ISSN 0026-2617, Microbiology, 2009, Vol. 78, No. 1, pp. 92–101. © Pleiades Publishing, Ltd., 2009.
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EXPERIMENTAL ARTICLES

Roseococcus suduntuyensis **sp. nov., a New Aerobic Bacteriochlorophyll** *a***-Containing Bacterium Isolated from a Low-Mineralized Soda Lake of Eastern Siberia**

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Received August 20, 2007

Abstract—A novel strain, SHET, of aerobic bacteriochlorophyll *a*-containing bacteria was isolated from the surface layer of bottom sediments from the soda lake Shuluutai-Ekhe-Torom (Chita oblast, Eastern Siberia, Russia). The lake water has a total mineralization of 30 g/l and a pH of 9.2. The cells of strain SHET are cocci or short rods, which reproduce by uniform division. The cells are motile by means of flagella. The cell wall structure is of the gram-negative type. Sparse intracytoplasmic membrane vesicles are located close to the cell wall. The new isolate is an obligate aerobe and facultative alkaliphile which grows in a pH range of 7.5–9.5 (with an optimum at pH 8.5–9.0). The best growth of strain SHET occurred at 2.0 g/l NaCl and 23-28°C. Photosynthetic pigments are represented by bacteriochlorophyll *a*, with the maximum absorption at 865 nm in the in vivo spectrum, and carotenoids (spirilloxanthin derivatives). Analysis of the 16S rRNA gene sequences demonstrated that strain SHET is closely related to *Roseococcus thiosulfatophilus* of the α-1 subclass of *Proteobacteria* (98.6 % similarity). The DNA G+C base content is 69.1 mol %. Unlike *Rsc. thiosulfatophilus*, strain SHET grows well on sugars and glycerol and is not capable of utilizing thiosulfate as an energy source. The new isolate is a facultative alkaliphile and reduces nitrates to nitrites. On the basis of its phenotypic and genetic characteristics, strain SHET was described as a new species of the genus *Roseococcus, Rsc. suduntuyensis* sp. nov.

Key words: Proteobacteria, anoxygenic phototrophs, aerobic bacteriochlorophyll *a*-containing bacteria, alkaliphily, soda lakes.

DOI: 10.1134/S0026261709010123

Aerobic bacteriochlorophyll *a*-containing (ABC) bacteria differ from purple bacteria in their inability to grow under anaerobic conditions at the expense of photosynthetic activity. However, they are capable of anoxygenic photosynthesis in the presence of oxygen, although respiration is the main type of their energy metabolism. All the known ABC bacteria belong to *Proteobacteria. Roseococcus thiosulfatophilus* is the first described representative of aerobic bacteriochlorophyll *a*-containing bacteria of the α-1 subclass of *Proteobacteria* [1]. In addition to this microorganism, five ABC species of the α-1 subclass of *Proteobacteria* are known, including freshwater neutrophilic and the mesophilic species *Craurococcus* and *Paracraurococcus*, the moderately thermophilic *Rubritepida flocculans* (growth optimum at 50° C), and freshwater acidophilic species of the genera *Acidisphera* and *Acidiphi-* *lum* [2]. This subclass also includes the moderately acidophilic, purple, nonsulfur bacterium *Rhodopila globiformis*.

There is only one validly described species of the genus *Roseococcus.* It was isolated from a cyanobacterial mat of an alkaline sulfide-containing thermal spring located in the Bolshaya River Valley (Eastern Siberia) [1, 3]. This species is characterized by a number of distinctive features. The cells of *Rsc. thiosulfatophilus* are coccoid; they contain bacteriochlorophyll *a* (Bchl *a*) with an absorption maximum at 855–858 nm in the core complex and highly polar carotenoids of an unusual composition, such as carotene-dioate (4,4' diapocarotene-4,4'-dioate) and a glycoside ester (di-β-D-glycopyranosil]- 4,4'-diapocarotene-4,4'-dioate) [3, 4]. Optimal growth was observed within a pH range of 7.0–8.0 and at $25-30$ °C. The microorganism does not require the presence of NaCl in the growth medium. The G+C base content of the DNA of the type strain

