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EXPERIMENTAL ARTICLES

Rhodobaculum claviforme gen. nov., sp. nov., a New Alkaliphilic Nonsulfur Purple Bacterium

I. A. Bryantseva, V. A. Gaisin, and V. M. Gorlenko[1]

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

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Abstract—Two alkaliphilic strains of nonsulfur purple bacteria (NPB), B7-4 and B8-2, were isolated from moderately saline alkaline steppe lakes in southeast Siberia with pH values above 9.0. The isolates were motile, polymorphous cells (from short rods to long spindly cells) 1–2.5 × 2.5–7 μm. Intracellular membranes of vesicular type were mostly located at the cell periphery. The microorganisms contained bacteriochlorophyll *a* and carotenoids of the spheroidene and spirilloxanthin series. The photosynthetic apparatus was represented by LH2 and LH1 light-harvesting complexes. In the presence of organic compounds, the strains grew aerobically in the dark or anaerobically in the light. Capacity for photo- and chemoautotrophic growth was not detected. The *cbbL* gene encoding RuBisCO was not revealed. Optimal growth of both strains occurred at 2% NaCl (range from 0.5 to 4%), pH 8.0–8.8 (range from 7.5 to 9.7), and 25–35°C. The DNA G+C content was 67.6–69.8 mol %. Pairwise comparison of the nucleotides of the 16S rRNA genes revealed that strains B7-4 and B8-2 belonged to the same species (99.9% homology) and were most closely related to the aerobic alkaliphilic bacteriochlorophyll *a*-containing anoxygenic phototrophic bacterium (APB) *Roseibacula alcaliphilum* De^T (95.2%) and to NPB strains *Rhodobaca barguzinensis* VKM B-2406^T (94.2%) and *Rbc. bogoriensis* LBB1^T (93.9%). The isolates were closely related to the NPB *Rhodobacter veldkampii* DSM 11550^T (94.8%) and to aerobic bacteriochlorophyll *a*-containing bacteria *Roseinatronobacter monicus* ROS 35^T and *Roseicitreum antarcticul* ZS2-28^T (93.5 and 93.9%, respectively). New strains were described as a new NPB genus and species of the family *Rhodobacteriaceae, Rhodobaculum claviforme* gen. nov., sp. nov., with B7-4^T (VKM B-2708, LMG 28126) as the type strain.

Keywords: nonsulfur purple bacteria, taxonomy, extremophiles, alkaliphiles, soda lakes, *Rhodobaculum claviforme*

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Nonsulfur purple bacteria (NPB) are a phylogenetically and physiologically diverse group of bacteria [1]. These organisms belonging to the *Alfa-* and *Betaproteobacteria* possess characteristic morphology and may grow by budding or division. Photosynthetic membranes of different species may be vesicular or lamellar; the pigments may include bacteriochlorophyll *a* or *b* and carotenoids of the spirilloxanthin or spheroidene series [2].

Five presently known alkaliphilic NPB species were isolated from soda lakes of different salinity. They belong to the *Alphaproteobacteria* (family *Rhodobacteriaceae*): *Rhodobaca bogoriensis* [3], *Rbc. barguzinensis* [4], *Rubribacterium polymorphum* [5], *Rhodovulum steppense*, and *Rvu. tesquicola* [6, 7].

Most NPB are capable of photoheterotrophic growth with various sources of organic carbon. Some species use sulfide as an electron donor for anoxygenic photosynthesis, similar to purple sulfur bacteria. Most NPB were shown to be capable of photoautotrophic growth with H_2. Many species are also capable of chemoheterotrophic growth in the dark due to respiration under aerobic or microaerobic conditions. While some species, such as *Rhodobaca* spp., *Rubribacterium polymorphum*, and *Charonomicrobium ambiphototrophicum*, preferentially grow aerobically in the dark [8], they retain capacity for anaerobic phototrophic growth. Enzymes of the Calvin cycle required for autotrophic CO_2 fixation were not detected in these species. This physiological feature resembles aerobic bacteriochlorophyll *a*-containing bacteria.

In the present work, investigation of two new strains of bacteriochlorophyll *a*-containing bacteria is reported. While both strains exhibited better aerobic growth on organic substrates in the dark, they were also capable of anaerobic photoheterotrophic growth. The isolates, which were phenotypically and phylogenetically similar, were identified as two strains of a new genus and species of the family *Rhodobacteriaceae*, *Rhodobaculum claviforme* gen. nov., sp. nov.

