877085)
Allochromatium minutissimum MSUT cbbL-2 (EU622786)
Allochromatium vinosum ATCC 17899T rbcA (M26396)
A. vinosum cbbL-2 cluster
Ectothiorhodosinus mangolicus DSM 15479T (EF199947)
Halorhodospira halophila DSM 244T (EF199945)
Halorhodospira halophila Mg3 (HQ877069)
Halorhodospira halophila DSM 1059T (EF199944)
Halorhodospira abdelmalekii DSM 2110T (EF199943)
Ecrothiorhodospira shaposhnikovii DSM 243T (AY450587)
Ecrothiorhodospira mobilis DSM 237T (EF199939)
Ecrothiorhodospira variabilis DSM 21381T (EU503123)
Ecrothiorhodospira haloalkaliphila BN 9903T (EF199941)
γ-PB
Allochromatium minutissimum MSUT cbbL-1 (EU622787)
Allochromatium vinosum ATCC 17899T rbcL (D90204)
Lamprobacter modestohalophilus ShNLb02 (HQ877067)
Lamprobacter modestohalophilus SivashNT (HQ877065)
Prochlorococcus marinus CCMP1375 (NC_005042)
'Ectothiorhodospira magna' M10 (HM149327)
'Ectothiorhodospira magna' B7-7 (HM149326)
A. vinosum cbbL-1 cluster
Thiocapsa roseopersieina DSM 217T (EU622785)
'Thiocapsa bogorovii' BBS (EU622784)
Thioalkalicoccus limnaeus B7-1 cbbL-1 (HQ877080)
Thioalkalicoccus limnaeus A-26T cbbL-1 (HQ877074)
Thioalkalicoccus limnaeus A-31 cbbL-1 (HQ877077)
Green (1A)
Rhodovulum sultidophilum DSM 1374T (AY656721)
Rhodovulum sultidophilum Rp-6 (HQ877081)
Rhodobacter blasticus IFO 16437T (AB062781)
Rhodovulum euryhalinum DSM 4868T (AY656722)
Rhodovulum adriaticum DSM 2781T (AY656723)
Rhodobacter azotoformans cbbL-2 IFO 16436T (AB062780)
Rhodobacter capsulotus IFO 16435T (L82000)
Rhodobacter veldkampii IFO 16458T (AB062782)
Rhodocista centenaria SW cbbL-2 (NC_011420)
Rhodopseudomonas palustris BisB5 cbbL-2 (NC_007958)
Thiorhodospiva sibirica ATCC 700588T (EF199948)
Thiorhodospiva sibirica B8-1 (HQ877084)
α-PB
γ-PB
Anabaena sp. PCC 7120 (J01540)
Cyanothece sp. ATCC 51142 (NC_010546)
Synechococcus sp. JA-3-3Ab (NC_007775)
Cyanobacteria
Green (1B)
Oscillochloris trichoides Dg-6T (DQ139406)
Chloroflexi
Rhodobacter azotoformans IFO 16436T cbbL-1 (AB062780)
Rhodobacter sphaeroides 2.4.1T (NC_007493)
α-PB
Rhodoblastus acidophilus 5-6 (HQ877068)
Rubrivivax gelatinosus DSM 149 (HQ877082)
β-PB
Rhodomicrobium vunnielii ATCC 17100T (ADFF01000013)
Rhodomicrobium vannielii K-1 (HQ877083)
Rhodocista centenaria SW cbbL-1 (NC_011420)
Rhodopseudomonas palustris TIE-1 (NC-011004)
Rhodopseudomonas palustris BisB5 cbbL-1 (NC_007958)
α-PB
Red (1C)
Form I
Rhodovulum sulfidophilum Rp-6 (HQ877080)
Rhodovulum sulfidophilum DSM 1374T (HQ877079)
Rhodobacter sphaeroides 2.4.1.(NC_007494)
Rhodobacter capsulatus ATCC 11166 (U23145)
Rhodopseudomonas palustris TIE-1 (NC_011004)
Rhodopseudomonas palustris BisB5 (NC_007958)
Rhodospirillum rubrum ATCC 11170T (NC_007643)
α-PB
Phaeospirillum fulvum 5K (HQ877086)
Form II
Lamprobacter modestohalophilus SivashNT (HQ877066)
γ-PB
Rhodoferax ferrireducus T118 (NC_007908)
β-PB
Thioalkalicoccus limnaeus B7-1 (HQ877072)
Thioalkalicoccus limnaeus A-26T (HQ877073)
Thioalkalicoccus limnaeus A-31 (HQ877078)
γ-PB

Fig. 2. Phylogenetic position of the studied strains (in boldface) of photoautotrophic bacteria of the phylogenetic tree constructed on the basis of the deduced amino acids sequences of form I and form II RuBisCO. The tree was constructed by the neighbor-joining method. The bar shows evolutionary distance, corresponding to 10 replacements per 100 amino acid residues. The numerals show the significance of the branching order as determined by bootstrap analysis of 1000 alternative trees (only bootstrap values above 80% were considered significant).

