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EXPERIMENTAL ARTICLES

The New Alkaliphilic Bacteriochlorophyll *a*–Containing Bacterium *Roseinatronobacter monicus* sp. nov. from the Hypersaline Soda Mono Lake (California, United States)

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Abstract—Two strains of pink-colored aerobic bacteriochlorophyll *a*–containing bacteria were isolated from aerobic (strain ROS 10) and anaerobic (strain ROS 35) zones of the water column of Mono Lake (California, United States). Cells of the bacteria were nonmotile oval gram-negative rods multiplying by binary fission by means of a constriction. No intracellular membranes were detected. Polyphosphates and poly-β-hydroxybutyric acid were the storage compounds. Pigments were represented by bacteriochlorophyll *a* and carotenoids of the spheroidene series. The strains were obligately aerobic, mesophilic (temperature optimum of 25–30°C), alkaliphilic (pH optimum of 8.5–9.5), and moderately halophilic (optimal NaCl concentration of 40 g/l). They were obligately heterotrophic and grew aerobically in the dark and in the light. Respiration was inhibited by light at wavelengths corresponding to the absorption of the cellular pigments. The substrate utilization spectra were strain-specific. In the course of organotrophic growth, the bacteria could oxidize thiosulfate to sulfate; sulfide and polysulfide could also be oxidized. The DNA G+C content was 59.4 mol % in strain ROS 10 and 59 mol % in strain ROS 35. In their phenotypic properties, the new strains were close but not identical to the alkaliphilic bacterium *Roseinatronobacter thiooxidans.* The distinctions in the nucleotide sequences of the 16S rRNA genes (2%) and low DNA–DNA hybridization level with *Rna. thiooxidans* (22–25%) allow the new strains to be assigned to a new species of the genus *Roseinatronobacter, Roseinatronobacter monicus* sp. nov. with the type strain ROS 35^T (=UNIQEM U-251^T = VKM B-2404^T).

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Key words: aerobic bacteriochlorophyll *a*–containing bacteria, alkaliphiles, *Roseinatronobacter monicus* sp. nov., phylogeny, soda lakes.

Aerobic bacteriochlorophyll *a*–containing (ABC) bacteria are chemoorganotrophs incapable of anaerobic phototrophic growth. These bacteria were first isolated from marine habitats in the 1970s [1]. The data obtained over the last several years show that these bacteria are widespread in marine and freshwater basins. More than 20 genera of ABC bacteria have been described to date. Most of them belong to phylum BXII *Proteobacteria*, class I *Alphaproteobacteria*, and a few belong to class II *Betaproteobacteria* [2]. As indicated by their phylogenetic position, ABC bacteria are the probable evolutionary link between purple nonsulfur bacteria and aerobic chemoorganotrophic bacteria [2]. Utilization of the energy of light for ATP synthesis has been shown for various species of ABC bacteria [3]. ABC bacteria possess photosynthesis reaction centers, light-harvesting pigment–protein complexes, and light-dependent electron transport chains similar to those of nonsulfur purple bacteria, to which they are related phylogenetically [3]. However, as distinct from purple bacteria, under no conditions can ABC bacteria use light as the sole source of energy. In the ABC bacteria studied so far, the synthesis of bacteriochlorophyll *a* (Bchl *a*) depends on the presence of molecular oxygen and is more intense in the dark. Permanent illumination results in the suppression of Bchl *a* synthesis; its content is rapidly restored in the dark. Under anaerobic conditions, ABC bacteria grow as chemoheterotrophs. With the exception of some *Acidiphilium rubrum* strains, they are incapable of chemolithoautotrophic

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growth [4]; in neither of the strains investigated in this respect was ribulose-bisphosphate carboxylase detected. Light suppresses aerobic respiration of ABC bacteria, which indicates the presence of competing sites in their respiratory and photosynthetic electron transport chains [5].

Some ABC bacteria can thrive under extreme conditions, such as elevated temperatures (but no higher than 48°C), high salt concentrations (up to 20%), low pH values (3.0–5.0), or in the presence of toxic metalloids (Te, Se, V) [2, 6]. Only one species of ABC bacteria is known to grow at high pH values (8.5–10.4); this is *Roseinatronobacter thiooxidans*, isolated from a weakly mineralized soda lake in Kunkur Steppe (Chita oblast, Russia) [7]. The present paper provides a description a new alkaliphilic species, *Roseinatronobacter monicus*, based on the study of two pigmented strains isolated from alkaline, hypersaline Mono Lake (California, United States).

MATERIALS AND METHODS

Isolation source and cultivation methods. Two strains of ABC bacteria, ROS 10 and ROS 35, were isolated from the meromictic hypersaline soda Mono Lake from depths of 10 m (aerobic zone) and 35 m (near-bottom anaerobic zone). Mono Lake is a closed body of water in the central part of California (United States). As a result of evaporative concentration, the salt content in the lake reaches 82–92 g/l. The mineral composition of the lake water is as follows (%): Na^+, 96.8; Mg^{2+}, 0.2; (CO_3^{2-} + HCO_3^-), 46.3; Cl^-, 37.8; SO_4^{2-}, 15.6; pH 9.8 [8]. The boundary of the aerobic and anaerobic zones occurs at a depth of 20 m; the maximal depth at the station is 35 m.

For cultivation, medium with pH 9.0–9.5 was used, which had the following composition (g/l): NH_4Cl, 0.4; KH_2PO_4, 0.5; $NaNO_3$, 0.4; $MgCl_2$, 0.2; Na_2SO_4. 0.5; yeast extract, 1; Na acetate, 1; Na pyruvate, 1; NaCl, 40; $Na_2S_2O_3 \cdot 5H_2O$, 1; KCl, 0.5; $NaHCO_3$, 10, Na_2CO_3, 5; vitamin B_{12}, 10 μg; trace element solution [7], 1 ml.