[1] Corresponding author; e-mail: vgorlenko@mail.ru

MATERIALS AND METHODS

Isolation and cultivation. Two NPB strains were isolated from moderately saline steppe soda lakes Doroninskoe and Zun Torei (southeast Siberia, Russia). The strains were isolated and grown in the medium containing the following (g/L): NH_4Cl, 0.4; KH_2PO_4, 0.5; $MgCl_2$, 0.2; Na_2SO_4, 0.5; KCl, 0.5; NaCl, 20; $NaHCO_3$, 5; yeast extract, 1; Na acetate, 1; Na pyruvate, 1; bacto pepton, 1; vitamin B_{12}, 20 μg/L; and 1 mL/L trace element solution [9]. Trace element soultion contained the following (g/L): trilon B, 5; $FeSO_4 \cdot 6H_2O$, 2; $ZnSO_4 \cdot 7H_2O$, 0.1; $MnCl_2 \cdot 4H_2O$, 0.03; H_3BO_3, 0.3; $CoCl_2 \cdot 2H_2O$, 0.2; $CuCl_2 \cdot 2H_2O$, 0.03; $NiCl_2 \cdot 6H_2O$, 0.02; $Na_2MoO_4 \cdot 2H_2O$, 0.03; pH 3.0–4.0. Solutions of trace elements and $NaHCO_3$ (10%) were prepared separately in 50–100-mL screw-capped vials, sterilized at 0.5 atm, and added to the medium prior to inoculation. The basal medium was sterilized at 1 atm in 0.5–1-L flasks. The final pH of the medium was 8.0.

Pure cultures were obtained by repeated transfers of individual colonies formed in agar stabs (0.8% agar) at 2000 lx and 25–35°C. Pure cultures were maintained in liquid media in the light under anaerobic conditions. The cultures were also grown in petri dishes (2% agar) and under aerobic conditions in the dark in 50–100-mL vials.

Morphology and ultrastructure. Bacterial morphology was investigated by light (Olympus BX 41, Japan) and electron microscopy (Jeol JEM-100C, Japan). Whole cells were contrasted with 1% phosphotungstic acid. Ultrathin sections were prepared as described previously [10].

Pigments. Absorption spectra of the pigments were recorded for whole cells (resuspended in 50% glycerol) and in acetone–methanol extracts (7 : 2) on an SF-56 spectrophotometer (LOMO, Russia).

Carotenoid composition was determined by HPLC [4].

Physiological properties. Phototrophic growth in the presence of various substrates was determined in mineral medium supplemented with yeast extract (0.05 g/L) as a growth factor. Tested compounds were sterilized as 5% solutions at 0.5 atm and added into the medium to the final concentration of 0.5 g/L. Requirement for inorganic electron donors, as well as growth under different pH values and NaCl concentrations, were studied using the basic media, with the parameters under study varying accordingly [10]. Growth was determined during the stationary growth phase as OD_{650} measured on a KFK-3 photometer (Russia). The concentrations of $S_2O_3^{2-}$, SO_3^{2-}, and $H_2S + HS^-$ were determined by separate iodometric titration [11].

Fatty acid composition was analyzed by gas chromatography and chromatography–mass spectrometry. Dry cell biomass (5 mg) was treated for 3 h at 80°C with 0.4 mL 1 N HCl in methanol (acid methanolysis). Fatty acid methyl esters and other lipid components were extracted with hexane and analyzed on a Sherlock gas chromatograph (Microbial identification system, MIDI Inc., United States) [12].

Molecular genetic study. DNA was isolated from pure cultures according to Marmur [13]. The DNA G+C content was determined by optical reassociation [14].

For amplification and sequencing of the 16S rRNA gene, the universal bacterial primers 27f and 1492r were used [15]. The *cbb*L gene fragment was amplified using the primers reported by Spiridonova et al. [16]. Amplification products were sequenced using the Big Dye Terminator v. 3.1 kit on an ABI 3730 automatic sequencer (Applied Biosystems, Inc., United States) according to the manufacturer's recommendations. The sequences were edited using BioEdit [17]. The presence of chimeral inserts was checked using the Pintail 1.0 software package [18]. Phylogenetic analysis and construction of the maximum-likelihood tree were carried out using the MEGA 5.1 software package [19].

Deposition of the nucleotide sequences. The 16S rRNA gene fragments of the strains B8-2 and B7-4 were deposited in GenBank under accession nos. KM077018 and KM077019, respectively.

RESULTS AND DISCUSSION

Sites of isolation. The culture of strain B7-4 was isolated in 2007 from a coastal mat formed on the silt surface of a stratified soda Lake Doroninskoe (51°25′N, 112°28′ E) with 32 g/L salinity and pH 9.72. The culture of strain B8-2 was isolated from a sediment sample from a closed lake Zun Torei (50°04′N, 115°48′E), a member of the Torei lake system. The maximal depth is 7 m, pH 9.5, and salinity 7 g/L.

