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EXPERIMENTAL ARTICLES

Roseibacula alcaliphilum gen. nov. sp. nov., a New Alkaliphilic Aerobic Anoxygenic Phototrophic Bacterium from a Meromictic Soda Lake Doroninskoe (East Siberia, Russia)

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Abstract—A bacterial strain De was isolated from the surface water layer of the meromictic soda lake Doroninskoe. When grown in the dark, it formed pink colonies on agar media. The cells were nonmotile, containing bacteriochlorophyll *a* and carotenoids. Stationary-phase cells contained intracellular vesicular membranes similar to the membranes of the photosynthetic apparatus of some nonsulfur purple bacteria. Aerobic growth did not occur. Sucrose, citrate, mannitol, sorbitol, casein hydrolysate, and yeast extract were the preferable substrates for aerobic growth. Xylose, lactose, aspartate, benzoate, malate, malonate, succinate, tartrate, formate, fumarate, glycerol, methanol, and ethanol were not utilized. Growth occurred at up to 50 g/L NaCl (optimum at 5 g/L) and pH 9.8. According to the 16S rRNA gene sequencing, similarity between the isolate and the known alkaliphilic genera of nonsulfur purple bacteria (*Rhodobaca*) and of aerobic anoxygenic phototrophic bacteria (*Roseinatronobacter*) was 96%, which was sufficient for the description of a new genus of aerobic anoxygenic phototrophic bacteria. The name *Roseibacula alcaliphilum* gen. nov., sp. nov. was proposed for the isolate.

Keywords: aerobic anoxygenic phototrophic bacteria, nonsulfur purple bacteria, alkaliphiles, soda lakes

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The first representative of aerobic anoxygenic phototrophic bacteria (AAPB) was isolated in 1978 from seawater [1]. This group comprises gram-negative bacteria that carry out anoxygenic photosynthesis due to the presence of carotenoids and bacteriochlorophyll *a* (BChl *a*) in their cells. Representatives of this group of phototrophs cannot use light as their only energy source. In the species examined thus far, bacteriochlorophyll *a* production depends on the presence of molecular oxygen and is more intensive in the dark. AAPB are heterotrophs and obligate aerobes unable to live in anoxic environments. RuBisCO has not been found in any of the studied strains. Organic compounds serve as carbon sources for the cells. AAPB are a phylogenetically heterogeneous group. Most of them belong to the phylum BXII *Proteobacteria*, class I *Alphaproteobacteria* and only few of them belong to class II *Betaproteobacteria*. Representatives of the *"Gammaproteobacteria"* are also known.

AAPB were isolated from various habitats: freshwater springs, thermal water, low-mineralized lakes, cold Antarctic waters, and soil. Nevertheless, only one genus, *Roseinatronobacter*, is known so far to include the species that can live at extremely high pH values. This genus belongs to the class *Alphaproteobacteria*. The genus *Roseinatronobacter* includes two species, *Rsn. thioooxidans* [2] and *Rsn. monicus* [3], which grow at pH 8.5–10.4 and in high salinity (up to 60 g/L NaCl). Representatives of this genus were isolated from soda lakes of East Siberia (Russia) and from a meromictic soda lake (Mono Lake, United States). These bacteria are gram-negative, nonmotile, and oval-shaped. They are obligate aerobes and heterotrophs containing no intracellular membrane structures. Anoxygenic photosynthesis is performed due to the presence of reaction centers (RC) II in the membranes. The cells were shown to contain carotenoids and bacteriochlorophyll *a*. The DNA G+C content is 59.0–59.4 mol % [2, 3]. The strain De discussed in the present work possesses the characteristics of this genus. However, according to the 16S rRNA gene sequencing, the newly isolated bacterium has only a 96% similarity to the previously described *Roseinatronobacter* species, which are its nearest relatives. This article presents the morphological physiological characteristics and taxonomic description of the new genus and species of alkaliphilic AAPB, *Roseibacula alcaliphilum* gen. nov., sp. nov., isolated from the meromictic soda lake Doroninskoye.

MATERIALS AND METHODS

Source of isolation. The novel AAPB strain was isolated from the samples of the upper water layer of the

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meromictic soda lake Doroninskoye, Chita oblast, Zabaykalsky Krai, Russia [4].

Cultivation techniques. The cultivation medium contained 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; Na acetate, 1; Na 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 Pfennig's trace element solution, 1 mL/L [3]. $NaHCO_3$ (10%), Na_2CO_3 (10%), yeast extract (5%), Na acetate (10%), Na pyruvate (10%), and Na thiosulfate (10%) were added to the basic medium as sterile solutions immediately before inoculation. The pH value of prepared medium was 9.0.

