ISSN 0026-2617, Microbiology, 2010, Vol. 79, No. 6, pp. 780–790. © Pleiades Publishing, Ltd., 2010.
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EXPERIMENTAL ARTICLES

Ectothiorhodospira magna sp. nov., a New Large Alkaliphilic Purple Sulfur Bacterium

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Received January 12, 2010

Abstract—Two strains of purple sulfur bacteria of the family *Ectothiorhodospiraceae* were isolated from moderately saline steppe lakes (with pH above 9.0) of the Transbaikal region (strain B7-7) and Mongolia (strain M10). The cells of the novel strains were spiral-shaped, 2.0–3.2 × 9.6–20.0 μm, motile due to a polar tuft of flagella. Photosynthetic pigments were represented by bacteriochlorophyll *a* and carotenoids of the spirilloxanthin series. Photosynthetic membranes were represented by long strands of lamellae distributed throughout the whole cell; unlike most *Ectothiorhodospiraceae* species, the membranes were not packed into regular stacks. Bacteria were capable of weak growth on sulfide and slow grow on hydrogen under photoautotrophic conditions. The best growth was noted on sulfide in the presence of acetate and bicarbonate. Thiosulfate did not stimulate phototrophic growth, even in the presence of organic substrates. The new isolates were alkaliphiles growing at a pH optimum of 9–10. Growth was possible within a salinity range of 0–80 g/l NaCl, with an optimum at 5–15 g/l NaCl. The morphology, the structure of the photosynthetic apparatus (strands of lamellae), and the physiology of the new strains were similar to those of *Thiorhodospira sibirica*. However, analysis of the 16S rRNA gene sequences demonstrated that the studied isolates were closely related to the type strain *Ectothiorhodospira shaposhnikovii* (99% similarity) of the family *Ectothiorhodospiraceae*, whereas the level of similarity between the new strains and *Thiorhodospira sibirica* was only 94–95%. According to the results of DNA–DNA hybridization, the DNA–DNA homology level between the tested strains was almost 100%; the similarity between the new isolates and the type strain *Ectothiorhodospira shaposhnikovii* was only 58%. The isolates differed from other representatives of the genus *Ectothiorhodospira* in the structure of the gene encoding the key enzyme of autotrophic CO_2 fixation, ribulose-1,5-bisphosphate carboxylase (RuBisCo), which was similar to the RuBisCo genes of members of another family of sulfur bacteria, *Chromatiaceae*. The new isolates of purple bacteria were described as a new species of the genus *Ectothiorhodospira, Ect. magna* sp. nov. with the type strain B7-7^T (= VKM B-2537 = DSM 22250).

Keywords: soda lakes, purple sulfur bacteria, family *Ectothiorhodospiraceae, Ectothiorhodospira magna* sp. nov., extremophiles, alkaliphiles.

DOI: 10.1134/S002626171006010X

Purple sulfur bacteria of the family *Ectothiorhodospiraceae* are typical representatives of the microbial communities inhabiting brackish, moderately saline, and hypersaline lakes, as well as soda lakes with a broad salinity range [1–5]. The characteristic trait of soda ecosystems with a water mineralization exceeding that of sea water is the presence of haloalkaliphilic species *Ectothiorhodospira haloalkaliphila* and *Ect. variabilis*, as well as of various species of the genus *Halorhodospira* [4–7]. The halotolerant or slightly halophilic species *Ect. shaposhnikovii, Ect. vacuolata, Thiorhodospira sibirica*, and *Ectothiorhodosinus mongolicus* are often found in moderately or slightly saline lakes [8–10]. *Trs. sibirica* differs from the other species of the family *Ectothiorhodospiraceae* by the large cell size and unusual topography of intracellular photosynthetic membranes (represented by randomly distributed strands of lamellae); in other members of this family, except for *Ets. mongolicus*, the lamellae are packed into compact stacks [8, 9]. Another characteristic trait of *Trs. sibirica* is their ability to accumulate the sulfur drops formed in the course of sulfide oxidation both outside the cells and in the cell periplasm. They look like intracellular sulfur under the light microscope. Large spirilla with "intracellular" sulfur drops, which are frequently mistaken for *Thiorhodospira* due to their morphological properties, are often found in the low-mineralized soda lake of Mongolia, the Transbaikal region, and Altai krai [5]. Two strains of large purple sulfur spirilla were isolated. These strains, according to the results of 16S rRNA gene sequencing, were found to be phylogenetically distant from bacteria of the genus *Thiorhodospira*.

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They were assigned to a new species of the genus *Ectothiorhodospira*, *Ectothiorhodospira magna* sp. nov.

The aim of the present work was to determine the physiological and biochemical characteristics and the phylogenetic position of the new species.

MATERIALS AND METHODS

Subjects of investigation. Two novel strains of spiral-shaped purple sulfur bacteria were isolated from two geographically distant moderately saline soda lakes. Strain B7-7 was isolated in 2008 from a water sample collected from the stratified soda Lake Doroninskoe (51°25′ N, 112°28′ E, Transbaikal region, Russia) with water mineralization of 32 g/l and pH 9.72 (near-bottom zone). Strain M10 was isolated in 1999 from a thin bacterial mat at the near-shore zone of the soda Lake Dzun-Uldziit (48°10'52"N, 114°39′37′′ E, Mongolia) with water mineralization of 61 g/l and pH 9.91. The morphological and physiological properties, pigment composition, and phylogeny of the new isolated were found to be similar. In addition to these two strains, the type strains of the purple bacteria *Trs. sibirica* ATCC 700588^T, *Ect. shaposhnikovii* DSM 243^T, and *Ect. variabitis* DSM 21381^T were used.