Morphology and ultrastructure. The cells of strains B7-4 and B8-2 were polymorphic (spindle-shaped, short rods, or long rods), 1–2.5 × 2.5–7 μm (Figs. 1a, 1b). Aerobically grown cells exhibited more pronounced polymorphism (Fig. 1b). While most of the cells were nonmotile, rare motile rod-shaped cells were observed in the initial transfers of the environmental material on agar media. Division occurred by binary fission with formation of a constriction. The cell wall structure was of the gram-negative type (Fig. 1c). Photosynthetic membranes were present only in the cells grown anaerobically in the light. They formed vesicles located mostly at the cell periphery, but also in the center of the cytoplasm (Fig. 1c).

Pigments. The colonies of the new strains grown anaerobically on solid media were orange–brown. Under oxic conditions, the colony coloration was pink under oxic conditions. The colonies formed in Petri dishes on the surface of agar media were initially of pale pink color and became deep purple in the course

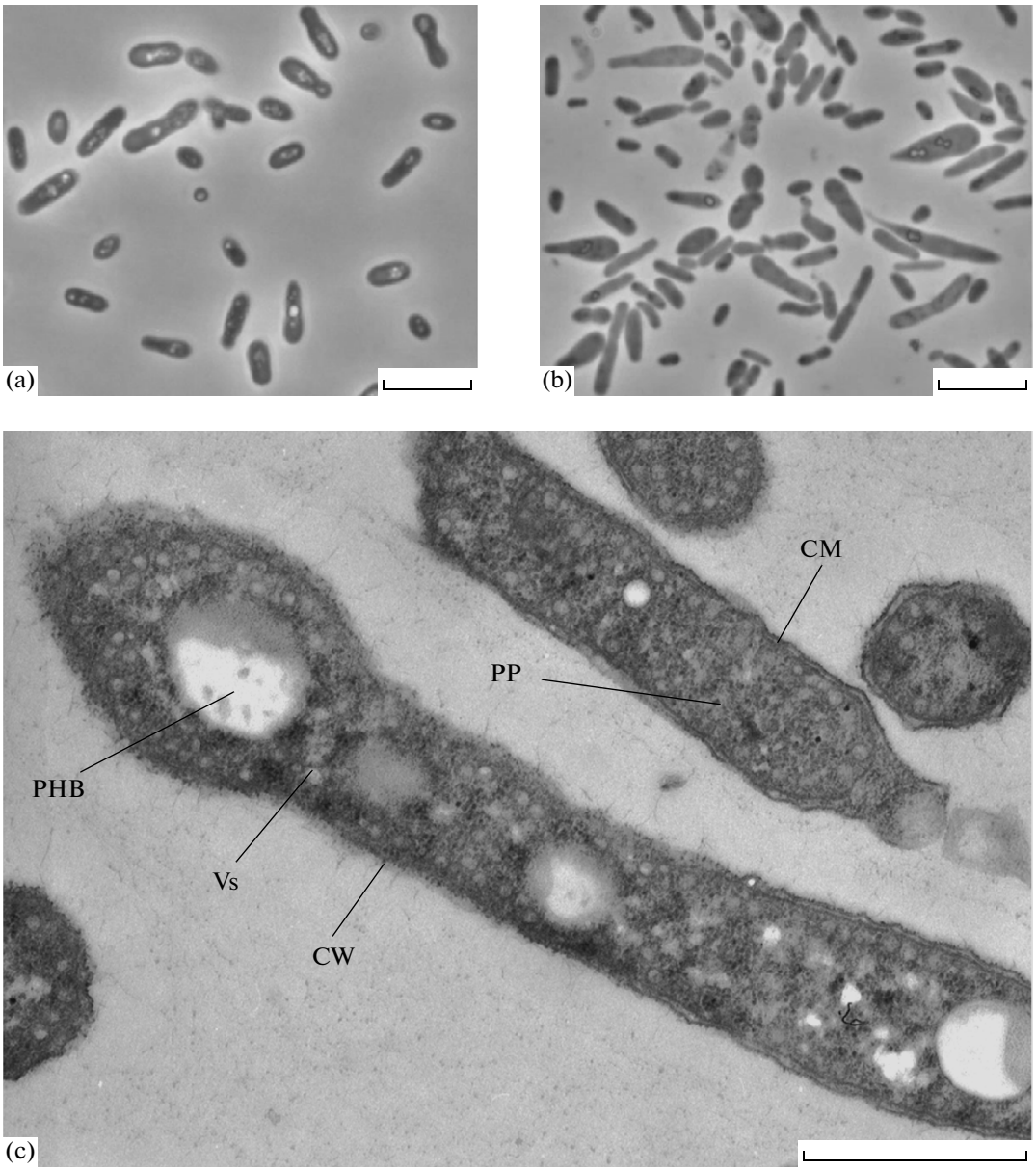

Fig. 1. Morphology (a, b) and ultrastructure (c) of the new nonsulfur purple bacterium *Rhodobaculum claviforme* strain B7-4^T grown anaerobically (a, c) and aerobically (b). Scale bar: 5 μm (a, b) and 1 μm (c). Designations: PP, polyphosphates; PHB, poly-β-hydroxybutyrate; CW, cell wall; Vs, vesicles; CM, cytoplasmic membrane.

of growth. Cell suspensions of the strains grown in liquid media under oxic or anoxic conditions were of the same color as the colonies obtained on agar media under the same conditions.

