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EXPERIMENTAL ARTICLES

Purple Nonsulfur Bacteria in Weakly and Moderately Mineralized Soda Lakes of the Southern Transbaikal Region and Northeastern Mongolia

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Abstract—Purple nonsulfur bacteria (PNB) are shown to be widespread in weakly and moderately mineralized soda lakes of the southern Transbaikal Region and northeastern Mongolia. PNB occurred in most samples and enrichments at all pH and salinity values recorded or tested. Of the 24 investigated lakes of the southern Transbaikal Region, they were found in 22. In addition, the presence of PNB was noted in most soda lakes of northeastern Mongolia with water mineralization from 3 to 60 g/l. In all of the lakes, PNB were represented by morphologically similar forms. These were motile rods measuring 0.3–0.5 × 1.2–2.5 μm, multiplying by binary fission and containing bacteriochlorophyll *a* and carotenoids of the spheroidene series. 17 pure cultures of PNB were isolated from soda lakes of Chita Oblast, Buryat Republic, Agin Buryat Autonomous District, and northeastern Mongolia. All isolates were weakly halophilic and alkaliphilic and grew in wide ranges of salinity (0.3–15%) and pH (7.5–9.5). The highest growth rate was recorded at a 1–3% salinity and a pH value of about 8.5. The bacteria failed to grow in freshwater medium or at pH 7. All of the isolates were assigned to the genus *Rhodovulum* according to their morphological and physiological properties. For more precise identification of the new isolates, their phylogenetic analysis was performed. According to the results of 16S rRNA gene sequencing and DNA–DNA hybridization, the isolates were most close to the species *Rhodovulum strictum*, from which they however differed at the species level (98.5–99.5% 16S rRNA gene identity and 42–44% DNA–DNA hybridization level). Moreover, the isolates fell into two groups, one of which was comprised by strain A-20s and its close relatives (100% 16S rRNA identity and 93–98% DNA–DNA hybridization level), and the other was represented by the phylogenetically distinct strain A-36s (98.7% 16S rRNA identity and 50–55% DNA–DNA hybridization level). In the nucleotide sequences of the 16S rRNA genes, sequence signatures were revealed that were specific to the isolates and the two closest species and distinguished them from other *Rhodovulum* representatives. Thus, the new PNB isolates represent two new species. Currently, their morphological and physiological investigation is in progress, aiming at their description as two new species of the genus *Rhodovulum.* The fact that PNB of weakly and moderately mineralized soda lakes are represented by new species is one more piece of evidence in favor of our earlier conclusion about the specificity of these habitats and of the autochthonous microflora characteristic of them.

Key words: purple nonsulfur bacteria, halophiles, alkaliphiles, soda lakes, taxonomy, DNA–DNA hybridization, 16S rRNA gene.

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In the cryoarid zone of the southern Transbaikal Region [1] and northeastern Mongolia [2], small shallow soda lakes are abundant. A specific feature of these lakes distinguishing them from most of the earlier investigated saline and hypersaline alkaline water bodies is their lower mineralization and smaller salt reserves. In addition, the limited drainage and the climatic conditions in this region result in a highly unstable hydrochemical regime. The water level, mineralization, alkalinity, and pH in these lakes undergo considerable variations, both seasonal and those occurring with a long term periodicity.

Earlier, for southern Transbaikal Region lakes as an example, we showed that weakly and moderately mineralized soda lakes are a peculiar type of habitat that has its specific autochthonous microflora; this habitat differs both from highly mineralized soda lakes and from shallow saline water bodies of thalassic origin [1]. The specificity of weakly and moderately mineralized soda lakes is also evidenced by the abundance of new

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Table 1. Characteristics of the studied soda lakes in which PNB were found