Isolation and cultivation. The purple sulfur bacteria were isolated and cultivated on a medium (pH 9.0) containing the following (g/l): NH_4Cl, 0.5; KH_2PO_4, 0.5; $MgCl_2$, 0.2; NaCl, 10; $NaHCO_3$, 10; Na_2CO_3, 5; yeast extract, 0.1; sodium acetate, 0.5; $Na_2S \cdot 9H_2O$, 0.5; vitamin B_{12}, 20 μg/l; and trace element solution, 1 ml [11]. Water solutions of $NaHCO_3$ (10%), Na_2CO_3 (10%), and sulfide (10%) were prepared and sterilized separately and added to the medium immediately before the inoculation, resulting in pH 9.0. Pure cultures were obtained using the method of terminal dilutions after repeated transfers of well-separated colonies grown on agarized media (0.8%). In liquid media, the cultures were grown under anaerobic conditions at 30–35°C and an illumination intensity of 2000 lx in screw-capped glass vials.

Cell morphology and ultrastructure. The morphology of bacterial cells was studied using light and electron microscopy. Whole cell specimens were stained with 1% phosphotungstic acid. Ultrathin sections were obtained using the previously described technique [8].

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

Physiology. The reaction of the studied bacteria to oxygen was assessed by their growth in agar (0.7%) columns in tubes with cotton plugs. After 2-day incubation, the distance between the growth zones and the surface of the agar columns was measured [12]. To determine the spectrum of substrates utilized under phototrophic conditions, mineral medium with sulfide (0.5 g/l) and yeast extract (0.05 g/l) was used. The tested compounds were added to the concentration of 0.5 g/l. The requirements of the studied microorganisms for mineral electron donors, as well as their reaction to various pH values and NaCl concentrations, were determined by the addition of different concentrations of the tested compounds to the medium [8]. The biomass yield was assessed by the optical density of the cell suspension at the stationary growth stage (measured with a KFK-3 photometer at 650 nm). At the end of the active growth stage, the content of elemental sulfur in the culture was almost depleted, making it possible to avoid a substantial error in nephelometric measurements of the biomass. The concentrations of $S_2O_3^{2-}$, SO_3^{2-}, and $H_2S + HS^-$ were determined by iodometric titration [13]. Sulfate was determined nephelometrically by the method of Dodgson [14].

Analysis of the fatty acid composition. The fatty acid composition was determined by chromatography and chromatography–mass spectrometry. Dry biomass (5 mg) was treated with 0.4 ml of 1 N HCl in methanol at 80°C for 3 h (acidic methanolysis). The methyl esters of fatty acids and other lipid components were extracted with hexane and analyzed on a Sherlock gas chromatograph (Microbial identification system, MIDI Inc., United States) [15].

Determination of the composition of isoprenoid quinones. Wet biomass was ground with liquid nitrogen in a mortar. Extraction was performed with cold acetone. The extract was subjected to thin-layer chromatography in a hexane–diethyl ether (85 : 15) system. The absorption bands visualized in UV light with an R_f of 0.65–0.75 corresponded to menaquinones; the absorption bands with an R_f of 0.2–0.3 corresponded to ubiquinones. The eluent was analyzed on an LCQ ADVANTAGE MAX tandem mass spectrometer using an atmospheric pressure chemical ionization (APCI) ion source [16].

Molecular genetic studies. The DNA of the pure cultures was isolated according to Marmur [17]. The DNA G + C content was determined using the thermal denaturation method according to Owen et al. [18]. The DNA homology was determined by the optical reassociation method [19].

Amplification and sequencing of the 16S rRNA genes was performed using universal bacterial primers [20]; amplification of the *cbbL* and *nifH* genes was carried out using the earlier designed system of primers [21, 22]. Sequencing of the PCR products was performed by the Sanger method 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 the BioEdit software package [http://jwbrown.mbio.ncsu.edu/BioEdit/bioedit.html]. Primary comparison of the de novo determined sequences with sequences within the GenBank database was performed using the NCBI BLAST software package [http://www.ncbi.nlm.