Sivash, whereas the genome of another strain of this species contained only the green-like form I gene. The level of interspecific similarity of the *cbbL* gene sequences of *L. modestohalophilus* strains was 97.7% (nucleotides) and 99.5% (amino acids). The level of homology between both the nucleotide and amino acid sequences of the *cbbL* genes of *Thc. limnaeus* strains was high as well (97.8% and 99.0%, respectively). At the same time, the level of homology between the *cbbL-1* and *cbbL-2* genes of each strain did not exceed 83%. The genes encoding form II enzyme in *Thc. limnaeus* strains were found to be identical (100% similarity).

Phylogenetic analysis (Fig. 2) showed that the *cbbL* genes of all the representatives of the family *Chromatiaceae* that have been studied thus far (mostly in this work) were clustered around each of the two *A. vinosum* genes forming the "*A. vinosum-cbbL-l* (*rbcL*)" and "*A. vinosum-cbbL-2*" clusters, respectively. The *cbbL* gene sequences of *Tc. roseopersicina, Tc. bogorovii*, and two *L. modestohalophilus* strains, along with the *cbbL-1* genes of three *Thc. limnaeus* strains were clustered together with the *rbcL* gene of *A. vinosum* and formed branches in the "*A. vinosum-cbbL-1*" cluster. The *cbbL-2* genes of *Thc. limnaeus* clustered together with the *cbbL* genes of *Thiocapsa* sp. 1R and *Trc. mannitoliphagus* around the *rbcA* gene of *A. vinosum*, expanding the '*A. vinosum-cbbL-2*" cluster.

The *cbbM* gene sequences of the representatives of the genera *Thioalcalicoccus* and *Lamprobacter* of the family *Chromatiaceae* were found to be more divergent. In the CbbM tree, they occupied two separate branches and were not closely related to the known *cbbM* sequences of photoautotrophic bacteria. Hence, it was demonstrated that phylogenetically close (at the genus level) purple sulfur photoautotrophic bacteria might differ considerably from each other in the structures of their RuBisCO genes, even when they encode the enzymes of the same type.

To summarize the results obtained, we propose a hypothetical scheme of evolution of the RuBisCO genes of photoautotrophic representatives of the order *Chromatiales* (Fig. 3). It is possible that the genome of the common ancestor of *Chromatiales* contained a single copy of the gene encoding form I enzyme and the RuBisCO form II gene. Members of the family *Ectothiorhodospiraceae* inherited the ancestral form of the *cbbL* gene and lost the *cbbM* gene. During the evo-

