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EXPERIMENTAL ARTICLES

Arhodomonas recens sp. nov., a Halophilic Alkane-Utilizing Hydrogen-Oxidizing Bacterium from the Brines of Flotation Enrichment of Potassium Minerals

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Abstract—A halophilic nonpigmented rod-shaped (0.8–1.0 × 2.0–2.5 μm), gram-negative bacterium with a single polar flagellum (strain RS91) was isolated from acidic brines of flotation enrichment of potassium minerals (Silvinit Co., Solikamsk, Russia). The strain grew in the media with 2 to 25% NaCl (optimum at 10–12%), 20–45°C (optimum at 37°C), and pH 5.5–8.5 (optimum 6.5–7.5). It was an aerobe or facultative anaerobe incapable of fermentation. The strain was characterized by the absence of growth on glucose, fructose, and citrate, extensive aerobic growth on *n*-hexadecane and in the mineral medium with $H_2 + O_2 + CO_2$ in the gas phase, anaerobic nitrate reduction with acetate or hydrogen (under $H_2 + CO_2 + N_2$), and variable fatty acid composition. The DNA G+C content was 68.2 mol %. Phylogenetic analysis based on 16S rRNA gene sequencing revealed that while strain RS91 was most closely related to *Arhodomonas aquaeolei* HA-1^T (98.3%) and *Nitrococcus mobilis* (98.1%), it was only remotely related to the halophilic phototroph *Halorhodospira halophila* (90.6%). Based on the combination of its phenotypic and genotypic characteristics, the organism was classified as a new species of the genus *Arhodomonas*, family *Ectothiorhodospiraceae* with the proposed name *Arhodomonas recens* sp. nov. The type strain is RS91^T (= IEGM 796^T = VKPM B-11280^T).

Keywords: Arhodomonas, new halophilic microorganism, phylogeny, alkane-utilizing bacteria, hydrogen-oxidizing bacteria, technogenic brines

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The family *Ectothiorhodospiraceae* comprises physiologically diverse halophilic and halotolerant, alkaliphilic and neutrophilic species, aerobic and anaerobic, photo- and chemolithoautotrophs, facultative autotrophs, and various chemotrophic species of gram-negative *Gammaproteobacteria* [1–7]. The subgroup of obligate chemotrophic *Gammaproteobacteria* is represented by two species with high homology of the 16S rRNA gene sequences and different types of metabolism: a halophilic chemoorganotroph *Arhodomonas aquaeolei* and a nitrite-oxidizing marine chemolithoautotroph *Nitrococcus mobilis* [3, 4]. From acidic industrial brines of the Silvinit Co. (Solikamsk, Russia), we isolated a new halophilic alkane-utilizing gram-negative bacterium *Arhodomonas* sp. RS91, which was capable of switching between organotrophic and lithotrophic (hydrogen-oxidizing) metabolism under aerobic or anaerobic conditions (in the presence of nitrates).

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The present work deals with an investigation of strain RS91 and its description as a new *Arhodomonas* species.

MATERIALS AND METHODS

Source of isolation and techniques for cultivation and maintenance of the isolates. The microflora of mother brines, foamy products, and solid waste of the SKRU-2 and SKRU-3 production divisions for flotation enrichment of potassium minerals (Silvinit Co., Solikamsk, Perm' krai, Russia) was investigated. The ore enrichment technology involves the application of a mixture of hydrochloride (C_8–C_{20}) monoamines, in which hexadecane amine hydrochloride $C_{16}H_{33}NH_2 \cdot HCl$ is usually the main component. In the samples presented in early November 2009, pH was 4.1–4.6, the content of ammonium and amino groups in the foamy products was 40–80 mg/L, and the numbers of bacteria-like cells (direct count) varied from 3–4 ×

10^6/mL in mother brines to 1.8–2.1 × 10^9/mL in foamy products.