Absorption spectra of the pigments of two strains were similar. Spectra of the membranes of strain B7-4 exhibited three major near-infrared maxima at 803, 851, and 886 nm (the latter one as a shoulder) (Fig. 2). They corresponded to bacteriochlorophyll *a* in light-harvesting complexes LH2 (803 and 851 nm) and LH1 (886 nm). The bands in the visual range (400–550 nm) belonged to carotenoids. The spectra of acetone–methanol cell extracts of strain B7-4 exhibited peaks of bacteriochlorophyll *a* (360 and 771 nm) and carotenoids with the major peak at 480 nm (Fig. 2).

Analysis of carotenoid pigments of strain B7-4 indicated the presence of spheroidenone (72.77%) and its derivatives (3.34%), as well as diketospirilloxanthin (18.68%) and rhodopin (2.94%) (Table 1). Spheroidene, rhodovibrine, anhydrorhodovibrin, spirilloxanthin, lycopene, and neurosporene constituted less than 1% of the total carotenoid content. Carotenoid composition of the strain was similar to that of alkaliphilic *Rubribacterium polymorphum*, which was also found to contain both spheroidene and spirilloxanthin [5].

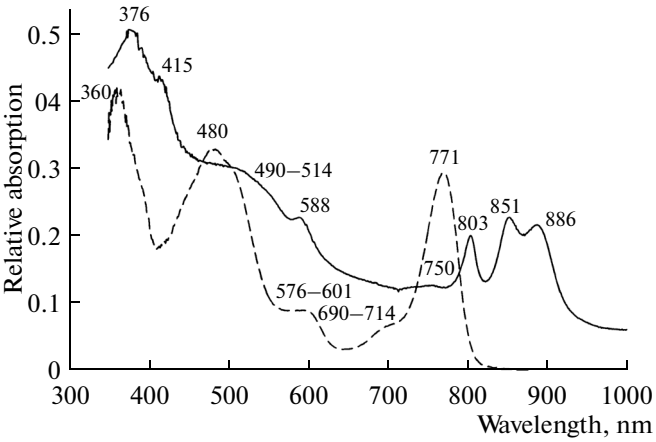

Fig. 2. Absorption spectra of whole cells (solid line) and acetone–methanol extract (broken line) of *Rhodobaculum claviforme* strain B7-4^T.

Thus, photosynthetic pigments of both strains were bacteriochlorophyll *a* and carotenoids of the spheroidene and spirilloxanthin series.

Physiology. The isolates were capable of anaerobic growth in the light (photoheterotrophic) and of aerobic growth in the dark (chemoheterotrophic). Both photoheterotrophic and chemoheterotrophic growth were poor in liquid medium. The isolates were shown to possess no *cbb*L gene encoding the RuBisCO L unit (Tourova, personal communication) and were incapable of photo- or chemoautotrophic growth. Sulfide and thiosulfate were not oxidized in the course of growth (both aerobic in the dark and anaerobic in the light), although they were used as sulfur sources.

The best growth occurred under heterotrophic conditions (aerobically in the dark) on solid media. Growth of both strains under anoxic photoheterotrophic conditions was slower. Acetate, propionate, pyruvate, yeast extract, peptone, and soyton were used as organic carbon sources. Malate, lactate, fumarate, and fructose supported weak growth (Table 2). No growth occurred with succinate, formate, mannitol, citrate, benzoate, butyrate, caproate, valerate, sorbitol, arginine, aspartate, casein hydrolysate, glucose, xylose, ribose, sucrose, glycerol, methanol, propanol, ethanol, or butanol. Yeast extract (0.05 g/L) provided the growth factors required by the strains.

Optimal growth occurred at 2% NaCl (growth range, 0.5 to 4%) (Fig. 3) and 25–35°C. Growth occurred at pH 7.5 to 9.7 with the optimum at pH 8.0–8.8 (Fig. 4). Almost no growth occurred at pH 7.0, which is typical of alkaliphiles.