The solutions of $NaHCO_3$ (10%), Na_2CO_3 (10%), yeast extract (5%), Na acetate (10%), Na pyruvate (10%), and Na thiosulfate (10%) were sterilized separately and introduced into the medium immediately before inoculation. The pH of the prepared medium was 9.0–9.5.

Cultivation of the pigmented strains was performed aerobically in the dark in 500-ml conic flasks. Pure cultures were obtained by picking and restreaking individual colonies grown in petri dishes with medium solidified with 2% agar. The purity of the cultures was checked by microscopy of the colonies.

Comparative physiological and molecular-genetic studies used the type strain of the alkaliphilic ABC bacterium *Roseinatronobacter thiooxidans*, the alkaliphilic nonsulfur purple bacterium *Rhodobaca bogoriensis* LBB1^T, and the alkalitolerant nonsulfur purple bacterium *Rhodobacter* sp. B6, isolated from a weakly mineralized soda lake in central Mongolia. The studies on the photoinhibition of respiration used, as a reference organism, strain *Rhodospirillum rubrum* KMMGU no. 301 (2R) from the collection of the Microbiology Department, Moscow State University.

Physiological tests and determination of the conditions of growth of the isolates. The ability of strains ROS 10 and ROS 35 to grow anaerobically at the expense of nitrate reduction was judged from the increase in biomass and production of N_2 and NO_2^- as the reduction products. N_2 formation was judged from the increase of pressure in the flasks due to gas production; NO_2^- formation was determined colorimetrically [9]. In the experiments with different NaCl concentrations, liquid medium of the above-specified composition was used, and the NaCl concentration was varied from 0 to 2.6 M at a 0.1 M background content of soda (0.06 M hydrocarbonates and 0.04 M carbonates). When determining the pH optimum, the pH values were adjusted by using phosphate buffer (within the pH range of 6.8–7.4) or Na carbonate and Na bicarbonate at different ratios (within the alkaline pH range); the buffer molarity was kept constant [10].

The bacterial biomass yield was judged from the increase in the culture optical density (measured on a KFK-3 photometer at 650 nm) by the end of the exponential growth phase.

To determine the spectrum of substrates utilized under aerobic conditions, mineral medium supplemented with 0.01 g/l yeast extract as a source of vitamins was used. The tested compounds were added in a concentration of 1 g/l. The ability of the bacteria to oxidize thiosulfate and other reduced sulfur compounds was tested as follows. The cultures were grown on medium with peptone and thiosulfate at pH 9.6. Cells were harvested by centrifugation and washed in a buffer with equal molar contents of soda and NaCl (0.6 M). To decrease endogenous respiration, the suspension was incubated on a shaker for 3 h. Respiration measurements were performed in a 5-ml polarographic cell with a Clark electrode (Yellow Spring Co, Ohio, United States). The ability to utilize a substrate was judged from the increase in oxygen consumption by cell suspensions (in comparison with endogenous respiration [7]). Sulfate was determined nephelometrically on a KFK-3 photometer at 400 nm after sample acidification and sulfate sedimentation with $BaCl_2$ in 50% glycerol [11].

The ability of the strains to grow aerobically was judged from the distance between the surface of the column of agarized (0.7%) medium in the tubes and the zone of growth.

To prove the photosynthesizing activity of cultures, photoinhibition of respiration (PIR) was studied polarographically by an earlier described method [12]. O_2 consumption was measured in samples illuminated by 1-s impulses of monochromatic light (0.2 μmol quanta/(m^2 s); spectral half width of the aperture, 3 nm) with 20- to 60-s dark intervals; the wavelength range scanned was 400–930 nm. The photoinhibition spectrum of the typical purple bacterium *Rhodospirillum rubrum* recorded under the same conditions was used for comparison.

Morphology and fine structure. The morphology of the bacterial cells was studied under a Reichert (Austria) light microscope equipped with a phase-contrast device and under an electron microscope in specimens of whole cells negatively stained with a 2% aqueous solution of uranyl acetate. Thin sections were obtained from cells grown in liquid medium. Cells were treated according to the Kellenberger procedure, dehydrated, and embedded in Epon. Thin sections cut with an ultramicrotome were placed on formvar-coated copper grids. Reynolds reagent was used for negative staining [13]. Whole cell specimens and thin sections were examined under a Jeol JEM 100C electron microscope (Japan) at an accelerating voltage of 80 kV.

Pigments. The pigment composition of bacterial cells was studied by recording absorption spectra of whole cells suspended in 50% glycerol on a Lomo SF-56A spectrophotometer (LOMO, Russia) and the spectra of acetone–methanol (7 : 2, vol/vol) cell extracts on a Hewlett Packard spectrophotometer.

Analysis of fatty acids. 5 mg of dry cells 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 acetals formed as a result of methanolysis were extracted with hexane and analyzed on a Sherlock gas chromatograph (Microbial identification system, MIDI Inc., United States) [14].

Molecular-genetic analysis. DNA was isolated according to the Marmur procedure [15]. The DNA G+C content was determined by the method of Owen et al. [16]. The DNA homology was determined by the optical reassociation method [17].