The samples were used to inoculate the liquid media containing the following (g/L): NaCl, 30, 150, or 240; K_2SO_4, 5; $MgSO_4 \cdot 6H_2O$, 20; $(NH_4)_2HPO_4$, 0.5; $CaCl_2 \cdot 2H_2O$, 0.1; yeast extract, 0.7; casein hydrolysate, 0.7; Na pyruvate, 0.7; ampicillin, 0 or 1; pH 6.5 or 7.5. Sequential transfers in liquid and solid media (2% Difco agar) were used to obtain 7 halophilic isolates. Based on their morphophysiological characteristics and initial results of BLAST analysis of the 16S rRNA gene sequences, the colorless, gram-negative motile isolates were classified as members of the genera *Arhodomonas* (2 strains) and *Chromohalobacter* (2 strains), while ampicillin-resistant, pigmented nonmotile ones (3 strains) belonged to halophilic archaea *Halarchaeum*. The taxonomic description of the new *Arhodomonas* species (type strain RS91) isolated from the SKRU-3 acidic mother brines is presented in this article.

For prolonged storage of strain RS91 (on agar slants in rubber-stoppered test tubes at 2–3°C), the complex medium was used which provided for stable aerobic growth at 37°C and contained the following (g/L): agar (Difco), 20; NaCl, 100; KCl, 1.5; $MgCl_2 \cdot 6H_2O$, 10; $MgSO_4 \cdot 7H_2O$, 2; $(NH_4)_2HPO_4$, 0.5; $CaCl_2 \cdot 2H_2O$, 0.2; $FeCl_3 \cdot 4H_2O$, 0.04; tryptone, 0.6; casein hydrolysate, 0.4; yeast extract, 0.3; $CH_3COONa \cdot 3H_2O$, 0.8; Na pyruvate, 0.4; pH 6.9–7.1.

Morphological and physiological characteristics. Cell morphology was studied under an OPTON ICM 405 phase contrast microscope (Germany) and a JEM-100B electron microscope (Jeol, Japan) on negatively stained preparations and ultrathin sections as previously described [8]. The cells for electron microscopic investigation were fixed, dehydrated, and embedded into a mixture of epoxy resins. Ultrathin sections were made on an LKB-III microtome (Sweden). Contrasted sections were examined under the 80 kV accelerating voltage and 8000–25000× magnification. Inclusions of high-molecular polyphosphates were revealed by light microscopy of the preparations stained with methylene blue (1 : 40). Lipoid granules were revealed by staining with Sudan III [9].

The effect of a number of factors (pH, temperature, gas phase composition, electron donors, and the concentrations of NaCl, KCl, $MgCl_2$, $MgSO_4$, vitamins, and growth substrates) on the growth characteristics of strain RS91 was determined. For anaerobic cultivation, potassium nitrate (3 g/L) or sodium nitrite (0.1 g/L) were used as electron acceptors, while glucose, tryptone, glutamate, lactate, pyruvate, citrate, acetate, formate, H_2, sulfide, and thiosulfate were the electron donors tested. Anaerobic fermentation of carbohydrates, lactate, pyruvate, and glutamate was tested in the absence of sulfate, nitrate, and other electron acceptors.

Catalase and oxidase activities, as well as the sensitivity to antibiotics, were determined using the standard bacteriological techniques [9]. Capacity for denitrification was assessed by N_2O accumulation in the gas phase of N_2 and acetylene (a specific inhibitor of N_2O reductase) in liquid medium with acetate and nitrate or nitrite [10]. Capacity for nitrogen fixation was determined from nitrogenase activity (acetylene reduction to ethylene) under microaerobic and anaerobic conditions in a nitrogen-free medium with acetate [10]. Gas mixtures were analyzed on a Chrom-5 chromatograph (Czechoslovakia).