Lake location	Lake name	pH	Mineralization, g/l	Total alkalinity (CO_3^{2-} + HCO_3^-), g/l	PNB abundance, logcells/ml	Designation of the PNB isolate
Chita Oblast	Barun-Torei	9.1	1.8	0.7	6	A-4s
	Ostozhe	9.2	2.2	1.1	3	–
	Ilim-Torom	9.5	2.5	1.8	5	A-9s
	Malyi Kasytui	9.5	2.0	1.6	4	A-12s
	Dabasa-Nur	9.4	10.0	1.0	6	A-14s
	Nizhnii Mukei	9.5	8.3	4.7	3	–
	Ulan-Nur	10.5	22.5	3.2	4	–
Agin Buryat Autonomous District	Gorbunka	9.8	6.5	1.1	7	A-18s
	Gonzogor	9.3	1.0	0.8	5	–
	Khilganta	9.5	40.0	1.5	6	A-20s
	Bezymyannoe	9.2	0.8	0.7	2	–
	Nozhei	8.9	0.5	0.4	2	A-23s
	Bortogo-Nur	9.6	3.5	1.5	3	–
	Khoito-Kholvo-Torom	9.5	14.5	1.1	4	A-44s
	Khuzhirta	10.0	5.6	1.1	3	A-46s
	Oshoroi-Nur	9.9	4.7	2.1	6	–
Buryat Republic	Verkhnee Beloe	10.1	7.5	4.1	6	A-26s
	Nizhnee Beloe	9.8	3.7	2.0	6	A-27s
	Tsaidam	10.2	15.8	5.2	3	A-30s
	Selendumskoe	8.5	0.7	0.3	4	–
	Sul'fatnoe	9.2	7.7	1.1	6	A-36s
	Nukhe-Nur	9.9	16.8	7.8	5	–
Mongolia	Tsaidam-Nuur	9.2	18.0	2.4	+	M-4s
	Barun-Uldziit-Nur	9.9	19.0	4.2	+	–
	Toson	9.8	7.6	ND	+	M-31s
	Dzun-Tukhem-Nur	9.1	27.5	ND	+	M-33s
	Barun-Erenii-Nur	9.8	10.0	ND	+	–
	Dzun-Erenii-Nur	9.6	19.0	3.6	+	M-46s

Notes: "–" means that isolation of PNB was not performed; "+" means that PNB were present but their cell number was not determined; "ND" stands for "no data."

forms of anoxygenic photosynthetic bacteria (purple sulfur bacteria and heliobacteria) that have never been found in other ecosystems and were described by us as new taxa, including taxa of the generic level [1]. However, no detailed investigation of the purple nonsulfur bacteria (PNB) inhabiting weakly and moderately mineralized soda lakes has been conducted.

The present work is devoted to the study of the PNB of weakly and moderately mineralized soda lakes of the southern Transbaikal Region and northeastern Mongolia and to determination of their taxonomic status.

MATERIALS AND METHODS

The subjects of this study were PNB of soda lakes of the southern Transbaikal Region (Dzhidinskii, Selenginskii, and Kurumkanskii Districts of Buryat Republic; Agin Buryat Autonomous District; Chita Oblast) and of northeastern Mongolia. Characteristics of the habitats and designations of the PNB isolates are given in Table 1.

The methods used in the field studies and those used for enumeration, isolation, and cultivation of pho-

totrophic bacteria have been described in detail in our previous paper [1].

Cell morphology was examined under an Olympus BX-41 light microscope equipped with a phase-contrast device.

DNA–DNA hybridization. The isolation and purification of DNA was carried out with the use of *N-N*-hexadecyltrimethylammonium bromide [3]. DNA–DNA hybridization was carried out on Hiiu Kalur (Estonia) micropore neylon membranes with the use of labeled deoxycytidine [1',2',5',-^{3}H]-triphosphate and a no. 5500 nick-translation kit (GE Healthcare Amersham, UK). Hybridization was performed under optimal conditions at 50°C in a Denhardt solution containing 50 vol % formamide [4]. The duration of hybridization was 24 h.

16S rRNA gene sequencing. DNA was isolated by a modified Birnboim–Doly alkaline extraction procedure [5] and according to the Promega (United States) Wizard technology. A universal primer system [6] was used to amplify 16S rRNA gene fragments. PCR products were analyzed by electrophoresis in 2% agarose at 6 V/cm. The gels were photographed using the BioDocII videodocumentation system (Biometra, Germany). Isolation and purification of PCR products from low-gelling temperature agarose was performed using a Wizard PCR Preps kit (Promega) according to the manufacturer's instructions. The amplification products were sequenced by the Sanger method [7] on an ABI PRIZM 3730 automatic sequencer (Applied Biosystems, Inc.) using the Big Dye Terminator v.3.1 kit (Applied Biosystems, Inc., United States) according to the manufacturer's instructions. Sequencing was performed in both directions using both external and internal primers.

