ISSN 0026-2617, Microbiology, 2008, Vol. 77, No. 2, pp. 206–218. © Pleiades Publishing, Ltd., 2008.
Original Russian Text © E.N. Boldareva, V.N. Akimov, V.A. Boychenko, I.N. Stadnichuk, A.A. Moskalenko, Z.K. Makhneva, V.M. Gorlenko, 2008, published in Mikrobiologiya, 2008, Vol. 77, No. 2, pp. 241–254.

EXPERIMENTAL ARTICLES

Rhodobaca barguzinensis sp. nov., a New Alkaliphilic Purple Nonsulfur Bacterium Isolated from a Soda Lake of the Barguzin Valley (Buryat Republic, Eastern Siberia)

E. N. Boldareva[a,1], V. N. Akimov[b], V. A. Boychenko[c], I. N. Stadnichuk[d], A. A. Moskalenko[c], Z. K. Makhneva[c], and V. M. Gorlenko[a]

[a] *Winogradsky Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia*

[b] *Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, pr. Nauki 5, Pushchino, Moscow oblast, 142290 Russia*

[c] *Institute of Fundamental Problems of Biology, Russian Academy of Sciences, ul. Institutskaya 2, Pushchino, Moscow oblast, 142290 Russia*

[d] *Bach Institute of Biochemistry, Russian Academy of Sciences, Leninskii pr. 33, Moscow, Russia*

Received February 6, 2007

Abstract—A novel strain, alga-05, of alkaliphilic purple nonsulfur bacteria was isolated from sediments of a small saline (60 g/l) soda lake near Lake Algin (Barguzin Valley, Buryat Republic, Russia). These bacteria contain bacteriochlorophyll *a* and carotenoids of the alternative spirilloxanthin group with predominating demethylspheroidenone. They are facultative anaerobes; their photosynthetic structures are of the vesicular type and arranged along the cell periphery. Growth of this strain is possible in a salinity range of 5–80 g/l NaCl, with an optimum at 20 g/l NaCl. Best growth occurred at 20–35°C. Analysis of the 16S rRNA gene sequences demonstrated that the studied isolate is closely related to the alkaliphilic purple nonsulfur bacterium *Rhodobaca bogoriensis* (99% similarity) isolated from soda lakes of the African Rift Zone. According to the results of DNA–DNA hybridization, strain alga-05 has a 52% similarity with the type species of the genus *Rhodobaca.* On the basis of the obtained genotypic data and some phenotypic properties (dwelling in a hypersaline soda lake of Siberia, moderate halophily, ability to grow at relatively low temperatures, etc.), the isolated strain of purple bacteria was described as a new species of the genus *Rhodobaca*, *Rca. barguzinensis* sp. nov.

Key words: soda lakes, extremophiles, alkaliphiles, purple nonsulfur bacteria, *Rhodobaca.*

DOI: 10.1134/S0026261708020148

Purple Nonsulfur bacteria (PNB) have been detected extremely rarely in the microbial communities of soda lakes. For instance, purple nonsulfur bacteria were detected in the highly mineralized soda lakes of Wadi Natrun (Egypt); however, no cultures of haloalkaliphilic species have been isolated from these habitats [1]. Alkalitolerant purple nonsulfur bacteria of the *Rhodobacter–Rhodovulum* group were later isolated from samples collected in lowly mineralized soda lakes of eastern Siberia and Mongolia [2]. The only known species of alkaliphilic nonsulfur bacteria, *Rhodobaca bogoriensis*, was recently described [3]. Three closely related strains have been isolated from bottom sediments of two soda lakes (Lake Bogoria and Crater Lake) of the African Rift Zone. These are soda lakes with relatively low mineralization where salt concentrations are about 5%.

The cells of *Rca. bogoriensis* are motile cocci or short rods arranged in chains; they reproduce by transverse division. The unique property of these PNB is the small number of photosynthetic vesicular membrane structures located only along the cell periphery. The cells of *Rca. bogoriensis* contain bacteriochlorophyll *a* (BChl *a*) and the carotenoid demethylspheroidenone, which have not been revealed in other purple nonsulfur bacteria. The photosynthetic apparatus of *Rca. bogoriensis* does not contain the peripheral antenna complex LH II (adsorption at 800 and 850 nm); on the contrary, it contains a pronounced light-harvesting complex LH-I (adsorption at 871 nm). *Rca. bogoriensis* is a photoheterotroph and a facultative chemoheterotroph. This microorganism is peculiar in its inability to grow under photolithoautotrophic conditions on hydrogen and sulfide and to fix molecular nitrogen; this is uncharacteristic of purple nonsulfur bacteria. This species shows good growth under anaerobic conditions

[1] Corresponding author; e-mail: boldareva@gmail.com

on media containing organic substrates. *Rca. bogoriensis* is a halotolerant alkaliphile which grows in a pH range of 7.5–10.0 with an optimum at about 9. No growth was detected at temperatures lower than 30°C and higher than 45°C. The optimum temperature of growth was 39°C.

So far, no strains of alkaliphilic purple nonsulfur bacteria have been isolated from lakes of temperate climatic zones.

The aim of the present work was to determine the physiological and biochemical characteristics, as well as the phylogenetic position, of the new species of alkaliphilic purple nonsulfur bacteria isolated from the steppe saline soda lake of the Barguzin Valley (Buryat Republic, Eastern Siberia), the region characterized by a sharply continental climate and relatively low summer temperatures.

MATERIALS AND METHODS

Cultivation methods. Bacteria were cultivated in a medium containing the following (g/l): NH_4Cl, 0.4; KH_2PO_4, 0.5; $MgCl_2$, 0.2; Na_2SO_4, 0.5; yeast extract, 1; sodium acetate, 1; sodium pyruvate,1; NaCl, 20; $Na_2S_2O_3 \cdot 5H_2O$, 1; KCl, 0.5; $NaHCO_3$, 10; Na_2CO_3, 5; vitamin B_{12}, 10 μg/l; and trace element solution (according to Pfennig), 1 ml/l (pH 9.0) [4].

Water solutions of $NaHCO_3$ (10%), Na_2CO_3 (10%), yeast extract (5%), sodium acetate (10%), sodium pyruvate (10%), and sodium thiosulfate (10%) were prepared and sterilized separately, and then added to the medium immediately before the inoculation.

The cells were cultivated under anaerobic conditions in the presence of light in 20-ml penicillin vials filled with liquid medium up to the brim and sealed with rubber stoppers, or under aerobic conditions in the dark in 500-ml conical flasks with 300 ml of the medium. Purification of the cultures obtained was carried out by repeated streaking of the individual colonies formed under aerobic conditions on the medium solidified with 2% (wt/vol) agar. The culture purity was confirmed by microscopic examination of the obtained colonies.

Morphology and ultrastructure. The morphology of bacterial cells was studied under an Olympus light microscope (Japan) with a phase-contrast device and also under an electron microscope in specimens of whole cells stained with a 0.2% aqueous solution of uranyl acetate. To obtain ultrathin sections, the cells were treated according to Kellenberger–Reyter, dehydrated, and embedded in Epon. Ultrathin sections cut with an ultramicrotome were placed on copper grids covered with formvar support and stained with Reynolds reagent [5]. Whole cell specimens and ultrathin sections were examined under a Jeol JEM 100C electron microscope (Japan) at an accelerating voltage of 80 kV.

Pigment composition. The pigment composition was determined using the absorption spectra of the sonicated cell suspension, as well as of the chromatophore fractions and acetone–methanol total extracts (7 : 2, vol/vol). Absorption spectra were determined with an SF 56A spectrophotometer (LOMO, Russia) or a UV-160 spectrophotometer (Shimadzu, Japan) in the 350–900 nm range. In addition, a differential (oxidized–reduced) spectrum of cytochromes was measured in the supernatant after precipitation of the chromatophores.