Polar lipids of the membranes were analyzed by one- and two-dimensional thin-layer chromatography (TLC) [11]. The cells were concentrated by centrifugation, washed with physiological saline, and used for lipid extraction by a polar solvent in the methanol–chloroform mixture (2 : 1). Two solvent systems were used for the separation of the lipids on the TLC silica gel plates (10 × 10 cm): A, chloroform–methanol–ammonia water (65 : 35 : 3) and C, chloroform–methanol–acetic acid–water (85 : 22 : 8 : 3). The chromatography was carried out in glass chambers with massive lids. The preparations were incubated to the time when the front of the A or C solvent approached the upper edge of the plate (usually 40–50 min). The plates were then removed from the chamber, dried in a fume hood, and treated with the relevant reagents to detect the different classes of the lipids. The nonspecific reagent causing charring of all lipids (black spots) was used, as well as specific reagents: Zinzade's reagent for phospholipids (blue spots), α-naphthol reagent for glycolipids (brown spots), and ninhydrin reagent for nitrogen-containing phospholipids (pink spots). Comparative analysis of the composition of the membrane polar lipids (according to the area and intensity of the spots) was thus carried out with 12 chromatograms per sample. Phosphatidic acid, phosphatidylglycerol (PG), diphosphatidylglycerol (cardiolipin, DPG), phosphatidylcholine (lecithin, PC), and phosphatidylethanolamine (PE) were used as phospholipid markers.

Fatty acid (FA) composition was analyzed on an Agilent 6890/5973N gas chromatography–mass spectrometry system (United States) according to the procedure recommended for the Microbial Identification System [12]. FA were extracted from the biomass, converted into methyl ethers in the methanol–HCl mixture, separated on a capillary chromatographic column with the HP-5MS nonpolar phase in the temperature programming mode, and analyzed on the mass spectrometer in the dynamic mode. The components were identified using the AMIDIS automated system for analysis of mass spectral data; the target components were identified using the NIST 98/NISTEPA.MSL library (USEPA) with at least 80% similarity.

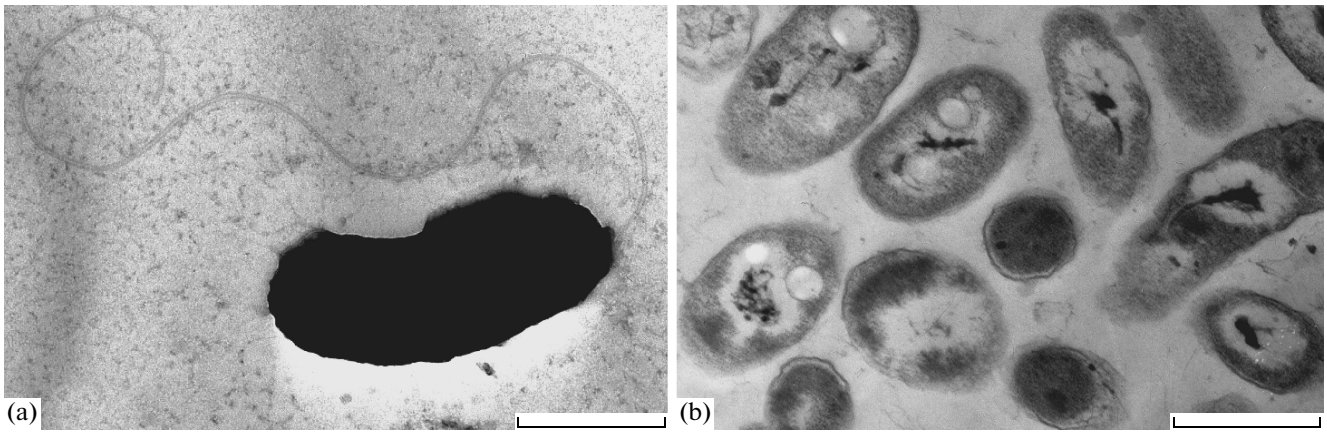

Fig. 1. Electron micrographs of strain RS91: negatively stained cell with a single polar flagellum (a) and ultrathin sections of the cells with a typical gram-negative bacterial cell wall, delineated hydroxyalkanoate inclusions, and an electron-dense fibrillar nucleoid (b). Scale bar is 1 μm.