Both strains were sensitive to amikacin, ampicillin, benzylpenicillin, gentamycin, kanamycin, lincomycin, polymyxin, rifampicin, streptomycin, and erythromycin. Both strains were resistant to neomycin, novobiocin, and penicillin. Strain B8-2 was weakly sensitive to vancomycin, while nalidixic acid

Table 1. Carotenoid composition of *Rhodobaculum claviforme* and closely related alkaliphilic NPB species

Carotenoid, % of total	*Rhodobaculum claviforme* B7-4^T	*Rhodobaca bogoriensis* LBB1^T [4]	*Rhodobaca barguzinensis* alga-05^T [4]	*Rubribacterium polymorphum* Green^T [5]
Diketospirilloxanthin	18.68			10.9
Diketomonodimethylspirilloxanthin				2.5
Rhodopin	2.84			
Rhodopin isomer	0.1			
Rhodovibrine	0.62			
Anhydrorhodovibrine	0.24			0.4
Spirilloxanthin	0.69			23.7
Spheroidenone	72.77	17.0	10.7	
Spheroidenone, 3 isomers	3.34			
Dimethylspheroidenone		40.0		
Spheroidene	0.22	7.0	4.8	12.7
Hydroxyspheroidene				23.4
Dimethylspheroidene and its isomer		19.0	74.6	25.3
Lycopene	0.21			0.8
Neurosporene	0.28	18.0	7.01	0.3
Neurosporene isomer			1.8	
Not identified			1.1	
Total, %	99.99	101	100.01	100

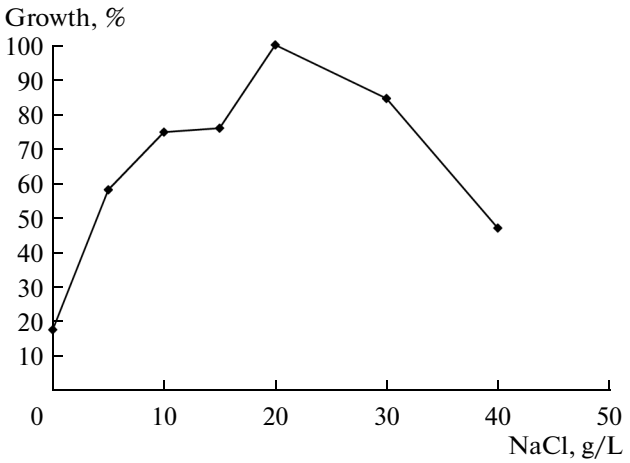

Fig. 3. Effect of NaCl concentration of the growth of *Rhodobaculum claviforme* strain B7-4^T.

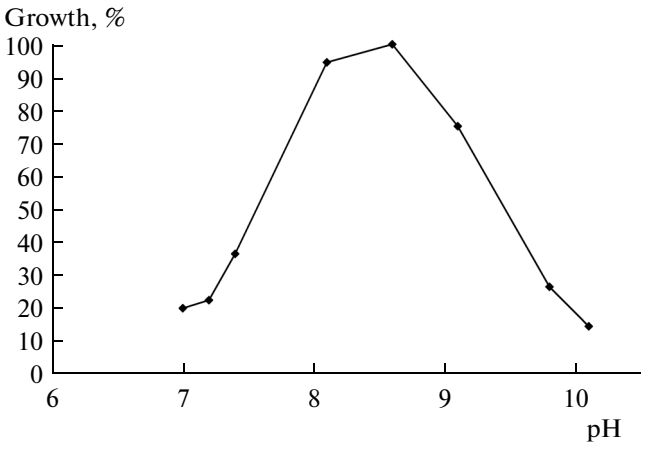

Fig. 4. Effect of pH of the growth of *Rhodobaculum claviforme* strain B7-4^T.

and tetracycline suppressed its growth completely. Strain B7-4 was not sensitive to vancomycin, nalidixic acid, and tetracycline (Table 3).

Fatty acid composition. Similar to other NPB of the family *Rhodobacteriaceae*, 11-octadecenoic acid ($C_{18:1\omega7}$) was the predominant fatty acid in the cells of strain B7-4 (72.02% of the total fatty acid content). Considerable amounts of the $C_{16:0}$ acid were also present (12.42%). Relatively high content of the $C_{18:0}$ acid (13.72%) differentiated this strain from other NPB species preferring aerobic growth (Table 4).

Genetic properties. The DNA G+C content of strains B7-4 and B8-2 was 69.8 and 67.6 mol % (Tm), respectively. Pairwise comparison of the 16S rRNA gene sequences revealed that the strains were almost identical (99.9% similarity), which confirmed their classification within the same species. The isolates were most closely related to alkaliphilic aerobic anoxygenic phototrophic bacteria *Roseibacula alcaliphilum* De (95.2%) [20], and to alkaliphilic NPB strains *Rbc. barguzinensis* VKM B-2406^T (94.2%) and *Rbc. bogoriensis* LBB1^T (93.9%). Similar levels of homology were determined for the NPB species *Rba. veldkampii* DSM 11550^T (94.8%) and for aerobic, bacteriochlorophyll *a*-containing bacteria *Roseinatronobacter monicus* ROS 35^T (93.5%) and *Roseicitreum antarcticum* ZS2-28^T (93.9%).