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RB-3 is 70.4 mol %. *Rsc. thiosulfatophylus* utilizes thiosulfate as an additional energy source and oxidizes it to sulfate. Importantly, the aerobic bacteriochlorophyll *a*containing bacterium *Rubritepida flocculans* [5] and some species of the genus *Roseinatronobacter* of the α-3 subclass of *Proteobacteria* [6, 7] utilize thiosulfate to sulfate as well. Recent investigations have demonstrated that ABC bacteria are widespread in marine and freshwater environments; from soda lakes, however, only two species of the genus *Roseinatronobacter* have been isolated [6, 7].

The aim of the present work was to determine the morphological and physiological characteristics, as well as the phylogenetic position, of *Rsc. suduntuyensis* sp. nov., a new species of aerobic bacteriochlorophyll *a*-containing bacteria belonging to the genus *Roseococcus.* The bacterium was isolated from a low-mineralized soda lake from the forest-steppe zone of the Chita oblast (Eastern Siberia).

MATERIALS AND METHODS

Field studies. The following water parameters were measured at the sampling site: pH (with a HANNA portable pH meter), temperature (with a VWR digital thermometer), and total mineralization (with a C-1 COMECTA refractometer). The samples were collected from the surface of the near-shore sediments (with algal films and cyanobacterial mats) into sterile, plastic, test tubes.

Cultivation methods and isolation of pure cultures. Aerobic bacteriochlorophyll *a*-containing bacteria were cultivated in a medium containing the following (g/l): NH₄Cl, 0.4; KH₂PO₄, 0.5; MgCl₂, 0.2; $Na₂SO₄, 0.5$; yeast extract, 1; sodium acetate, 1; sodium pyruvate, 1; NaCl, 1; Na₂S₂O₃ · 5H₂O, 1; KCl, 0.5; vitamin B_{12} , 10 μ g/l; and Pfennig trace element solution [8], 1 ml/l.

The medium pH was adjusted either to 7.5 (with 1 g/l NaHCO₃), or to 9.0 (with 4 g/l NaHCO₃ and 1 g/l Na_2CO_3), by changing the ratio of sodium carbonate and bicarbonate. The molar concentration of carbonates was maintained at the same level. Water solutions of NaHCO₃ (10%), Na₂CO₃ (10%), yeast extract (5%), sodium acetate (10%), sodium pyruvate (10%), and sodium thiosulfate (10%) were prepared and sterilized separately, then added to the medium immediately before inoculation.

The cells were cultivated under aerobic conditions in the dark in 500-ml conical flasks with 200 ml of the nutrient medium. Purification of the cultures obtained was carried out under aerobic conditions on petri dishes by repeated streaking of well isolated pigmented colonies formed on the medium solidified with 2% (wt/vol) agar. The culture purity was confirmed by microscopic

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examination and by the absence of colonies of other microorganisms.

Physiological characteristics and growth conditions. Experiments with various NaCl concentration and pH values were carried out using liquid media. To determine the optimal NaCl content, NaCl concentrations from 0 to 30 g/l were used. The background soda concentration in the media was 0.05 M (0.035 M and 0.015 M of hydrocarbonates and carbonates, respectively). Within the alkaline range, the optimum pH of the medium was adjusted by adding sodium carbonate or bicarbonate. To elucidate the reaction of the studied bacteria to the pH values from 6.8 to 7.4, we used aphosphate buffer [9].

The biomass yield was assessed by the optical density of the cell suspension at the end of exponential growth (measured with a KFK-3 photometer at 650 nm).

To determine the spectrum of utilized substrates under aerobic conditions, a mineral medium was used that did not contain any organic compounds; yeast extract (0.1 g/l) was added as a vitamin source. The tested compounds were added to the concentration of 1g/l. In addition, we tested the ability of the strains to oxidize thiosulfate and other reduced sulfur compounds. The cultures were grown on the medium (pH 8.5) with peptone (1 g/l) and thiosulfate (1 g/l) . Sulfate was determined nephelometrically; the content of thiosulfate was determined by iodometric titration [10].