Molecular genetic investigation. The G+C content in purified DNA preparations was determined by the thermal denaturation method [13]. The fragment of the 16S rRNA gene of the isolate RS91 was amplified in a polymerase chain reaction (PCR) with universal eubacterial primers 11f and 1492r [14]. Amplification was carried out on a DNA Engine Tetrad 2 Peltier Thermal Cycler (Bio-Rad, United States). After electrophoretic separation in 1% agarose gel, PCR products were detected on a BioDOc Analyzer (Biometra, Germany). The PCR fragment was purified using the Wizard PCP Preps kit (Promega, United States) according to the manufacturer's recommendations and sequenced on a DNA Analyzer ABI 3730 automatic sequencer (Applied Biosystems, United States). Primary comparison of the nucleotide sequence to GenBank sequences was carried out using the BLAS software package (http://www.ncbi.nlm.nih.gov/BLAST). The sequences were compared using the ClustalX software package [15]. Phylogenetic analysis was carried out using the neighbor-joining method and the algorithms implemented in the TREECONW software package [16]. The sequence of the 16S rRNA gene of the isolate RS91 was deposited to GenBank under accession no. HQ 833040.

RESULTS AND DISCUSSION

The cells of strain RS91 were gram-negative, nonspore-forming rods, 0.8–1.0 × 2.0–2.5 μm. Division occurred by binary fission in one plain. The cells were motile, with a single polar flagellum (Fig. 1). Intracytoplasmic membrane structures were not revealed. The electron-dense fibrillar nucleoid and electron-transparent inclusions (presumable of poly-3-hydroxybutyrate) surrounded by single-layer membrane were present. Intracellular lipoid granules were especially abundant at the early stationary phase in the complex medium with acetate and tryptone. Metachromatic polyphosphate granules were not formed. After 3–4 days of growth on complex solid media with 10–12% NaCl at 37°C, smooth, convex, round, nonpigmented colonies with a smooth edge 0.5–1.0 mm in diameter, were formed.

The organism was halophilic, neutrophilic, and mesophilic. Growth occurred at NaCl concentrations from 2 to 25% with the optimum at 10–12% at 20–45°C (optimum at 37°C), and pH 5.5–8.5 (optimum at 6.5–7.5). Although Na^+ ions were required, this moderate halophile could grow in the media with potassium or magnesium chloride substituted for sodium salts: 3% NaCl + 12% KCl or $MgCl_2$. The organism was resistant to neomycin (30 μg/disk) and streptomycin (10 μg/disk) and sensitive to ampicillin (10 μg/disk) and erythromycin (15 μg/disk).

The organism grew as an aerobe or a facultative anaerobe and was incapable of fermentation; it was catalase- and oxidase-positive. Casamino acids, tryptone (and, to a lesser degree, peptone and yeast extract), glutamate (to a lesser degree glutamine), acetate, pyruvate (to a lesser degree succinate, lactate, propionate, glycerol, and ethanol), *n*-hexadecane, *n*-tetradecane (to a lesser degree *n*-decane), and the mixture of (C_8–C_{20}) monoamine hydrochlorides were used as substrates for aerobic organotrophic growth. Acids and H_2S were not produced from these substrates. The minimal requirement in growth factors was satisfied with 0.1 g/L of yeast extract. In media with 0.3 g/L yeast extract, xylose, formate, benzoate, phenol, and methanol supported growth, but not glucose, fructose, galactose, sucrose, mannitol, γ-hydroxybutyrate, malate, citrate, urea, methane, propane, or methylamine. No growth occurred at high concentrations of organic matter (OM) in the medium, which should not exceed 2 g C/L for proteinaceous OM. Casein, gelatin, and starch were not hydrolyzed. Ammonium, nitrates, and glutamate were

used as nitrogen sources. Molecular nitrogen was not fixed and acetylene was not reduced to ethylene.