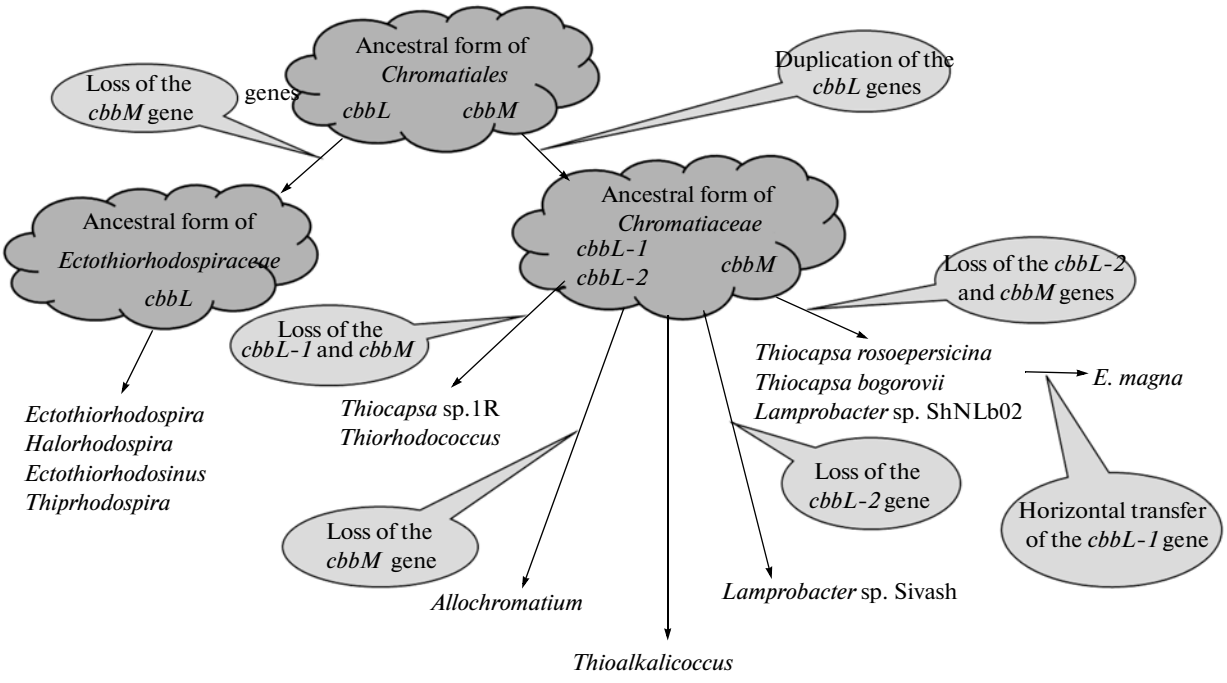


Fig. 3. Hypothetical scheme of the evolution of the RuBisCO genes of photoautotrophic representatives of the order *Chromatiales*.

lution of another branch, the family *Chromatiaceae*, the *cbbL* gene was duplicated. As a result, representatives of *Thioalkalicoccus* carry a triple set of RuBisCO genes (*cbbL-l, cbbL-2* and *cbbM*). The fact that the frequency of codon usage in two genes of *A. vinosum* and their G + C content are virtually the same, as well as the fact that both genes code for the completely active enzymes with different constant values and CO_2 specificity [19], confirm the suggestion that the *cbbL* genes resulted from duplication, rather than from lateral transfer. Subsequently, during the evolution of other *Chromatiaceae* species, the *cbbM* genes, as well as one of the two ancestral *cbbL* genes were selectively lost, which resulted in the division of the family into two groups carrying each their own set of genes. During the formation of the CbbL cluster around the *rbcL* gene of *A. vinosum* ("*A. vinosum-cbbL-1*"), horizontal gene transfer could have occurred, since this cluster includes chemoautotrophic *Gammaproteobacteria* [16, 20], as well as a new strain of the photoautotrophic bacterium *Ectothiorhodospira* recently described as *E. magna* [21].

Unlike cyanobacteria and most purple sulfur *Gammaproteobacteria*, the previously studied purple nonsulfur *Alphaproteobacteria* were highly diverse regarding the composition of their RuBisCO genes. The genomes of these photoautotrophic bacteria could contain these genes as a single copy of the *cbbL* gene (green- or red-like form II) or the *cbbM* form II gene, or in the form of a multiple set (double or triple) of these genes in various combinations. This is also true for the purple nonsulfur *Alphaproteobacteria* studied in this work. The genomes of strains 5-6 and K-1, identified respectively as *Rhodoblastus acidophilus* and *Rhodomicrobium vanniellii* of the order *Rhizobiales*, contained one red-like form I *cbbL* gene each. Strain *R. acidophilus* 5-6 occupies a new branch within the IC cluster of this form, whereas *Rm. vanniellii* K-1 is closely related to the type strain of this species (92.0 and 97.3% similarity of nucleotides and amino acids, respectively).

The purple nonsulfur *Alphaproteobacteria* carrying the green-like form I *cbbL* gene form a separate subcluster within the 1A cluster of this form (Fig. 2). Among other strains, this subcluster includes the type strains of purple nonsulfur bacteria of the genus *Rhodovulum* belonging to the order *Rhodobacterales*, whose *cbbL* genes were previously studied in [22]. In this work, the *cbbM* gene encoding form II RuBisCO was detected in the genome of the type strain of *Rhodovulum sulfidophilum* and sequenced. This gene, like the *cbbL* genes, was close to the corresponding genes of the *Rhodobacter* species. Another strain (Rp-6) of this species was also studied. Its genome contained two RuBisCO genes close to those of the type strain (*cbbL*, 96.0 and 96.3% similarity of nucleotides and amino acids, respectively, and *cbbM*, 93.0 and 97.0% similarity of nucleotides and amino acids, respectively).