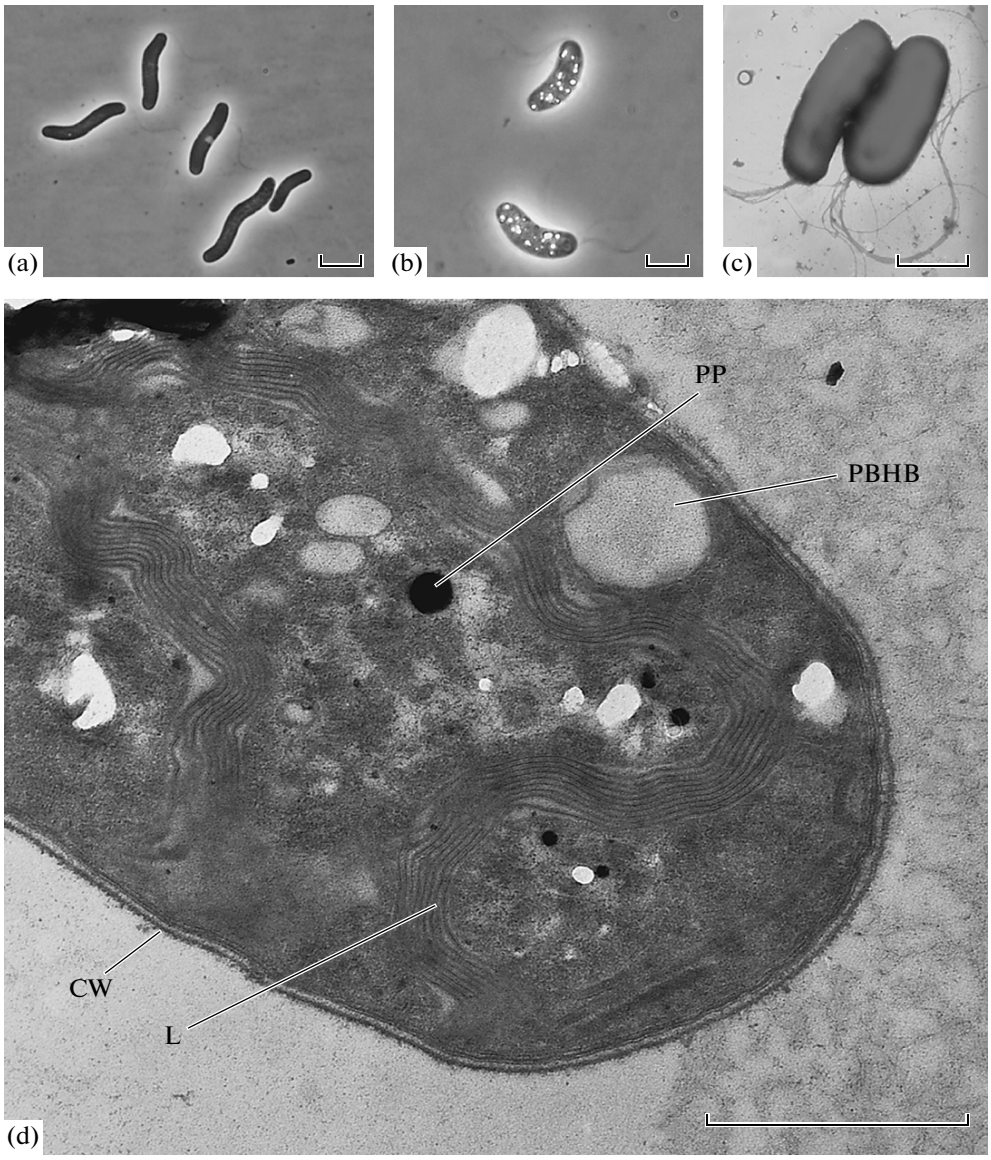

Fig. 1. Morphology (a-c) and ultrastructure (d) of *Ect. magna* B7-7. Scale bar: 9 (a), 6 (b), 3 (c), 1 μm (d). Designations: CW, cell wall; L, lamellar photosynthetic structures, PP, polyphosphates; PHB, poly-β-hydroxybutyric acid.

nih.gov/blast]. The nucleotide sequences and the deduced amino acid sequences of the studied genes were aligned with appropriate sequences from the closest relatives using the CLUSTAL W v 1.75 software package. The phylogenetic trees of the studied bacteria were constructed by the methods implemented in the TREECONW software package [http://bioc-www.uia.ac.be/ u/yvdp/treeconw.html].

Deposition of the nucleotide sequences. The 16S rRNA, *cbbL*, and *nifH* gene fragments of strain B7-7 were deposited in the GenBank database under accession numbers HM149323, HM149326, and HM149325, respectively; the 16S rRNA and *cbbL* gene fragments of strain M10 were deposited in GenBank under accession numbers HM149324 and HM149327, respectively.

RESULTS AND DISCUSSION

Phenotypic characteristics. The newly obtained strains of purple sulfur bacteria (B7-7 and M10) were similar with respect to their morphology, physiology, and pigment absorption spectra. Their cells were large (2.0–3.2 × 9.6–20.0 μm) and spiral-shaped (Figs. 1a–1c). The cells were motile due to a tight polar tuft of flagella (Fig. 1c), which was clearly visualized under a phase-contrast light microscope (Fig. 1b). The cells reproduced by binary division. At the early stage of phototrophic growth, numerous sulfur drops were formed on the cell surfaces. Light microscopy gives an impression of intracellular accumulation of sulfur as a result of sulfide oxidation (Fig. 1b). A similar pattern was observed in phototrophically growth of *Trs. sibir-*

ica, another member of the family *Ectothiorhodospiraceae*. It was demonstrated that, at the first stage of growth, the medium contained extracellular sulfur that was not associated with bacterial cells. Upon the onset of the stationary phase, sulfur was oxidized to sulfate and disappeared. It should be noted that, in the course of numerous transfers, extracellular sulfur not associated with bacterial cells was the main intermediate product of sulfide oxidation.

In ultrathin sections, the gram-negative type of strain B7-7 cell wall can be clearly seen (Fig. 1d). Intracytoplasmic membranes (ICMs) were represented by long strands of lamellae distributed throughout the whole cell. They were not packed into regular stacks as in most *Ectothiorhodospiraceae* species. Ultrathin sections demonstrated that ICM extended from the cytoplasmic membrane (Fig. 1d). Small dense rounded inclusions in the cytoplasm were probably storage compounds (polyphosphates); poly-β-hydroxybutyrate granules appeared as large light-colored inclusions.

The cell suspensions of both strains grown an aerobically in the presence of light were of deep red color. Bacteriochlorophyll *a* and carotenoids of the spirilloxanthin series were the main pigments. The presence of bacteriochlorophyll *a* was confirmed by the maximum absorption of cell suspensions at 373, 593, 797, 853 nm and a shoulder at 885 nm (Fig. 2). The presence of carotenoids of the spirilloxanthin series was supported by the maximum absorption within a range of 485–514 nm and a shoulder at 545 nm (Fig. 2). A similar in vivo pigment absorption spectrum with three long-wave maxima is typical of many *Ectothiorhodospiraceae* species, except for *Trs. sibirica* with four bacteriochlorophyll *a* absorption maximums observed in the near-infrared spectral region at 799, 830, 858, and 901 nm [8, 23].