To obtain a carotenoid extract, one ml of bacterial chromatophore suspension (with an optical density of 40–50 units at 850 nm) was added to 10 ml of an acetone–methanol mixture (7 : 2, vol/vol) under constant stirring. The obtained extract was subsequently supplemented with 2–4 ml of petroleum ether and 20–25 ml of water. The resultant mixture was then stirred. The extracted pigments accumulated in petroleum ether in the upper layer of the mixture, which was removed with a pipette, placed in a penicillin vial, and dried under nitrogen flow. The resultant pigment film was dissolved in an acetone–methanol mixture (7 : 2, vol/vol). Then 25 μl of the extract was applied to a chromatographic column.

The pigment composition was determined by high-performance gas–liquid chromatography (HPLC) on a Spherisorb ODS2 column (4.6 × 250 mm, 5 μm pore size) (Waters; United Kingdom), as described earlier [6]. The carotenoid concentration was determined on the basis of the relevant extinction coefficients and the area of the obtained fraction in the 415–550 nm range using the LC-solution software (Shimadzu, Japan), as described in [7].

Chromatophores were isolated by differential centrifugation of sonicated cells [8].

Photoinhibition of respiration. Photoinhibition of respiration (PIR) was studied polarographically by illumination of cells with light at various wavelengths [9]. A decrease in the O_2 consumption rate in cell suspensions was registered in samples illuminated by 1-s impulses of monochromatic light (0.2 μmol quanta m^{-2} s^{-1}; the spectral half-width of the slit, 3 nm) with 20- to 60-s dark intervals; the wavelength range scanned was 400–930 nm.

Analysis of fatty acids. The fatty acid composition was determined by gas chromatography–mass spectrometry (GC–MS). Dry biomass (5 mg) was treated with 0.4 ml of 1 N HCl in methanol at 80°C for 1 h (acidic methanolysis). The methyl esters of fatty acids and dimethyl acetate formed as a result of methanolysis were extracted with hexane and analyzed on a Sherlock gas chromatograph (Microbial identification system, MIDI Inc., United States) [10].

Physiological characteristics and growth conditions. To determine the spectra of utilized organic substrates and nitrogen sources under anaerobic conditions in thelight and under aerobic conditions in the dark, the cultures were grown on the above medium with NaCl,

20 g/l, B_{12}, 10 μg/l; and yeast extract, 0.1 g/l as a vitamin source. The medium did not contain any other organic substrates or nitrogen sources (depending on the goal of the experiment). The pH of the medium was adjusted within the range of 8.5–9.0 by adding sodium carbonate or bicarbonate. The tested organic compounds and nitrogen sources were added to concentrations of 1 and 0.5 g/l, respectively.

To determine the resistance of the studied bacteria to sodium sulfide and their capacity for its utilization by photosynthesis, the cultures were grown on organics-containing media at various $Na_2S \cdot 9H_2O$ concentrations (0, 500, 700, and 1000 mg/l). Changes in sulfide concentration were determined colorimetrically using the standard technique, as previously described [11].

The ability of the isolated strain to grow under anaerobic conditions in the dark due to nitrate reduction was determined by the biomass yield and production of N_2 and NO_2^-. The presence of N_2 was determined from gas production in the flasks, whereas the presence of NO_2^- was determined qualitatively by using the Griess reagent [12].

Experiments with various NaCl concentration and pH values were carried out under anaerobic conditions in the presence of light. To elucidate the reaction of the studied bacteria to various pH values, ranging from 6.8 to 7.4, we used phosphate buffer; to obtain media with alkaline pH values (8.0–9.5), carbonate buffer solutions were used [13]. The background NaCl concentration in these media was 10 g/l.

The temperature favorable for the growth was determined using a gradient thermostat at temperatures ranging from 10 to 50°C.

The biomass yield was assessed by the optical density measured with a KFK-3 photometer at 650 nm.

Sensitivity to antibiotics was determined under aerobic conditions by applying filter discs containing the antibiotic in question on cell lawns on solid medium; after incubation, the zone of growth inhibition was registered.

The capacity for aerotaxis was determined in a liquid medium in sealed Pasteur pipettes open at the wide ends. The medium contained an active culture of motile cells of the new strain, alga-05, grown under anaerobic conditions in the presence of light.

The presence of ribulose bisphosphate carboxylase was detected by the previously described technique [14]. The catalase activity was assayed by monitoring the production of gas bubbles after the addition of a 3% hydrogen peroxide solution to the suspension [15].

The reactions of strains alga-05 and LBB1 to tellurium and selenium were determined on agarized media supplemented with Na_2TeO_3 or Na_2SeO_3 (1 g/l) on petri dishes and in agar columns.

Molecular-genetic analysis. The DNA of the new strain was isolated according to the Marmur procedure [16]. The content G+C base pairs in the DNA was determined by the method of Owen et al. [17]. The DNA homology was determined by the optical reassociation method [18]. Amplification and sequencing of the 16S rRNA gene of the isolate was performed using universal primers [19]. The obtained nucleotide sequences were aligned with the corresponding sequences of most closely related bacteria using the CLUSTALX software package. The unrooted phylogenetic trees of the studied bacteria were constructed by the methods implemented in the TREECON software package [20]. The obtained 16S rRNA gene sequences of strain alga-05 have been deposited in the GenBank under the accession number EF554833.

RESULTS AND DISCUSSION

Characteristics of the habitat. Bacteria were isolated from thin shallow-water algo–bacterial mats of a small nameless soda lake (53°38′26.07″ N; 109°57′20.19″ E) located at a distance of 500–700 m from the freshwater Lake Algin (Barguzin Valley, Buryat Republic). There are numerous soda patches on the lake shores. At the time of sampling (September), the total mineralization of the water in the near-shore zone of the lake was 60 g/l; the temperature of the surface of littoral sediments was 31°C; the pH value of the water was 9.62.

Cultural properties, morphology, and fine structure. The pure culture of strain alga-05 was obtained by successive transfers of individual colonies grown on the surface of the agarized medium. Under aerobic conditions, bacteria form light pink rounded convex colonies that turn deep red after prolonged exposure to light. The strain grown in agar columns forms small beige (in the anaerobic zone) and pink (in the aerobic zone) spherical colonies. The cultures grown in liquid medium under anaerobic conditions were beige, flaky, and slimy. The cells of strain alga-05 were represented by ovoid short rods (Fig. 1a, 1b), 1.0×1.5 μm. Individual cells are motile (data not shown). The cells divide by constriction. Prior to division, the cells become elongated and assume an ovoid form. The division often appears to be nonuniform (Fig. 1) and resembles the pattern of *Rhodobacter blasticus* budding. The cell wall structure is of the gram-negative type. The ultrathin sections of bacteria grown anaerobically in the presence of light show intracytoplasmic vesicular structures arranged only along the cell periphery (Fig. 1f). The cells grown under aerobic conditions in the dark do not have intracytoplasmic membranes (Fig. 1d). Storage compounds are represented by electron-transparent rounded inclusions typical of poly-β-hydroxybutyric acid (Fig. 1f). The cells are diffusely surrounded by a slimy substance (data not shown).