Cultivation was performed aerobically in the dark in 500-mL conical flasks. The cultures were isolated and purified on Petri dishes by successive transfers of well-isolated aerobic colonies on solid medium with 2% (wt/vol) agar.

Morphology and ultrastructure. The overall morphology of bacterial cells was studied under an Axio Imager D1 phase contrast microscope (Carl Zeiss, Germany) and by electron microscopy of total preparations contrasted with 0.2% aqueous solution of uranyl acetate. Cell structure was studied in ultrathin sections prepared from the material treated according to the Kellenberger procedure, dehydrated, and embedded in Epon. The sections were placed on Formvar-coated copper grids. The cells were contrasted with Reynolds' reagent [5]. Total preparations and ultrathin sections were examined under a Jeol JEM-100C electron microscope (Japan) at an accelerating voltage of 80 kV.

Pigment apparatus. Pigment composition and involvement in photosynthesis were determined by recording the spectra of cell membrane suspension and of acetone–methanol total extracts (7 : 2, vol/vol) in a SF 56A spectrophotometer (LOMO, Russia) in the wavelength range of 350–900 nm.

Fatty acid analysis. Fatty acids were analyzed by gas chromatography–mass spectrometry (GC-MS). A dry cell biomass sample (5 mg) was treated with 0.4 mL of 1 N hydrogen chloride in methanol at 80°C for 1 h (acidic methanolysis). Methyl esters of fatty acids and dimethylacetate were extracted with hexane and injected into a Sherlock gas chromatograph (Microbial Identification System, MIDI Inc., United States) [6].

Physiological properties and growth conditions. The experiments with different NaCl concentrations and pH values were carried out under aerobic conditions in the dark. Phosphate buffer was used to determine the effects of different pH values within the range of 6.8–7.4; carbonate buffer solutions were used to prepare the media with alkaline pH values (8.0–9.5) [7]. The background NaCl content in the medium in these experiments was 10 g/L. The temperature favorable for growth of the isolate was determined using a gradient thermostat in the temperature range from 10 to 50°C.

The spectrum of organic compounds consumed under aerobic conditions in the dark was determined in the above growth medium with 20 g/L of NaCl, B_{12} (10 μg/L) and yeast extract (0.1 g/L) as a vitamin additive containing no other organic substrates. The pH value of the medium was set up at 9.0–9.5 by varying the sodium carbonate or bicarbonate ratio. The tested organic substances and nitrogen sources were added at concentrations of 1 and 0.5 g/L, respectively. In addition, the ability of the cells to oxidize thiosulfate and other reduced sulfur compounds was determined.

The ability of the isolated microorganism to grow under aerobic conditions in the dark due to nitrate reduction was determined by the increment of cell biomass and production of N_2 and NO_2^-. N_2 was detected by gas evolution in the vials, while NO_2^- was qualitatively determined using the Griess reagent [8].

Bacterial biomass was monitored by optical density measurements at 650 nm with a KFK-3 photometer.

Antibiotic sensitivity was assessed during aerobic growth of the cells inoculated as lawn plates on an agarized medium by the size of growth inhibition zones around the discs with the relevant antibiotic.

Molecular genetic studies. The RuBisCO gene was detected using the DNA isolated with DNA Isolation Kit (Sigma). Two forms of the RuBisCO gene were identified as described [9].

The DNA for phylogenetic analysis was isolated according to Marmur [10]. The DNA G+C content was determined by the common method [11]. The level of DNA homology was determined by the method of optic reassociation [12]. The 16S rRNA gene of the new isolate was amplified and sequenced using the universal primers [13]. The amplification buffer contained 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, pH 8.3, and 0.001% gelatin. Reaction mixtures (100 μL) contained standard dNTP concentrations and equimolar amounts of the pA and pH' primers. Thirty amplification cycles of the following temperature profile were performed: DNA denaturation, 94°C, 30 s; primer annealing, 40°C, 1 min; and elongation at 72°C, 2 min 30 s. The 16S rRNA gene sequencing after purification on fusible agarose and Promega chromatography columns was performed in both directions using forward and reverse universal primers and Sequenase (Biochemicals, United States). The identified nucleotide sequences were aligned with the respective sequences of the most closely related bacterial species using CLUSTALX. Unrooted phylogenetic trees were constructed for the bacteria under study by the methods implemented in the TREECON software package [14].