The new isolates were strict anaerobes and phototrophs. They were capable of weak phototrophic growth with sulfide and of slow growth on hydrogen. The best growth of both strains occurred in mineral medium with sulfide as an electron donor, supplemented with acetate, fumarate, malate, or succinate (Table 1). Weak growth was observed in the presence of propionate, pyruvate, and yeast extract. Strains B7-7 and M10 did not utilize arginine, aspartate, benzoate, butyrate, caprylate, caproate, casein hydrolysate, ethanol, formate, fructose, glucose, glutamate, glycerol, lactate, malonate, mannitol, methanol, propanol, sorbitol, tartrate, citrate, and valerate as carbon sources. It was established that yeast extract (0.05 g/l) completely satisfied all requirements for growth factors. On media with sulfide and bicarbonate as sole carbon sources, growth was very weak, not exceeding 20% of the maximum growth, which was observed on the media additionally supplemented with acetate. In the presence of light, bacteria oxidized sulfide to elemental sulfur, which probably accumulated in the periplasm and gave an impression of intracellular sulfur. During growth, sulfur accumulated outside the cells as well and was then oxidized to sulfate. The new purple bacteria did not utilize thiosulfate as an electron donor; however, they utilized it as a sulfur source for biosynthesis. No growth occurred on organic substrates in the presence of sulfates. Growth on sodium thiosulfate or sulfite was very weak; oxidation of reduced sulfur compounds was not detected. Hence, it may be concluded that the isolated strains were not capable of assimilatory sulfate reduction.

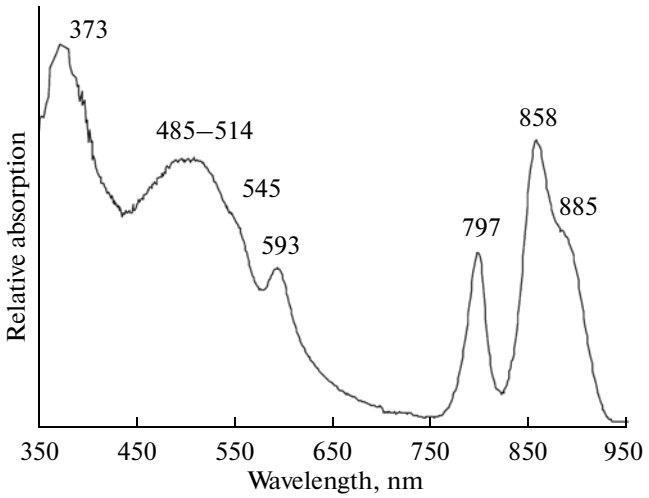

Fig. 2. In vivo pigment absorption spectrum of strain B7-7.

The new bacteria were mesophilic (the temperature range for growth was 20–45°C, with an optimum at 30–35°C), alkaliphilic (growing within a pH range of 8–11, with an optimum at pH 9–10), and halotolerant (growing at a NaCl concentration of 0–80 g/l, with an optimum at 5–15 g/l) microorganisms (Figs. 3 and 4).

The cells of strain B7-7 contained menaquinone MK_7 and ubiquinone Q_7. The compositions of quinones and major fatty acids of strain B7-7 (18 : 1, 71%; 16 : 1, 12.6%; 16 : 0, 14.2%) were similar to those of *Ect. shaposhnikovii* (Table 1).

Taxonomic position. The results of phylogenetic analysis indicated that the new strains belonged to the genus *Ectothiorhodospira* (Fig. 5). Comparative analysis of the16S rRNA gene sequences revealed that two strains (B7-7 and M10) were almost identical (99.9% similarity). The level of similarity between these strains and the known species of the genus *Ectothiorhodospira* was 93.4–99.0%. The highest similarity was observed with *Ect. shaposhnikovii* and *Ect. variabilis*. However, the DNA–DNA homology indicated an interspecific level of relatedness: 58 and 47% similarity between strain B7-7 and *Ect. shaposhnikovii* DSM 243^T and *Ect. variabilis* DSM 21381^T, respectively (Table 2). The content of the G + C base pairs in the DNA of the new strains was only 59.2 mol %, whereas it was 62–64 mol % in *Ect. shaposhnikovii*.

Table 1. Comparison of the characteristics of the new species *Ect. magna* with those of the phenotypically and genotypically close species *Ect. shaposhnikovii*, *Ect. vacuolata*, and *Trs. sibirica*

Characteristics	***Ect. magna***	*Ect. shaposhnikovii* [25]	*Ect. vacuolata* [25]	*Trs. sibirica* [8]
Cell shape	Vibrioid or spirilloid	Rod-shaped, usually slightly curved vibrios or short spirilla	Rod-shaped, sometimes slightly curved rods	Vibrioid or spirilloid
Size, μm	2.0–3.2 × 9.6–20.0	0.8–0.9 × 1.5–2.5	1.5 × 2–4	3–4 × 7–20
Flagellation	monopolar tuft of flagella	polar tuft of flagella	polar tuft of flagella	monopolar tuft of flagella
pH optimum	9–10	8–9	7.5–9.5	9–9.5
NaCl optimum, %	0.5–1.5	3	1–6	0–1
NaCl range, %	0–8	0–7	0.5–10	0–6
Chemolithotrophic growth	–	+	ND	–
Utilized substrates	Hydrogen, sulfide, sulfur, acetate, malate, succinate, and fumarate; weak growth on yeast extract, pyruvate, and propionate	Hydrogen, sulfide, thiosulfate, sulfur, sulfite, acetate, pyruvate, propionate, butyrate, lactate, fumarate, succinate, malate, fructose	Hydrogen, sulfide, thiosulfate, sulfur, sulfite ND, acetate, pyruvate, propionate, fumarate, succinate, and malate, weak growth on fructose	Hydrogen N/D, sulfide, sulfur, sulfite N/D, acetate, pyruvate, propionate, fumarate, succinate, and malate
DNA G + C content of the species, mol %	59.2 (T_m)	62–64	61.4–63.6	56–57.9
DNA G + C content of the type strain, mol %	59.2 (T_m)	62 (T_m)	63.6 (T_m)	56–57.4 (T_m)
Fatty acids, % of total				
12:0		1.3–1.7	N/D	
14:0				0.3
16:0	14.2	24.4–26.4	21.6	18.1
16:1ω7c	12.58	4.1–4.6	3.6	21.3
18:0	1.53	5.6–6.8	5.5	3.7
18:1ω7c	70.58	57.1–59.0	65.3	52.9
19:0cyc		N/D	N/D	
total, %	98.89	92.5–98.5	96	96
Quinones	MK_7, Q_7	MK_7, Q_7	MK_7, Q_7	N/D

Notes: "+" means that the characteristics is positive; "–" means that the characteristics is negative; "N/D" stands for "not determined"; ND stands for "no data"; (T_m), G + C in the DNA was determined by the thermal denaturation method.