The reaction of the studied bacteria to oxygen was assessed by the distance between the growth zones and the surface of agar (0.7%) columns.

Sensitivity to antibiotics was determined by applying filter discs containing the antibiotic in question; after incubation, the zone of growth inhibition was registered.

The capacity of the isolated microorganism for nitrate reduction accompanied by NO_2^- production was determined with the Griess assay [11].

Catalase activity was assayed by monitoring the formation of gas bubbles on addition of a 3% hydrogen peroxide solution to the suspension.

Morphology and ultrastructure. The morphology of bacterial cells was studied under an Olympus light microscope (Japan) with a phase-contrast device and also under a Jeol JEM 100C electron microscope (Japan) at an accelerating voltage of 80 kV. Whole cell preparations were stained with a 0.2% aqueous solution of uranyl acetate. To obtain ultrathin sections, the cells were grown in liquid medium. The cells harvested by centrifugation were treated according to Kellenberger, dehydrated, and embedded in Epon. Ultrathin sections were prepared using an LKB ultramicrotome (Swe-

Fig. 1. Morphology and ultrastructure of strain SHET: a, light micrographs (bar, 10 µm); b, whole-cell specimen of a dividing cell (bar, 1 µm); c, ultrathin sections, electron micrograph (bar, 1 µm); outer membrane (*1*), vesicles (*2*), poly-β-hydroxybutyric acid (*3*); polyphosphates (*4*); slime (*5*); septum (*6*).

den), placed on copper grids covered with formvar support, and stained with the Reynolds reagent [12].

Pigment composition. The pigment composition was determined using sonicated cell suspensions, as well as acetone–methanol (7 : 2) extracts. Absorption spectra were determined with an SF 56A spectrophotometer (LOMO, Russia) or a Cary 50 spectrophotometer equipped with an SPD M-20A diode array detector (Shimadzu, Japan). The carotenoid composition was determined by high-performance liquid chromatography (HPLC) as described earlier [13, 14].

Analysis of fatty acids. 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) [15].

Molecular genetic analysis. The DNA of the new strain was isolated according to the Marmur procedure [16]. The content of G+C base pairs in the DNA was determined by the method of Owen et al. [17]. Amplification and sequencing of the 16S rRNA gene of the isolate was performed using universal primers [18]. The obtained nucleotide sequences were aligned with

the corresponding sequences of the most closely related bacteria using the CLUSTALX software package. Unrooted phylogenetic trees of the studied bacteria were constructed by the methods implemented in the TREECON software package [19].

The obtained 16S rRNA gene sequence of strain SHET was deposited in the GenBank under the accession number EU012448.

RESULTS

Characteristics of the habitat. Strain SHET was isolated from the surface layer of the near-shore, silt sediments of the soda lake Shuluutai-Ekhe-Torom (near the Suduntui settlement, forest-steppe zone of the Chita oblast). At the time of sampling, the total mineralization of the lake water was 30 g/l; pH of the water was 9.24. On the surface of the dark silt sediments, a biofilm consisting of diatoms and cyanobacteria was observed, as well as fragments of microbial films consisting of purple bacteria.

Cultural properties. On the surface of agarized media, strain SHET grown in the dark forms milkypink rounded convex colonies with even edges. The cultures grown in liquid medium are flaky; bacteria

Fig. 2. Absorption spectrum of strain SHET pigments in vivo (*1*) and of the acetone–methanol extract (*2*).

form milky- pink suspensions which later turn deep pink.

Cell morphology and ultrastructure. The cells of the new strain are coccoids or short rods, $0.8-1.1 \times 1.2 1.7 \mu m$ (Fig. 1a), which reproduce by binary division. In young cultures, motile cells are observed; their motility patterns are typical of the cells with polar flagella. The cell wall structure revealed on ultrathin sections is of the gram-negative type (Fig. 1c). Intracyto-

plasmic membrane vesicles are located along the cell periphery. The cells are surrounded by a mucous capsule (Fig. 1c). Storage compounds are represented by electron-dense, rounded inclusions, presumably polyphosphates, and electron-transparent ovoid inclusions typical of poly-β-hydroxybutyric acid (Fig. 1c).