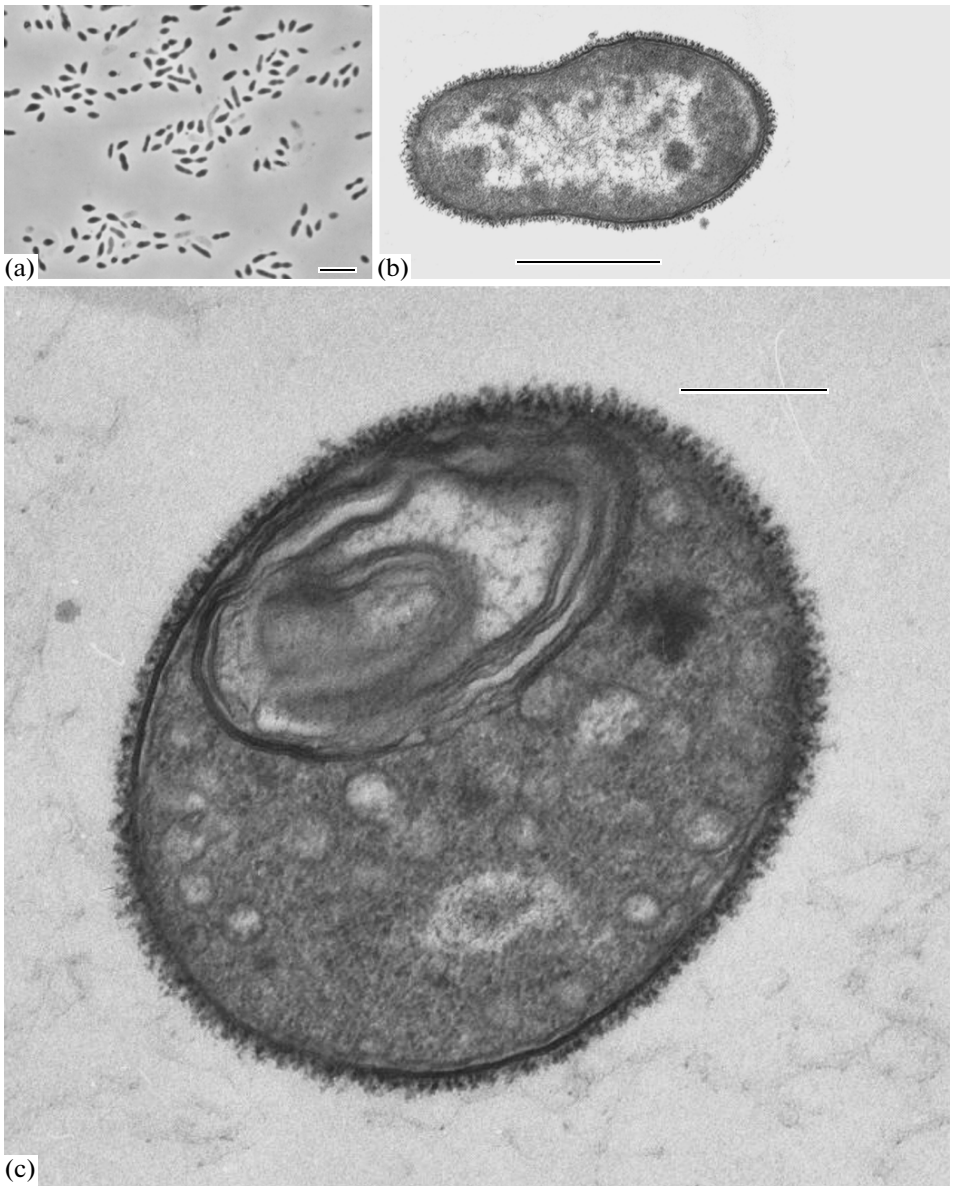

Fig. 1. Cell morphology and ultrastructure of the strain De: cell morphology, phase contrast (scale bar is 5 μm) (a); sections of aerobically grown young cells, electron microscopy (scale bar is 1 μm) (b); sections of aerobically grown cells, mature culture (membrane vesicles observed in the cells are presumably the photosynthesizing apparatus), electron microscopy (scale bar is 0.3 μm) (c).

RESULTS AND DISCUSSION

Habitats. The meromictic soda lake Doroninskoye (East Siberia, Russia) is situated in the Zabaykalsky Krai in the permafrost area (51°25' N; 112°28'E) [4]. The maximum depth of the lake is presently ~6 m; pH of the water is 9.72. In the summer period, the upper oxygen-containing layer of the water column is desalinated to 26.5 g/L and reaches to the depth of 3.5–4.0 m, where a sharp transition to the anaerobic layer occurs. In the latter, sulfide concentration is up to 12.5 g/L near the bottom and water mineralization is 32.3 g/L. Density stratification of the water column usually provides stability of aerobic and anaerobic conditions. The strain of aerobic anoxygenic phototrophic bacteria De was isolated from the surface water layer in contact with air.