Analysis of the nucleotide sequences of 16S rRNA genes. Primary comparison of the de novo determined sequences with GenBank sequences was performed using the NCBI BLASTN program (http://www.ncbi.nlm.nih.gov/blast) [8]. Editing of sequence spectrograms was done with the Chromas v. 1.45 editor (http://www.techelysium.com.au/chromas.html). The 16S rRNA gene sequences of the type strains of valid species of the genus *Rhodovulum* were retrieved from RDP II release 9.59 (http://rdp.cme.msu.edu/) [9]; they had the following GenBank accessions: *Rhodovulum strictum*, D16419; *Rdv. sulfidophilum*, D16423; *Rdv. euryhalinum*, D16426; *Rdv. iodosum*, Y15011; *Rdv. robiginosum*, Y15012; *Rdv. imhoffii*, AM180953; *Rdv. adriaticum*, D16418; *Rdv. visakhapatnamense*, AM180707; *Rhodobacter sphaeroides*, X53853. Nucleotide sequences were aligned using Multalin program (http://bioinfo.genopole-toulouse.prd.fr/multalin) [10]. The positions of sequence signatures were determined in *E. coli* numbering [11]. The phylogenetic trees were constructed using methods implemented in the TREECONW 1.3b software package [12]: calculation of evolutionary distances with Jukes & Cantor's correction and neighbor-joining.

The newly determined nucleotide sequences of 16S rRNA genes have been deposited in GenBank with the accession numbers EU741680–EU741685 and EU918391.

RESULTS AND DISCUSSION

Distribution of PNB in the Soda Lakes Investigated

In terms of the mineralization level of their water, the investigated lakes of the southern Transbaikal Region [1] and northeastern Mongolia [2] varied from fresh (less than 1 g/l) to moderately saline (25–60 g/l); the majority of the lakes were brackish (1.8–16 g/l) (Table 1). The lake water was alkaline: the pH values were from 8.5 to 10.5; the total alkalinity varied from 0.3 to 7.8 g/l (5–130 mM) but was 1–2 g/l (16–33 mM) in most lakes.

In the warm season, the conditions in the soda lakes of the cryoarid zone are on the whole favorable for the development of phototrophic microbial communities. In most of the lakes studied, massive development of phototrophic organisms in the form of mats or films was observed. The communities of anoxygenic phototrophic bacteria were diverse and exhibited evenness of the species composition [1]. In most of the lakes, representatives of the family *Ectothiorhodospiraceae* predominated. However, the anoxygenic phototrophic bacteria of other groups (*Chromatiaceae, Rhodobacteraceae*, the green filamentous bacteria *Oscillochloris* sp., and heliobacteria) were also quite abundant. According to our preliminary data, no less than 15 species of anoxygenic phototrophic bacteria occurred in the lakes that we studied. At the present moment, three new genera and four new species of purple sulfur bacteria and heliobacteria have already been described [1].

Among anoxygenic phototrophs, PNB turned out to be the most eurybiotic; they occurred in samples from most of the lakes studied (Table 1). In addition, in our laboratory experiments on the effect of pH on the structure of phototrophic communities, PNB occurred in most of the enrichments at all pH and salinity values [13]. Pronounced capacity for adaptation to unstable environmental conditions is a characteristic feature of most representatives of this microbial group. In the southern Transbaikal Region (Dzhidinskii, Selenginskii, and Kurumkanskii Districts of Buryat Republic; Agin Buryat Autonomous District; Ononskii District of Chita Oblast), PNB were found in 22 of the 24 lakes studied; their population density was 10^2 to 10^7 cells/ml and they were often dominant (together with ectothiorhodospiras) [1]. In lakes of northeastern Mongolia, enumeration of phototrophic bacteria was not performed; however, regular occurrence of PNB was noted in lakes with a water mineralization of 3 to 60 g/l [2]. We found PNB in six samples from Mongolian lakes

(Table 1), kindly provided to us by V.M. Gorlenko. On the whole, the data obtained show that PNB are a permanent and essential component of the haloalkaliphilic communities of weakly and moderately mineralized soda lakes.

Morphological and Physiological Characterization of PNB of Soda Lakes

In spite of the wide distribution of PNB in soda lakes of the studied type, no visually detectable diversity of these microorganisms was observed. A sole PNB morphotype (motile spheroidene-containing rods) occurred in all soda lakes of all regions.