Since autotrophic CO_2 fixation via the Calvin cycle, as well as nitrogen fixation, are the main metabolic processes of all members of the genus *Ectothiorhodospira*, detection, amplification, and phylogenetic analysis of the key genes (encoding ribulose-1,5-bisphosphate carboxylase and nitrogenase) responsible for these processes was carried out. In this study, the *nifH* gene fragment (about 450 bp) was amplified on the DNA template of strain B7-7. After conceptual translation of this fragment, it was aligned with the relevant sequences available from the GenBank database (149 positions). Although the topology of the "nitrogenase" tree (Fig. 6) differed from that of the "ribosomal" tree (in particular, the genus *Ectothiorhodospira* did not form a single phylogenetic cluster), the position of strain B7-7 on this tree remained unchanged. The studied strains exhibited the highest level of similarity of the *nifH* gene fragments to *Ect. shaposhnikovii* (100.0%); the similarity level between the studied strains and other species of the genus *Ectothiorhodospira* was considerably lower (94.7–99.6%).

As in other members of the family *Ectothiorhodospiraceae*, we detected only *cbbL* genes encoding form I (greenlike) RuBisCO (about 750 bp) in the DNA of strains B7-7 and M10; the obtained sequences were identical. After conceptual translation of these fragments, they were aligned with the relevant sequences available from the GenBank database (231 positions), including those of the phototrophic bacteria of the families *Ectothiorhodospiraceae* and *Chromatiaceae*. However, unlike the "ribosomal" and "nitrogenase" trees, the position of the new strains on the *cbbL* tree (Fig. 7) was unusual. They fell into a cluster with representatives of another family of purple

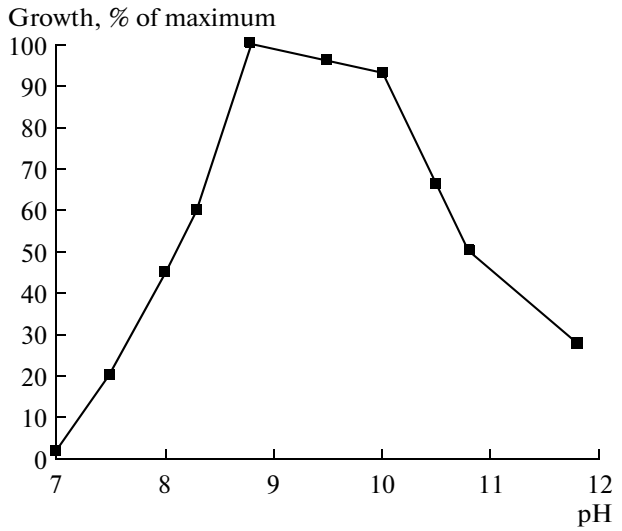

Fig. 3. Effect of pH on the growth of strain B7-7. The medium contained 15 g/l NaCl and 5 g/l of carbonates.

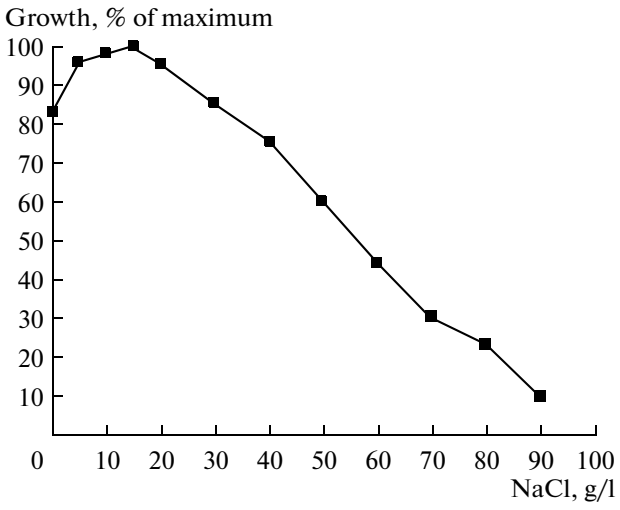

Fig. 4. Effect of NaCl on the growth of strain B7-7 at pH 9 in the presence of 5 g/l carbonates.

sulfur bacteria, *Chromatiaceae*, rather than into the cluster of the genus *Ectothiorhodospira*. The similarity level between their deduced amino acid sequences and those of their closest relative, *Thiocapsa roseopersicina*, was 92.2%. It has been previously demonstrated that this cluster includes some chemoautotrophic representatives of the family *Ectothiorhodospiraceae* [24]; however, this phenomenon, which may be attributed to horizontal transfer of the *cbbL* genes, was observed for the first time for the phototrophic bacteria of this family. The data obtained provide another confirmation of the unusual position of the new strains within the genus *Ectothiorhodospira*.

Although many phenotypic properties of the new bacteria were similar to those of *Trs. sibirica* (cell shape and size, structure of the photosynthetic membranes, response to NaCl, and spectra of utilized substrates), it was demonstrated that the new isolates were not closely related to *Trs. sibirica*, according to the results of 16S rRNA, *nifH*, and *cbbL* gene sequencing (94–95, 97.7, and 68.3%, respectively).

