ISSN 0026-2617, Microbiology, 2012, Vol. 81, No. 5, pp. 589–595. © Pleiades Publishing, Ltd., 2012.
Original Russian Text © A.I. Saralov, R.V. Baslerov, E.M. Reutskikh, B.B. Kuznetsov, 2012, published in Mikrobiologiya, 2012, Vol. 81, No. 5, pp. 638–644.

EXPERIMENTAL ARTICLES

Halarchaeum solikamskense sp. nov., a Thermotolerant Neutrophilic Haloarchaeon from the Foamy Products of Flotation Enrichment of Potassium Minerals

A. I. Saralov[a, 1], R. V. Baslerov[b], E. M. Reutskikh[a], and B. B. Kuznetsov[b]

[a] *Institute of Ecology and Genetics of Microorganisms, Ural Branch, Russian Academy of Sciences, Perm, Russia*
[b] *Bioengineering Center, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 1, Moscow, 117312 Russia*

Received December 23, 2011

Abstract—Three pigmented strains of halophilic archaea, RS94–RS96, were isolated from acidic foamy products of flotation enrichment of potassium minerals (Silvinit Co., Solikamsk, Russia). The cells were gram-negative, nonmotile, pleomorphic ovoids, 1.0–1.5 × 1.5–2.5 μm. The isolates were chemoorganotrophic, obligately aerobic, and catalase-positive. A range of carbohydrates and organic acids was used, as well as amino acids and peptides. The strains were halophiles and thermotolerant neutrophiles. They grew in the media with 15 to 30% NaCl (optimum at 20–22%) and 0.005–0.7 M Mg^{2+} (0.1–0.2 M), at pH 5.0–8.2 (optimum 7.0–7.2) and 25–55°C (optimum at 35–50°C). The major fatty acids were $C_{16:0}$, $C_{18:1}$, $C_{18:0}$, and $C_{16:1}$. The membranes contained carotenoid pigments of the bacterioruberin series and polar lipids, mostly as C_{20},C_{20} isoprenoid derivates: phosphatidylglyceromethylphosphate, phosphatidylglycerol, and three unidentified sulfated glycolipids of the S-DGD type. The DNA G+C content was 65.1–66.4 mol %. Phylogenetic analysis based on the 16S rRNA gene sequencing revealed that the thermotolerant neutrophilic isolate RS94 (DNA G+C content of 66.4 mol %) was most closely related to the nonpigmented moderate acidophile *Halarchaeum acidiphilum* MH1-52-1^T (97.3%). Based on its phenotypic and genotypic characteristics, the organism was classified as a new species of the genus *Halarchaeum* with the proposed name *Halarchaeum solikamskense* sp. nov. The type strain is RS94^T (= VKPM B-11282^T).

Keywords: Halarchaeum, new halophilic archaeon, technogenic brines

DOI: 10.1134/S0026261712050141

The family *Halobacteriaceae* of the order *Halobacteriales* is comprised of cultured aerobic, extremely halophilic archaea (halobacteria). As of 2001, it included representative species of the following genera: *Haloarcula, Halobacterium, Halobaculum*, *Halococcus, Haloferax, Halogeometricum, Halorubrum, Haloterrigena, Natrialba, Natrinema, Natronobacterium, Natronococcus, Natronomonas*, and *Natronorubrum* [1]. During the last decade, species of 14 more genera of halobacteria were described: *Haladaptatus, Halalkalicoccus, Halarchaeum, Halobiforma, Halomicrobium, Halopiger, Haloplanus, Haloquadratum, Halorhabdus, Halosarcina, Halosimplex, Halostagnicola, Halovivax*, and *Natronolimnobius* [2–15]. After revision of the type genus *Halobacterium*, new species were additionally described in 2004–2008 [16–18].

Investigation of the microflora of production divisions for flotation enrichment of potassium minerals (Silvinit Co., Solikamsk, Russia) resulted in isolation of ten halophilic strains. According to phylogenetic analysis of the 16S rRNA gene sequences of three pink haloarchaeal isolates obtained directly from the monoliths of motley sylvinite (type strain RS82), they exhibited high similarity (99.5–99.6%) to *Halobacterium jilantaiense* [17]. Gram-negative colorless motile isolates from mother brines, foamy products, and solid waste were classified as *Chromohalobacter salexigens* (99.2–99.6%) of the family *Halomonadaceae* and *"Arhodomonas recens"* sp. nov. of the family *Ectothiorhodospiraceae* [19]. Three pigmented neutrophilic isolates of halophilic archaea, RS94–RS96 (with RS94 as the type strain) were most closely related to the nonpigmented moderate acidophile *Halarcheum acidiphilum* (97.3–98.0%) [15].