For further study of the PNB, 17 pure cultures were isolated from soda lakes of the Chita Oblast, Buryat Republic, Agin Buryat Autonomous District, and northeastern Mongolia (Table 1). All of the isolates shared a number of morphological properties. In natural samples and during culture isolation, these were thin elongated rods (0.3–0.5 $\times$ 1.2–2.5 μm) multiplying by binary fission and containing bacteriochlorophyll *a* and carotenoids of the spheroidene series. Anaerobically grown cultures had yellow–brown coloration; in the presence of oxygen, they turned red. In the process of cultivation, especially in the presence of yeast extract (0.1 g/l), the cells gradually changed their initial shape, becoming shorter and thicker. Cell morphology depended on the organic substrate used, degree of anaerobiosis, and pH and salinity of the medium. Thus, under anaerobic conditions at optimal pH and salinity values, the cells were short rods with rounded ends (Figs. 1a–1d), and, in the presence of oxygen (Figs. 1e, 1f), the cell ends sharpened and the bacteria became morphologically similar to *Rhodobacter capsulatus*. Under all cultivation conditions, the size of cells depended on the organic substrate: on media with propionate, valerate, or some other compounds (Figs. 1a, 1e), the cells were smaller (0.4–0.7 $\times$ 1.0–2.5 μm) than on media with acetate and/or malate (0.8–1.1 $\times$ 2.0–3.5 μm, Figs. 1b, 1f). In the presence of casein hydrolysate or certain amino acids, the bacteria grew in the form of aggregates of small oval cells 0.5–0.7 μm in diameter. For most strains, formation of tiny polymorphic microcolonies (Fig. 1d) was typical, whereas strain A-36s formed chains, including branching ones (Fig. 1c).

All of the studied strains turned out to be weak halophiles and moderate alkaliphiles capable of growing in wide ranges of salinity (0.3 to 10–15%) and pH (7.5–9.5). The highest growth rate was observed at a salinity of 1–3% and pH of about 8.5. They did not grow on fresh medium and/or at pH 7. The metabolism type of the PNB studied was primarily photoorganoheterotrophic, with utilization of a wide range of organic compounds. In addition, they could grow photolithoautotrophically, oxidizing sulfide or thiosulfate to sulfate, and, under aerobic conditions, they could grow chemoorganoheterotrophically.

Based on the results of the morphological and physiological studies, all our PNB isolates could be assigned to the genus *Rhodovulum*.

Results of the 16S rRNA Gene Sequence Analysis

To elucidate the taxonomic status of the new isolates, their phylogenetic analysis was performed. We sequenced 16S rRNA genes of seven *Rhodovulum* sp. strains isolated from soda lakes of different regions (Table 1): A-20s and A-18s (Agin Buryat Autonomous District); A-26s and A-27s (Dzhidinskii District of Buryat Republic); A-36s (Selenginskii District of Buryat Republic); and M-33s and M-46s (Mongolia). Nearly complete sequences were determined for strains A-20s (1349 nucleotides) and A-36s (1370 nucleotides), and partial sequences were determined for the five other strains: A-18s, A-26s, A-27s, M-33s, and M-46s (761, 999, 840, 923, and 831 nucleotides, respectively, beginning approximately at *E. coli* position 50).

Analysis of 16S rRNA gene sequences (Fig. 2) confirmed the affiliation of our isolates with the genus *Rhodovulum*; the most closely related species (98.5–99.5% identity) was *Rdv. strictum*, widespread in coastal marine ecosystems of Japan [14]. Interestingly, although *Rdv. strictum* is a marine species, it prefers, like our isolates, alkaline media (pH growth range of 7.5–9.0 with a pH optimum of 8–8.5).

In the phylogenetic dendrogram, our new isolates formed two clusters (Fig. 2): six strains (A-20s, A-18s, A-26s, A-27s, M-33s, and M-46s) shared 100% 16S rRNA gene identity, whereas strain A-36s was phylogenetically distinct (98.7% identity).

Members of the first cluster were very close to *Rdv. strictum* (99.5% 16S rRNA gene identity). However, they were still closer to each other, exhibiting 100% identity, with the only exception of strain A-27s, which differed by a single nucleotide substitution (C for T in position 590 in *E. coli* numbering). It should be said that, although in five of the six strains of this group only partial 16S rRNA gene sequences (761–999 nucleotides) were determined, it was this region (more precisely, the first 600 nucleotides of the molecule) that accommodated most of the distinctions observed between the nearly complete sequences of strains A-20s, A-36s, and *Rdv. strictum* JCM 9220^T. Therefore, the incompleteness of sequences in this case should not have affected our evaluation of the relatedness of the organisms studied.