Amplification and sequencing of the 16S rRNA genes of the new isolates was performed using universal primers [18]. The buffer used for amplification was composed of 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris–HCl (pH 8.3), and 0.001% gelatine. The reaction mixture (100 μl) contained standard concentrations of dNTP and equimolar amounts of the pA nd pH' primers. The amplification regime included 30 cycles of DNA denaturation at 94°C for 30 s, primer annealing at 40°C for 1 min, and primer extension at 72°C for 2.5 min. The amplificate was purified using low-gelling temperature agarose and Promega columns. Sequencing was performed in both directions using forward and reverse universal primers and Sequenase (Biochemicals, Cleveland, Ohio, United States). The nucleotide sequences determined were aligned with corresponding sequences of most closely related bacteria with the help of the CLUSTALX program. Unrooted phylogenetic trees were constructed by the methods implemented in the TREECON software package [19]. The nucleotide sequences of strains ROS 10 and ROS 35 were deposited in GenBank under the accession numbers DQ659237 and DQ659236, respectively.

RESULTS AND DISCUSSION

Isolation of bacteria. Inoculation of solid medium in petri dishes by samples of water taken from different depths of the meromictic hypersaline soda Mono Lake (California, United States) showed that the population density of ABC bacteria was highest in the aerobic zone of the water column (60% of the total CFU number). Six strains were isolated, and two of them (strain ROS 10 from the aerobic zone and strain ROS 35 from the anaerobic zone) were chosen for further studies.

Morphology and fine structure. On the surface of the solid medium, strains ROS 10 and ROS 35 formed round convex pink colonies 1–3 mm in diameter. The cells of the two strains were morphologically similar. They were oval-shaped, nonmotile, and measured 0.5–0.7×1.2–1.7 μm (Figs. 1a, 1b). Cell division occurred by binary fission by means of a constriction (Fig. 1c). In whole cell specimens, electron microscopy revealed thin filaments, most probably pili (Fig. 1c). The cell wall of both strains had a gram-negative-type structure, exhibiting a wavy two-layered outer membrane. In some cells of strain ROS 35, the periplasmic space was extended at the cell poles (Figs. 1d, 1e). Electron-dense polyphosphate granules and electron-transparent inclusions of poly-β-hydroxybutyrate were deposited as storage compounds (Fig. 1e). No intracellular membranes were revealed in the thin sections.

Pigments. Photosynthetic pigments were represented by bacteriochlorophyll (Bchl) *a* and carotenoids, whose presence was evidenced by the peaks at 590, (804), and 870 nm (Bchl *a*) and 480, 525, and 550 nm (carotenoids) in the in vivo spectra (Fig. 2b). The spectra of acetone–methanol (7 : 2, vol/vol) extracts of cells of both strains were identical and exhibited absorption maxima at 360, 383, 482, 506, 708, and 769 nm (Fig. 2a). The peaks at 482 and 506 nm are characteristic of the carotenoid spheroidene, and the peaks at 360, 383, 708, and 769 nm are characteristic of Bchl *a*.

Fatty acid composition. The fatty acid composition of the new isolates of ABC bacteria and of the type strain of *Rna. thiooxidans* is presented in Table 1. The new isolates were essentially similar in their fatty acid compositions and exhibited certain distinctions from other ABC bacteria belonging to the class *Alphaproteobacteria.* The fatty acid predominant in the new isolates