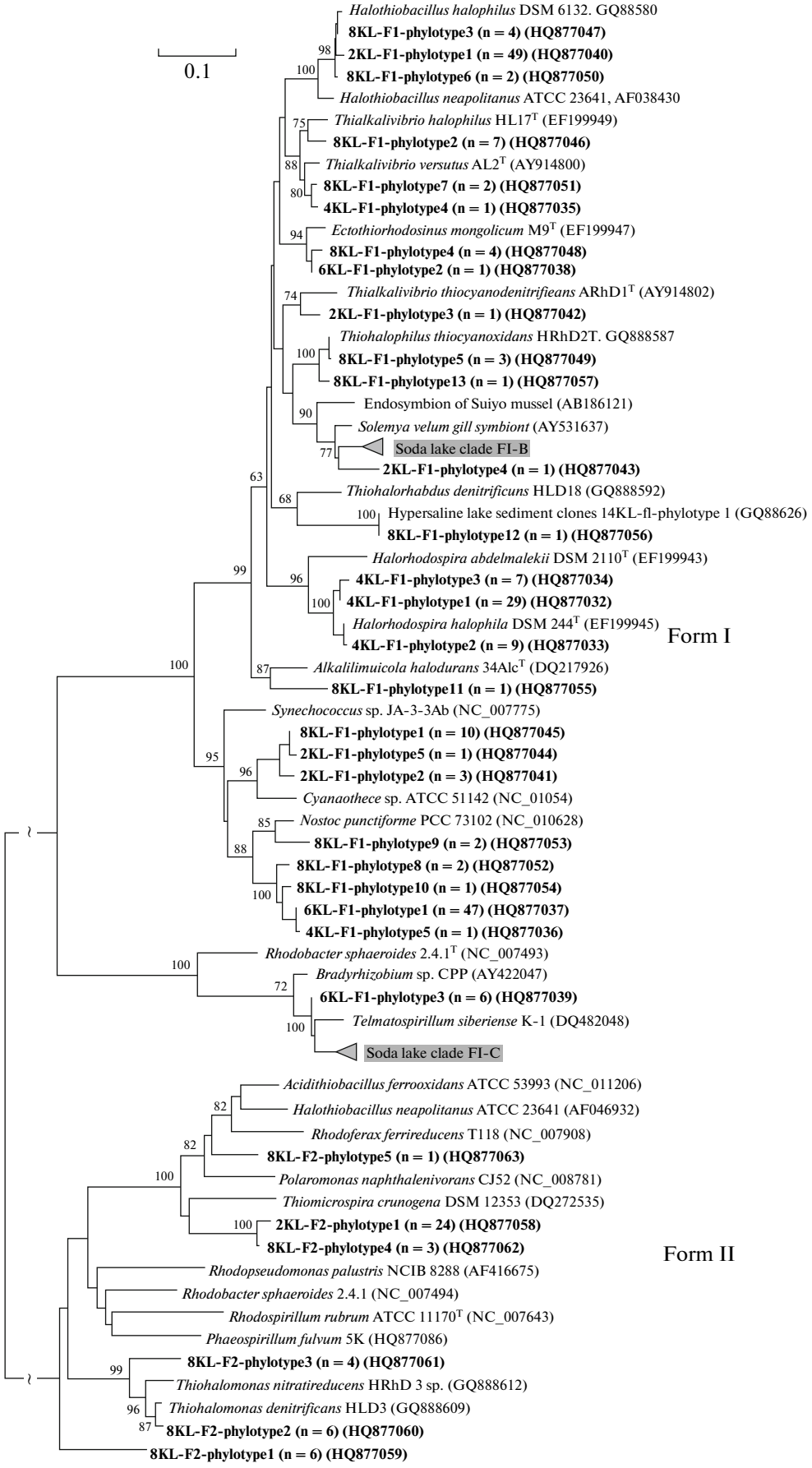
Finally, the genome of strain 5K, identified as *Phaeospirillum fulvum* of the order *Rhodospirillales*, similar to the genomes of the previously studied chemo- and photoautotrophic representatives of this order, contained a single *cbbM* gene encoding form II RuBisCO and occupying a separate branch within the CbbM cluster.

The genome of the only strain of purple nonsulfur betaproteobacteriua studied in this work, *Rubrivivax gelatinosus* DSM 149 of the order *Burkholderiales* contains only the red-like form I *cbbL* gene, which occupies a separate branch within the IC cluster of this form. At the same time, only the *cbbM* gene encoding form II RuBisCO was previously detected in the sequence of the total genome of another phototrophic representative of this order, *Rhodoferax ferrireducens* T118, which demonstrates the variety of RuBisCO forms in the genomes of photoautotrophic *Betaproteobacteria*.

Although, on the whole, RuBisCO genes are less conservative than 16S rRNA genes, the level of their intraspecific divergence is relatively low (0–10% for nucleotides and 0–8% for amino acids). Therefore, despite the fact that some peculiarities of the RuBisCO gene evolution lead to certain discrepancies with the "ribosomal" phylogeny in the topology of the relevant phylogenetic trees, identification of microorganisms at the species level is quite reliable, and RuBisCO genes can be used as functional markers for molecular ecology studies of natural microbial communities.