Although representatives of the first cluster (A-20s etc.) differed from the *Rdv. strictum* type strain in only 0.5% of the 16S rRNA gene sequence positions, we supposed that they might represent an independent species, since, despite their different geographic origins, the isolates belonging to this cluster had 100% identical 16S rRNA genes.

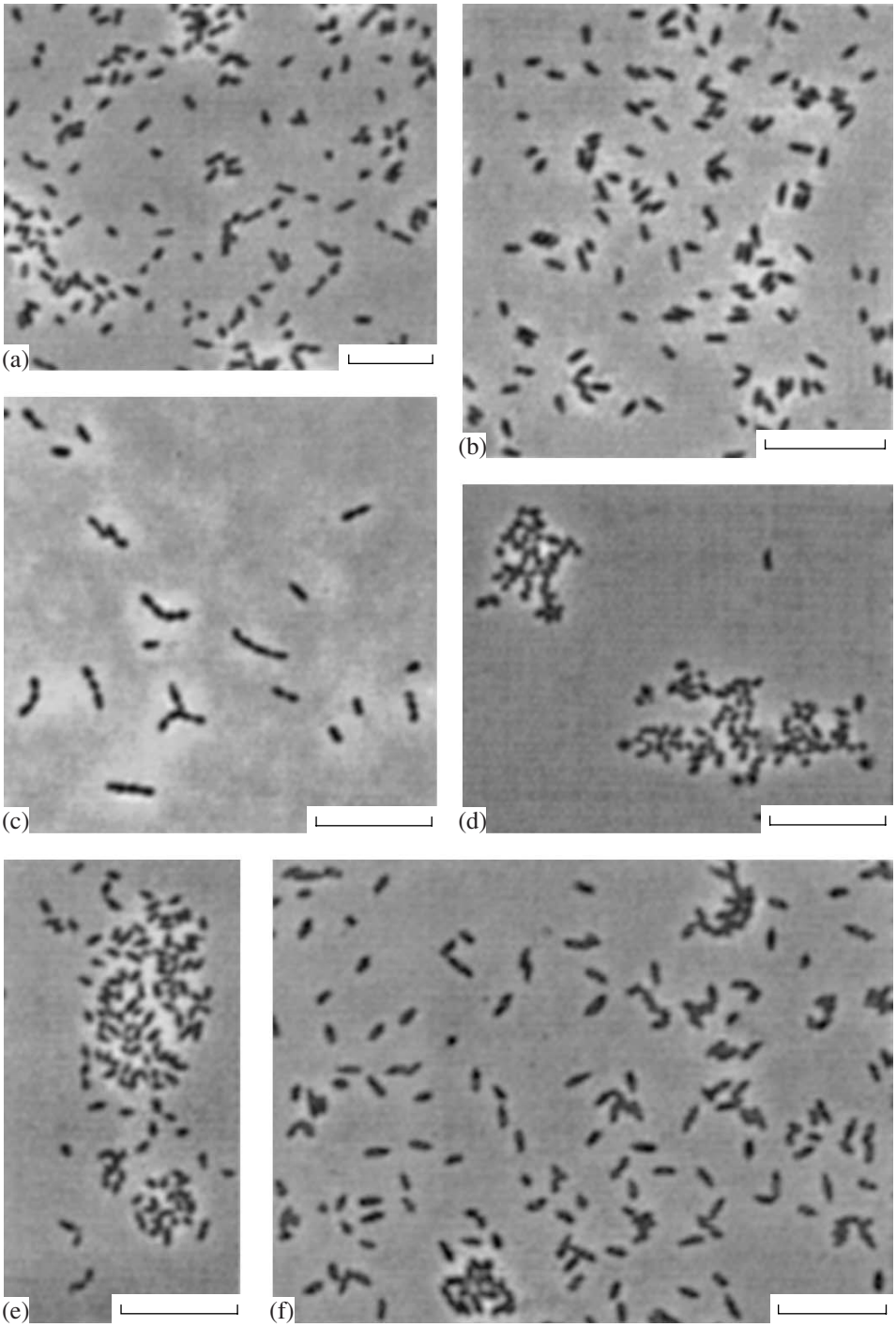

Fig. 1. Cell morphology of the purple nonsulfur bacteria *Rhodovulum* sp. A-20s (a, b, d, f) and *Rhodovulum* sp. A-36s (c, e) grown under various conditions: (a) anaerobically with propionate; (b) anaerobically with acetate; (c, d) anaerobically with casein hydrolysate; (e) aerobically with propionate; and (f) aerobically with acetate. Light microscope with phase-contrast device. Bar, 10 μm.