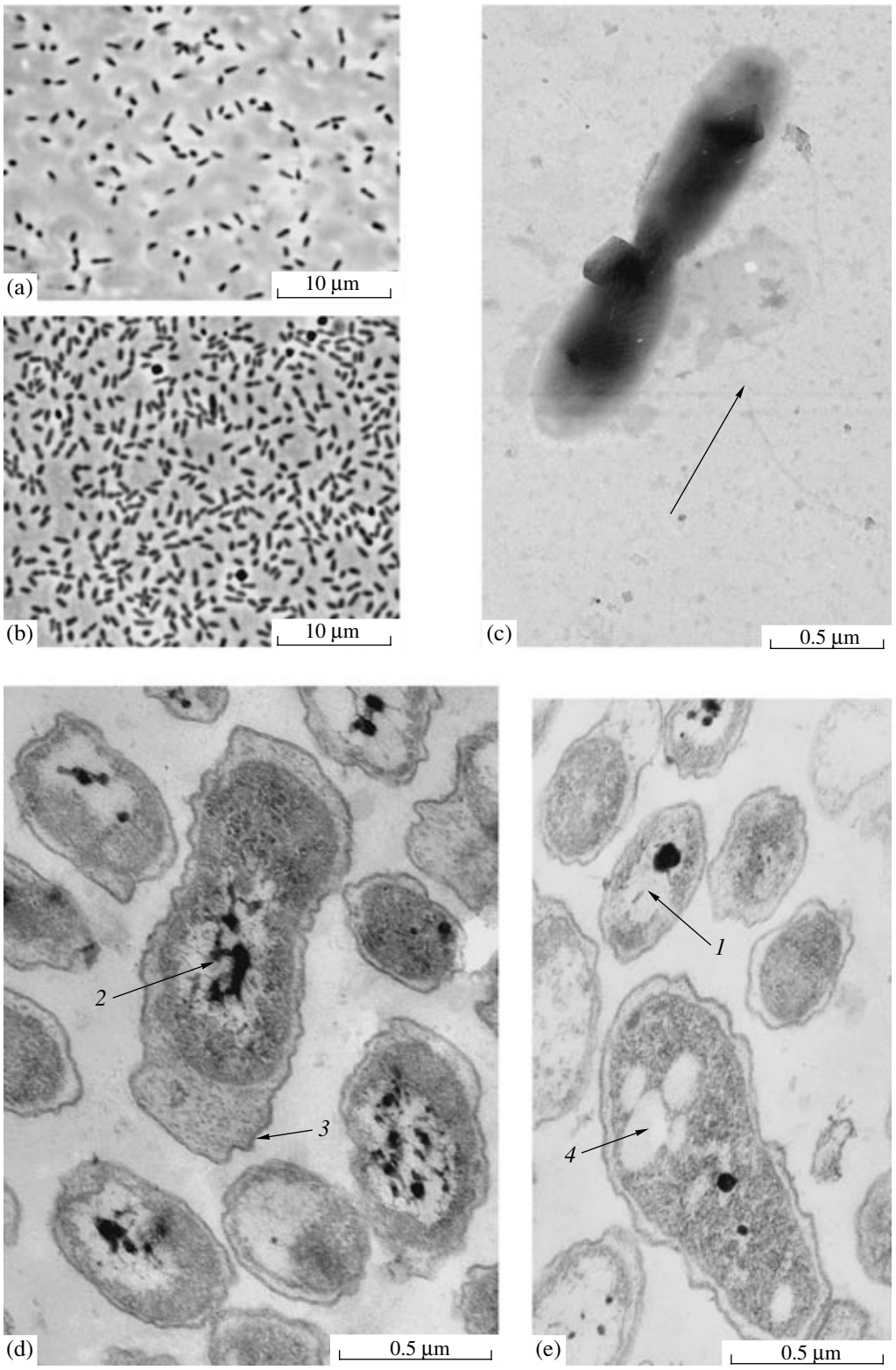

Fig. 1. Morphology and ultrastructure of the cells of strains ROS 10 and ROS 35: light micrographs (bar, 10 μm) of (a) ROS 10 and (b) ROS 35 cells, (c) electron micrograph of ROS 10 whole cells (pili can be seen; bar, 0.5 μm), and electron micrographs of thin sections (bar, 0.5 μm) of (d) ROS 35 cells and (e) ROS 10 cells. *1*, storage compounds (polyphosphates); *2*, nucleoid; *3*, outer membrane; *4,* poly-β-hydroxybutyrate.

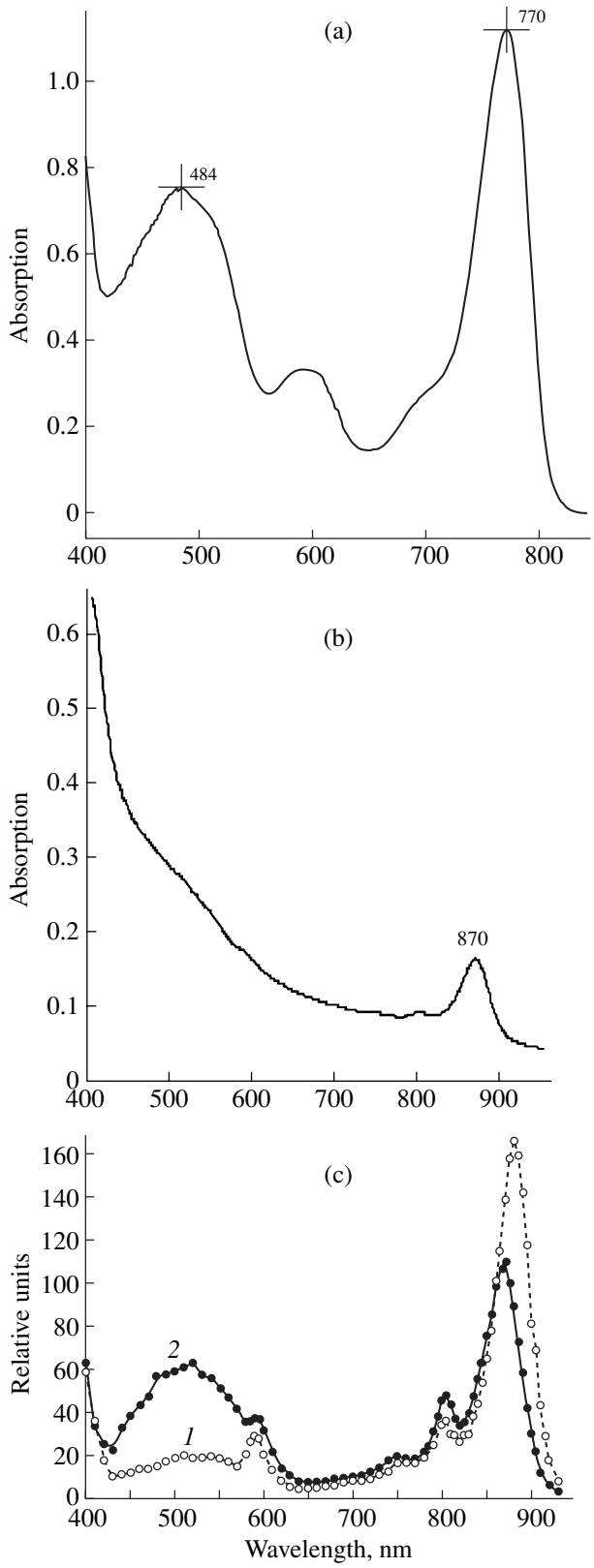

Fig. 2. Absorption spectrum of the pigments of strain ROS 35 (a) extracted with acetone–methanol and (b) in vivo, and (c) spectrum of photoinhibition of respiration for strain ROS 35 in comparison with the spectra of pigments of the purple nonsulfur bacterium *Rh. rubrum: 1, Rh. rubrum; 2*, strain ROS 35.

was an isomer of the monounsaturated C18:1ω7 acid (73.8–84.84% of total fatty acids). 6,9-octadecadienoic acid (6.9-18:2) also occurred in a significant amount (5.87–12.24%). The Mono Lake isolates differed from *Rna. thiooxidans* in a lower content of 11-methyl-octadecanoic acid (2.62–2.93% in strains ROS 10 and ROS 35 and 3.69% in *Rna. thiooxidans*). It is evident that fatty acid composition cannot be used as a reliable criterion for the differentiation of *Roseinatronobacter* species.