Pigments. Photosynthetic pigments are represented by carotenoids and bacteriochlorophyll *a*. The presence of Bchl *a* was supported by the maximum absorption at 865 nm in the in vivo spectrum (Fig 2) and at 770 nm in the spectrum of the acetone–methanol extract. The presence of carotenoids was supported by absorption peaks at 410, 480, 509, and 540 nm in the in vivo spectrum (Fig. 2) and at 466 , 493 , and 522 nm in the spectrum of the acetone–methanol extract.(Fig. 2). Analysis of the acetone–methanol extract by high-performance liquid chromatography has shown the presence of four major carotenoids (Fig. 3). According to the absorption spectrum, all carotenoids showed a similarity to each other and to spirilloxanthin (Fig. 4). The fact that the pigments had different retention times indicates that carotenoids of the studied bacteria differed insignificantly from spirilloxanthin. According to S. Takaichi (personal communication), in the carotenoids of the studied strain, the spirilloxanthin methoxy groups are replaced by glycosides. Similar replacements were observed in the carotenoids of the type strain of *Rsc. thiosulfatophilus.*

Fig. 3. Results of the HPLC analysis of the pigments of *Rsc. suduntuyensis* (a) and *Allochromatium* (*Alc.*) *minutissimum* (b): *1*−*4*, spirilloxanthin glycoside and its derivatives, *5*, bacteriochlorophyll; *6*, dehydrorhodopin; *7*, rhodopin; *8*, spirilloxanthin; *9*, anhydrorhodovibrin; *10*, lycopene.

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Fig. 4. Absorption spectra of spirilloxanthin glycoside and spirilloxanthin of *Rsc. suduntuyensis* (*1*) and *Alc. minutissimum* (*2*). The spectra were recorded in the ethyl acetate– acetonitrile–water mixture (80 : 18 : 2).

Fig. 5. Effect of pH on the growth of strain SHET (maximum growth was taken as 100%) during 24-h (solid line) and 50-h (broken line) incubation.

Fig. 6. Effect of NaCl on the growth of strain SHET (maximum growth was taken as 100%).

Physiological properties. Bacteria of strain SHET are obligate aerobes and organoheterotrophs; respiration is their main type of energy metabolism. Catalase activity was detected in the cells. The strain was shown to reduce nitrate to nitrite under aerobic conditions.

A wide range of organic compounds was tested as carbon sources (Table 1). The best growth of SHET was noted in the presence of glucose, fructose, sucrose, maltose, glycerol, and yeast extract. Weaker growth occurred on ribose, arabinose, acetate, pyruvate, butyrate, malate, citrate, succinate, lactate, formate, fumarate, propionate, tartrate, mannitol, and casein hydrolysate. No growth occurred on glycolate, ethanol, and methanol as a sole carbon source.

The new isolate was found to be incapable of utilizing thiosulfate both under autotrophic and heterotrophic conditions.

During the first 24 h of incubation, best growth was observed at pH 9.5, in the pH range 8.0–9.8 (Fig. 5). On the second day of incubation, high biomass yield was detected within a wider pH range (6.8–9.5). Hence, the new isolate can be considered a facultative alkaliphile. The isolate grew at a NaCl concentration of 0–20 g/l, with an optimum at $2-5$ g/l (Fig. 6) The new strain is mesophilic and has a growth optimum at $25-30^{\circ}$ C (Fig. 7). No growth was detected at temperatures lower than 10° C and higher than 50° C.

Strain SHET was sensitive to the following antibiotics: ampicillin, benzylpenicillin, vancomycin, lincomycin, nalidixic acid, polymyxin, rifampicin, tetracycline, and erythromycin; the new isolate was resistant to amikacin, gentamicin, kanamycin, neomycin, novobiocin, and streptomycin (Table 2).

Table 3 shows the fatty acid composition of the cells grown under standard conditions. As the other aerobic bacteriochlorophyll *a*-containing bacteria, the main fatty acid in the cells of strain SHET is 11-octadecenoic acid (66.73%).