Cultural properties and morphology. Bacteria of the strain De formed slightly colored (beige), round, sharp-edged colonies on the surface of solid medium. The colonies turned slightly pink during long-term exposure in the dark. The cells were citron-shaped, oval, 0.5–1.0 × 1.5–1.7 μm (Fig. 1a), and reproduced by binary division. The cell wall was of the gram-negative type. Young cells had no intracellular membranes (Fig. 1b). However, intracellular membranes of vesicular type similar to the photosynthesizing vesicles of

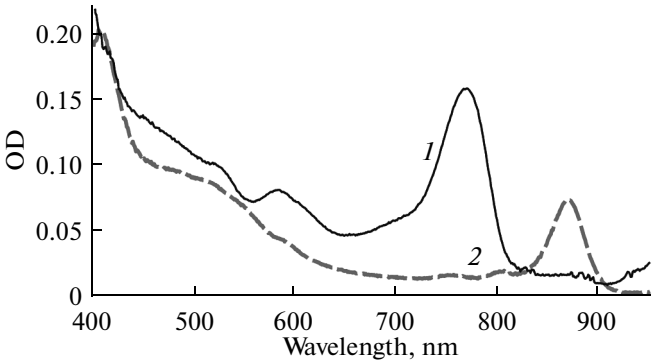

Fig. 2. Pigment composition in the cells of bacterial strain De: (*1*) acetone–methanol extract; (*2*) cells in vivo.

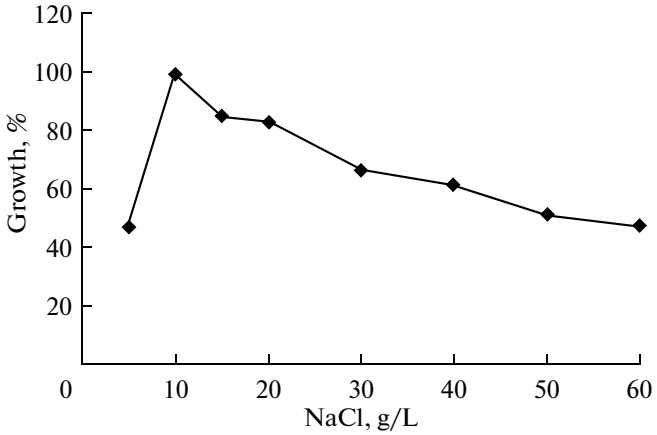

Fig. 3. Effect of NaCl on the growth of bacterial strain De cultivated under aerobic conditions in the dark (the maximum growth is taken as 100%).

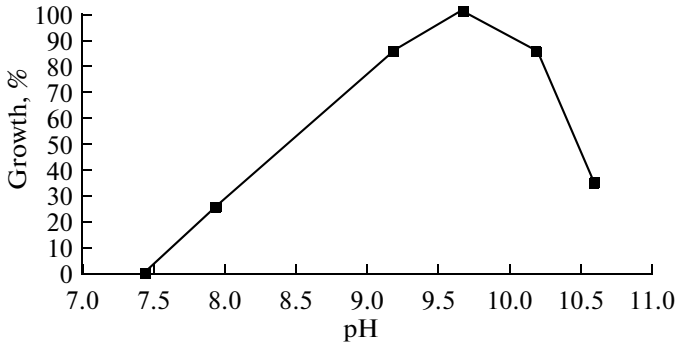

Fig. 4. Effect of pH on the growth of bacterial strain De cultivated under aerobic conditions in the dark (the maximum growth is taken as 100%).

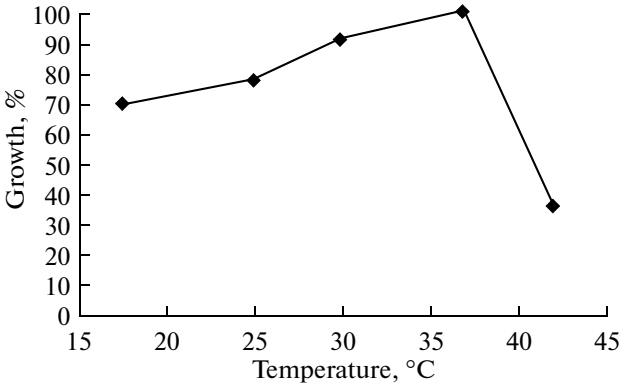

Fig. 5. Effect of temperature on the growth of bacterial strain De cultivated under aerobic conditions in the dark (the maximum growth is taken as 100%).

purple nonsulfur bacteria (PNSB) were observed in some cells of mature cultures (Fig. 1c). Storage compounds were accumulated as polyhydroxybutyrate.