Capacity for aerotaxis. Purple bacteria are characterized by phobotaxis which manifests itself as random movements in bright light. Phototaxis, directed movement of an organism toward a source of light, has been

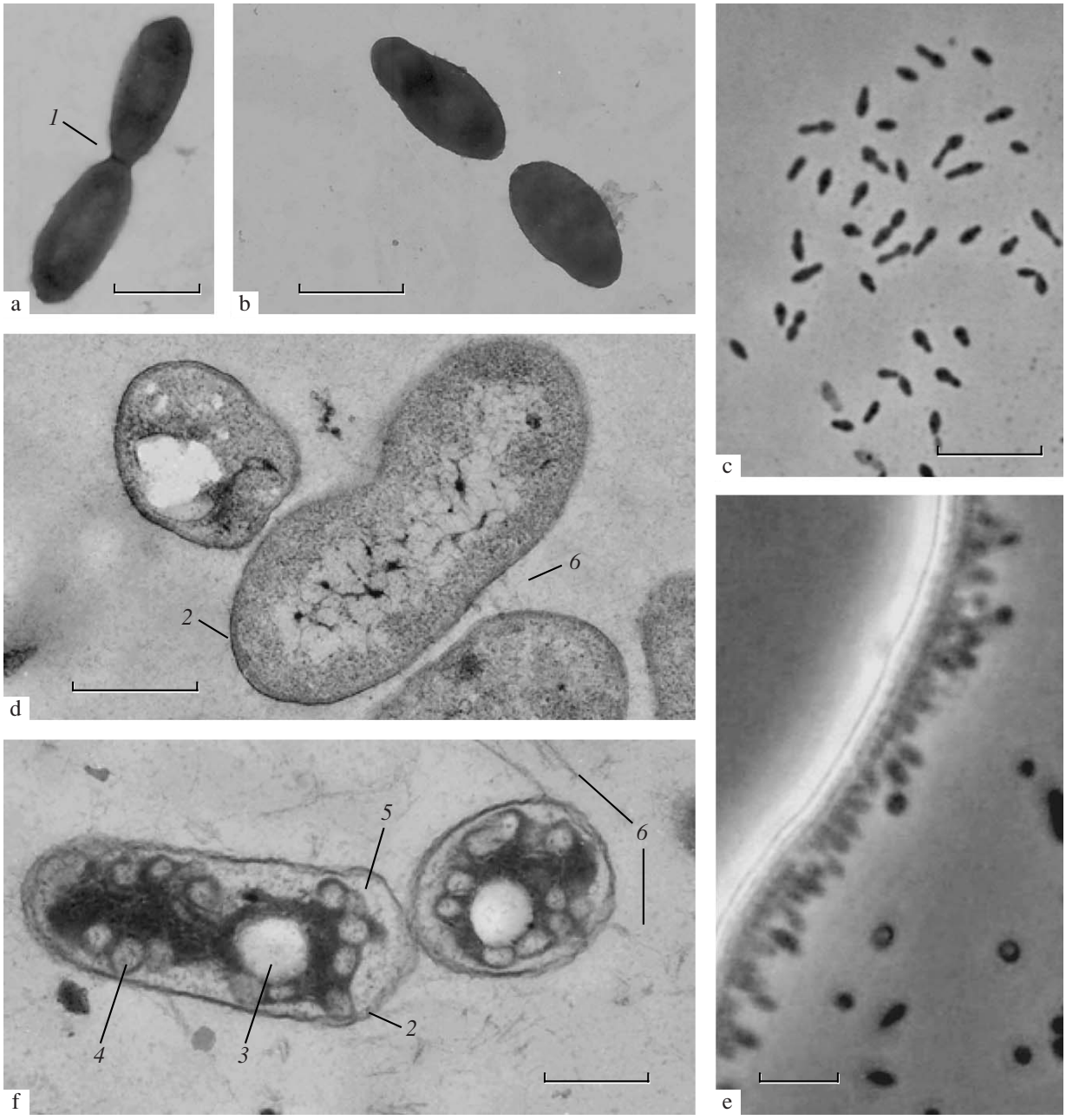

Fig. 1. Morphology and ultrastructure of strain alga-05 cells: (a, b) whole-cell specimen (bar, 1 μm); (c) light micrographs (bar, 10 μm), (d) ultrathin sections of the cells grown under aerobic conditions in the dark, electron micrograph (bar, 0.5 μm); (e) aerotaxis cell concentrated at the air buble boundary (bar, 5 μm); (f) ultrathin sections of the cells grown under anaerobic conditions in the presence of light, electron micrograph (bar, 0.5 μm); (*1*) septum, (*2*) outer membrane, (*3*) reserve compounds; (*4*) vesicular intracytoplasmic membrane; (*5*) periplasmic space; (*6*) slime.

reported for only one species of purple nonsulfur bacteria, *Rhodocista centenaria* [21]. The studied bacteria did not exhibit phototaxis; however, under aerobic conditions, they displayed active positive aerotaxis. After one hour, motile cells accumulated in the open ends of Pasteur pipettes, where the oxygen concentration was highest. Illumination did not prevent the cells from moving to the zone of the highest oxygen concentration. Microscopic examination (Fig. 1e) demonstrated that the cells accumulated at the interface of the liquid and gas phases.

Physiological properties. Bacteria of the strain alga-05 are able to grow under both aerobic conditions in the dark and anaerobic conditions in the presence of light. A wide range of organic compounds were tested as carbon sources for bacterial growth under phototrophic and chemotrophic conditions (Table. 1). The cells of both *Rca. bogoriensis* and strain alga-05 grew

Table 1. Utilization of organic compounds as carbon sources by the new PNB strain and *Rca. bogoriensis*

Substrate	*Rca. bogoriensis*		*Rca. barguzinensis*	
	LBB1* (under anaerobic condition)	LBB1 (under aerobic conditions)	alga-05 (under anaerobic conditions)	alga-05 (under aerobic conditions)
Succinate	4	3	3	4
Malate	4	3	4	4
Fumarate	4	3	4	4
Pyruvate	4	4	2	3
Lactate	2	4	2	4
Acetate	3	4	3	4
Propionate	4	3	2	2
Butyrate	4	3	2	2
Valerate	2	3	2	1
Caproate	4	–	2	1
Heptanoic acid	4	ND	ND	ND
Glucose	4	ND	ND	ND
Fructose	4	4	2	4
Sucrose	4	4	4	4
Ribose	1	2	1	3
Xylose	4	2	ND	2
Mannitol	4	3	4	3
Yeast extract	2	4	4	4
Peptone	1	4	3	4
Casein hydrolysate	2	4	3	4
Soeton	2	4	3	4
Methanol	–	–	–	–
Ethanol	–	–	–	–
Propanol	–	–	–	–
Butanol	–	–	–	–
Pentanol	–	ND	ND	ND
Lactose	–	–	1	2
Citrate	–	–	–	–
Formate	–	–	–	–
Benzoate	–	–	–	–

Note: "–", the compound is not utilized; ND, no data; 1, weak growth; 2, evident growth; 3, good growth; 4, very good growth; *, [3].

on succinate, malate, fumarate, pyruvate, lactate, acetate, propionate, butyrate, valerate, caproate, fructose, and sucrose under anaerobic conditions in the presence of light. No growth occurred on methanol, ethanol, propanol, lactose, citrate, and benzoate as a sole carbon source. The new isolate grows well on yeast extract, peptone, casein hydrolysate, and soeton, whereas the growth of the type strain *Rca. bogoriensis* on these substrates was very weak [3]. The range of substrates utilized aerobically in the dark by the strains under comparison was similar to the range of substrates utilized under phototrophic conditions (Table 1).

Under photoheterotrophic conditions, both *Rca. bogoriensis* and the new strain utilize NH_4Cl, urea, serine, and glutamate as a source of nitrogen. It should be noted that sodium nitrate significantly inhibited growth, probably due to the formation of nitrite (Table 2). Reduction of nitrates to nitrites was observed both under anaerobic conditions in the presence of light and under aerobic conditions in the dark.

Strain alga-05 is not capable of nitrogen fixation, as demonstrated by the absence of the *nifH* gene (personal communication of E.S. Boulygina).

Under anaerobic conditions in the presence of light, bacteria were able to oxidize sulfide (Fig. 2) in the presence of acetate (1 g/l) and yeast extract (1 g/l). Sulfide was not consumed completely. Microscopic examination revealed the presence of sulfur drops in the medium, which disappeared probably due to the reaction with the excess sulfide resulting in polysulfide formation. Sulfate as an oxidation product was not detected. The data in Table 3 demonstrate that the maximum amount of sulfide was utilized at a $Na_2S \cdot 9H_2O$ concentration of 700 mg/l. Strain alga-05 was found to be less tolerant to sulfide than the previously described species *Rca. bogoriensis*. The strain did not utilize thiosulfate both under anaerobic and aerobic conditions. Under chemolithoheterotrophic conditions, the new strain was not able to grow autotrophically on reduced sulfur compounds. It should be noted that the gene encoding the synthesis of ribulose bisphosphate carboxylase (RuBisCo), the key enzyme responsible for carbon dioxide fixation, was not revealed in either the studied or type strains (personal communication of T.V. Kalganova).