During growth of *n*-decane, *n*-tetradecane, and especially of *n*-hexadecane (10–15 mL/L), ultrasound-emulsified hydrocarbons were infested by numerous small cells (0.7–0.8 × 1.8–2.0 μm). Local aggregations of these cells decomposed eventually, including even the larger spherical micelles. After four days of growth, the cell numbers were as high as 6–9 × 10^9/mL. Ribbon-shaped strands sometimes united the pairs of dividing cells. Free-floating cell chains and microcolonies in the bacterial suspension formed mucous capsules of surfactants. Abundant formation of mucus was observed in the cultures growing on emulsified alkane amines.

High yields of bacterial cells (up to 3–10 × 10^9/mL) were observed in the cultures of strain RS91 in mineral medium (organic-free) with bicarbonate in hermetically sealed vials with $H_2 + O_2 + CO_2$ (7 : 2 : 1) in the headspace. In this case, mucous capsules were not formed; the cells became thicker, so that a significant part of the population became ovoid (1.0–1.2 × 2.0–2.2 μm) after three days of cultivation. Repeated addition of the gas mixture in order to compensate for the negative pressure in the vials resulted in accumulation of the biomass of the new hydrogen-oxidizing bacterium RS91. Almost no growth occurred in the control with hydrogen replaced by nitrogen in the gas mixture.

The strain did not use reduced sulfur compounds for chemoautotrophic growth.

Low concentrations of nitrate or nitrite (but not sulfate or thiosulfate) could be used as electron acceptors for anaerobic organotrophic and lithoautotrophic growth of strain RS91 with hydrogen. In the case of organotrophic growth, nitrate reduction was most active with acetate and was significantly lower with pyruvate, lactate, and tryptone. During the first 4–5 days of growth in liquid medium in hermetically sealed vials with acetate + NO_3^-, nitrites were accumulated up to 150 mg N/L, while by days 8–10 their concentration decreased to 0.02 mg N/L. Accumulation of N_2O in the gas phase (N_2) did not occur, however, under anaerobic or microaerobic conditions, at elevated pH or salinity, and in the case of N_2O reductase inhibited by acetylene (in the case of denitrification). While growth of the culture in agar media resulted in formation of small colonies, as well as in formation and consumption of nitrites, no gas ruptures typical in the case of denitrification were observed.

Being a facultative anaerobe, strain RS91 could grow by nitrate reduction coupled to hydrogen oxidation. In semisolid mineral medium with nitrates and the headspace of $H_2 + CO_2 + N_2$ (7 : 1 : 2) in high cylinders, the zone of active growth initially formed at the depth of 1–2 cm; nitrites were accumulated in the medium. Small colonies and extensive gas ruptures developed subsequently throughout the agar layer. Anaerobic growth with nitrates was facilitated by bicarbonate and the initial dose of yeast extract (0.3 g/L) in the medium (for mixotrophic growth) and by repeated addition of H_2 to the gas phase (for lithoautotrophic growth).

The fatty acid composition varied significantly depending on the medium composition and cultivation conditions (Tables 1 and 2). These variations were especially pronounced in the case of transition from aerobic growth on proteinaceous OM to anaerobic nitrate reduction with acetate. While the content of the relatively low-melt $C_{16:0}$ increased from 12–14 to 26–42% and $C_{18:2}$ and $C_{18:1}$ 2OH appeared, the ratios of $C_{19:0cyclo}$ and $C_{18:1}$ decreased from 17–22 to 3–11% and from 30–33 to 13–21%, respectively and the high-melting $C_{20:0}$ and $C_{22:0}$ disappeared. This tendency in the variations in the FA composition was observed during transition from aerobic growth under $H_2 + O_2 + CO_2$ to anaerobic growth with nitrates under $H_2 + CO_2$. Significant variation in FA composition were reported for the nitrite-oxidizing autotroph *Nitrococcus mobilis* at the strain level ($C_{18:1}$, 16–65%) and for anaerobic phototrophs of the genus *Ectothiorhodospira* at the interspecific level ($C_{18:1}$, 12–59%; $C_{16:0}$, 21–56%; $C_{19:0cyclo}$, 0–38%) [1].