The second cluster was represented by a single strain, A-36s. It was sufficiently remote both from *Rdv. strictum* JCM 9220^T (98.5%) and from other our isolates (98.7%) to be assigned to a new species according to the recent Stackebrandt's work [15] which showed that 1–1.3% difference between 16S rRNA gene sequences is sufficient to assume affiliation with different species.

Thus, in the weakly and moderately mineralized soda lakes studied by us, we found two presumably new PNB species.

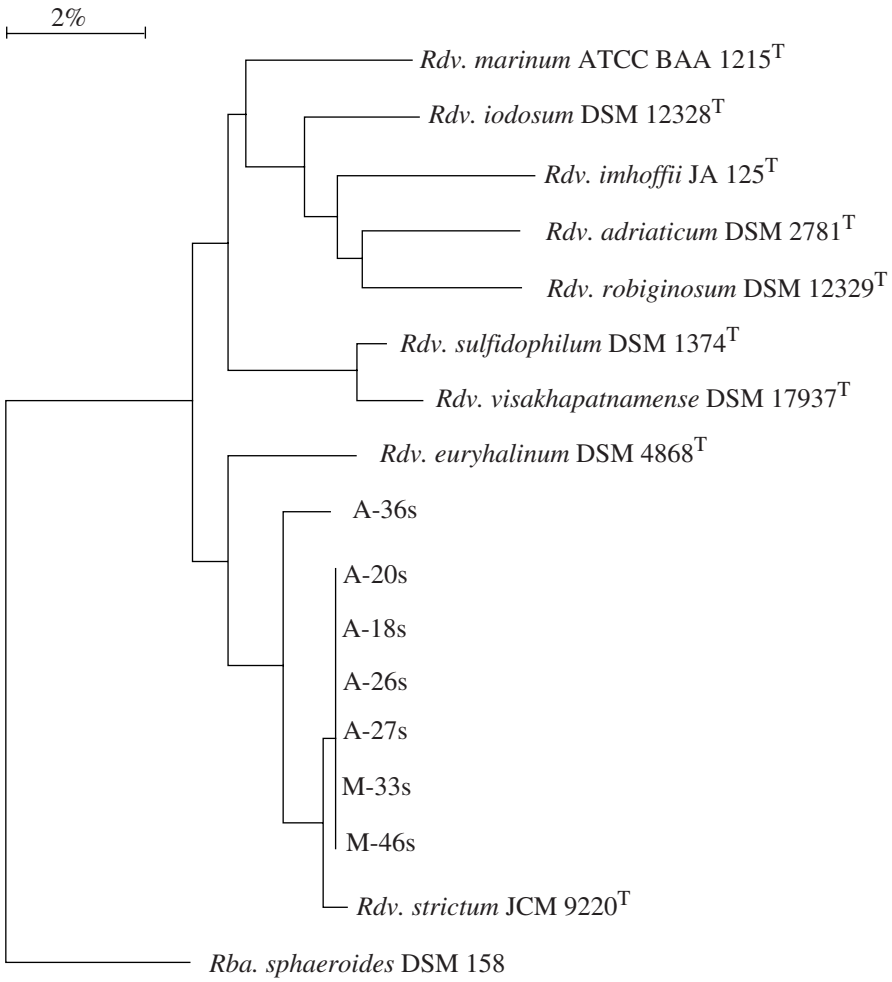

Fig. 2. Dendrogram constructed based on homologies of nucleotide sequences of the 16S rRNA genes of seven PNB isolates from soda lakes and of the type strains of recognized species of the genus *Rhodovulum*.

After aligning with the use of the Multalin program of the 16S rRNA gene sequences of our isolates and sequences retrieved from GenBank, we conducted a search for 16S rRNA sequence signatures characteristic of our isolates and the two closest *Rhodovulum* species (Table 2). We revealed sequence signatures of different ranks, both characteristic of the group as a whole and those typical of individual taxa or pairs of taxa.