Genetic characteristics. The content of the G+C base pairs in the DNA of strain SHET is 69.1 mol %.

To elucidate the phylogenetic position of the new isolate, a nucleotide sequence of 16S rRNA genes (about 1400 nucleotides between *E. coli* positions 37−1483) was determined. According to phylogenetic analysis of the 16S rRNA gene sequences, strain SHET belongs to the family *Rhodospirillaceae* (α-1 subclass of *Alphaproteobacteria*). The phylogenetic tree (Fig. 8) shows that strain SHET occupies a position which is closest to the unidentified strain SL4.26 isolated from an ephemeral lake in the Mohave Desert (99.6% similarity), as well as to the type strain $RB-3^T$ of the species *Rsc. thiosulfatophylus* (98.6% similarity) and two strains isolated from a thermal spring in Colorado (99.1% similarity). On the basis of similarity between their 16S rRNA gene sequences, the latter two isolates were affil-

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iated with the species *Rsc. thiosulfatophylus* however, their properties have not been described. In the constructed phylogenetic tree, all strains closely related to *Rsc. thiosulfatophylus* RB-3, including strain SHET, form a single cluster with high bootstrap support (100%). The phylogenetic similarity between other representatives of anoxygenic, phototrophic bacteria of the α-1 subclass of *Alphaproteobacteria* was significantly lower ((90.1–94.9 %); the type strain of the moderately thermophilic aerobic bacteriochlorophyll *a*-containing bacterium *Rubritepida flocculans* was found to be closest to the studied strain (94.9% similarity).

DISCUSSION

The characteristic traits of the studied strain SHET allow us to assign it to the physiological group of aerobic bacteriochlorophyll *a*-containing bacteria. The new isolate contains Bchl a ; it is a heterotroph, an obligate aerobe incapable of phototrophic growth under anaerobic conditions. Analysis of the 16S rRNA gene sequences demonstrated that the studied isolate is closely related to the type strain *Rsc. thiosulfatophylus* RB-3 (98.6% similarity). The extent of phylogenetic divergence (1.4%) revealed by 16S rRNA gene analysis corresponds to the interspecific level of relatedness [20]. A significant difference $(5%)$ in the DNA G+C base content was observed. Some differences were observed in the spectrum of antibiotic sensitivity, as well as in the minor components of fatty acids. Strain SHET differs considerably from the closely related species *Rsc. thiosulfatophylus* in a number of phenotypic properties. The new strain was isolated from a low-mineralized soda lake (*Rsc. thiosulfatophylus* RB-3 was isolated from an alkaline thermal spring). Its DNA G+C base content is lower (69.1 mol $\%$). The strain is an alkaliphile; it grows in a salinity range of 2–5 g/l NaCl, differs in the range of utilized organic substrates, and does not utilize thiosulfate. Unlike *Rsc. thiosulfatophylus* RB 3, strain SHET is capable of reducing nitrate to nitrite. Thus, the phenotypic and phylogenetic characteristics of strain SHET allow us to describe it as a new species belonging to the genus *Roseococcus*, *Roseococcus suduntuyensis* sp. nov.

Taxonomic description of *Roseococcus suduntuyensis* **sp. nov.** *su.dun.tuy.en.sis* N.L. fem. adj., discovered near the Suduntui settlement (Chita oblast).

The cells are motile cocci or short rods $(0.8-1.1 \times$ 1.2–1.7 µm). The cells reproduce by binary division. Intracytoplasmic membrane vesicles are attached to the cell wall. Storage compounds are represented by electron-dense rounded inclusions (polyphosphates) and electron-transparent poly-β-hydroxybutyrate inclusions. Bacterial cells form a pink suspension. Photosynthetic pigments are represented by bacteriochlorophyll *a*, with the maximum absorption at 865 nm in the

Table 1. Utilization of organic compounds as a carbon source by strain SHET and *Roseococcus thiosulfatophilus* RB-3T