Detection and phylogenetic analysis of RuBisCO genes in the samples collected from the surface horizons of silt sediments of saline and soda lakes. From all the studied samples, the relevant amplification products were obtained using the primers specific to the green-like form I RuBisCO genes. The obtained clone libraries (about 50 for each sample) contained 2–13 unique *cbbL* phylotypes (the level of similarity between the nucleotide sequences within each phylotype was at least 97%). The compositions of the clone

Fig. 4. Phylogenetic tree constructed on the basis of the deduced amino acid sequences of the gene encoding form I and form II RuBisCO and depicting the taxonomic positions of uncultured microorganisms isolated from the bottom sediments of soda and hypersaline lakes with neutral pH. The form I RuBisCO clusters detected previously in the integral samples of bottom sediments of the studied soda lakes are shown in gray. The RuBisCO phylotypes isolated from the bottom sediments of saline and soda lakes are in boldface; for each phylotype, the number of clones is given in parentheses. The tree was constructed by the neighbor-joining method. The bar shows evolutionary distance, corresponding to 10 replacements per 100 amino acid residues. The numerals show the significance of the branching order as determined by bootstrap analysis of 1000 alternative trees (only bootstrap values above 70% were considered significant).


0.1
Halothiobacillus halophilus DSM 6132. GQ88580
8KL-F1-phylotype3 (n = 4) (HQ877047)
2KL-F1-phylotype1 (n = 49) (HQ877040)
8KL-F1-phylotype6 (n = 2) (HQ877050)
Halothiobacillus neapolitanus ATCC 23641, AF038430
Thialkalivibrio halophilus HL17T (EF199949)
8KL-F1-phylotype2 (n = 7) (HQ877046)
Thialkalivibrio versutus AL2T (AY914800)
8KL-F1-phylotype7 (n = 2) (HQ877051)
4KL-F1-phylotype4 (n = 1) (HQ877035)
Ectothiorhodosinus mongolicum M9T (EF199947)
8KL-F1-phylotype4 (n = 4) (HQ877048)
6KL-F1-phylotype2 (n = 1) (HQ877038)
Thialkalivibrio thiocyanodenitrifieans ARhD1T (AY914802)
2KL-F1-phylotype3 (n = 1) (HQ877042)
Thiohalophilus thiocyanoxidans HRhD2T. GQ888587
8KL-F1-phylotype5 (n = 3) (HQ877049)
8KL-F1-phylotype13 (n = 1) (HQ877057)
Endosymbion of Suiyo mussel (AB186121)
Solemya velum gill symbiont (AY531637)
Soda lake clade FI-B
2KL-F1-phylotype4 (n = 1) (HQ877043)
Thiohalorhabdus denitrificuns HLD18 (GQ888592)
Hypersaline lake sediment clones 14KL-fl-phylotype 1 (GQ88626)
8KL-F1-phylotype12 (n = 1) (HQ877056)
Halorhodospira abdelmalekii DSM 2110T (EF199943)
4KL-F1-phylotype3 (n = 7) (HQ877034)
4KL-F1-phylotype1 (n = 29) (HQ877032)
Halorhodospira halophila DSM 244T (EF199945)
4KL-F1-phylotype2 (n = 9) (HQ877033)
Form I
Alkalilimuicola halodurans 34AlcT (DQ217926)
8KL-F1-phylotype11 (n = 1) (HQ877055)
Synechococcus sp. JA-3-3Ab (NC_007775)
8KL-F1-phylotype1 (n = 10) (HQ877045)
2KL-F1-phylotype5 (n = 1) (HQ877044)
2KL-F1-phylotype2 (n = 3) (HQ877041)
Cyanaothece sp. ATCC 51142 (NC_01054)
Nostoc punctiforme PCC 73102 (NC_010628)
8KL-F1-phylotype9 (n = 2) (HQ877053)
8KL-F1-phylotype8 (n = 2) (HQ877052)
8KL-F1-phylotype10 (n = 1) (HQ877054)
6KL-F1-phylotype1 (n = 47) (HQ877037)
4KL-F1-phylotype5 (n = 1) (HQ877036)
Rhodobacter sphaeroides 2.4.1T (NC_007493)
Bradyrhizobium sp. CPP (AY422047)
6KL-F1-phylotype3 (n = 6) (HQ877039)
Telmatospirillum siberiense K-1 (DQ482048)
Soda lake clade FI-C
Acidithiobacillus ferrooxidans ATCC 53993 (NC_011206)
Halothiobacillus neapolitanus ATCC 23641 (AF046932)
Rhodoferax ferrireducens T118 (NC_007908)
8KL-F2-phylotype5 (n = 1) (HQ877063)
Polaromonas naphthalenivorans CJ52 (NC_008781)
Thiomicrospira crunogena DSM 12353 (DQ272535)
2KL-F2-phylotype1 (n = 24) (HQ877058)
8KL-F2-phylotype4 (n = 3) (HQ877062)
Form II
Rhodopseudomonas palustris NCIB 8288 (AF416675)
Rhodobacter sphaeroides 2.4.1 (NC_007494)
Rhodospirillum rubrum ATCC 11170T (NC_007643)
Phaeospirillum fulvum 5K (HQ877086)
8KL-F2-phylotype3 (n = 4) (HQ877061)
Thiohalomonas nitratireducens HRhD 3 sp. (GQ888612)
Thiohalomonas denitrificans HLD3 (GQ888609)
8KL-F2-phylotype2 (n = 6) (HQ877060)
8KL-F2-phylotype1 (n = 6) (HQ877059)