Pigments. In the cells of the strain De grown aerobically in the light/dark cycle cultivation mode, the pigments were represented by BChl *a* and carotenoids. In the spectrum of intact cells (Fig. 2, dash line), the absorption maxima at 483–550 nm demonstrated the presence of carotenoids, while those at 596, 810 and 874 nm indicated the presence of BChl *a*. In the spectrum of acetone–methanol extracts (Fig. 2, solid line), the maxima of carotenoid absorption were recorded at 455 and 530 nm, while the major BChl *a* maximum was recorded at 773 nm.

Physiological properties. Optimal cell growth was observed in the dark under aerobic conditions at 10 g/L of NaCl; the culture grew at salt concentrations up to 50 g/L (Fig. 3). The isolate was an obligate alkaliphile, with the optimal growth at pH 9.8. Cell growth was absent at pH below 8 (Fig. 4). The aerobic culture grew well within the temperature range from 20 to 40°C (Fig. 5).

A broad range of organic compounds were tested as carbon sources (Table 1). Cell growth of the strain De was observed on sucrose, citrate, mannitol, sorbitol, casein hydrolysate, and yeast extract, which was typical of the group of aerobic anoxygenic phototrophic bacteria.

The new isolate was unable to oxidize thiosulfate even in the presence of organic substrates. Unlike *Roseinatronobacter* species, the strain demonstrated no ability to reduce nitrate to nitrite. The RuBisCO gene responsible for carbon dioxide fixation was not

Table 1. Utilization of organic compounds as carbon sources by the strain De, the purple nonsulfur bacteria *R. bogoriensis* LBB1 and *R. barguzinensis* alga-05, and the aerobic anoxygenic phototrophic bacterium *R. monicus* (strains ROS 10 and ROS 35)

Substrate	De	*R. bogoriensis* LBB1*	*R. barguzinensis* alga-05**	*Rna. monicus* ROS 10***	*Rna. monicus* ROS 35***
Xylose	–	++	++	n.d.	n.d.
Lactose	–	–	+–	–	+
Ribose	+–	+–	+–	n.d.	n.d.
Sucrose	++	++	++	+	+
Acetate	+	++	++	+	+
Aspartate	–	++	+	–	–
Benzoate	–	–	–	–	–
Butyrate	+–	++	+	–	–
Valerate	+–	+	+	n.d.	n.d.
Glutamate	+	n.d.	n.d.	+	–
Malate	–	++	++	+	+
Malonate	–	n.d.	n.d.	–	–
Succinate	–	++	++	–	–
Pyruvate	+	++	++	+	+
Propionate	+–	++	+	+	+
Tartrate	–	n.d.	n.d.	–	–
Citrate	++	–	–	–	+
Formate	–	–	–	n.d.	n.d.
Fumarate	–	++	++	n.d.	n.d.
Glycerol	–	n.d.	n.d.	+	+
Methanol	–	–	–	–	–
Ethanol	–	–	–	–	–
Mannitol	++	++	++	–	+
Sorbitol	++	n.d.	n.d.	–	+
Casein hydrolysate	++	++	–	+	+
Yeast extract	++	++	++	+	+

Designations: "–", no growth; "+–", weak growth; "+", good growth; "++", very good growth; n.d. stands for no data.
* [15].
** [16].
*** [3].

Table 2. Antibiotic sensitivity of the strain De and PNSB *Rodobaca barguzinensis* alga-05

Antibiotic	De	*R. barguzinensis* alga-05*
Benzylpenicillin, 10 U	+	+
Amikacin, 30 μg	+	
Ampicillin, 10 μg	+	+
Kanamycin, 30 μg	+	–
Vancomycin (VA), 30 μg	+	+
Lincomycin, 15 μg	+	+
Gentamycin (GM), 10 μg	+	–
Rifampicillin, 5 μg	+	+
Streptomycin, 300 μg	+	+
Tetracycline, 30 μg	–	+
Novobiocin, 30 μg	–	+
Neomycin, 30 μg	–	+
Nalidixic acid, 30 μg	+	–
Polymyxin, 300 U	+	+
Erythromycin		+

Designations: "+", utilizes or is sensitive; "–", does not utilize or resistant.
* [16].

found, as they weren't in other representatives of the AAPB group.