Our study has revealed that both strains, alga-05 and *Rca. bogoriensis*, are obligate alkaliphiles growing in a pH range 7.5–9.0 with an optimum at 8.2 (Fig. 3).

The optimal NaCl concentration for bacterial growth was 20 g/l (Fig. 4). However, the cells were not able to grow without sodium chloride, being highly tolerant to this compound. The inability to grow in the absence of sodium chloride was demonstrated and confirmed by triple transfers of the new strain to a salt-free medium. The obligate requirement for sodium chloride distinguishes strain alga-05 from *Rca. bogoriensis*, which grows well in media without NaCl.

Table 2. Utilization of various nitrogen sources by the PNB strain under study and *Rca. bogoriensis* under photoheterotrophic conditions

Substrate	LBB1 (under anaerobic condition)*	alga-05 (under anaerobic condition)
NH_4Cl	4	4
Urea	2	4
Serine	2	3
Glycine	2	ND
Aspartate	4	ND
Glutamate	4	4
$NaNO_3$	–	–

Note: "–", the compound is not utilized; ND, no data; 1, weak growth; 2, evident growth; 3, good growth; 4, very good growth; *, [3].

Under anaerobic conditions, the best growth of strain alga-05 occurred at 23–35°C (Fig. 5). At temperatures below 10°C and above 45°C, no growth occurred. It should be noted that the previously studied *Rca. bogoriensis* strains did not grow at temperatures lower than 30°C and had a growth optimum at 39°C.

Some differences between the new isolate and the type strain *Rca. bogoriensis* were revealed in their sensitivity to antibiotics (Table 4). Both strains were sensitive to the following antibiotics: ampicillin, benzylpenicillin, vancomycin, lincomycin, neomycin, novobiocin, polymyxin, rifampicin, streptomycin, tetracycline, and erythromycin. Unlike *Rca. bogoriensis*, the new isolate was resistant to gentamicin, kanamycin, and nalidixic acid.

For the first time, it has been shown that both strain alga-05 and *Rca. bogoriensis*, is able to reduce soluble

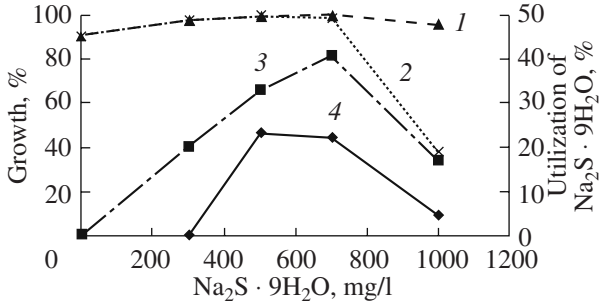

Fig. 2. Reaction of strains alga-05 and LBB1 to various concentrations of $Na_2S \cdot 9H_2O$ under photoheterotrophic conditions: (*1*) maximal growth of strain LBB1; (*2*) maximal growth of strain alga-05; (*3*) utilization of $Na_2S \cdot 9H_2O$ by alga-05 in the experiments with various $Na_2S \cdot 9H_2O$ concentrations; (*4*) utilization of $Na_2S \cdot 9H_2O$ by strain LBB1 in the experiments with various $Na_2S \cdot 9H_2O$ concentrations (0, 500, 700 1000 mg/l; X-axis).

Table 3. Changes in sulfide concentration in the growth media of the strains under study after seven days of incubation

Initial concentration of $Na_2S \cdot 9H_2O$, mg/l	Final concentration of $Na_2S \cdot 9H_2O$ mg/l (%), strain LBB1	Final concentration of $Na_2S \cdot 9H_2O$, mg/l (%) strain alga-05
500	384 (76.8%)	336 (67.2%)
700	544 (77.7%)	415 (59.3%)
1000	952 (95.2%)	832 (83.2%)

selenite (Na_2SeO_3) and tellurite (Na_2TeO_3) to elemental selenium and tellurium, respectively. The reduction of Na_2SeO_3 and Na_2TeO_3 was most intense under aerobic conditions in the presence of light, whereas it slowed down under anaerobic conditions in the dark. Metalloids accumulated mostly outside the cells. Selenium and tellurium inclusions were sometimes detected within the cells grown under aerobic and microaerobic conditions.

Pigments. In strain alga-05 cells grown under anaerobic conditions in the presence of light, the pigments are represented by BChl *a* and carotenoids; their presence was supported by the maximum absorption at 475 and 507 nm (carotenoids) and 870 and 590 nm (BChl *a*) in the in vivo spectra (Fig. 6a). The absorption spectra of the acetone–methanol extract (Fig. 6b) exhibited absorption maxima at 490 and 520 nm (carotenoids) and 770 nm (BChl *a*). Analysis of the pigments by high-performance liquid chromatography has shown the presence of the following carotenoids: hydroxyspheroidene, neurosporene, spheroidene, and demethylspheroidene (which is the principal carotenoid of the new isolate with the content above 70%) (Table 6). On the whole, the pigment composition of the new isolate is similar to that of *Rca. bogoriensis*; however, demethylspheroidenone, whose share in the total bulk of caro-

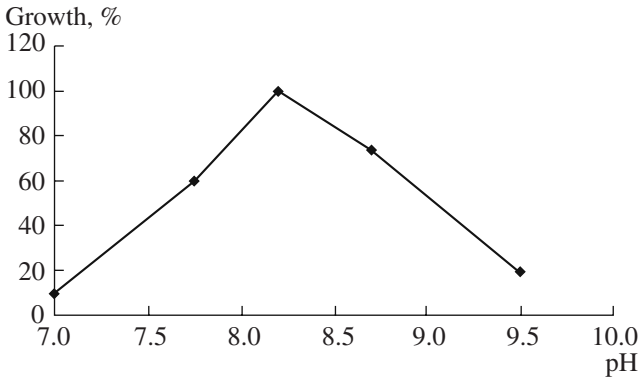

Fig. 3. Effect of pH on the growth of strain alga-05 under anaerobic conditions (maximum growth was taken as 100%).

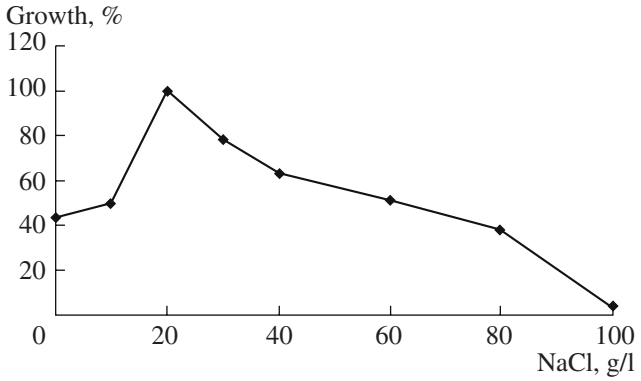

Fig. 4. Effect of NaCl on the growth of strain alga-05 under anaerobic conditions (maximum growth was taken as 100%).

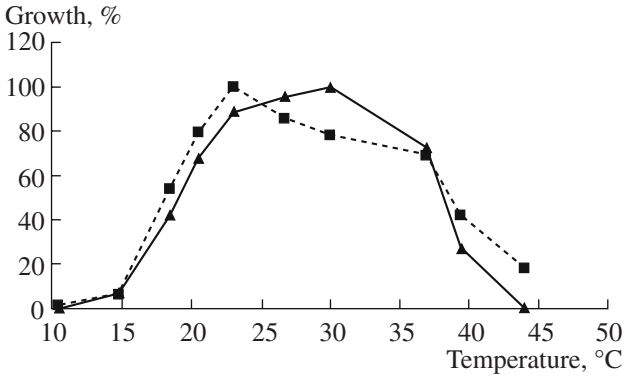

Fig. 5. Effect of temperature on the growth of alga-05 under aerobic (solid line) and anaerobic (broken line) conditions (maximum growth was taken as 100%).

tenoids of the latter strain was 40%, was not detected in the new isolate.