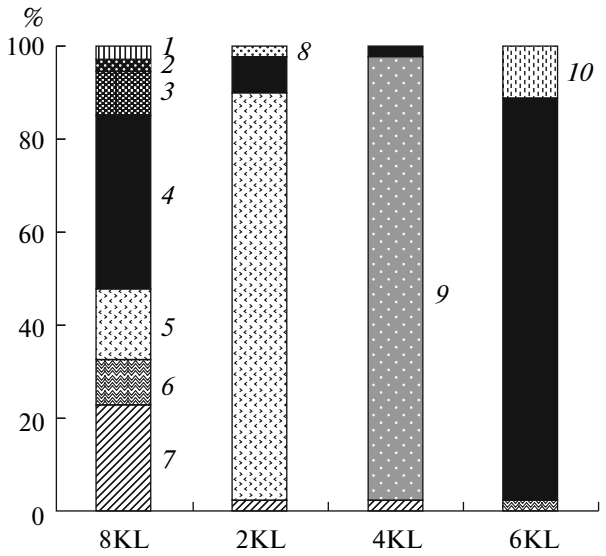


Fig. 5. Taxonomic distribution of form I RuBisCO phylotypes isolated from the bottom sediments of the studied soda and hypersaline lakes with neutral pH. The numerals indicate: *Alkalispirillum* (*1*); *Thiohalorhabdus* (*2*); *Thiohalophilus* (*3*); *Cyanobacteria* (*4*); *Halothiobacillus* (*5*); Ectothiorhodosinus (*6*); *Thioalkalivibrio* (*7*); Soda lake clade FI-B (*8*); *Halorhodospira* (*9*); and Soda lake clade FI-C (*10*).

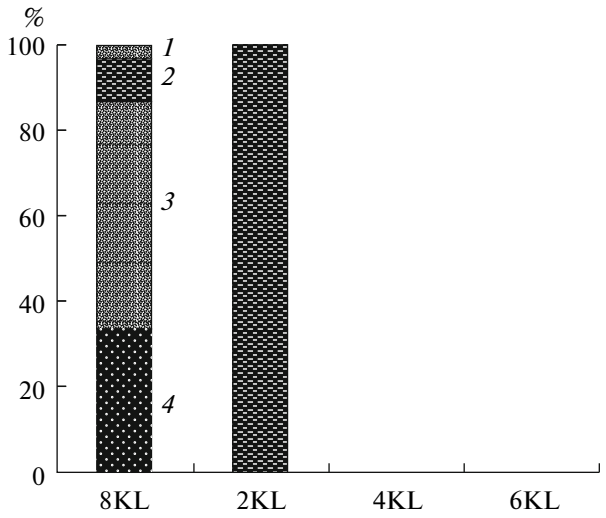


Fig. 6. Taxonomic distribution of form II RuBisCO phylotypes isolated from the bottom sediments of the studied soda and hypersaline lakes with neutral pH. The numerals indicate: Group C (*1*); Group B (*2*); Group A (*3*); *Thiohalomonas* (*4*).

libraries constructed for each sample differed considerably.

The libraries constructed for the samples collected in the saline Lake Petukhovo (2KL-09), as well as in the soda lakes Gorchina-1 (4KL-09) and Tanatar-5 (6KL-09), were virtually monotype and characterized by the overwhelming predominance of the phylotypes belonging to one taxon; these taxa, however, differed for each sample (Figs. 4 and 5). In the clone library 2KL-F1, a representative of the chemolithotrophic halophilic genus *Halothiobacillus* was the main phylotype (88% of the total number of clones). In the clone library 4KL-F1, three close phylotypes (87% of the total number of clones) were represented by members of the phototrophic genus *Halorhodospira* (most probably, *H. halophila*), whereas, in the clone library 6KL-F1, cyanobacteria represented the main phylotype (98% of the total number of clones). The cyanobacterial phylotypes were detected in the clone libraries 2KL-F1 and 4KL-F1 (as minor components) as well. The phylotypes belonging to the genera *Thioalkalivibrio* (2KL-F1 phylotype 3 and 4KL-F1 phylotype 4), *Ectothiorhodosinus* (6KL-F1 phylotype 2), and to the group of uncultured *Gammaproteobacteria* Soda lake clade F1-B (2KL-F1 phylotype 4) detected in the bacterial community of soda lake sediments [9], were other minor components of these CbbL libraries.