The present work deals with an investigation of strains RS94–RS96 and RS82 and description of strain RS94^T as a new *Halarchaeum* species.

MATERIALS AND METHODS

Source of isolation and cultivation techniques. The source material from the SKRU-2 and SKRU-3 production divisions for flotation enrichment of potassium minerals (Silvinit Co.) and the major investigation techniques were described in our previous article [19]. Strains RS94 and RS96 were isolated from

[1] Corresponding author; e-mail: saralov@iegm.ru

acidic foamy products (pH 4.6) of the SRKU-3 and SKRU-2 divisions, respectively, while strain RS95 was isolated from mother brines of SKRU-2. For prolonged storage of the cultures (on agar slants in rubber-stoppered test tubes at 2–3°C), the complex medium was used, which provided for stable aerobic growth at 35–50°C and contained the following (g/L): agar (Difco), 20; NaCl, 200; $MgCl_2 \cdot 6H_2O$, 25; K_2SO_4, 5; $CaCl_2 \cdot 2H_2O$, 0.1; $NaH_2PO_4 \cdot 2H_2O$, 0.2; $Na_2HPO_4 \cdot 12H_2O$, 0.4; Na pyruvate, 2; $CH_3COONa \cdot 3H_2O$, 1.0; casein hydrolysate, 2.0; yeast extract, 1.0; Pfennig trace element solution, 1 mL/L; pH 7.1.

Strain RS82 was isolated from the inner part of a freshly removed piece of a massive aggregate of motley sylvinite with inclusions of halite and clay–anhydrite material. Sylvinite samples were collected in the SKRU-3 mine (250 m) in the B layer of the sylvinite–carnallite zone of the Permian Verkhnekamsk deposit. The isolate RS82 was maintained on the complex media which provided for rapid aerobic growth at 40°C and contained the following (g/L): agar (Difco), 20; NaCl, 180; KCl, 2; $MgSO_4 \cdot 7H_2O$, 20; $CaCl_2 \cdot 2H_2O$, 0.2; ampicillin, 0.5; casein hydrolysate, 5; yeast extract, 5; Na_3 citrate $\cdot 2H_2O$, 3; trace elements, 1 mL/L; pH 7.0–7.5.

Pigment composition of strains RS94–RS96 and RS82 was determined from absorption spectra (300–700 nm) obtained on a Schimadzu UV mini-1240 single-beam scanning spectrophotometer (Japan). The cell suspensions were concentrated by centrifugation, washed with 10% NaCl solution, and used for carotenoid extraction with acetone–methanol (1 : 1) or lysed in distilled water to reveal the hydrophilic chromoprotein complexes.

Polar lipids of the membranes were analyzed by one- and two-dimensional thin-layer chromatography (TLC). 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 until 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.

Molecular genetic investigation was carried out using the techniques described previously [19]. The fragments of the 16S rRNA genes of the halophilic archaea were amplified in a polymerase chain reaction (PCR) using the original primer system [20]. Amplification was carried out on a DNA Engine Tetrad 2 Peltier Thermal Cycler (Bio-Rad, United States). The purified PCR fragment was sequenced on a DNA Analyzer ABI 3730 automatic sequencer (Applied Biosystems, United States) using the BigDye v. 3.1 reagent kit. The sequence of the 16S rRNA gene of the archaeal isolates were deposited to GenBank under accession nos. JN227878, JN227879, JN227881, and JN227880 for strains RS94, RS95, RS96, and RS82, respectively.