Variability of the FA composition in the members of *Ectothiorhodospiraceae* is probably an important mechanism of adaptation to changing conditions. Comparative composition of the polar lipids of the membranes is, however, relatively similar (Table 2). Among the seven polar lipids of isolate RS91, five glycerophosphatides predominate: phosphatidylglycerol, diphosphatidylglycerol, a phosphoglycolipid (presumably phosphatidylinositol), phosphatidylethanolamine, and phosphatidylcholine. The DNA G+C content was 68.2 mol %.

According to phylogenetic analysis of the 16S rRNA gene sequences, strain RS91 fell into the isolated subgroup of *Arhodomonas aquaeolei–Nitrococcus mobilis* within the family *Ectothiorhodospiraceae*, class *Gammaproteobacteria* (Fig. 2). While its similarity to *A. aquaeolei* and *N. mobilis* was as high as 98.3 and 98.1%, respectively, the similarity to the haloalkaliphilic species *Alkalispirillum mobile, Alkalilimnicola ehrlichii*, and *Alkalilimnicola halodurans* was lower (93.4, 93.2, and 93.1%, respectively), while the similarity to the phototrophic purple bacterium *Halorhodospira halophila* was still lower (90.6%). Relative remoteness of the alkaliphilic *Aquisalimonas asiatica* from the neutrophilic *Arhodomonas aquaeolei* (91.6%) and isolate RS91 (92.1%) correlates with certain of its morphophysiological traits, such as a different flagellation type, growth in a broad pH range (6.0–10.8), higher heterotrophic potential (uses also glucose, citrate, and γ-hydroxybutyrate), H_2S production from proteinaceous OM, $C_{12:0}$ instead of $C_{18:0}$ among the dominant FA, and resistance to penicillin and tetracycline (Table 2). Its physiological, biochemical, and

Table 1. Fatty acid composition of strain RS91 (% of the total FA) grown at 37°C aerobically (O_2) or anaerobically (NO_3^-) in media with 10 and 20% NaCl

Fatty acid	Tryptone, peptone + NaCl (%)		Acetate + NO_3^- + NaCl (%)		$H_2 + CO_2$ + NaCl (10%)	
	10	20	10	20	O_2	NO_3^-
$C_{12:0}$	1.85	1.73	0.30	0.32	3.42	4.04
$C_{15:0}$	0.34	0.42	1.27	3.07	0.98	1.26
$C_{16:1\omega7c}$	9.31	4.55	4.31	2.11	1.24	4.27
$C_{16:0}$	11.81	14.10	25.54	41.86	12.40	16.89
$C_{18:2}$			1.23	2.77	1.65	1.54
$C_{18:1\omega9c}$	10.04	5.93	8.16	4.65	12.86	8.15
$C_{18:1\omega7c}$	20.08	27.31	12.72	8.81	10.90	10.94
$C_{18:0}$	22.46	24.20	34.35	23.75	24.33	27.02
$C_{19:0cyclo}$	22.20	17.20	2.61	11.37	23.56	21.07
$C_{18:0}$ 3OH	1.31	1.37	6.90	0.75	6.13	2.92
$C_{18:1}$ 2OH			2.61	0.54	2.53	1.90
$C_{20:0}$	0.30	0.80				
$C_{22:0}$	0.31	1.69				

chemotaxonomic characteristics, as well as phylogenetic analysis of its 16S rRNA gene sequence, unequivocally support classification of the isolate RS91 as a new *Arhodomonas* species.

Description of *Arhodomonas recens* sp. nov.

Arhodomonas recens (re. cen's, N.L. masc. adj., *recens*, recent, modern).

On complex agar medium, nonpigmented, semitransparent, rounded colonies up to 0.5–1.0 mm in diameter are formed. The cells are gram-negative rods, 0.8–1.0 × 2.0–2.5 μm. No intracytoplasmic membranes are present. The cells are motile, with a single polar flagellum. Intracellular polyhydroxyalkanoate inclusions may be present. Mucous capsules may be formed. No spores or other dormant forms are produced.