The absorption spectrum of the crude extract of cell membranes and purified chromatophore fraction (Fig. 7a) reveals the presence of two principal absorption maxima at 414 nm (cytochrome) and 871 nm (LH1 complex). The content of cytochrome is approximately 5 times higher than the content of bacteriochlorophyll, indicating its important functional role (Table 5). The additional absorption band at 800 nm confirms the presence of the standard core complex (LH1–RC complex). After precipitation, the chromatophore fraction barely contained cytochrome, which was all released into the supernatant; cytochrome is therefore not bound to the membranes. In the chromatophore absorption spectrum (Fig. 7a), a small cytochrome shoulder is present, and the infrared region of the spectrum is similar to that of *Rhodospirillum rubrum*. The cytochrome belongs to the c_{550} type, which is demonstrated by the differential oxidized–reduced spectrum (Fig. 7b).

Table 4. Sensitivity of strains alga-05 and LBB1 to antibiotics

Antibiotic	Strain alga-05	Strain LBB1
Ampicillin	+	+
Benzylpenicillin	+	+
Vancomycin	+	+
Gentamycin	–	+
Kanamycin	–	+
Lincomycin	+	+
Nalidixic acid	–	+
Neomycin	+	+
Novobiocin	+	+
Polymyxin	+	+
Rifampicin	+	+
Streptomycin	+	+
Tetracycline	+	+
Erythromycin	+	+

Note: "+", the compound is utilized or the strain is sensitive to the compound; "–", the compound is not utilized or the strain is resistant to the compound.

Photoinhibition of respiration (PIR). The new purple nonsulfur bacterium alga-05 grows well under aerobic conditions. It was shown that light (strictly in a wavelength range corresponding to absorption spectra of the photosynthetic pigments of cells) reversibly inhibits respiration phototrophically grown cells; similar phenomena have been demonstrated in our experiments with *Rs. rubrum* [25] and aerobic bacteriochlorophyll *a*-containing bacteria *Roseinatronobacter thiooxidans* (ALG1), and *Rna. monicus* (ROS35) [4]. The PIR spectrum of strain alga-05 exhibited BChl *a* bands at 590, 800, and 870 nm and carotenoid bands at 475, 507, and 550 nm (Fig. 8a). A comparison with the PIR spectrum of strain ALG1 (Fig. 8b) demonstrates the difference of carotenoid qualitative composition in the light-harvesting antenna complexes of these species. However, the efficacy of the excitation energy transition from demethylspheroidene (strain alga-05) and spheroidene (aerobic anoxygenic phototrophic bacteria of the genus *Roseinatronobacter*) to the photosynthesis reaction centers is similar and several times higher than that in the case of the excitation energy transition from spirilloxanthin to the reaction centers of *Rs. rubrum*. A short-wave 10-nm shift of the main bacteriochlorophyll *a* band observed in strains alga-05 and ALG1 (as compared to *Rs. rubrum*) can be attributed to the peculiarities of the interaction between the antenna molecules of this pigment and the carotenoids of pigment–protein complexes. The data on the photoinhibition of alga-05 respiration indicate the presence of the photosynthetic electron transport chain (ETC) intersecting with the respiratory ETC of this purple bacterium at the sites of the common electron carriers.

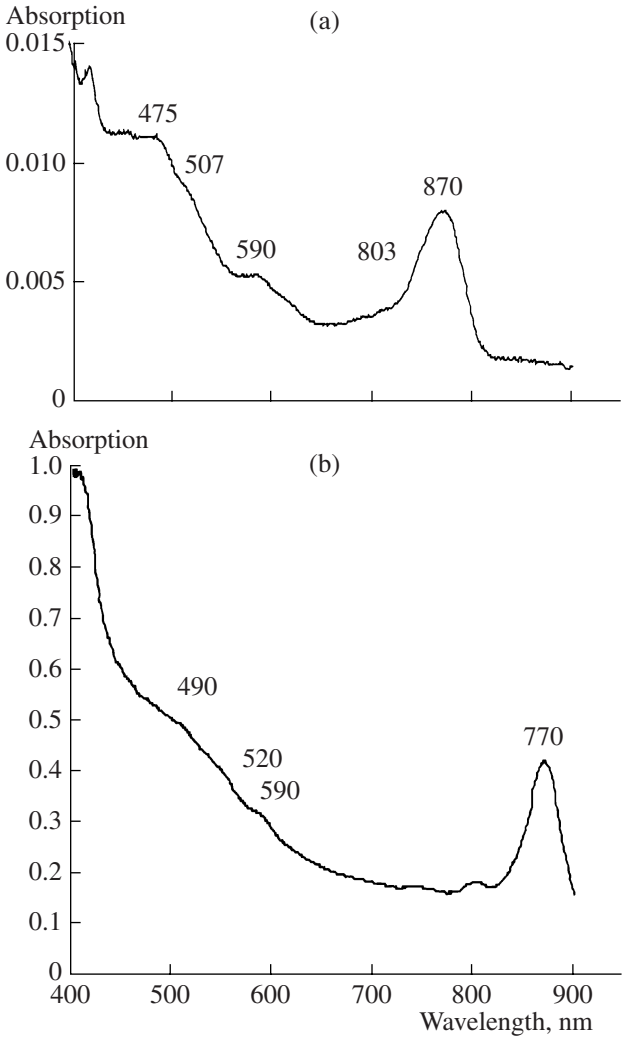

Fig. 6. In vivo pigment absorption spectrum of strain (a) alga-05 and (b) acetone–methanol total extract.

Fatty acid composition. An isomer of the monounsaturated fatty acid C18:1ω7 was predominant both in the studied isolate and in the type strain *Rca. bogoriensis* LBB1 (79.32% and 67.28% of total fatty acids, respectively). Significant quantities of hexadecanoic acid (16:0) were found in both strains (9.69% and 18.66% in strains alga-05 and LBB1, respectively). The cells of both strains contained similar amounts of tetradecenoic (14:1) and 7-hexadecenoic (16:1ω7) acids (Table 1). Unlike the new isolate, the type strain LBB1contained also the following fatty acids: 12:0, 14:0, 2h14, i15, a15, 2h15, and 18:1ω9) (Table 7). Hence, the fatty acid composition of the studied strains differs mainly in the minor constituents.

Phylogenetic position. Members of the genus *Rhodobaca*, including strain alga-05, occupy a phylogenetic position in a cluster with spheroidene-containing species of the genera *Rhodovulum* and *Rhodobacter*; however, they are rather distant from these species (92.2–94.5% similarity) (Fig. 9). Members of the genus *Rhodobaca* are characterized by a low G+C content (59 mol %), while the content of G+C content of the members of the *Rhodovulum–Rhodobacter* group varies between 62.1 and 69 mol %. In addition to purple nonsulfur bacteria, this cluster includes alkaliphilic aerobic bacteriochlorophyll *a*-containing bacteria of the genus *Roseinatronobacter* and the facultatively chemoautotrophic bacterium *"Natronohydrobacter thiooxidans,"* which does not contain bacteriochlorophyll *a*. From the evolutionary perspective, it is noteworthy that members of the genus *Rhodobaca* are closely related to alkaliphilic chemoorganotrophic aerobic BChl *a*-containing bacteria of the genus *Roseinatronobacter.*

To elucidate the phylogenetic position of the new isolate, the nucleotide sequences of 16S rRNA genes were determined (Table 8). The species *Rca. bogoriensis* was found to be most closely related to strain alga-05