RESULTS AND DISCUSSION

The cells of all investigated strains (RS94–RS96) were gram-negative, nonmotile, pleomorphic, of disk or ovoid shape, 1.0–1.5 × 1.5–2.5 μm (Fig. 1). The electron-dense, fibrillar nucleoid was present. Spores, dormant forms, gas vacuoles, and intracytoplasmic membranes were not formed. Growth occurred on media with 15 to 30% NaCl (with an optimum at 20–22%) and 0.05–0.7 M Mg^{2+} (optimum at 0.1–0.2 M), at pH 5.0–8.2 (optimum at 7.0–7.2), and 25–55°C (optimum at 35–50°C). The organisms were thermotolerant, with the growth rate changing insignificantly within the 35–50°C range. The strains were chemoorganotrophs, obligate aerobes, catalase-positive, and incapable of fermentation or anaerobic growth. A broad spectrum of organic compounds (a mixture of amino acids and peptides, organic acids, carbohydrates, and alcohols) was used as carbon and energy sources (Table 1). The strains were resistant to ampicillin (>500 μg) and streptomycin (>100 μg) and sensitive to rifampicin (5 μg) and novobiocin (30 μg).

On agar media, strains RS94–RS96 formed convex colonies with creamy, pink, or red coloration. Cell pigmentation depended significantly upon the NaCl and $MgCl_2$ levels in the medium. At NaCl concentrations close to the lower growth limit (150 g/L) and high concentration of $MgCl_2 \cdot 6H_2O$ (107 g/L), a significant amount of carotenoids was synthesized. In the acetone–methanol extract, intense absorption bands with the maxima at 472, 497, and 532 nm were observed (Table 2). This pronounced "red shift" of absorption maxima is only typical of carotenoids with a system of 13 conjugated binary bonds, e.g., C_{50} molecules of α-bacterioruberin and its derivatives [21, 22]. At Mg^{2+} deficiency and high Na^+ concentration, the content of C_{50} carotenoids decreased. In the absence of growth during prolonged storage on solid media at 2–20°C, the content of carotenoid pigments in the membranes of these halophilic archaea increased gradually. Under Mg^{2+} limitation in saline medium (1 g/L $MgCl_2$ and 240 g/L NaCl), a relatively intense absorption band at 320–430 nm with the maximum at ~372 nm was detected in suspensions of the membranes and cell walls in distilled water. This band is typical of chromoproteins, including bacteriorhodopsins of halophilic archaea.

Relatively low activity of the enzymes of fatty acid biosynthesis has been previously reported for