Substrate		Strain SHET Rsc. thiosulfatophilus*
Glucose	$^{++}$	$+-$
Fructose	$^{++}$	
Sucrose	$^{++}$	
Ribose	$^{+}$	
Maltose	$^{++}$	$+ -$
Arabinose	$+-$	
Acetate	$^{+}$	$^{+}$
Pyruvate	$\qquad \qquad +$	$^{+}$
Glutamate	ND	$^+$
Butyrate	$\begin{array}{c} + \end{array}$	
Malate	$^{+}$	$^{+}$
Citrate	$+ -$	$^{+}$
Succinate	$^{+}$	$\begin{array}{c} + \end{array}$
Lactate	$\,^+$	$\begin{array}{c} + \end{array}$
Formate	$+ -$	
Fumarate		
Propionate	$^+$	
Benzoate		
Tartrate	$+-$	
Ethanol		
Methanol		
Glycerol	++	
Mannitol		
Casein hydrolysate	$^{+}$	$^{++}$
Yeast extract	$^{++}$	$^{++}$
Glycolate		

Note: "-" indicates no growth detected; "+-", weak growth; "+", good growth; " $++$ ", very good growth; ND, no data; $*$, [1].

Fig. 7. Effect of temperature on the growth of strain SHET (maximum growth was taken as 100%).

in vivo spectrum, and carotenoids (spirilloxanthin glycoside and its derivatives) with absorption peaks at 410, 480, 509, and 540 nm in the in vivo spectrum. Bacteriochlorophyll *a* is synthesized under aerobic conditions

Table 2. Sensitivity of the new strain SHET and *Roseococcus thiosulfatophilus* RB-3T to antibiotics

in the dark. The organism is an aerobic chemoorganoheterotroph and a facultative photoheterotroph. The organism is a facultative alkaliphile; best growth was observed within the pH range of 8.5–9.5; the optimal NaCl concentration was from 2 to 5 g/l; growth occurs in a salinity range of 0–20 g/l NaCl. The organism is mesophilic; optimal growth was observed within the 20–35°ë temperature range. Glucose, fructose, sucrose, maltose, glycerol, ribose, arabinose, acetate, pyruvate, butyrate, malate, citrate, succinate, lactate, formate, fumarate, propionate, tartrate, mannitol, casein hydrolysate, and yeast extract are utilized as carbon sources. No growth occurs with glycolate, ethanol, or methanol as sole carbon sources. The strain does not utilize thiosulfate in the presence of organic substrates either in the dark or in the presence of light. The strain is capable of reducing nitrate to nitrite. The strain is resistant to amikacin, gentamicin, kanamycin, neomycin, novobiocin, and streptomycin; it is sensitive to ampicillin, benzylpenicillin, vancomycin, lincomycin, nalidixic acid, polymyxin, rifampicin, tetracycline, and

Table 3. Fatty acid composition of the new strain SHET

μ <i>mosuruophinis</i> KD-9 to antionally					Rsc. thiosulf-	
Antibiotic	Strain SHET	Rsc. thiosulfatophilus*	Acid	Symbol	Strain SH- ET, $%$	atophilus $RB-3^i, \%$
Amikacin	$+$	$+$	Dodecanoic	12:0	0.28	
Ampicillin	-		iso-Tetradecanoic	i14	0.40	
Benzylpenicillin	$\overline{}$	ND	Tetradecanoic	14:0	0.78	
Vancomycin	-	$+$	3-Hydroxytetrade- canoic	14:0 3OH		3.0
Gentamicin	$+$	$\overline{}$	Isopentadecanoic	i15	0.43	
Kanamycin	$+$	$+$	Anteisopentade- canoic	a15	2.60	
Lincomycin	$\overline{}$		7-Hexadecenoic	$16:1\omega$ 7	4.29	0.9
	Nalidixic acid $\overline{}$		5-Hexadecenoic	16:105	1.71	0.5
			Hexadecanoic	16:0	7.92	7.6
Neomycin	$^{+}$	ND	Anteisoheptade- canoic	a17	0.45	
Novobiocin	$^{+}$	ND	11-Octadecenoic	$18:1\omega$ 7	66.73	65.1
Polymyxin	-	$+$	13-Octadecenoic	$18:1\omega$ 5	1.89	1.3
Rifampicin	$\overline{}$	ND	Octadecanoic	18:0	2.57	0.7
Streptomycin	$+$	$+$	3-Hydroxyoctade- canoic	18:0 3OH		2.4
Tetracycline	$\overline{}$	$+$	11-Methyl-octade- cenoic	11Me18:1	3.23	
Erythromycin	$\overline{}$	$+$	2-Hydroxyoctade-	18:1 2OH	6.73	15.5
$\mathbf{A} \mathbf{y}$ and \mathbf{z} and \mathbf{y} and \mathbf{y} and \mathbf{y} and \mathbf{y}	the common	$1.66 - 22.61$	cenoic			