Phylogenetic analysis based on the results of the 16S rRNA gene sequencing showed strains B7-4 and B8-2 form a separate branch in the cluster of the genera *Roseibacula, Roseibaca*, *Rhodobaca*, and *Roseinatronobacter* (Fig. 5).

Based on molecular genetic and phenotypic properties of the new isolates, they were described as a new PNB genus and species of the family *Rhodobacteriaceae, Rhodobaculum claviforme* gen. nov., sp. nov. with the type strain B7-4^T. The type strain B7-4^T was deposited in the international collections under designations VKM B-2708 (All-Russian Collection of Microorganisms) and LMG 28126 (Belgian Coordinated Collection of Microorganisms).

Description of the genus *Rhodobaculum* gen. nov.

Rho.do.ba'cu.lum. Gr. n. *rhodon*, the rose, L. neut. dimin. n. *baculum*, small rod, N. L. neut. dimin. n. *Rhodobaculum*, small pink rod.

The cells are polymorphic (from short rods to long spindle-shaped ones) and motile. Division is by binary constriction. The cells are gram-negative. The genus belongs to Class I *Alphaproteobacteria*, order *Rhodobacterales*, family *Rhodobacteraceae*. Phototrophically grown cells form vesicular photosynthetic membranes. Anaerobically and aerobically grown cell suspensions are orange-brown and pink, respectively. Bacteriochlorophyll *a* and carotenoids of both the spheroidene and spirilloxanthin series are the photosynthetic pigments.

Both anaerobic photoheterotrophic growth and aerobic chemoheterotrophic growth in the dark are possible on a number of organic substrates. Growth factors are required. The organism is alkaliphilic and mesophilic.

Its habitats are alkaline soda lakes with moderate mineralization.

The DNA G+C content is 67.6–69.8 mol %.

Type species is *Rhodobaculum claviforme*.

Description of the species *Rhodobaculum claviforme* sp. nov.

Rhodobaculum claviforme; cla.vi.for'me. L. fem. n. clava, a club (as weapon), L. neut. suffix *-forme*, shaped like, L. neut. adj. *claviforme*, shaped like a club. *Rhodobaculum claviforme*, small pink rod shaped like a club.

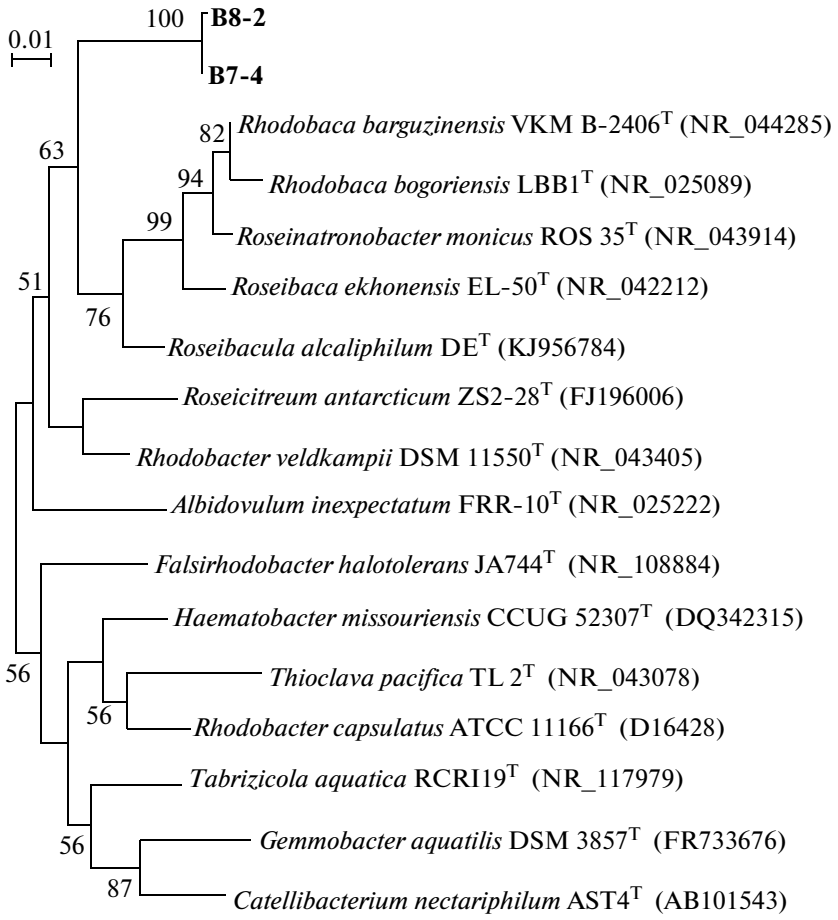

Fig. 5. Phylogenetic position of strains B7-4 and B8-2 determined by analysis of the 16S rRNA gene sequences. The tree was constructed using the maximum likelihood algorithm. The branching order was determined by analysis of 1000 alternative trees. Scale bar corresponds to 1 replacement for 100 nucleotides.