Physiological properties. Strains ROS 10 and ROS 35 were obligately aerobic and mesophilic (growth optimum at 25–30°C). The strains grew heterotrophically under aerobic conditions in the dark and in the light. Cell suspensions of the strains grown aerobically in the dark were pink-colored.

Light inhibited respiration at wavelengths corresponding the absorption maxima of the pigments. Thus, the PIR spectrum of strain ROS 35 exhibited bands at 800 and 860–870 nm, determined by Bchl *a*, and a wide band in the 450- to 550-nm region, determined by carotenoids (Fig. 2c). The similarity of the PIR spectra of the new isolates of ABC bacteria and *Rh. rubrum* (Fig. 2c) may be considered direct evidence of the operation of photosynthesis in their cells. The distinctions from the PIR spectrum of *Rh. rubrum* are mainly related to greater involvement of the carotenoids of ABC bacteria, as compared to carotenoids of nonsulfur purple bacteria, in the process of photoinhibition of respiration. This conclusion is in agreement with the fact that, in ABC bacteria, the content of carotenoids is higher and their portion associated with the photosynthetic antenna is larger [20, 21]. The decreased intensity of the main Bchl *a* band at 860–870 nm indicates a smaller size of the photosynthetic antenna in cells of strain ROS 35. The data on the PIR spectra show the operation in both strains of ABC bacteria under study of a light-dependent electron-transport chain some segments of which compete with the respiratory chain.

The new strains of ABC bacteria were incapable of anaerobic growth. As distinct from strain ROS 35, strain ROS 10 could reduce nitrates with the production of nitrites under anaerobic conditions; however, virtually no biomass increment could be recorded (data not presented). The type strain of *Rna. thiooxidans*, taken as a reference, also performed active reduction of nitrates to nitrites. At an initial nitrate concentration of 4.3 mM, strain ROS 10 and the type strain of *Rna. thiooxidans* formed 2.2–3.2 mM nitrites over six days of incubation. Dimethyl sulfoxide (0.5 g/l) failed to support the growth of strains ROS 10 and ROS 35 under anaerobic conditions.

A wide range of organic compounds were tested as potential sources of carbon under aerobic conditions (Table 2). Both of the new isolates utilized acetate, casein hydrolysate, glycerol, yeast extract, lactate, malate, propionate, pyruvate, rhamnose, maltose, and

Table 1. Composition of fatty acids in the ABC bacteria under study and *Roseinatronobacter thiooxidans* ALG1 (% of total)

№	Fatty acid	Designation	AlG 1	ROS 10	ROS 35	DSM 8510*	DSM 10594**
1	Dodecenoic	12:1	Tr.	Tr.	Tr.	ND	ND
2	Tetradecenoic	14:1	1.88	2.35	1.84	ND	ND
3	Tetradecanoic	14:0	0.37	0.18	0.13	0.2	0.2
4	2-Hydroxitetradecanoic	2-OH 14:0	0.00	0.00	0.00	4.5	9.2
5	Pentadecanoic	15:0	0.00	0.00	0.00	2.1	0.1
6	2-Hydroxipentadecanoic	2-OH 15:0	0.00	0.00	0.00	4.6	0.3
7	9-Hexadecenoic	16:1ω9	Tr.	0.13	0	ND	ND
8	7-Hexadecenoic	16:1ω7	1.76	1.27	1.58	2.0	2.1
9	Hexadecanoic	16:0	2.49	1.85	1.81	9.5	6.8
10	2-Hydroxihexadecanoic	2-OH 16:0	0.00	0.00	0.00	1.8	1.3
11	Heptadecanoic	17:0	0.00	0.00	0.00	4.7	0.3
12	Iso-heptadecanoic	17:0*iso*	0.00	0.00	0.00	0.6	0.0
13	Heptadecenoic	17:1ω6*cis*	0.00	0.00	0.00	22.4	2.1
14	6,9-Octadecadienoic	6.9-18:2	12.24	10.03	5.87	ND	ND
15	9-Octadecenoic	18:1ω9	0.51	0.41	0.26	ND	ND
16	11-Octadecenoic	18:1ω7	73.80	79.12	84.84	41.8	68.5
17	13-Octadecenoic	18:1ω5*cis*	0.00	0.00	0.00	0.6	2.0
18	Octadecanoic	18:0	0.55	0.52	0.76	0.2	1.1
19	11-Methyl-octadecenoic	11Me18:1	6.39	2.62	2.93	ND	ND
20	11-Eicosenoic	20:1ω9	Tr.	1.01	Tr.	ND	ND
21	9-Eicosenoic	20:1ω11	Tr.	0.50	Tr	ND	ND

Note: "Tr." – traces; "ND" stands "no data".
* *Erythromicrobium ramosum* [24].
** *Porphyrobacter tepidarius* [24].

sucrose. Strain 35, as distinct from strain ROS 10, also showed good growth on media with mannitol, sorbitol, fructose, citrate, arabinose, galactose, lactose, and inositol. Strain 10 utilized glutamate, which was not used by strain ROS 35. Neither of the strains grew on arginine, aspartate, butyrate, benzoate, glycolate, glucose, caproate, malonate, methanol, succinate, tartrate, ethanol, or propanol. The only alkaliphilic species of ABC bacteria currently recognized, *Rna. thiooxidans* [7], utilized, as distinct from the new isolates of ABC bacteria, benzoate, glycolate, glucose, caproate, malonate, and tartrate.