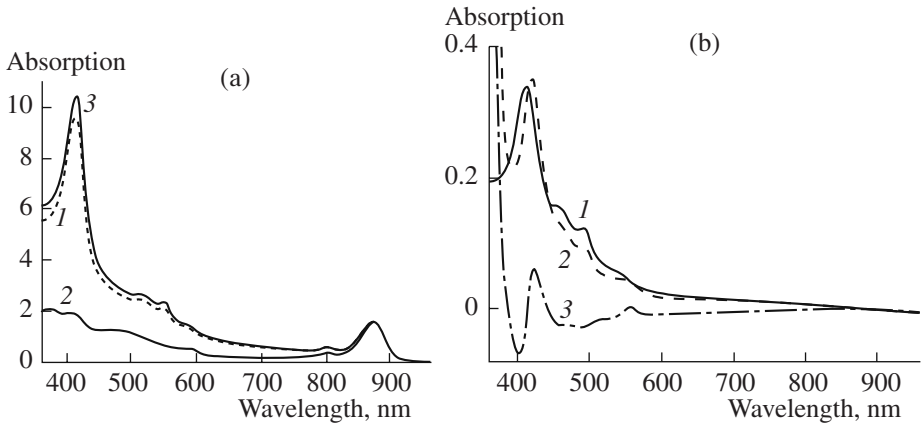

Fig. 7. Absorption spectra of various fractions of strain alga-05 cells: (a) (*1*) crude fraction of the membranes of disintegrated cells; (*2*) purified chromatophore fraction; (*3*) supernatant after chromatophore precipitation; (b) spectra of cytochrome absorption; (*1*) oxidized cytochrome; (*2*) reduced cytochrome; (*3*) difference absorption spectrum.

Table 5. Content of the major membrane components in strain alga-05 cells grown under anaerobic conditions in the presence of light

Membrane component	Quantity
Protein, mg/ml	21.2 (absorption at 280 nm)
Cytochrome, nmol/ml	140.0
BChl, nmol/ml	36.0
BChl/carotenoids, mol/mol	1.3
BChl/protein, nmol/mg	1.7
Carotenoids/protein, nmol/mg	1.27

Table 6. Carotenoid composition in strain alga-05 and strain LBB1 cells grown under anaerobic conditions in the presence of light

Carotenoid	Content, %	
	alga-05	LBB1*
UC	1.1	–
Spheroidenone	10.7	17.0
Demethylspheroidene and its isomer	74.6	19.0
Demethylspheroidenone	0	40.0
Spheroidene	4.8	7.0
Neurosporene and its isomer	7.01 1.8	18.0

Note: UC stands for "unidentified carotenoid".
* [24].

(99.0–99.11% similarity); therefore, the new isolate undoubtedly belongs to the genus *Rhodobaca*. Stackebrandt et al. [23] have shown that a 1% difference is typical of the species differentiation. According to the results of DNA–DNA hybridization, the similarity between strain alga-05 and the type strain *Rhodobaca bogoriensis* LBB1 was 52%. This gives us an additional reason to describe strain alga-05 as a new species belonging to the genus *Rhodobaca*. The G+C contents

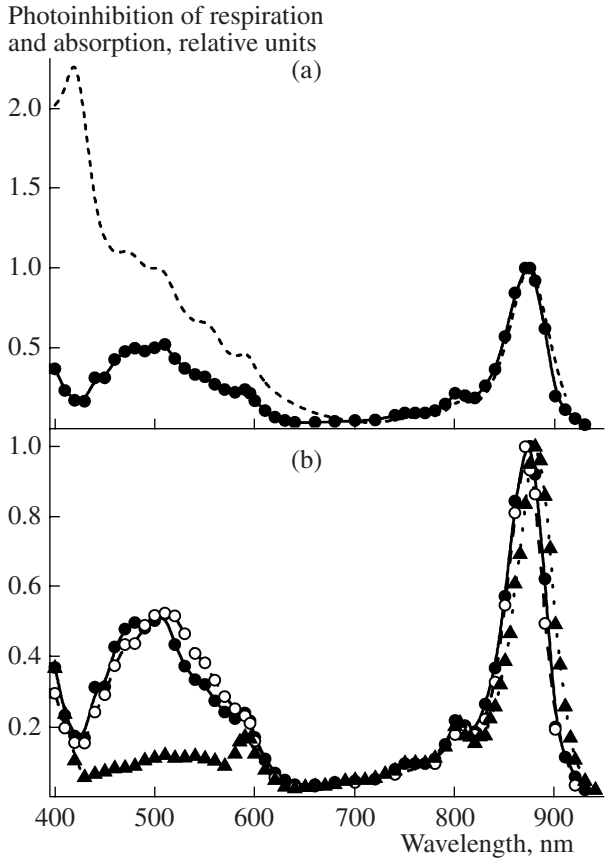

Fig. 8. Spectra of absorption and photosynthetic activity of intact cells: (a) action spectrum of photoinhibition of respiration (black dots and solid line) and absorption spectrum (broken line) for strain alga-05; (b) action spectrum of photoinhibition of respiration for strain alga-05 (black dots), *Roseinatronobacter thiooxidans* ALG1 (white dots), and *Rhodospirillum rubrum* (black triangles).

of the DNA of alga-05 and *Rhodobaca bogoriensis* LBB1 are 59.8 mol % and 58.8 mol %, respectively.

Both organisms share a number of phenotypic characteristics. They are alkaliphiles, their pigments are represented by bacteriochlorophyll *a* and carotenoids (with the prevalence of demethylspheroidene and spheroidenone); they are facultative aerobes and are able to utilize similar groups of organic compounds. For the first time, it has been shown that both the type species of the genus *Rhodobaca* and the isolated strain reduce nitrate to nitrite. Both strains exhibit significant tolerance to sodium sulfide and oxidize it to sulfur under photoheterotrophic conditions. At the same time, there are a number of interspecific differences between the new microorganisms. The type species was isolated from the soda lake of the African Rift Zone characterized by a hot arid climate. Strain alga-05 was isolated from the steppe soda lake of the southeastern Siberia, a region characterized by a sharply continental climate and daily temperature fluctuations in the summer from 44°C to 10°C. As a consequence, the isolated strain shows best growth at relatively low temperatures (with an optimum at 25–30°C), whereas the type strain has a growth optimum at 39°C. Although both organisms are moderately halophilic, the optimal NaCl concentrations are 20–30 g/l and 10–15 g/l for alga-05 and LBB1, respectively. Unlike strain LBB1, strain alga-05 requires the presence of NaCl in the growth medium. There are also some differences in the carotenoid composition and the minor constituents of their fatty acids. Thus, the phenotypic and genetic differences of the new isolate allow us to classify it as a new species, *Rhodobaca barguzinensis* sp. nov., within the family *Rhodobacteriaceae* of the class of *Alphaproteobacteria*.

Taxonomic description of *Rhodobaca barguzinensis* sp. nov.

bar.gu.zin.en'sis, discovered in the Barguzin Valley.

Table 7. Fatty acid composition of the studied strain alga-05 and the purple nonsulfur bacterium LBB1 (*Rca. bogoriensis*))

Acid	Symbol	LBB1	alga-05
Dodecanoic	12:0	0.19	–
Tetradecenoic	14:1	2.12	2.21
Tetradecanoic	14:0	1.02	–
2-Hydroxytetradecanoic	2h14	0.75	–
Isopentadecanoic	i15	0.15	–
Anteisopentadecanoic	a15	0.34	–
7-Hexadecenoic	16:1ω7	2.41	2.64
Hexadecanoic	16:0	18.66	9.69
2-Hydroxypentadecanoic	2h15	0.57	–
9-Octadecenoic	18:1ω9	0.21	–
11-Ctadecenoic	18:1ω7	67.28	79.32
Octadecanoic	18:0	2.40	1.56
11-Methyl-octadecenoic	11Me18:1	3.89	4.58

Note: "–", not detected.