The species belongs to anoxygenic phototrophic nonsulfur purple bacteria. The cells are gram-negative, polymorphic (from short rods to long spindle-shaped ones), 1–2.5 × 2.5–7 μm, and motile. The flagellation type was not determined. Division is by binary constriction. Anaerobically and aerobically grown cells from solid and liquid media are orange-brown and pink, respectively. Photosynthetic membranes of vesicular type located mostly in the periphery of the cytoplasm are formed only under anaerobic conditions in the light. Photosynthetic pigments are bacteriochlorophyll *a* (in vivo peaks at 376, 588, (750), 803, 851, (886) nm) and carotenoids of both the spheroidene and spirilloxanthin series (in vivo peaks at 415, 490–514 nm): spheroidenone and its derivatives (over 70%), diketospirilloxanthin (18.68%) and rhodopin (2.94%), as well as spheroidene, rhodovibrine, anhydrorhodovibrin, spirilloxanthin, lycopene, and neurosporene. LH1 (886 nm) and LH2 (803 and 851 nm) light-harvesting complexes are formed. The major fatty acids are $C_{18:1}$, $C_{18:0}$, and $C_{16:0}$.

Chemoheterotrophic aerobic growth in the dark and photoheterotrophic anaerobic growth in the light are both possible. The organism is incapable of photo- and chemoautotrophic growth; the *cbb*L gene encoding the RuBisCO L-subunit was not revealed. Sulfide and thiosulfate are not used as electron donors for photosynthesis and are not oxidized during aerobic growth in the dark. Best growth occurs aerobically in the dark, on solid media with organic substrates. Acetate, yeast extract, pyruvate, peptone, soyton, and propionate are used as organic carbon sources in the course of photosynthesis. Weak growth occurs on malate, lactate, fumarate, and fructose. No growth occurred with succinate, formate, mannitol, citrate, benzoate, butyrate, caproate, valerate, sorbitol, arginine, aspartate, casein hydrolysate, glucose, xylose, ribose, sucrose, glycerol, methanol, propanol, ethanol, or butanol. Sulfide and thiosulfate are used as sulfur sources for biosynthesis. Yeast extract provides the required growth factors. Best growth occurs at 2% NaCl 2% (range, 0.5–4%) and 25–35°C. The organism is mesophilic and alkaliphilic, growing at pH 7.5–9.7 with the optimum at pH 8.0–8.8.

The organism is sensitive to amikacin, ampicillin, benzylpenicillin, gentamycin, kanamycin, lincomy-

Table 2. Comparison of characteristics of the new bacterium *Rhodobaculum claviforme* and the phenotypically and genetically close genera of anoxygenic phototrophic bacteria (APB)

Characteristics	*Rhodobaculum claviforme*	*Rhodobaca* [3, 4]	*Rhodobacter* [1]	*Rubribacterim* [5]	*Roseibacula* [20]
Habitat	Soda lakes	Soda lakes	Freshwater and terrestrial environments	Soda lakes	Soda lakes
Cell shape	Polymorphic: short rods and spindle-shaped	Ovoid to very short rods	Ovoid to rod-shaped	Oval or polymorphic	Oval or citron-shaped
Size, μm	1–2.5 × 2.5–7	0.8–1.0 × 1.0–1.5	0.5–1.2 × 0.9–3.5	0.7 × 1.0–3	0.5–1.0 × 1.5–1.7
Motility	+	+	±	+	–
Intracellular membrane system	Vesicles	Vesicles	Vesicles or lamellae	Vesicles	Vesicles
Cell division	Binary fission	Binary fission	Binary fission or budding	Binary fission	Binary fission
Pigments	Bchl *a*, carotenoids of both the spheroidene and spirilloxanthin groups	Bchl *a*, carotenoids of the spheroidene group	Bchl *a*, carotenoids of the spheroidene group	Bchl *a*, carotenoids of both the spheroidene and spirilloxanthin groups	Bchl *a*, carotenoids of the spheroidene group
Light-harvesting complex	LHI and LHII	LHI	LHI and LHII	LHI and LHII	ND
pH optimum (range)	8–8.8 (7.5–9.7)	8.2–9 (7.0–10.0)	6.5–7.5	8.5–9.5 (7.4–10)	9.8 (8.0–10.0)
NaCl optimum (range), %	2 (0.5–4)	1–3 (0–8)	0 (0–3)	1 (0.5–4)	1 (0.5–5)
Sulfide oxidation to	No oxidation	S^0	S^0/SO_4	No oxidation	ND
Autotrophic growth	–	–	+	–	–
Anaerobic growth in the light	+	+	+	+	–
Aerobic growth in the dark	+	+	+	+	+
DNA G+C, mol %	67.6–69.8	58–59.8	64–73.2	69.9	64.4

Designations: "+" and "–" indicate the presence and absence of a feature, respectively. Bchl stands for bacteriochlorophyll. ND stands for no data.