The cells of the strain De were sensitive to most of the tested antibiotics: benzylpenicillin, amikacin, kanamycin, vancomycin, lincomycin, gentamycin, rifampicillin, streptomycin, polymyxin, and nalidixic acid (Table 2).

Fatty acid composition. The major fatty acid in bacteria under study, as in other representatives of AAPB related to the *Alphaproteobacteria*, was an isomer of monounsaturated $C_{18:1w7}$ acid, making up 82.71% of the total acid content in the new isolate. Hexadecanoic $C_{16:0}$ (2.53%), *iso*-octadecanoic C_{i18} (2.46%), 11-methyl-octadecenoic $C_{11Me18:1}$ (1.91%), 9-hexadecenoic $C_{16:1w7}$ (1.86%), and nonadecenoic $C_{19:1}$ (1.81%) acids were also found in minor amounts. The 3.41% content of an unidentified fatty acid was shown. Some fatty acids were found in trace amounts, below 1% (Table 3).

Phylogenetic position. The DNA G+C content of strain De was 64.4 mol %. The 16S rRNA gene sequences (about 1540 nucleotides, approximately at *E. coli* positions 3–1535) were identified to specify the phylogenetic position of the new isolate. According to the data of phylogenetic analysis, the bacterium proved to be a member of the family *Rhodobacteriaceae* belonging to the class *Alphaproteobacteria*. On the phylogenetic tree, the 16S rRNA sequences of the strain De fell into the phylogenetic cluster comprising the members of the genera *Roseinatronobacter* and *Rhodobaca* (Fig. 6) [15, 16]. The level of similarity between nucleotide sequences of the 16S rRNA gene of the strain De and all members of this phylogenetic cluster was 96%. The new isolate was closest to the group of aerobic anoxygenic phototrophic bacteria by phenotypic properties (Table 4) and relation to oxygen. As Figure 6 shows, the bacteria of the strain De were the nearest relatives of PNSB (purple nonsulfur bacteria) of the genus *Rhodobaca* and AAPB of the genus *Roseinatronobacter*. One more rather close relative of the new isolate was the neutrophilic AAPB *Roseibaca ekhonensis* EL50 [17] isolated from an Antarctic salt lake. The same cluster, in addition to the purple nonsulfur bacterium *Rhodobaca*, includes the facultative chemoautotrophic alkaliphilic bacterium "*Natronohydrobacter thiooxidans*" not containing bacteriochlorophyll *a*. The fact of close relationship between these three groups of microorganisms, NPSB, alkalihalophilic and halophilic AAPB species, and an obligate chemotrophic bacterium, is interesting in the context of evolution.

According to the 16S rRNA data, the new isolate had a 96% similarity to its nearest relatives, which is sufficient for describing a novel genus of aerobic anoxygenic phototrophic bacteria. The proposed name for the novel genus and species is *Roseibacula alcaliphilum* gen. nov. sp. nov., belonging to the group of aerobic anoxygenic phototrophic bacteria.

Taxonomic description of *Roseibacula* gen. nov. (Ro.se.i.ba.cu.la. L. adj.; L. fem. n N.L. fem. N, pink-colored rod)

The cells are nonmotile, citron- or oval-shaped, 0.5–1.0 × 1.5–1.7 μm in size; they reproduce by binary division. The cell wall is gram-negative. The pigments are represented by BChl *a* and carotenoids. The organisms are aerobic heterotrophs. Cells are alkaliphilic, and grow with sucrose, citrate, mannitol, sorbitol, casein hydrolysate, or yeast extract as carbon sources. The dominant fatty acid is an isomer of monounsaturated $C_{18:1w7}$. The type species is *Roseibacula alcaliphilum* sp. nov.

Taxonomic description of *Roseibacula alcaliphilum* sp. nov. (al.ca.li.phi.lum N.L. n. *alcali* from French

Table 3. Fatty acid composition in the cells of the strains De, PNSB *Rodobaca barguzinensis* alga-05, and AAPB *Roseinatronobacter monicus* ROS 10