Experiments with growing cultures showed that, in the presence of organic substrates, both strains of ABC bacteria oxidized thiosulfate with the production of sulfate (Table 3). Additional polarographic studies showed that, apart from thiosulfate, strains ROS 10 and ROS 35 could oxidize sulfide, polysulfide, and elemental sulfur at a rate characteristic of lithoheterotrophs [23]. As distinct from strain ROS 10, strain ROS 35, isolated from an anaerobic environment, oxidized sulfide at a rate that was higher than the rate of thiosulfate oxidation and exhibited a low oxidation rate of elemental sulfur. It should be mentioned that *Rna. thiooxidans* can oxidize sulfite in addition to sulfide, thiosulfate, and elemental sulfur.

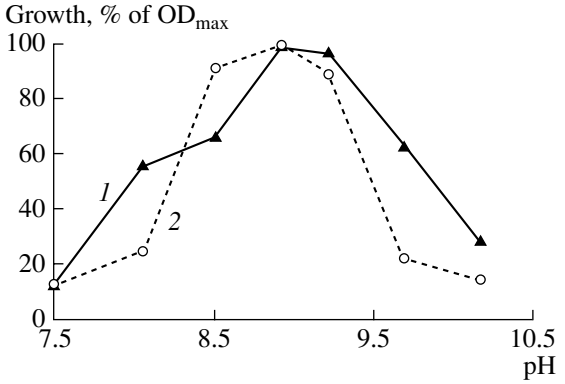

Fig. 3. Effect of pH on the growth of strains (*1*) ROS 10 and (*2*) ROS 35. Maximum value of optical density (OD) was taken as 100%.

Table 2. Utilization of organic compounds as the carbon sources by the ABC bacteria under study and *Roseinatronobacter thiooxidans* ALG1

Substrate	*Rna. thiooxidans*, ALG 1 [7]	ROS 10	ROS 35
Acetate	+	+	+
Casein hydrolysate	+	+	+
Glycerol	–	+	+
Yeast extract	+	+	+
Lactate	+	+	+
Malate	+	+	+
Propionate	+	+	+
Pyruvate	+	+	+
Rhamnose	ND	+	+
Maltose	ND	+	+
Sucrose	ND	+	+
Glutamate	+	+	–
Mannitol	+	–	+
Sorbitol	+	–	+
Fructose	+	–	+
Citrate	+	–	+
Arabinose	ND	–	+
Galactose	ND	–	+
Lactose	ND	–	+
Inositol	ND	–	+
Arginine	–	–	–
Aspartate	+	–	–
Butyrate	–	–	–
Benzoate	+	–	–
Glycolate	+	–	–
Glucose	+	–	–
Caproate	+	–	–
Malonate	–	–	–
Methanol	–	–	–
Succinate	+	–	–
Tartrate	–	–	–
Ethanol	–	–	–
Propanol	–	–	–

Note: "+" means that the compound is utilized; "–" means that the compound is not utilized; "ND" stands for "not determined."

Investigation of the pH dependence of the growth of strains ROS 10 and ROS 35 showed that the strains were obligate alkaliphiles with a pH growth range of 8.0–10.0 and a pH optimum of 8.5 to 9.1–9.5 (Fig. 3). At unfavorable pH values, the cells exhibited an atypical morphology: at pH 7.0, the cells were swollen and formed spheroplasts; at pH 10.0, chains of deformed cells were observed, indicating impairment of cell division.

Strains ROS 10 and ROS 35 showed good growth at NaCl concentrations from 0 to 80 g/l; the optimum NaCl concentration was 40 g/l (Fig. 4). Thus, strains ROS 10 and ROS 35 can be classified as moderate halophiles and natronophiles.

Genetic characteristics. The DNA G+C content of strains ROS 10 and ROS 35 was 59.4 and 59.0 mol %, respectively. The DNA–DNA hybridization level of the strains was 95% (Table 4), suggesting their affiliation with the same species.

In order to elucidate the taxonomic position of strains ROS 10 and ROS 35, we determined almost complete nucleotide sequences of their 16S rRNA genes (about 1540 nucleotides corresponding to positions 3–1535 in *E. coli* numbering). The high level of similarity between these sequences (99.2%) was in agreement with the results of DNA–DNA hybridization. According to the results of the phylogenetic analysis that we performed, both strains proved to be members of the family *Rhodobacteriaceae* within the class *Alphaproteobacteria.* In the phylogenetic tree, the 16S rRNA genes of both strains fell into the cluster formed by members of the genera *Roseinatronobacter* and *Rhodobaca* (Fig. 5). This cluster also included the species *"Natronohydrobacter thiooxidans,"* an unidentified strain of *Alphaproteobacteria*, and uncultured organisms from Mono Lake. The similarity of the 16S rRNA genes of strains ROS 10 and ROS 35 to other genes belonging to this cluster was rather high (96.2–98.5%). The species closest to the strains under study was *Rna. thiooxidans* (98.1% similarity). In their phenotypic properties, strains ROS 10 and ROS 35 were also most close to this alkaliphilic species. At the same time, strains ROS 10 and ROS 35 differed from *Rna. thiooxidans* in a 1.6–2.0 mol % lower G+C content of DNA. The DNA–DNA hybridization level between the new strains and *Rna. thiooxidans* was 22–25%, which did not allow them to be assigned to this species. A similar evolutionary distance separated the new strains and the alkaliphilic purple bacterium *Rbc. bogoriensis* (Table 4). The alkalitolerant strain of purple bacteria *Rhodobacter* sp. B6 showed a very low hybridization level (3%) with our new isolates.