Table 3. Antibiotic sensitivity of strains B7-4 and B8-2

Antibiotic	Strain B7-4	Strain B8-2
Amikacin	+	+
Ampicillin	+	+
Benzylpenicillin	+	+
Vancomycin	–	+–
Gentamycin	+	+
Kanamycin	+	+
Lincomycin	+	+
Nalidixic acid	–	+
Neomycin	–	–
Novobiocin	–	–
Polymyxin	+	+
Penicillin	–	–
Rifampicin	+	+
Streptomycin	+	+
Tetracycline	–	+
Erythromycin	+	+

The "+" and "–" signs indicate sensitivity and resistance, respectively.

cin, polymyxin, rifampicin, streptomycin, and erythromycin. It is resistant to vancomycin, nalidixic acid, neomycin, novobiocin, penicillin, and tetracycline.

Its habitats are steppe soda lakes with moderate mineralization.

Type strain B7-4^T was isolated from coastal silt of the stratified soda lake Doroninskoe (32 g/L mineralization and pH 9.72).

The DNA G+C content is 67.6–69.8 mol %.

The DNA G+C content of the type strain is 69.8 mol %.

Strain B7-4^T was deposited to international collections of microorganisms as VKM B -2708 and LMG 28126. The GenBank accession no. for its 16S rRNA gene sequence is KM077019.

The phototrophic bacterium *Rhodobaculum claviforme* gen. nov., sp. nov. is a new member of a small group of alkaliphilic NPB. Physiologically it is a heterotroph incapable of autotrophic CO_2 fixation via the Calvin cycle. Importantly, this organism prefers aerobic growth via a respiratory mechanism and is highly tolerant to oxygen. In this respect, it is similar to the known species *Rhodobaca* spp., *Rubribacterium polimorphum*, and *Charonomicrobium ambiphototrophicum* [3, 5, 8]. These species, as well as *Rhodobaculum claviforme*, occupy an intermediate position between facultatively anaerobic NPB and obligately

Table 4. Fatty acid composition of *Rhodobaculum claviforme* and closely related species of anoxygenic phototrophic bacteria (APB)

Fatty acids, % of total	*Rhodobaculum claviforme* B7-4^T	*Rhodobaca bogoriensis* LBB1^T [4]	*Rhodobaca barguzinensis* alga-05^T [4]	*Rhodobacter capsulatus* [21][b]	*Rhodobacter veldkampii* [21]	*Rubribacterium polymorphum* Green^T [5]	*Roseibacula alcaliphilum* De^T [20]
$C_{12:0}$	–	0.19	–	ND	ND	–	–
$C_{14:0}$	–	1.02	–	0.1–0.4	0.1	–	–
$C_{14:1}$	–	2.12	2.21	ND	ND	–	–
$C_{14:0}$ 3OH	0.398	–	–	ND	ND	–	–
$C_{16:0}$	12.418	18.66	9.69	4.1–5.0	4.3	9.0	2.53
$C_{16:1}\omega7$	0.566	2.41	2.64	5.1–7.4	17.5	2.01	1.86
$C_{17:0}$	0.203	–	–	ND	ND	–	–
$C_{18:0}$	13.719	2.4	1.56	3.8–9.3	6.5	6.84	0.84
$C_{18:1}\omega7$	72.024	67.28	79.32	78.1–84.2	69.4	74.86	82.71
$C_{18:1}\omega9$	0.498	0.21	–	ND	ND	–	0.21
C_{11}Me18:1	–	3.89	4.58	ND	ND	2.04	1.91
$C_{18:0}$ 3OH	0.173	–	–	ND	ND	–	–
$C_{20:2}\omega6$	–	–	–	ND	ND	0.38	0.2
$C_{20:3}\omega6$	–	–	–	ND	ND	1.89	–
Others	–	1.81[a]		ND	ND	2.98[c]	9.74[d]
Total, %	99.999	99.99	100	91.2–106.3	97.8	100	100

Not detected and no data are designated by "–" and ND, respectively. [a] 2h14, i15, a15, and 2h15 fatty acids were found; [b] range of fatty acid content in six *Rhodobacter capsulatus* strains; [c] h15, i19, and 19cyc fatty acids were found; [d] 9:0, 11:0, 12:1, i18, 19:1, 10Me19, and i20 fatty acids were found.

aerobic anoxygenic phototrophs. Emergence of obligately chemotrophic aerobic bacteria phylogenetically related to anoxygenic phototrophs was probably the next evolutionary stage.

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