No.	Acid	Symbol	De	*R.*barguzinensisalga-05*	*Rna. monicus* strain ROS 10**
1	Nonanoic	$C_{9:0}$	0.10	–	–
2	Undecenoic	$C_{11:0}$	0.5	–	–
3	Dodecenoic	$C_{12:1}$	0.17	–	N.d.
4	Tetradecenoic	$C_{14:1}$	–	2.21	2.35
5	Tetradecanoic	$C_{14:0}$	–	–	0.18
6	7-Hexadecenoic	$C_{16:1w7}$	1.86	2.64	1.27
7	9-Hexadecanoic	$C_{16:1w9}$	–	–	0.13
8	Hexadecanoic	$C_{16:0}$	2.53	9.69	1.85
9	*Iso*-Octadecanoic	C_{i18}	2.46	–	–
10	6,9-Octadecadienoic	$C_{6.9-18:2}$	–	–	10.03
11	9-Octadecenoic	$C_{18:1w9}$	0.21	–	0.41
12	7-Octadecenoic	$C_{18:1w7}$	82.71	79.32	79.12
13	Octadecanoic	$C_{18:0}$	0.84	1.56	0.52
14	11-Methyl-octadecanoic	$C_{11Me18:1}$	1.91	4.58	2.62
15	Not identified	N.d.	3.41	–	–
16	Nonadecenoic	$C_{19:1}$	1.81	–	–
17	10-Methyl-nonadecenoic	C_{10Me19}	0.20	–	–
18	*Iso*-Eicosanoic	C_{i20}	0.26	–	–
19	6-Eicosadienoic	$C_{20:2w6}$	0.20	–	–
20	9-Eicosenoic	$C_{20:1w9}$	0.83	–	1.01
21	11-Eicosenoic	$C_{20:1w11}$	–	–	0.5
		Total	100.00	100.00	100.00

Designations: N.d., no data; t.a., trace amount; "–", absent.

* [16].

** [3].

n. *alcali*; Gr. adj. *philos* loving; N.L. neut. adj. *alcaliphilum* loving alkaline conditions)

The cells are oval-shaped, 0.5–1.0 × 1.5–1.7 μm in size, and nonmotile. Division is binary, by constriction. The cell wall is gram-negative. Intracellular membrane structures are absent or vesicular. The cells contain bacteriochlorophyll *a* and carotenoids of the spheroidene series. The absorption spectrum of acetone–methanol (7 : 2, vol/vol) extracts has the bands at 455, 530, 588, and 773 nm. The organism is an obligate aerobe and heterotroph. Sucrose, citrate, mannitol, sorbitol, casein hydrolysate, and yeast extract are utilized as carbon and energy sources. Obligate alkaliphiles, the cells grow well in the pH range of 8.5–10.0, with the optimum at pH 9.8. They are moderately halophilic. Growth occurs at 0 to 50 g/L of NaCl, with the optimum at 5–10 g/L. The storage compounds are poly-β-hydroxybutyric acid and polyphosphates. Growth occurs on sucrose, citrate, mannitol, sorbitol, casein hydrolysate, and yeast extract. The cells are sensitive to a number of antibiotics: benzylpenicillin, amikacin, kanamycin, vancomycin, lincomycin, gentamycin, rifampicillin, streptomycin, polymyxin, and nalidixic acid. The bacteria are not capable of nitrate reduction, and are incapable of thiosulfate oxidation. RuBisCO was not detected. The DNA G+C content is 64.4 mol %. The type strain De was registered in UNIQEM as U978.

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Table 4. Comparative characterization of the tested strain De, PNSB *Rodobaca barguzinensis* alga-05, and AAPB of the genus *Roseinatronobacter*

Characteristics	De	*R. barguzinensis* alga-05*	*Rna. monicus* ROS 10**	*Rna. thiooxidans* ALG 1***
Habitat	Surface of the meromictic soda lake Doroninskoye, salinity 26.5 g/L	Steppe soda lake, Siberia	Soda lake Mono (California), aerobic zone, salinity 70 g/L	Soda lake Gorbunka, Chita oblast, salinity 18 g/L
Cell shape and size	Citron-shaped, oval; 0.5–1.0 × 1.5–1.7 μm	Short rods; 1.0 × 1.5	Short rods; 0.5–0.7 × 1.2–1.7	Citron-shaped; 0.5–0.8 × 0.8–2.2
Motility	–	+	–	–
Carotenoids: peaks in vivo, nm	483–550	475, 501, 590, 870	480, 525, 550	(410), 483, (511)
Carotenoids:	In acetone–methanol (7 : 2) 455, 530	In acetone–methanol (7 : 2) 490, 520	In acetone–methanol (7 : 2) (459), 481, (518)	In hexane (450), 481, (505)
Bacteriochlorophyll *a*, major peaks in vivo, nm	596, 810, and 874	870	(805), 870	803, 870
Bacteriochlorophyll *a* in acetone–methanol (7 : 2) extract	773	770	360, 708, 770	765, 772
Autotrophic growth on thiosulfate	–	+	–	–
Heterotrophic growth	+	+	+	+
Thiosulfate utilization during heterotrophic growth	–	+	+	+
pH (optimum) range	(9.8) 8–10	(8.2) 7.5–9.0	(8.5–9.5) 8–10	(10) 8.5–10.4
NaCl, g/L (optimum), range	0–50	(20–30) 10–80	(40) 0–100	(30) 10–100
Presence of RuBisCO	–	–	–	–
DNA G+C content, mol %	64.4	59.8	59.4	61

Designations: "+", the character is present; "–", the character is absent.
* [16].
** [3].
*** [2].