Cells are spherical or oval-shaped, motile by means of flagella, and measure 1.0 × 1.0 or 1.0 × 1.5 μm. Reproduce by division (often nonuniform). The cell wall structure is of the gram-negative type. The cells are surrounded by a loose slimy substance. The strain is capable of anoxygenic photosynthesis under anaerobic conditions. Intracytoplasmic photosynthetic structures are presented by sparse vesicles arranged along the cell periphery. The strain does not form intracytoplasmic membrane structures under aerobic conditions. In the in vivo pigment absorption spectrum, the peaks were observed at 492, 525, 593, 803, and 870 nm. The cells

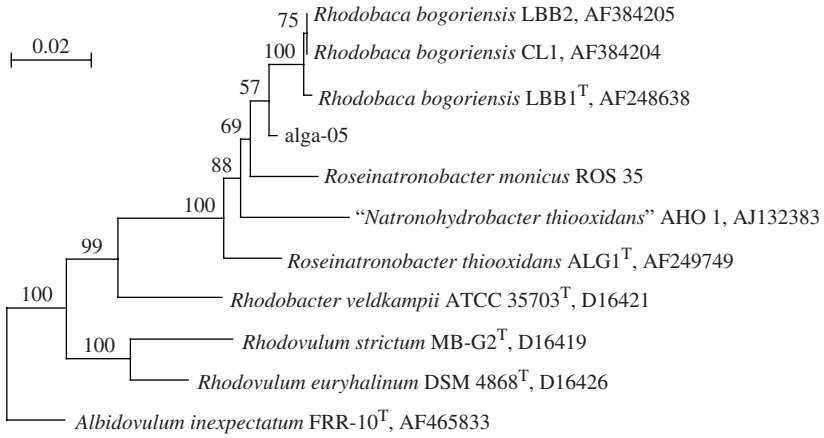

Fig. 9. 16 rRNA-based phylogenetic tree showing the position of strains alga-05 and ROS35 within the family *Rhodobacteraceae*. Numerals at the branching points indicate the bootstrap values. The bar shows the number of nucleotide substitutions in relation to the compared sequences of the homologous site.

Table 8. Similarity matrix (%) of the nucleotide sequences of 16S rRNA genes from the reperesentatives of the family *Rhodobacteraceae*

	1	2	3	4	5	6	7	8	9	10
1. *Roseinatronobacter monicus* ROS35	100									
2. *Roseinatronobacter thiooxidans* ALG1^T	96.0	100								
3. *Rhodobaca bogoriensis* CL1	97.2	95.9	100							
4. *Rhodobaca bogoriensis* LBB2	97.2	95.9	100	100						
5. *Rhodobaca bogoriensis* LBB1^T	96.8	95.9	99.9	99.9	100					
6. alga-05	98.6	96.1	99.0	99.0	99.1	100				
7. "*Natronohydrobacter thiooxidans*" AHO 1	96.1	94.7	96.4	96.4	96.6	96.8	100			
8. *Rhodovulum euryhalinum* DSM 4868^T	92.2	91.6	92.8	92.8	93.0	92.8	91.6	100		
9. *Rhodovulum strictum* MB-G2^T	91.6	91.1	92.2	92.2	92.4	92.2	91.3	96.5	100	
10. *Rhodobacter veldkampii* ATCC 35703^T	93.8	93.5	93.9	93.9	94.1	94.5	93.0	93.8	93.9	100
11. *Albidovulum inexpectatum* FRR-10^T	92.1	91.8	93.3	93.3	93.5	93.3	92.2	95.5	93.8	94.2

contain only the light-harvesting complex LH I. Photosynthetic pigments are represented by bacteriochlorophyll (BChl) *a* and carotenoids of the spheroidene series (hydroxyspheroidene, neurosporene, spheroidene, and demethylspheroidene). Demethylspheroidenone was not detected. Photoheterotrophic. The key enzyme of CO_2 assimilation, ribulose bisphosphate carboxylase (RuBisCO), is absent. Facultative aerobes. Capable of reducing nitrate to nitrite, both under anaerobic and anaerobic conditions. Succinate, malate, fumarate, pyruvate, lactate, acetate, propionate, butyrate, valerate, caproate, aspartate, fructose, sucrose, yeast extract, peptone, casein hydrolysate, and soeton are utilized as carbon sources. The strain utilizes NH_4Cl, urea, serine, and glutamate as nitrogen sources; it is not capable of nitrogen fixation and does not contain the *nifH* gene. The strain utilizes sulfide in the presence of organic substrates and oxidizes it to sulfur or polysulfide. Under aerobic conditions, the organisms utilize the same organic compounds as they do in the presence of light. Obligate alkaliphiles growing in a pH range of 7.5 to 9.0 with an optimum at 8.2. Moderate halophiles growing at a NaCl concentration of 10–80 g/l, with an optimum at 20–30 g/l. The strain requires NaCl. Optimal growth was observed within the 25– 35°C temperature range. The DNA G+C content is 59.8 mol %. The fatty acids are represented by 14:1, 16:0, 16:1ω7, and 18:1ω7 acids. The strain is sensitive to the following antibiotics: ampicillin, benzylpenicillin, vancomycin, lincomycin, neomycin, novobiocin, polymyxin, rifampicin, streptomycin, tetracycline, and erythromycin; it is resistant to gentamycin, kanamycin, and nalidixic acid.

The species belongs to the Phylum BXII of *Proteobacteria*, Class I *Alphaproteobacteria*.

GenBank accession no. EF554833; the type strain was registered in VKM(B2406) and DSMZ(19920).

Habitat is the soda lake of the Barguzin Valley (Buryat Republic, Eastern Siberia). Water mineralization is 60 g/l, pH 9.6.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research, project nos. 07-04-00651, 03-04-49061, 05-04-48058, 06-04-48668, 06-04-49304, and 06-04-48516 and by the programs of the Presidium of the Russian Academy of Sciences "Molecular and Cell Biology," "Evolution of the Biosphere," and "Support of Young Scientists."

We are very grateful to A.M. Lysenko for performing DNA–DNA hybridization, E.S. Barinova and A.Ya. Mikhachev for electron microscopic studies, E.S. Boulygina for detection of the *nifH* gene, I.I. Berg and T.V. Kalganova for detection of ribulose biphosphate carboxylase, G.A. Osipov for the analysis of fatty

Table 9. Comparative characterization of the studied strain alga-05 and *Rca. bogoriensis* LBB1

Trait	*Rhodobaca bogoriensis*, LBB1*	"*Rhodobaca bogoriensis*," strain alga-05
Habitat	Soda Lake Bogoria, African Rift Zone	Steppe soda lake, Siberia (sharply continental climate)
Shape and size, μm	Cocci, short rods, 0.8–-1.0 × 1.0–1.5 μm	Short rods, 1.0 × 1.5 μm
Division type	Constriction	Nonuniform division by constriction, resembles budding
Motility	+	+
Reaction to O_2	Facultative aerobe	Facultative aerobe
Carotenoids in vivo	450, 485, 525	475, 507, 590
BChl a in vivo	870	870
Requirement for NaCl	No	Yes
NaCl (optimum) range, %	(1–1.5) 0–6	(2–3) 1–8
pH (optimum) range	(9.0) 7.0–10.0	(8.2) 7.5–9.0
t°C optimum	39	25–35
DNA G + C content, mol	58.8	59.8
Catalase activi	+	+
Presence of *nifH* gene	–	–
Presence of ribulose biphosphate carboxylase **	–	–
Photoheterotrophic utilization of sulfide in the presence of light	+	+
Resistance to sulfide	+	+

Notes: "+", The trait was observed; "–", the trait was not observe.
* [3].
** Data obtained by E.S. Boulygina.
*** Data obtained by T.V. Kalganova and I.I Berg.

acid composition, and M.T. Madigan for kindly providing strain LBB1. We thank our colleagues from the Laboratory of Microbiology, Institute of General and Experimental Biology, Siberian Division, Russian Academy of Sciences (Ulan-Ude) for their help and support with the expedition to the Barguzin Valley.