At the same time, strains B7-7 and M10 differed from their closest relative *Ect. shaposhnikovii* (99% similarity between their 16S rRNA genes and 100% similarity between the deduced amino acid sequences of their *nifH* genes) in some phenotypic characteristics (Table 1) [25]. The cells of the new isolates were two to three times wider and ten times longer than the cells of *Ect. shaposhnikovii*. They also differed from *Ect. shaposhnikovii* by the structure of photosynthetic membranes, weak growth on sulfide under photoautotrophic conditions, and low DNA G + C content, as well as in their inability to utilize thiosulfate as an electron donor and grow under aerobic or microaerobic conditions in the dark even in the presence of organic substrates.

According to the results of comparative analysis of the 16S rRNA, *cbbL*, and *nifH* gene sequences and

Table 2. Results of DNA–DNA hybridization of strain B7-7 with the reference species

Bacteria	DNA G + C content, mol %	Strain B7-7	*Ect. shaposhnikovii* DSM 243^T	*Ect. variabilis* DSM 21381^T
Strain B7-7	59.2	–	58	47
Ect. shaposhnikovii DSM 243^T	62	58	–	47
Ect. variabilis DSM 21381^T	62.7	47	47	–

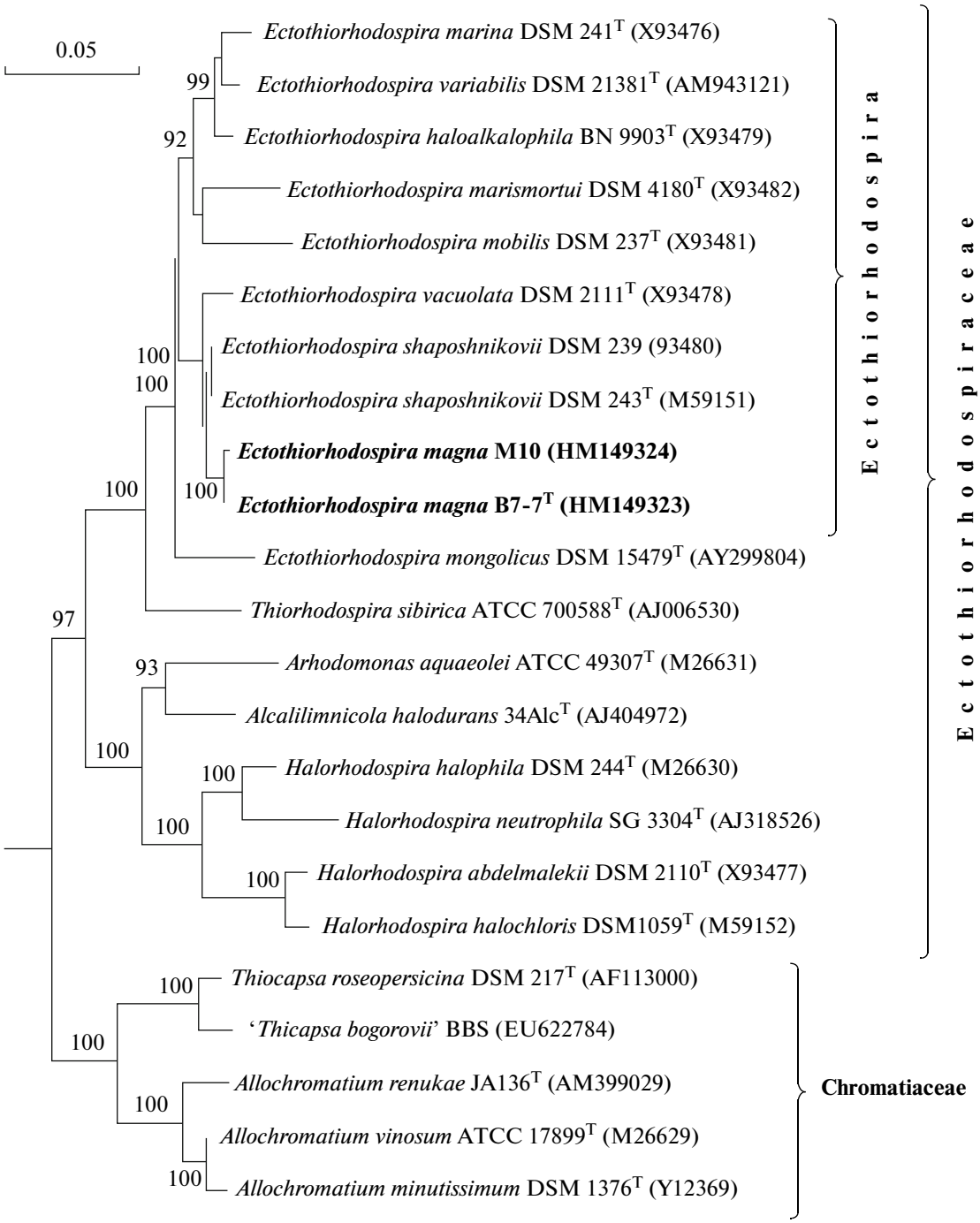

Fig. 5. Phylogenetic position of strains B7-7 and M10 among members of the family *Ectothiorhodospiraceae* according to the 16S rRNA gene sequence analysis. The phylogenetic tree was constructed using the neighbor-joining algorithm with the *E. coli* sequence taken as an outgroup. The sequences in bold were determined in this work. The numerals show the significance of the branching order as determined by bootstrap analysis. Only bootstrap values above 75 were considered as significant. The bar shows evolutionary distance, corresponding to five replacements per 100 nucleotides.

DNA–DNA hybridization and on the basis of their phenotypic properties, we propose that strains B7-7 and M10 should be classified as a novel species of the genus *Ectothiorhodospira, Ectothiorhodospira magna* sp. nov.