Unlike these libraries, the CbbL library constructed for the sample collected in the hypersaline Lake Lomovoe (8KL-09) exhibited a considerable diversity (Figs. 4 and 5). As in the case of the 6KL-F1 library, cyanobacteria prevailed; however, they belonged to four different phylotypes (37.5% of the total number of clones). The next largest groups were represented by two phylotypes belonging to the genus *Thioalkalivibrio* (most probably *T. halophilus*) (22.0% of the total number of clones) and two phylotypes belonging to the genus *Halothiobacillus* (15% of the total number of clones). In addition, the phylotypes belonging to the genera *Ectothiorhodosinus, Thiohalophilus*, and *Thiohalorhabdus*, as well as a phylotype belonging to the distantly related group *Alkalispirillum–Alkalilimnicola* were present in this community as minor components.

PCR amplification with the primers specific to the red-like form I RuBisCO genes have yielded positive results only for the 6KL sample. The clones obtained formed a single phylotype closely related to the group of uncultured *Alphaproteobacteria* Soda lake clade FI-C detected in soda lake sediments [9].

The *cbbM* genes encoding form II RuBisCO were detected only in the samples collected in hypersaline lakes with neutral pH (Figs. 4 and 6). The obtained clone libraries (about 30 for each sample) contained 1 and 5 unique *cbbM* phylotypes, respectively; the level of similarity between the nucleotide sequences was at least 97%. Unlike CbbL libraries, CbbM libraries consisted primarily of the phylotypes of unidentified bacteria. In the clone library 8KL-F2, only two out of five phylotypes were identified as representatives of the genus *Thiohalomonas* (denitrifying chemotrophic halophilic SOB; [23]), which corresponded to 33% of the total number of clones. Other phylotypes of this clone library were not related to any of the *cbbM* gene sequences within the GenBank database and occupied

separate branches in the phylogenetic tree. The group A phylotype predominated (53% of the total number of clones). The next largest phylotype (10% of the total number of clones), the group B phylotype, was close to the only phylotype detected in the clone library 2KL-F2.

The use of the primer pairs specific to the RuBisCO genes of the autotrophic filamentous nonsulfur bacteria of the genus *Oscillochloris* did not yield positive results for any of the studied samples.

As in the previous studies [8–10] of microbial communities of hypersaline lakes, RuBisCO genes were detected in all the studied samples, indicating the obligatory presence of autotrophic bacteria. However, the diversity of the studied phylotypes was even more restricted than that observed in previous studies, which confirmed the suggestion of Giri et al [8], that this is the result of stable extreme ambient conditions, which give an advantage to one or two best adapted microorganisms. This suggestion is especially probable for the three studied lakes, where the surface horizons of silt sediments were inhabited by almost monotype communities of autotrophic bacteria. At the same time, it is possible that seasonal or local weather fluctuations may significantly alter the ambient conditions and, consequently, affect the composition of these uniform communities, giving an advantage to other groups of autotrophs, for example by diluting them during an extremely rainy year. According to our 10-year observations of the lakes of the Kulunda Steppe, a similar phenomenon occurred in the soda lakes of the Solyanoozernaya Steppe (Mikhailovskoe raion), but not in hypersaline lakes.

The composition of the autotrophic community inhabiting the surface sediment horizons of the soda and chloride–sulfate lakes of the Kulunda Steppe was virtually the same as that of the autotrophic community detected in the integral samples of the sediments of similar lakes [9] and included representatives of the chemo- and photoautotrophic genera *Thioalkalivibrio, Halorhodospira, Halothiobacillus*, and *Ectothiorhodosinus*, as well as cyanobacteria. It should be emphasized that some *cbbL* phylotypes of *Thioalkalivibrio, Ectothiorhodosinus*, and cyanobacteria were detected in the soda Mono Lake as well [8]. Two groups of unidentified organisms: the *Alphaproteobacteria* carrying the red-like form I RuBisCO gene, and SOB of the class *Gammaproteobacteria* closely related to the *Solemia velum* symbiont, were detected in the communities isolated from both the integral samples [9] and the surface sediment horizons of the studied lakes. Hence, the comparatively wide distribution of uncultured microorganisms of these groups under haloalkaliphilic conditions was confirmed; however, since no pure cultures of these microorganisms were isolated, the type of their metabolism is yet to be determined. It may be suggested that they are difficult-to-cultivate microaerophilic sulfide-oxidizing bacteria.