Note: "+", the strain is sensitive to the compound; "-", the strain is resistant to the compound; ND, no data; *, [1]

Note: "-", not detected; $*$, [1].

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Fig. 8. 16S rRNA-based phylogenetic tree showing the position of strain SHET 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.

erythromycin. The principal fatty acid is 11-octadecenoic acid $(66.73 \%).$

The DNA G+C base content is 69.1 mol %.

The 16S rRNA gene sequence of the strain was deposited with GenBank under accession number EU012448.

The type strain SHET was registered in VKM (B-2453T) and DSMZ (19979).

The habitat is the soda lake Shuluutai-Ekhe-Torom (mineralization 3.0 g/l, pH 9.24) located in the foreststeppe zone of the Chita oblast (Eastern Siberia).

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ACKNOWLEDGMENTS

We are very grateful to G.A. Osipov for the analysis of fatty acid composition, N.A. Kostrikina for electron microscopic research, and S. Takaichi for help in analyzing the results of HPLC carotenoid analysis. We thank our colleagues from the Laboratory of Microbiology, Institute of General and Experimental Biology, Siberian Branch, Russian Academy of Sciences (Ulan-Ude) for their help and support of the expedition.

This work was supported by the Russian Foundation for Basic Research, project nos. 07-04-00651, 05-04-

Characteristics	Strain SHET	Roseococcus thiosulfatophilus*
Habitat	Low-mineralized steppe soda lake (pH 9.2), Eastern Siberia	Cyanobacterial mat from an alkaline sulfide-containing thermal spring, Eastern Siberia
Cell shape and size, μ m	Cocci, short rods, $0.8-1.1 \times 1.2-1.7$	Cocci, $0.9-1.3 \times 1.3-1.6$
Division	Binary	Binary
Motility	$\mathrm{+}$	$^{+}$
Reaction to oxygen	Obligate aerobe	Obligate aerobe
Carotenoids in vivo	410, 480, 509, 540	482, 510, 538
Carotenoids in the acetone–methanol 466, 493, 522 extract		(466) , 494, $(518-522)$
Bchl a in vivo	865	855-858
NaCl (optimum) range, %	$(0.2 - 0.5)$ $0 - 1.5$	(0)
pH (optimum) range	$(8.5 - 9.5)$ $7.5 - 9.5$	$(7-8)$
Temperature (optimum) range, °C	(25) $20 - 35$	$(25 - 30)$
Utilization of thiosulfate		$\ddot{}$
Reduction of $NO3$ to $NO2$	$^{+}$	ND
DNA G+C content, mol $%$	69.1	70.4

Table 4. Comparative characterization of the studied strain SHET and *Roseococcus thiosulfatophilus* RB-3^T

Note: "+", the trait was observed; "–", the trait was not observed; ND, no data.

48058, 06-04-48668, 06-0449304, 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."