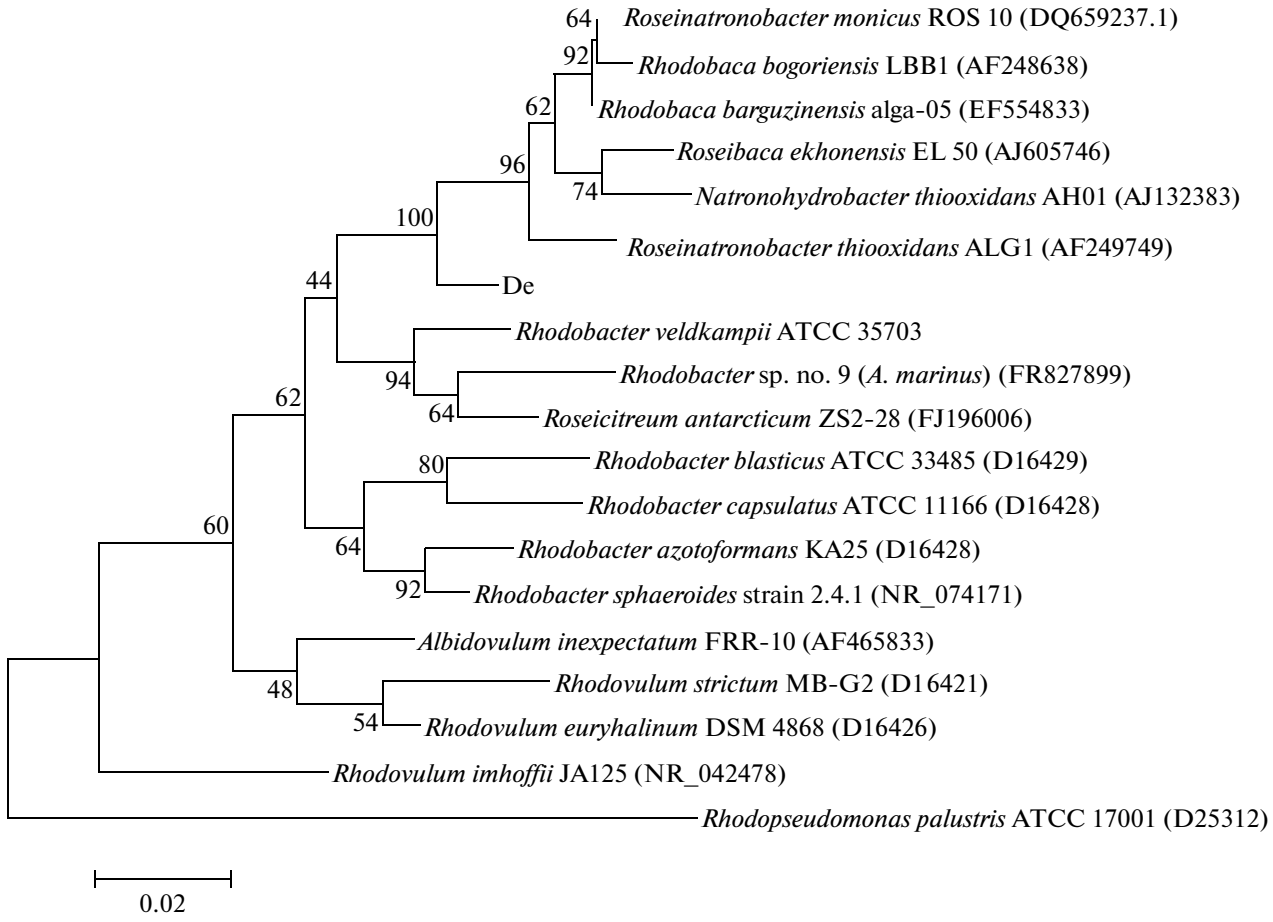

Fig. 6. The phylogenetic tree based on the 16S rRNA gene analysis, showing the position of strain De among the genera of the family *Rhodobacteraceae*. Bootstrap analysis values are given at the tree nodes. The distance marker shows the number of nucleotide substitutions per homologous site of the sequences being compared.

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