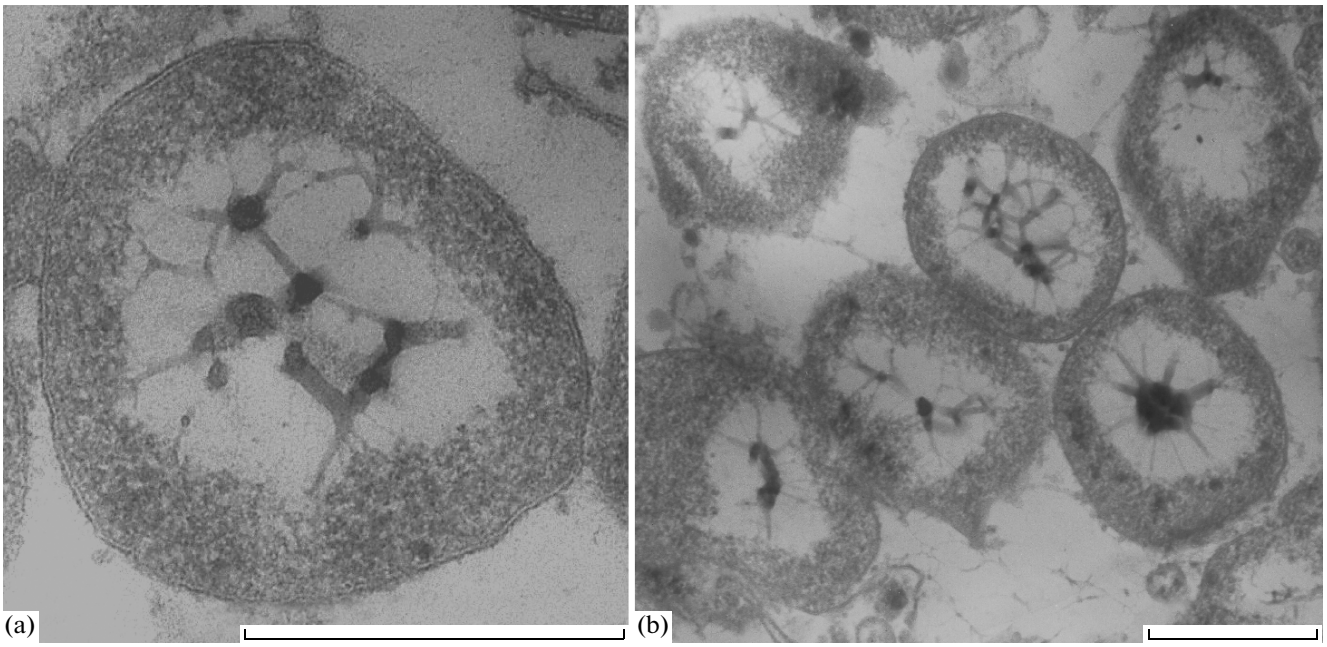

Fig. 1. Electron micrographs of ultrathin sections of the cells of strains RS94 (a) and RS96 (b). A typical gram-negative cell wall and an electron-dense fibrillar nucleoid are visible. Scale bar is 1 μm.

Natrinema pallirubrum (initially described as *Halobacterium cutirubrum* var. *proteolyticum*) [23, 24]. This phenomenon is associated with the inhibition of FA synthetases by high concentrations of NaCl and KCl accumulated in the cytoplasm of halobacterial cells. Strains RS94–RS96 and RS82, however, were capable of relatively active production of FA with even numbers of carbon atoms. Their composition varied depending on the cultivation conditions and was generally similar to that of the moderately halophilic gram-negative gammaproteobacterium *"Arhodomonas recens"* [19]. In strain RS94, under favorable growth conditions in complete medium, the predominant FA were (% of the total): hexadecanoic $C_{16:0}$ (32.6), *iso*-hexadecanoic $C_{16:0}$ (7.5), 11-octadecenoic $C_{18:1\omega7c}$ (15.3), 9-octadecenoic $C_{18:1\omega9c}$ (4.8), octadecanoic $C_{18:0}$ (17.2), 9-hexadecenoic $C_{16:1\omega7c}$ (11.3), tetradecanoic $C_{14:0}$ (4.7), and eicosanoic $C_{20:0}$ (1.8). At the temperature close to the lower growth limit (27–28°C), the content of saturated FA $C_{16:0}$ and $C_{18:0}$ decreased, while the ratio of the unsaturated $C_{18:1}$ increased.

Unlike FA and carotenoids, the composition of polar lipids of the membranes of the strains studied did not depend noticeably on the cultivation conditions. Unlike *"A. recens"*, they contained C_{20},C_{20} isoprenoid derivates: three unidentified sulfated glycolipids (S-GL) of the sulfated diglycosyl diether (S-DGD) type and phosphatidylglyceromethylphosphate (PGP-Me), but no nitrogen-containing phospholipids (phosphatidylethanolamine or phosphatidylcholine). Unlike strains RS94–RS96, strain RS82 contains phosphatidylglycerosulfate (PGS), triglycosyl diether (TGD-1), sulfated triglycosyl diether (S-TGD-1), and sulfated tetraglycosyl diether (S-TeGD).

High similarity of the 16S rRNA gene sequences (99.5%) of strain RS82 and *Halobacterium jilantaiense* (Table 2), together with the similar composition of the polar lipids and morphophysiological characteristics, suggested that it was probably a strain of this species [17]. Strains RS94–RS96 fell into an isolated subcluster within the genus *Halarchaeum* with the single validly reported species *Halarchaeum acidiphilum* [15], which was adjacent to the group of the *Halobacterium* type species (Fig. 3). The similarity of the 16S rRNA gene sequence of strain RS94^T to the sequences of the taxonomically unidentified uncultured Dead Sea clone Halophilic archaeon MH1-136-2, RS96, RS95, *Halarchaeum acidiphilum* MH1-52-1^T, *Halobacterium jilantaiense* RS82, and *Haloarcula japonica* was 100.0, 99.8, 98.6, 97.3, 91.4, and 89.0%, respectively (Table 2). The DNA G+C content of strains RS94, RS96, RS95, *Hb. jilantaiense* RS82, and *H. acidiphilum* MH1-52-1^T was 66.4, 66.3, 65.1, 64.3, and 61.4 mol %, respectively. Unlike the moderately thermophilic acidophile *H. acidiphilum*, strains RS94–RS96, were neutrophilic, thermotolerant, capable of production of catalase, carotenoids, and cellular fatty acids, and exhibited significant morphological and metabolic differences from *Hb. jilantaiense* RS82 (Table 1). Based on their phenotypic and genotypic characteristics, strains RS94–RS96 were classified as members of the genus *Halarchaeum* within the family *Halobacteriaceae* as a new species with the proposed