REFERENCES

- 1. Yurkov, V.V. and Gorlenko, V.M., *Roseococcus* gen. nov., a New Genus of Freshwater Aerobic, Bacteriochlorophyll *a*-Containing Bacteria, *Mikrobiologiya*, 1991, vol. 60, no. 5, pp. 902–907.
- 2. Yurkov, V.V. and Csotonyi, J.T., New Light on Aerobic Anoxygenic Phototrophs, *Adv. Photosynthesis and Respiration*, Govindjee, Ed., Berlin: Springer (in press).
- 3. Yurkov, V., Stackebrand, E., Holmes, A., Fuerst, J., and Hugenholtz, P., Golecki, J., God'on, N., Gorlenko, V., and Kompantseva, E. Phylogenetic Position of Novel Aerobic, Bacteriochlorophill *a*-Containing Bacteria and Description of *Roseococcus thiosulfatophilus* gen. nov., sp. nov., *Erythromicrobium ramosum* gen. nov., sp. nov., and *Erythrobacter litorales* gen. nov., sp. nov, *Int. J. Syst. Bacteriol.*, 1994, vol. 44, no. 3, pp. 427–443.
- 4. Takaichi, S, Carotenoids and Carotenogenesis in Anoxygenic Phototrophic Bacteria, in *The Photochemistry of Carotenoids*, Frank, H.A., Young, A.J., Britton, G., and Cogdell, R.J., Eds., Dordrecht: Kluwer Acadmic Publishers, 1999, vol. 8, pp. 39–69.
- 5. Alarico, S., Rainey, F.A., Empadinhas, N., Schumann, P., Nobre, M.F., and Da Costa, M.S., *Rubritepida flocculans* gen. nov., sp. nov., a New Slightly Thermophilic Member of the α1 Subclass of the *Proteobacteria, Syst. Appl. Microbiol.*, 2002, vol. 25, pp. 198–206.
- 6. Sorokin, D.Yu., Tourova, T.P., Kuznetsov, B.B., Bryantseva, I.A., and Gorlenko, V.M., *Roseinatronobacter thiooxidans* gen. nov., sp. nov., a New Alkaliphilic Aerobic Bacteriochlorophyll a-Containing Bacterium Isolated from a Soda Lake, *Mikrobiologiya*, 2000, vol. 69, no. 1, pp. 89–97 [*Microbiology* (Engl. Transl.), vol. 69, no. 1, pp. 75–82].
- 7. Boldareva, E.N., Bryantseva, I.A., Tsapin, A., Nel'son, K., Sorokin, D.Yu., Tourova, 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].
- 8. Pfennig, N., *Rhodocyclus purpureus* gen. nov. and sp. nov., a Ring-Shaped Vitamin B_{12} Requiring Member of the Family *Rhodospirillaceae, Int. J. Syst. Bacteriol.*, 1978, vol. 28, pp. 283–288.
- 9. Dawson, R., Elliott, D., Elliott, W., and Jones, K., *Data for Biochemical Research*, Oxford: Clarendon, 1986 [Russ. Transl. Moscow: Mir, 1991].
- 10. Reznikov, A.A., Mulikovskaya, E.P., and Sokolov, I.Yu., *Metody analiza prirodnykh vod.* (Methods of Analysis of Natural Waters), Moscow: Nedra, 1970.
- 11. Carret, R.H. and Nason, A., Further Purification and Properties of *Neurospora* Nitrate Reductase, *J. Biol. Chem.*, 1969, vol. 244, pp. 2870–2882.
- 12. Reynolds, E.S., The Use of Lead Citrate at High pH as an Electron Opaque Stain in Electron Microscopy, *J. Cell Biol.*, 1963, vol. 17, pp. 208–218.
- 13. Moskalenko, A.A., Britton, G., Konnor, A., Iang, A., and Toropygina, O.A., Carotenoid Composition in Chro-

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matophores and Pigment–Protein Complexes Isolated from *Chromatium minutissimum* Cells Grown in the Presence of Diphenylamine, *Biol. Membr.*, 1991, vol. 8, pp. 249–260.

- 14. Makhneva, Z.K. and Moskalenko, A.A., Pigment–Protein Complexes from the New Sulfur Photosynthetic Bacterium *Ectothiorhodosinus mongolicum* Strain M9 with Normal and Inhibited Carotenoid Synthesis, *Biol. Membr.*, 2004, vol. 21, pp. 196–207.
- 15. 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.
- 16. Marmur, J., A Procedure for the Isolation 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. 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.
- 19. 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.
- 20. Stackebrandt, E. and Ebers, J., Taxonomic Parameters Revisited: Tarnished Gold Standards, *Microbiology today*, 2006, vol. 6, pp. 152–155.