The organism is halophilic, neutrophilic, and mesophilic. Growth occurs at 2 to 25% NaCl (optimum at 10–12%), 20–45°C (optimum at 37°C), and pH 5.5–8.5 (optimum at 6.5–7.5). It is sensitive to penicillin G (10 U), ampicillin (10 μg), erythromycin (15 μg), and tetracycline (30 μg) and resistant to neomycin (30 μg) and streptomycin (10 μg).

The organism is catalase- and oxidase-positive, grows as an aerobe or facultative anaerobe and is incapable of fermentation. The metabolic type is oxidative, aerobic respiration and anaerobic nitrate respiration. It is capable of aerobic chemoorganoheterotrophy, hydrogen autotrophy in a mineral medium under the $H_2 + O_2 + CO_2$ gas mixture, and of anaerobic nitrate reduction with acetate (to a lesser degree also with pyruvate, lactate, and tryp-

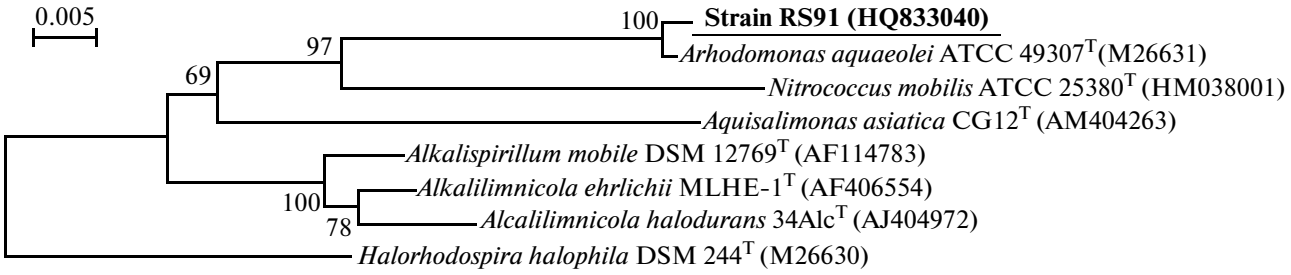

Fig. 2. A dendrogram depicting the phylogenetic position of strain RS91 among the type strains of colorless nonphototrophic *Gammaproteobacteria* of the family *Ectothiorhodospiraceae*. The tree was constructed with 1360 nucleotide sequences of the 16S rRNA gene using the neighbor-joining method. The numerals indicate reliability of the branching order determined by bootstrap analysis. Scale bar is 5 replacements per 1000 nucleotides. The sequence of the type strain of the phototrophic halophile *Halorhodospira halophila* was used as the outgroup.

Table 2. Differentiating characteristics of strain RS91 and the type strains of phylogenetically related species of four *Ectothiorhodospiraceae* genera