Table 1. Differentiating characteristics of strain RS94 and members of the genera *Halarchaeum* and *Halobacterium*

Characteristics	Strain RS94	*Halarchaeum acidiphilum* MH1-52-1^T	*Halobacterium jilantaiense* RS82
Cell size, μm	1.0–1.5 × 1.5–2.5	1.5–2.0 × 2.0–2.5	0.5–1.0 × 1.0–4.0
Motility	–	–	+
Range (optimum):			
NaCl, %	15–30 (20–22)	18–30 (21–24)	15–30 (18–21)
Mg, M	0.005–0.7 (0.1–0.2)	0.001–0.5 (0.05)	0.01–0.4 (0.1–0.2)
Temperature, °C	25–55 (35–50)	15–45 (37)	20–55 (40)
pH	5.0–8.2 (7.0–7.2)	4.0–6.0 (4.4–4.5)	6.0–8.5 (7.0–7.5)
Anaerobic growth	–	–	+
NO_2^- from NO_3^-	–	–	+
Hydrolysis of casein, gelatin	–	–	+
Oxidase	–	–	+
Catalase	+	–	+
Carotenoids	+	–	+
Oxidized substrates:			
Citrate	+	–	+
Acetate, lactate, malate, pyruvate	+	ND	+
Casein hydrolysate, tryptone	+	+	+
Glucose, ylose, fructose	+	+	+
Major membrane polar lipids	PG, PGP-Me, S-GL	PG, PGP-Me, S-GL	PG, PGP-Me, PGS, TGD, S-TGD, S-TeGD
Major cellular fatty acids (% of the totalFA)	$C_{16:0}$ (40), $C_{18:1}$ (20), $C_{18:0}$ (17), $C_{16:1}$ (11)	ND	$C_{16:0}$ (56), $C_{18:1}$ (23), $C_{18:0}$ (12), $C_{16:1}$ (6)
DNA G+C content, mol %	66.4	61.4	64.3

Note: "+" indicates positive results, "–" indicates negative results, ND stands for no data. PG, PGP-Me, S-GL, PGS, TGD, S-TGD, S-TGD, and S-Te-GD stand for phosphatidylglycerol, phosphatidylglyceromethylphosphate, sulfated glycolipid, phosphatidylglycerosulfate, triglycosyl diether, sulfated triglycosyl diether, and sulfated tetraglycosyl diether, respectively.

name *Halarchaeum solikamskense* sp. nov. (type strain RS94^T).

Description of *Halarchaeum solikamskense* sp. nov.

Halarchaeum solikamskense (soli. kam. sk. en'se, N.L. neut. adj., *solikamskense*, from the town Solikamsk).

The colonies are pigmented (creamy to red), convex, round, and up to 1 mm in diameter. The cells are gram-negative, nonmotile, pleomorphic, discoid and ovoid, 1.0–1.5 × 1.5–2.5 μm. Spores, dormant cells, and gas vacuoles are not formed.

The organism is extremely halophilic, grows in the presence of 15–30% NaCl (optimum at 20–22%), 0.01–20% KCl (optimum at 0.2–0.4%), or 0.005–0.7 M Mg^{2+} (optimum at 0.1–0,2 M); the cells are lysed in distilled water. It is thermotolerant, growing within the temperature range from 25 to 55°C (optimum at 40–45°C), with the growth rate practically unchanging at the temperature increase from 35 to 50°C. The organism is neutrophilic, growing at pH 5.0–8.2 (optimum at 7.0–7.2).