In this work, no phylotypes of anoxygenic phototrophs belonging to the purple nonsulfur bacteria were detected, although one of the previously studied communities inhabiting the bottom sediments of soda lakes included representatives of the *Alphaproteobacteria*, and, in particular, *Rhodovulum* species as minor components [9]. This indicates that growth of these of purple sulfur bacteria ceases at high salinity levels, which was confirmed by the cultural techniques [24].

The most diverse community, 8KL, inhabiting a hypersaline lake, includes the phylotypes belonging to the novel species of halophilic SOB *Thiohalorhabdus, Thiohalomonas*, and *Thiohalophilus*. These microorganisms are difficult-to-cultivate chemolithoautotrophs, and their numbers in the studied environmental samples may be so small that they are not detected by the most widely-used method of the 16S rRNA gene analysis. Analysis of specific gene markers, such as *cbbL* and *cbbM* genes is most suitable for detection of such organisms, since it makes it possible to elucidate their distribution in natural ecosystems. For example, in the case of the genus *Thiohalophilus*, only one type strain of the type species *T. thiocyanatoxidans* HRhD2 is known [25], whereas, in this study, the *cbbL* phylotypes closely related to this strain have been isolated from natural ecosystems for the first time.

However, despite the qualitative similarity of their composition, the quantitative characteristics of the autotrophic communities isolated from the integral samples and surface layers of bottom sediments of the studied saline and soda lakes were quite the opposite. In the communities from the integral samples of bottom sediments, the identified predominant phylotypes were represented by chemoautotrophic halophilic SOB of the genera *Thiohalorhabdus* (saline lake with neutral pH) and *Thioalkalivibrio* and *Halothiobacillus* (soda lakes). The phylotypes of photoautotrophic organisms (*Halorhodospira, Ectothiorhodosinus, Rhodovulum*, and *Cyanobacteria*) were identified only in the soda lake communities and were minor components [9, 10]. On the contrary, in the present work, phototrophic microorganisms were found to prevail in the bacterial communities of soda lakes and of the hypersaline soda Lake Lomovoe, except for the sample collected from the hypersaline Lake Petukhovo, in which chemoautotrophs were predominant. Chemotrophic microorganisms were minor components. Unlike the integral samples, in the communities inhabiting the surface sediment horizons, one taxonomic group, namely, cyanobacteria, was found to be universal and predominant in the bacterial communities of the soda Lake Tanatar-5 and the hypersaline Lake Lomovoe and was a minor component in the bacterial communities of lakes Petukhovo and Gorchina-1. The cyanobacterial phylotypes detected in different samples did not form a single group and, at the same time, did not reveal any close relations with the RuBisCO sequences of cultivable cyanobacteria from the GenBank database. Therefore, it can be

hypothesized that the microorganisms represented by these phylotypes are confined to soda and saline lakes, which indicates the necessity to focus on aerobic phototrophs inhabiting hypersaline lakes.

Our results do not contradict the microbiological data obtained during previous investigations, according to which planktonic cyanobacteria making a major contribution to primary production [26, 27] are the predominant photosynthetic microorganisms in the studied ecosystems. Anoxygenic phototrophic bacteria, together with sulfur-oxidizing alkaliphilic chemoautotrophs (including the *Thioalkalivibrio* species isolated in this study) are secondary producers and play a key role in the microbial community of saline and soda lakes by participating in the sulfur cycle. The taxonomic composition of anoxygenic phototrophs inhabiting soda lakes depends primarily on the salinity level. High salinity and pH of 9–10 are optimal for the obligate phototrophic haloalkaliphiles of the genus *Halorhodospira*, which was confirmed by the results of this study. However, the phylotypes of alkali(halo)phlic representatives of the genera *Ectothiorhodospira* and *Thiorhodospira* (including their new members, the RuBisCO phylogeny of which was studied in this work) were detected neither in this study of the surface layers of bottom sediments, nor in the integral samples collected in the bottom sediments of soda lakes. Moreover, no purple sulfur bacteria of the family *Chromatiaceae* and green filamentous nonsulfur bacteria of the family *Oscillochloridaceae*, which, according to the type of their metabolism, are alkali(halo)tolerant, rather than alkali(halo)phlic, microorganisms [5]. At the same time, alkaliphilic purple sulfur bacteria of the genus *Ectothiorhodosinus*, the phylotypes of which were detected both in the integral samples and the samples collected from the surface layers of bottom sediments, frequently occurred in the soda and saline lakes of the Kulunda Steppe.

Unlike previous studies of soda and saline lakes [8–10], we failed to detect any new unidentified *cbbL* phylotypes in the autotrophic communities of the surface layers of bottom sediments of the studied soda and saline lakes. However, the fact that new *cbbM* phylotypes were detected in two out of four studied samples characterized by high salinity indicates the presence of unknown specific autochthonous autotrophic bacteria whose taxonomy and metabolic properties requires further study.

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