Characteristics	Strain RS91	*Arhodomonas aquaeolei* [4]	*Aquisalimonas asiatica* [7]	*Alcalilimnicola halodurans* [5, 6]	*Nitrococcus mobilis* [1, 3]
Cell size, μm	0.8–1.0 × 2.0–2.5	0.8–1.0 × 2.0–2.5	0.7–0.9 × 2.0–10.0	1.5 × 2.0–6.0	1.5–1.8 (cocci)
Flagella	1 polar	1 polar	+	1 subpolar	2 subpolar
Range (optimum):					
NaCl, %	2–25 (10–12)	6–20 (15)	1–20 (7–10)	0–28 (3–8)	0–3 (0.1–0.5)
pH	5.5–8.5 (6.5–7.5)	6.0–8.0 (6.5–7.5)	6.0–10.8(7.5–8.5)	6.5–11.0 (9.5)	6.8–8.5 (7.5–8.0)
Temperature, °C	20–45 (37)	20–45 (37)	20–50 (37)	20–55 (35)	14–40 (25–30)
Autotrophic growth on:					
Hydrogen	+	–	–	–	–
Sulfides	–	–	–	+	–
Nitrites	–	–	–	–	+
NO_2^- from NO_3^-	+	+	+	+	–
Oxidized substrates:					
D-galactose	–	–	–	+	–
D-fructose	–	–	–	+	–
D-mannitol	–	–	–	+	–
D-glucose	–	–	+	+	–
γ-Hydroxybutyrate	–	–	+	+	–
Citrate	–	–	+	+	–
Acetate	+	+	+	+	+
Propionate	+	+	+	–	–
Glycerol	+	+	+	–	–
L-Glutamate	+	+	+	–	–
Formate	+	–	+	–	–
Benzoate	+	–	+	–	–
n-Hexadecane, *n*-decane	+	ND	ND	–	–
Casein hydrolysate	+	+	+	+	–
H_2S production	–	–	+	–	–
Resistance to penicillin, tetracycline	–	–	+	–	–
Major membrane polar lipids	PG, DPG, PGL, PE, PC	ND	PG, DPG, PE, PC, PGL	PG, DPG, PE, PC	ND
Major cellular fatty acids (%)	$C_{18:1}$ (29.7), $C_{18:0}$ (24.2), $C_{19:0cyclo}$ (20.4), $C_{16:0}$ (12.3), $C_{16:1}$ (7.0)	$C_{18:1}$ (21.7), $C_{16:0}$ (21.7), $C_{19:0cyclo}$ (12.7), $C_{16:1}$ (12.6), $C_{18:0}$ (11.2)	$C_{18:1}$ (38), $C_{16:0}$ (17), $C_{12:0}$ (15)	$C_{18:1}$ (54.0), $C_{16:0}$ (23.4), $C_{18:0}$ (12.1), $C_{14:0}$ (4.1)	$C_{18:1}$ (16–60), $C_{16:0}$ (>16), $C_{16:1}$ (>16), $C_{19:0cyclo}$ (<5), $C_{18:0}$ (<5)
DNA G+C content, mol %	68.2	67.0	63.6	65.6	61.2

Note: "+" indicates growth, "–" indicates absence of growth, ND stands for no data. PG, DPG, PGL, PE, and PC stand for phosphatidylglycerol, diphosphatidylglycerol, phosphoglycolipid, phosphatidylethanolamine, and phosphatidylcholine, respectively.

tone) or H_2 + CO_2 (more actively in the presence of bicarbonate and 0.3 g/L of yeast extract). Casamino acids, tryptone (to a lesser degree peptone and yeast extract), glutamate (to a lesser degree glutamine), acetate, pyruvate (to a lesser degree succinate, lactate, propionate, glycerol, and ethanol), *n*-hexadecane, and *n*-tetradecane (to a lesser degree *n*-decane) are used as substrates for aerobic organotrophic growth. In media with yeast extract (0.3 g/L), xylose, formate, benzoate, phenol, and methanol are used as growth substrates. Glucose, fructose, galactose, sucrose, mannitol, γ-hydroxybutyrate, malate, citrate, urea, methane, propane, and methylamine do not support growth. Casein, gelatin, and starch are not hydrolyzed. Acids and H_2S are not produced. The organism does not fix molecular nitrogen.

The membrane polar lipids are glycerophosphatides: phosphatidylglycerol, diphosphatidylglycerol, a phosphoglycolipid, phosphatidylethanolamine, and phosphatidylcholine. Under favorable conditions of cultivation in complex media, the following fatty acids predominate (% of total FA): $C_{18:1}$ (29.7), $C_{18:0}$ (24.2), $C_{19:0cyclo}$ (20.4), $C_{16:0}$ (12.3), $C_{16:1}$ (7.0), and $C_{12:0}$ (3.9).

The DNA G+C content is 68.2 mol %.

The type strain is *Arhodomonas recens* RS91^T.

The organism was isolated from acidic solutions of flotation enrichment of potassium minerals, SKRU-3, Silvinit Co., Solikamsk, Russia.

The strain was deposited to the regional collection of alkane-utilizing microorganisms (www.iegm.ru/iegmcol) (=IEGM 796^T) and to the All-Russian Collection of Industrial Microorganisms (=VKPM B-11280^T).

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