In the GenBank database, three 16S rRNA gene sequences of strains identified as *Rdv. strictum* are available. Our 16S rRNA gene sequence analysis showed that, apart from the type strain, none of these strains can be with confidence assigned to *Rdv. strictum.* The 16S rRNA gene sequences of the two other strains (AB079635 and AM696709) exhibit the same sequence signatures as the first cluster of our isolates (strain A-20s etc.); this fact suggests their affiliation to the same species. The species *Rdv. euryhalinum* is represented in GenBank by 16S rRNA gene sequences of two strains; these sequences are very close to each other. In GenBank, there is also a 16S rRNA gene sequence (EF153294) which has a high homology level and common signatures with our strain A-36s (second cluster); this sequence belongs to an unidentified strain, which evidently belongs to the same species as A-36s.

DNA–DNA Hybridization Results

Since the results of the 16S rRNA gene sequencing showed a rather high level of similarity between our new isolates and the type strain of *Rdv. strictum*, DNA–DNA hybridization was carried out (Table 3) to elucidate their taxonomic status. The hybridization experiments involved DNA of four strains: A-20s and A-26s, which had identical 16S rRNA gene sequences and belonged to the first cluster, strain A-36s, which formed a separate cluster, and the type strain *Rdv. strictum* JCM 9220T.

The results of DNA–DNA hybridization agreed with the results of the 16S rRNA sequencing. The DNA–DNA hybridization level between our isolates and the type strain *Rdv. strictum* JCM 9220T was 42–

Table 2. Sequence signatures of 16S rRNA genes of different representatives of the genus *Rhodovulum*

Position in *E.coli* numbering [11]	*Rdv. strictum* JCM 9220^T	*Rhodovulum* sp. A-20s, A-18s, A-26s, A-27s, M-33s, M-46s[1]	*Rhodovulum* sp. A-36s	*Rdv. euryhalinum* DSM 4868^T	Other species of the genus *Rhodovulum*
70	G	G	G	G	A
91	C	C	C	C	T
122	C	G	G	G	G/A
138–141	TTCT	TTCT	AAAG	AAAG	TTCT/TTCA
142	G	G	G	G	C
166	A	A	A	A	G/T
168	G	A	A	A	T/A[2]
181	A	A	G	G	A
186	C	C	T	A	C
197	T	C	C	C	C/T[3]
221	C	C	C	C	G
222–225	AGAA	AGAA	CTTT	CTTT	AGAA/TGAA
636	C	C	T	T	T
839	G	G	G	A	A
846	C	C	C	T	T
999	T	T	T	T	C/G
1009	–	–	–	–	T
1035–1037	ATA	ATA	ATA	ATA	GAT/GAC
1040	A	A	A	A	G/C
1043	–	–	–	–	G/C

[1] Until position 846, the data pertains to all six strains; further, the data are for strains A-20s and A-26S.

[2] *Rdv. marinum.*

3 *Rdv. imhoffii.*

44%, and it was 96% between strains A-20s and A-26s of the first cluster and 50% between these strains and strain A-36s (the second cluster) (Table 3). The 96% DNA–DNA hybridization level indicates affiliation of the microorganisms to the same species, whereas the 40–50% level is characteristic of separate species.

The results of the 16S rRNA gene sequencing and DNA–DNA hybridization demonstrate that the haloakaliphilic PNB strains under study form two phylogenetic clusters either of which differs from other *Rhodovulum* representatives at the species level. Currently, we continue their morphological and physiological studies with the aim to describe them as two new species of the genus *Rhodovulum* with the type strains A-20s and A-36s.

Table 3. DNA–DNA hybridization levels between the new isolates and the type strain *Rhodovulum strictum* JCM 9229^T (%)

Strains	Reference strains (^{3}H-CTP)			
	A-20s	A-26s	A-36s	*Rdv. stricrum* JCM 9220^T
A-20s	100	98	54	42
A-26s	93	100	53	39
A-36s	53	50	100	48
Rdv. stricrum JCM 9220^T	43	44	40	100
Controls without DNA	0	0	0	0

Thus, in weakly and moderately mineralized soda lakes of the cryoarid zone of the southern Transbaikal Region and northeastern Mongolia, we revealed wide distribution of haloalkaliphilic PNB belonging to at least two new species of the genus *Rhodovulum*. It may be assumed that the species most widely spread in lakes of this type is that represented by the group of strains closely related to strain A-20s (the first cluster). Our phylogenetic analysis shows that six out of seven isolates from soda lakes of different regions belong to this species. The second species, represented in our study by a single strain (A-36, the second cluster), is less widely distributed.