Taxonomic description of *Ectothiorkodospira magna* sp. nov. ***magna***, **L. *adj.*,** *magna* large.

Cells are spiral-shaped, slightly coiled, sometimes vibrioid, motile by means of a polar tuft of flagella, and measure 2.0–3.2 × 9.6–20.0 μm. The microorganism

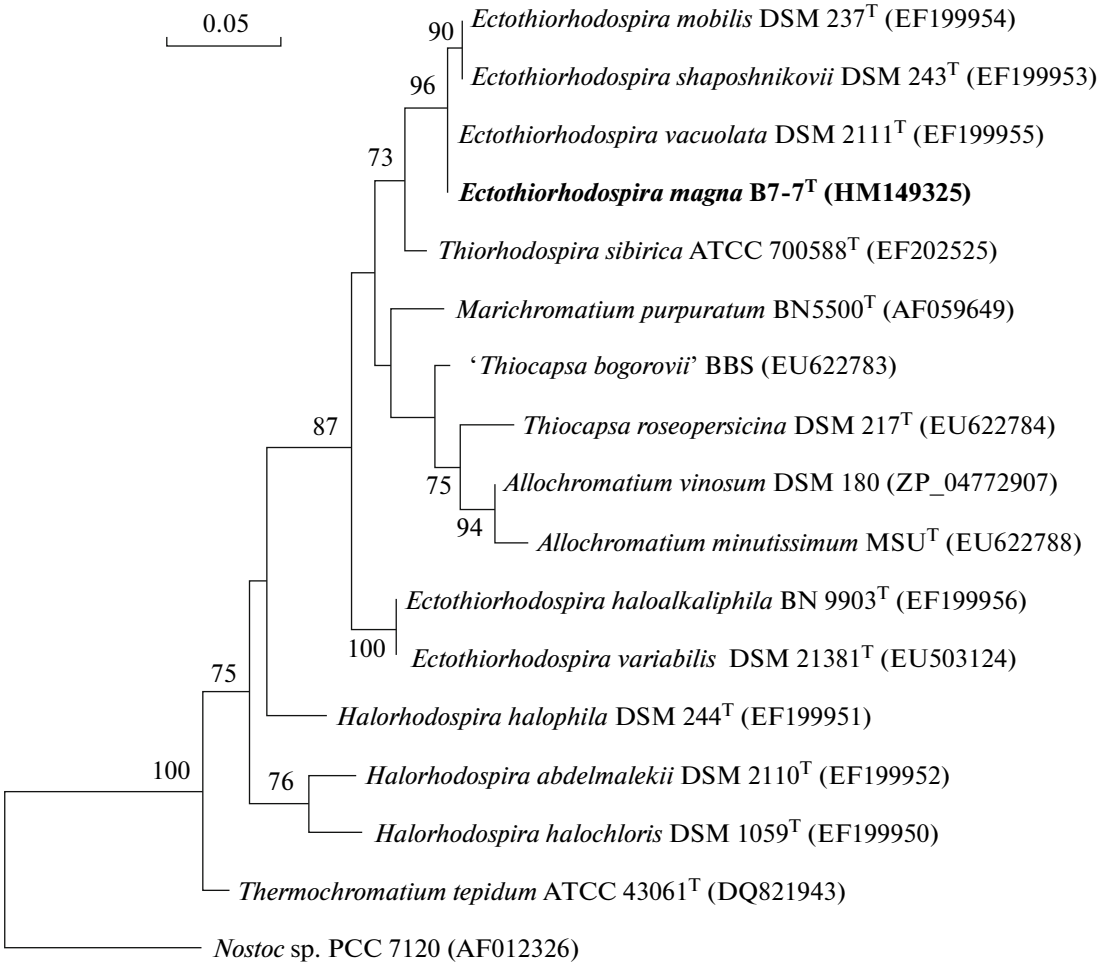

Fig. 6. Phylogenetic position of strain B7-7 among members of the family *Ectothiorhodospiraceae* according to analysis of deduced amino acid sequences encoded by *nifH*. The phylogenetic tree was constructed using the neighbor-joining algorithm with the *Nostoc* sp. PCC 7120 sequence taken as an outgroup. The sequences in bold were determined in this work. The numerals show the significance of the branching order as determined by bootstrap analysis. Only bootstrap values above 75 were considered as significant. The bar shows evolutionary distance, corresponding to five replacements per 100 amino acid residues.

reproduces by binary division. The cell wall structure is of the gram-negative type. Intracytoplasmic lamella-type membrane structures are present. Lamellae are distributed freely in the cytoplasm and are not packed into stacks. The cell suspensions are red to brownish-red. Absorption peaks of the cell suspension occur at 373, 485–514, (545), 593, 797, 858, and (885) nm. Photosynthetic pigments are represented by bacteriochlorophyll *a* and carotenoids of the spirilloxanthin series. Growth on sulfide or hydrogen as electron donors and on bicarbonate as a carbon source is weak. Sulfide is oxidized to sulfur and then to sulfates. Sulfur usually accumulates outside the cells; however, in freshly isolated cultures, sulfur drops are accumulated abundantly in the cell periplasm, which produces an appearance of their intracellular location. The best growth occurs upon addition of acetate, malate, succinate, or fumarate to the medium with sulfide and bicarbonate. Growth on yeast extract, pyruvate, and propionate is weak. Arginine, aspartate, benzoate, butyrate, caproate, caprylate, casein hydrolysate, citrate, ethanol, formate, fructose, glucose, glutamate, glycerol, lactate, malonate, mannitol, methanol, propanol, sorbitol, tartrate, and valerate are not utilized as carbon sources. The bacterium is incapable of assimilatory sulfate reduction and cannot grow aerobically in the dark. Ammonium is utilized as a nitrogen source. Yeast extract satisfies all requirements for growth factors. The organism is alkaliphilic and halotolerant. Optimal growth at pH 9.0–10.0 (with the growth range of pH 8–11); the bacterium grows at a NaCl concentration of 0–8%, with an optimum at 0.5–1.5%; the temperature range for growth is 20–45°C, with an optimum at 30–35°C.