Taxonomic status. Thus, strains ROS 10 and ROS 35 considerably differ from related species in the nucleotide sequence of the 16S rRNA genes and DNA G+C content; they also show a low level of total DNA

Table 3. Respiratory activity of the alkaliphilic strains of ABC bacteria

Substrate	ROS 10		ROS 35		*Rna. thiooxidans* ALG 1	
	V	product	*V*	product	*V*	product
No substrate	12		12		ND	ND
Peptone + acetate	45		96		220	ND
Thiosulfate	66	Sulfate	24	Sulfate (95%)	350	Sulfate
Sulfide	108	Sulfate	$V_1 = 320$ $V_2 = 30$	Sulfur Sulfate	240	Sulfate
Polysulfide	120	Sulfate	$V_1 = 150$ $V_2 = 64$	Sulfate + sulfur Sulfate + sulfur	ND	ND
Sulfite	–	–	–	–	140	Sulfate
Elemental sulfur (in acetone)	63	Sulfite	28	Sulfite	145	Sulfate

Note: *V* is the respiration rate in nmol O_2/(min mg protein); V_1 is the respiration rate during the initial oxidation of HS^- to S^0; and V_2 is the respiration rate during further oxidation of S^0 to sulfate. "ND" stands for "not determined".

Table 4. Results of DNA–DNA hybridization of the strains under study and reference strains

Strains	G+C, mol %	*Rna. thiooxidans* ALG 1	*Rbc. bogoriensis* LBB1	ROS 10	ROS 35	*Rhodobacter* sp. B6
Rna. thiooxidans ALG 1	61.5	100				
Rbc. bogoriensis LBB1	60.2	18	100			
ROS 10	59.4	22	27	100		
ROS 35	59.0	25	27	95	100	
Rhodobacter sp. B6	67.1	7	8	3	0	100

homology with these species. Phylogenetically, the new strains occupy an intermediate position between the purple bacterium *Rbc. bogoriensis* and the alkaliphilic ABC bacterium *Roseinatronobacter thiooxidans.* Taking into account that the new isolates, unlike *Rbc. bogoriensis*, are incapable of anaerobic phototrophic growth, they should be assigned to the genus *Roseinatronobacter.* Certain phenotypic distinctions between strains ROS 10 and ROS 35 should be viewed as strain-specific. Thus, based on the complex of phenotypic and genotypic properties, strains ROS 10 and ROS 35 should be assigned to the same new species of the genus *Roseinatronobacter*, for which we propose the name *Roseinatronobacter monicus* sp. nov.

Description of *Roseinatronobacter monicus* sp. nov.

mo'ni.cus. – M.L.n. *monicus*, living in Mono Lake.

Cells are oval-shaped, nonmotile, and measure 0.5–0.7×1.2–1.7 μm. Cell division occurs by binary fission by means of a constriction. The cell wall has the gram-negative-type structure. No intracellular membranes are present. Cells contain bacteriochlorophyll *a* and carotenoids of the spheroidene series. The spectra of acetone–methanol (7 : 2, vol/vol) extracts of cells exhibit absorption maxima at 360, 383, 482, 506, 708, and 769 nm. The metabolism is obligately aerobic and heterotrophic. Light suppresses synthesis of bacteriochlorophyll *a*. Photoinhibition of respiration occurs at wavelengths corresponding the absorption maxima of the pigments in whole cells. Reduction of nitrates to nitrites occurs but does not provide for anaerobic growth. Organic acids, sugar alcohols, acetate, casein hydrolysate, glycerol, yeast extract, lactate, malate, propionate, pyruvate, rhamnose, maltose, and sucrose are utilized as

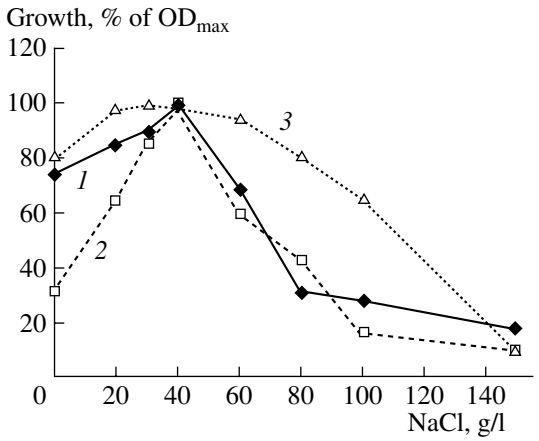

Fig. 4. Effect of NaCl concentration on the growth of (*1*) strain ROS 10, (*2*) strain ROS 35, and (*3*) *Rna. thiooxidans.* Maximum value of optical density (OD) was taken as 100%.

carbon and energy sources. Arginine, aspartate, butyrate, benzoate, glycolate, glucose, caproate, malonate, methanol, succinate, tartrate, ethanol, and propanol are not utilized. Thiosulfate, sulfide, polysulfide, and elemental sulfur can be oxidized. Oxidation of thiosulfate occurs with the production of sulfate. The species is obligately alkaliphilic (growth occurs in a pH range of 8.5–10.0 with an optimum at pH 9.1–9.5), moderately halophilic, and natronophilic (the NaCl concentration range suitable for growth is 0–80 g/l, and the optimal concentration is 40 g/l). Poly-β-hydroxybutyric acid and polyphosphate are the storage compounds. The DNA G+C content is 59.0–59.4 mol %. The species belongs to Phylum BXII *Proteobacteria*, Class I *Alphaproteobacteria.*

The type strain is ROS 35 = UNIQEM U-251^T = VKM B-2404^T, DSM 18423. The nucleotide sequence of the