REFERENCES

1. Imhoff, J.F., Hashwa, F., and Trüper, H.G., Isolation of Extremely Halophilic Phototrophic Bacteria from the Alkaline Wadi Natrun, Egypt, *Arch. Hydrobiol.*, 1978, vol. 84, pp. 381–388.
2. 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].
3. Milford, A.D., Achenbach, L.A., Jung, D.O., and Madigan, M.T., *Rhodobaca bogoriensis* gen. nov. and sp. nov., Alcaliphilic Purple Nonsulfur Bacterium from African Rift Valley Soda Lakes, *Arch. Microbiol.*, 2000, vol. 174, pp. 18–27.

4. Boldareva, E.N., Bryantseva, I.A., Tsapin, A., Nel'son, K., Sorokin, D.Yu., Turova, T.P., Boichenko, V.A., Stadnichuk, I.N., and Gorlenko, V.M., The New Alkaliphilic Bacteriochlorophyll *a*-Containing Bacterium *Roseinatronobacter monicus* sp. nov. from the Hypersaline Soda Mono Lake (California, United States), *Mikrobiologiya*, 2007, vol. 76, no. 1, pp. 95–106 [*Microbiology* (Engl. Transl.), vol. 76, no. 1, pp. 82–92].

5. Reynolds, E.S., The Use of Lead Citrait at High pH as an Electron Opaque Stain in Electron Microscopy, *J. Cell Biol.*, 1963, vol. 17, pp. 208–218.

6. Moskalenko, A.A., Toropygina, O.A., and Kuznetsova, N.Yu., The Isolation of the B812 Subcomplex of the B880 Core Complex and the B800-850 Complex from Membranes of *Chromatium minutissium* with Extracted Carotinoids; Structural Role of Carotenoids, *J. Photochem. Photobiol.*, 1995, vol. 27, pp. 199–207.

7. Moskalenko, A.A., Britton, G., Konnor, A., Iang, A., and Toropygina, O.A., Carotenoid Composition in the Chromatophores and Pigment–Protein Complexes Isolated from *Chromatium minutissimum* Cells Grown in the Presence of Diphenylamine, *Biol. Membr.*, 1991, vol. 8, pp. 249–260.

8. Moskalenko, A.A., Toropygina, O.A., Zhuravleva, Z.A., and Erokhin, Yu.E., Effect of Carotenoids on the Interaction between Pigment-Protein Complexesin Membranes of the Sulfur Photosynthetic Bacterium *Chromatium* minutissimum, *Dokl. Akad. Nauk*, 2001, vol. 381, pp. 1–4 [*Dokl. Biochem. Biophys.* (Engl. Transl.), vol. 381, pp. 423–426].

9. Boichenko, V.A., Study of the Organization of Photosynthetic Units by Action Spectra of Functional Activity, *Biofizika*, 2004, vol. 49, pp. 254–264 [*Biophysics* (Engl. Transl., vol. 49, no. 2, pp. 238–247.

10. Tsaplina, I.A., Osipov, G.A., Bogdanova, T.I., Nedorezova, T.P., and Karavaiko, G.I., Fatty Acid Composition of the Lipids of Thermoacidophilic Bacteria of the Genus *Sulfobacillus, Mikrobiologiya*, 1994, vol. 63, no. 5, pp. 821–830.

11. Trüper, H.G. and Schlegel, H.G., Shulphur Metabolism in Thiorhodaceae. 1. Quautitative Measurements on Growing Cells of *Chromacium okenii, Antonie van Leeuwenhoek J. Microbiol. Serol.*, 1964, vol. 30, no. 3, pp. 225–238.

12. Carret, R.H. and Nason, A., Further Purification and Properties of *Neurospora* Nitrate Reductase, *J. Biol. Chem.*, 1969, vol. 244, pp. 2870–2882.

13. Dawson, R., Elliott, D., Elliott, W., and Jones, K., *Data for Biochemical Research*, Oxford: Clarendon, 1986 [Rus. Transl. Moscow: Mir, 1991].

14. Spiridonova, E.M., Berg, I.A., Kolganova, T.V., Ivanovskii, R.N., Kuznetsov, B.B., and Turova, T.P., An Oligonucleotide Primer System for Amplification of the Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase Genes of Bacteria of Various Taxonomic Groups, *Mikrobiologiya*, 2004, vol. 73, no. 3, pp. 377–387 [*Microbiology* (Engl. Transl.), vol. 73, no. 3, pp. 316–325].

15. Netrusov, A.I., Egorova, M.A., Zakharchuk, L.M., Kolotilova, N.N., Kotova, I.B., Semenova, E.V., Tatarinova, N.Yu., Ugol'nikova, N.V., Tsavkelova, E.A., Bobkova, A.F., Bogdanova, A.G., Danilova, I.V., Dinarieva, T.Yu., Zinchenko, V.V., Ismailov, A.D., Kurakov, A.V., Maksimov, V.N., Mil'ko, E.S., Nikitina, E.P., Ryzhkova, E.P., Semenov, A.M., Khomyakova, D.V., Cherdyntseva, T.A., and Yudina, T.G., *Praktikum po mikrobiologii: Ucheb. posobie dlya stud. vyssh. ucheb. zavedenii* (Practical Manual of Microbiology), Moscow: Izdatel'skii tsentr "Akademiya," 2005.

16. Marmur, J., A Procedure for the Isolaition of Deoxyribonucleic Acid from Microorganisms, *J. Mol. Biol.,* 1961, vol. 3, pp. 203–218.

17. Owen, R.J., Hill, L.R., and Lapage, S.P., Determination of DNA Base Composition from Melting Profiles in Dilute Buffers, *Biopolymers*, 1969, vol. 7, pp. 503–516.

18. De Ley, J., Cattoir, H., and Reynaerts, A., The Quantitative Measurements of DNA Hybridizaition from Renaturation Rates, *Eur. J. Biochem.*, 1970, vol. 12, pp. 133–140.

19. Edwards, U., Rogall, T., Bloeker, H., Ende, M.D., and Boeettge, E.C., Isolation and Direct Complete Nucleotide Determination of Entire Genes, Characterization of Gene Coding for 16S Ribosomal RNA, *Nucleic Acids Res.*, 1989, vol. 17, pp. 7843–7853.

20. 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.

21. Kawasaki, H., Hoshino, Y., Kuraishi, H., and Yamasato, K., *Rhodocista centenaria* gen. nov., sp. nov., a Cyst-Forming Anoxygenic Photosynthetic Bacterium and Its Phylogenetic Position in the *Proteobacteria* Alpha Group, *J. Gen. Appl. Microbiol.*, 1992, vol. 38, pp. 541–551.

22. Iba, K., Takamiya, K., Toh, Y., and Nishimura, M., Roles of Bacteriochlorophyll and Carotenoid Synthesis in Formation of Intracellular Membrane Systems and Pigment-Protein Complexes in An Aerobic Photosynthetic Bacterium, *Erythrobacter* sp. Strain OCh114, *J. Bacteriol.*, 1988, vol. 170, pp. 1843–1847.

23. Stackebrandt, E. and Ebers, J., Taxonomic Parameters Revisited: Tarnished Gold Standarts, *Microbiology Today*, no. 6, pp. 152–155.

24. Takaichi, S, Carotenoids and Carotinogenesis in Anoxygenic Photosynthetic Bacteria, in *The Photochemistry of Carotenoids*, Frank, H.A., Young, A.J., Britton, G., and Cogdell, R.J., Eds., *Advances in Photosynthesis*, Dordrecht–Boston–London: Kluwer Academic Publishers, 1999.

25. Boichenko, V.A. and Makhneva, Z.K., Light-Induced Respiration Inhibition and Organization of Photosynthetic Units in Whole Cells of Purple Bacteria, *Biokhimiya*, 1994, vol. 59, pp. 1184–1193.