Menaquinone MK_7 and ubiquinone Q_7 are present. The main fatty acids are: 18 : 1 (71%), 16 : 1 (12.6%), and 16 : 0 (14.2%).

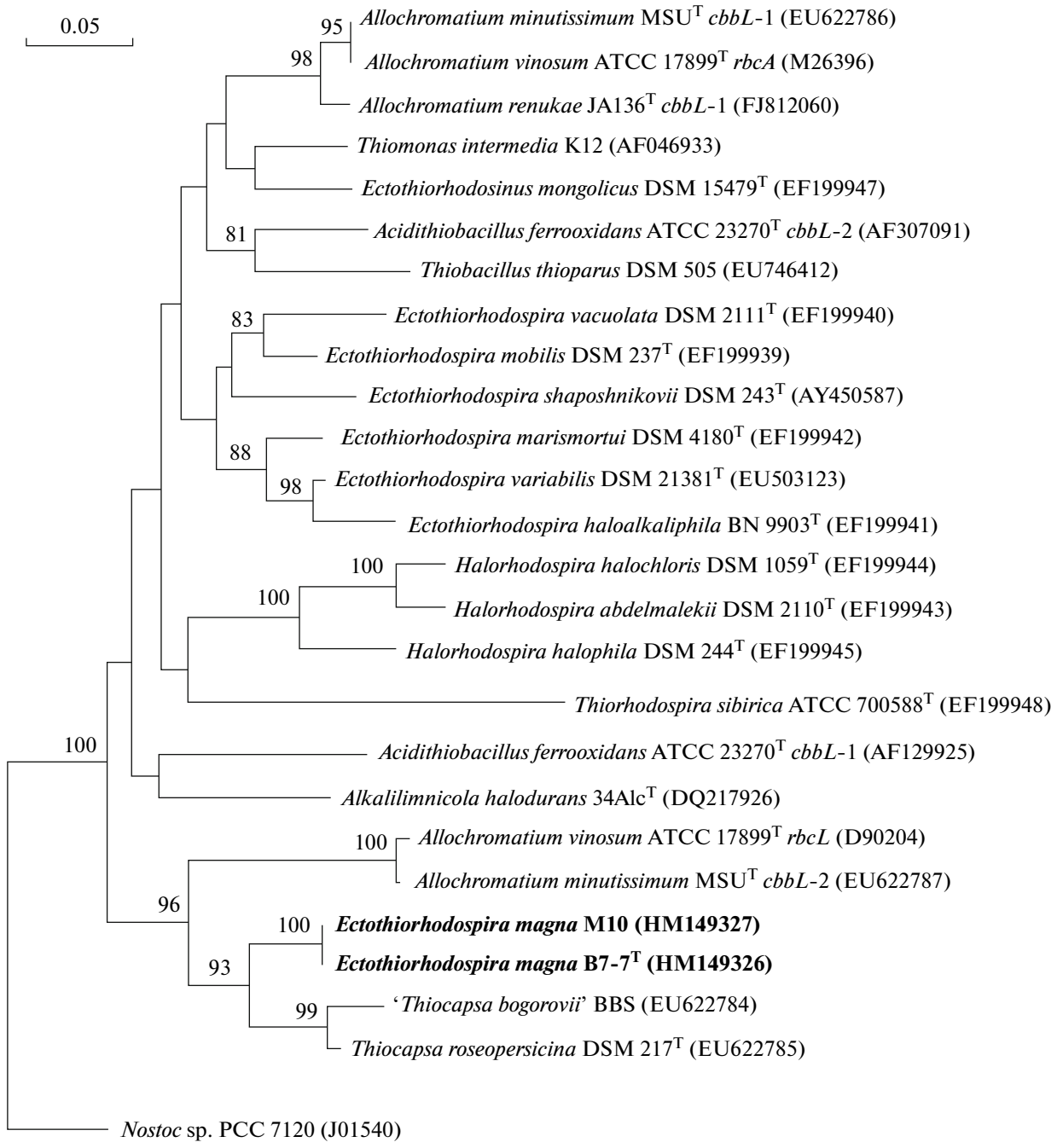

Fig. 7. Phylogenetic position of strains B7-7 and M10 among members of the order *Chtomatiales* according to the analysis of deduced amino acid sequences encoded by *cbbL*. The phylogenetic tree was constructed using the neighbor-joining algorithm with the *Nostoc* sp. PCC 7120 sequence taken as an outgroup. The sequences in bold were determined in this work. The numerals show the significance of the branching order as determined by bootstrap analysis. Only bootstrap values above 75 were considered as significant. The bar shows evolutionary distance, corresponding to five replacements per 100 amino acid residues.

Storage compounds are represented by polyphosphates and poly-β-hydroxybutyric acid.

The DNA G + C base content is 59.2 mol % (T_m).

The type strain B7-7^T (= VKM B-2537 = DSMZ 22250) was isolated from the chemocline of the stratified soda Lake Doroninskoe (Transbaikal region, Russia).

The obtained 16S rRNA, *cbbL*, and *nifH* gene sequences of strain B7-7^T were deposited with GenBank under the accession numbers HM149323, HM149326, and HM149325, respectively.

The authors thank E.N. Detkova for determining the DNA G + C base content and for performing DNA–DNA hybridization, G.A. Osipov for analysis of fatty acid composition, and B.P. Baskunov for determining the quinone content.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research (projects nos. 10-04-01500 and 08-04-0005), by the program of the Presidium of the Russian Academy of Sciences "Origin and Evolution of the Biosphere," and by the Russian Science Support Foundation.

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