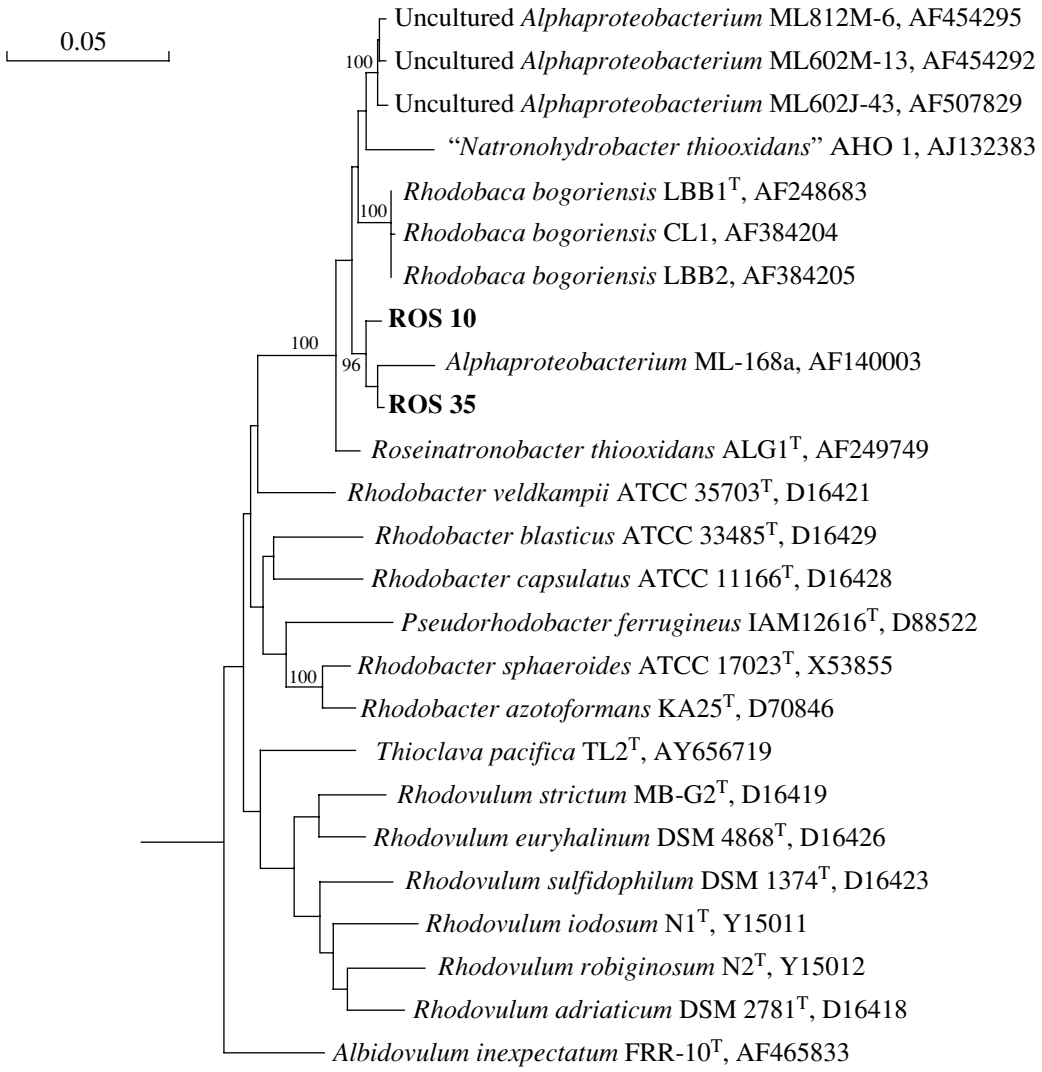

Fig. 5. 16 rRNA-based phylogenetic tree showing the position of the alkaliphilic strains ROS 10 and ROS 35 within the family *Rhodobacteriaceae* of the alphaproteobacteria. Numerals at the branching points indicate the bootstrap values (values lower than 95 are not shown). Scale bar corresponds to 5 nucleotide substitutions per 100 nucleotides (evolutionary distances).

Table 5. Comparison of the characteristics of strains ROS 10 and ROS 35 and *Roseinatronobacter thiooxidans*

Characteristic	ROS 10	ROS 35	*Rna. thiooxidans* ALG 1
Habitat	Soda Mono Lake (California), aerobic zone; salinity, 70 g/l	Soda Mono Lake (California), anaerobic zone; salinity, 100 g/l	Soda Lake Gorbunka (Chita oblast); salinity, 18 g/l
Cell shape and size, μm	Short rods, 0.5–0.7 × 1.2–1.7	Short rods, 0.5–0.7 × 1.2–1.7	Lemon-shaped, 0.5–0.8 × 0.8–2.2
Motility	–	–	–
Carotenoids (*in vivo* peaks, nm)	480, 525, 550	480, 525, 550	(410), 483, (511)
Carotenoids	In acetone–methanol (7 : 2) (459), 481, (518)	In acetone–methanol (7 : 2) (460), 484, (523)	In hexane (450), 481, (505)
Bacteriochlorophyll *a*, main in vivo peaks, nm	(805), 870	(804), 870	803, 870
Bacteriochlorophyll *a* in acetone–methanol (7 : 2) extract	360, 708, 770	360, 383, 708, 769	765, 772
Autotrophic growth on thiosulfate	–	–	–
Heterotrophic growth	+	+	+
Utilization of thiosulfate during heterotrophic growth	+	+	+
DNA G+C content, mol %	59.4	59	61
pH optimum and range	(9.0–9.5), 8–10	(8.5–9.1), 8–10	(10), 8.5–10.4
NaCl optimum and range, g/l	(40), 0–80	(40), 0–80	(30), 10–100

16S rRNA gene of the type strain has been deposited in GenBank under the accession number DQ659236.

The habitat is hypersaline soda Mono Lake (90 g/l NaCl, pH 9.6; California, United States).

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