The broad distribution of *Rhodobacteraceae* representatives in soda lakes with different physicochemical parameters is evidently related to the eurybiotic nature of the microorganisms of this group and to their adaptation to the unstable environmental conditions typical of these lakes. The fact that PNB of weakly and moder-

ately mineralized soda lakes are represented by new species is one more piece of evidence in favor of our earlier conclusion about the specificity of these habitats and of the autochthonous microflora characteristic of them.

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REFERENCES

1. Kompantseva, E.I., Bryantseva, I.A., Komova, A.V., and Namsaraev, B.B., The Structure of Phototrophic Communities of Soda Lakes of the Southeastern Transbaikal Region, *Mikrobiologiya*, 2007, vol. 76, no. 2, pp. 243–252 [*Microbiology* (Engl. Transl.), vol. 76, no. 2, pp. 211–219].
2. Sorokin, D.Yu., Gorlenko, V.M., Namsaraev, B.B., Namsaraev, Z.B., Lysenko, A.M., Eshinimaev, B.Ts., Khmelenina, V.N., Trotsenko, Yu.A., and Kuenen, J.G., Procaryotic Communities of the North-Eastern Mongolian Soda Lakes, *Hydrobiologia*, 2004, vol. 522, pp. 235–248.
3. Wolfrum, T., Gruner, G., and Stolp, H., Nucleic Acid Hybridization of Pink-Pigmented Facultative Methylotrophs and Pseudomonads, *Int. J. Syst. Bacteriol.*, 1986, vol. 36, no. 1, pp. 24–28.
4. Tijssen, P., Hybridization with Nucleic Acids Probes. Part II. Probe Labeling and Hybridization Techniques, *Laboratory Techniques in Biochemistry and Molecular Biology*, Van Der Vliet, P.C., Ed., Amsterdam: Elsevier Science, 1993, vol. 24, pp. 391–406.
5. Birnboim, H.C. and Doly, J., A Rapid Alkaline Extraction Procedure for Screening Recombinant Plasmid DNA, *Nucleic Acids Res.*, 1979, vol. 7, no. 6, pp. 1513–1523.
6. Lane, D.J., 16S/23S rRNA Sequencing, *Nucleic Acid Techniques in Bacterial Systematics*, Stackebrandt, E. and Goodfellow, M., Eds., Chichester: Wiley, 1991, pp. 115–175.
7. 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.
8. Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J., Basic Local Alignment Search Tool, *J. Mol. Biol.*, 1990, vol. 215, pp. 403–410.
9. Cole, J.R., Chai, B., Farris, R.J., Wang, Q., Kulam-Syed-Mohideen, A.S., McGarrell, D.M., Bandela, A.M., Cardenas, E., Garrity, G.M., and Tiedje, J.M., The Ribosomal Database Project (RDP-II): Introducing MyRDP Space and Quality Controlled Public Data, *Nucleic Acids Res.*, 2007, vol. 35, Database issue, pp. D169-D172
10. Corpet, F., Multiple Sequence Alignment with Hierarchical Clustering, *Nucleic Acids Res.*, 1988, vol. 16, pp. 10881–10890.
11. Brosius, J., Palmer, M.L., Kennedy, P.J., and Noller, H.F., Complete Nucleotide Sequence of a 16S Ribosomal RNA Gene from *Escherichia coli, Proc. Natl. Acad. Sci. USA*, 1978, vol. 75 P, pp. 4801–4805.
12. Van de Peer, Y. and De Wachter, R., TREECON for Windows: a Software Package for the Construction and Drawing of Evolutionary Trees for the Microsoft Windows Environment, *Comput. Applic. Biosci.*, 1994, vol. 10, pp. 569–570.
13. Kompantseva, E.I., The Effect of the pH Level on the Communities of Anoxygenic Phototrophic Bacteria of Soda Lakes of the Southeastern Transbaikal Region, *Mikrobiologiya*, 2007, vol. 76, no. 6, pp. 872–878 [*Microbiology* (Engl. Transl.), vol. 76, no. 6, pp. 775–781].
14. Hiraishi, A. and Ueda, Y., Isolation and Characterization of *Rhodovulum strictum* sp. nov. and Some Other Purple Nonsulfur Bacteria from Colored Blooms in Tidal and Seawater Pools, *Int. J. Syst. Bacteriol.*, 1995, vol. 45, no. 2, p. 319–326.
15. Stackebrandt, E. and Ebers, J., Taxonomic Parameters Revisited: Tarnished Gold Standards, *Microbiol. Today*, 2006, Nov 06, pp. 152–155.