The organism is a chemoorganotroph, obligate aerobe, and catalase-positive. The oxidase reaction is negative. Nitrates are not reduced to nitrites. No aerobic growth occurs with fumarate, nitrate, dimethyl sulfoxide, or thiosulfate. H_2S is not produced. The organism grows on a mixture of amino acids and peptides and, to a lesser extent, in synthetic media with individual substrates (acetate, lactate, malate, pyruvate, succinate, glycerol, galactose, glucose, xylose, sucrose, and fructose). The highest yield and growth rate occur in a complex saline medium containing pyruvate, acetate, casein hydrolysate, and yeast extract. Arginine, glutamate, citrate, and ethanol support growth in media with 0.1 g/L yeast extract. Methanol, formate, methylamine, benzoate, and hexadecane do not support growth. Casein, gelatin, urea, starch, and Tween 80 are not hydrolyzed. Dinitrogen is not fixed. The organism is sensitive to rifampicin

Table 2. Similarity between 16S rRNA gene sequences of the studied *Halarchaeum* strains (RS94, RS95, RS96) and the physiologically related species and isolates

No.	Strain	1	2	3	4	5	6	7	8	9	10	11
1	*Haloarcula japonica* JCM 7785^T (AB355986)	1.000										
2	*Halarchaeum* RS94 (JN227878)	0.890										
3	*Halarchaeum* RS95 (JN227879)	0.888	0.987									
4	*Halarchaeum* RS96 (JN227881)	0.890	0.998	0.986								
5	*Halobacterium jilantaiense* RS82 (JN227880)	0.894	0.914	0.914	0.913							
6	*Halobacterium noricense* DSM 15987^T (AJ548827)	0.900	0.913	0.915	0.913	0.975						
7	*Halobacterium jilantaiense* JCM 13558^T (AB477970)	0.896	0.915	0.915	0.914	0.995	0.974					
8	*Halobacterium salinarum* DSM 3754^T (AJ496185)	0.897	0.913	0.911	0.912	0.983	0.970	0.982				
9	*Halobacterium piscisalsi* HPC1-2^T (AB285020)	0.884	0.900	0.899	0.900	0.970	0.958	0.970	0.987			
10	*Halarchaeum acidiphilum* MH1-52-1^T (AB371717)	0.887	0.973	0.980	0.973	0.911	0.915	0.911	0.911	0.900		
11	Halophilic archaeon MH1-136-2 (AB372515)	0.890	1.000	0.987	0.998	0.914	0.913	0.915	0.913	0.900	0.973	
12	*Halarchaeum* sp. HY-204-1 (AB550130)	0.890	0.966	0.970	0.965	0.923	0.925	0.923	0.917	0.905	0.963	0.966

(5 μg) and novobiocin (30 μg) and resistant to ampicillin (>500 μg), gentamycin (100 μg), streptomycin (>100 μg), and ciprofloxacin (30 μg).

The membrane polar lipids are mainly C_{20}, C_{20} isoprenoid derivates: phosphatidylglyceromethylphosphate, phosphatidylglycerol, and three unidentified sulfated glycolipids of the sulfated diglycosyl diether type. The purple membranes contained carotenoids of the bacterioruberin series and chromoproteins. The following fatty acids predominate (% of total FA):

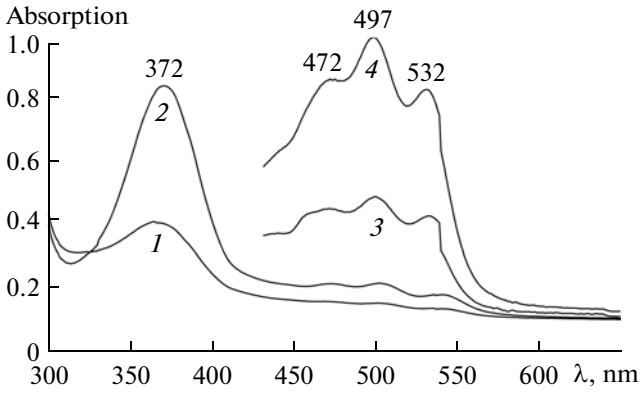

Fig. 2. Effect of Na^+ and Mg^{2+} ions on the pigment composition of strain RS94. Absorption spectra of disintegrated cells in distilled water (*1*, *2*) and of acetone–methanol extracts (*3*, *4*). The numerals indicate absorption maxima, nm. The complex medium contained 150 g/L NaCl and 50 g/L $MgCl_2$ (*1*, *4*) or 240 g/L NaCl and 1 g/L $MgCl_2$ (*2*, *3*).

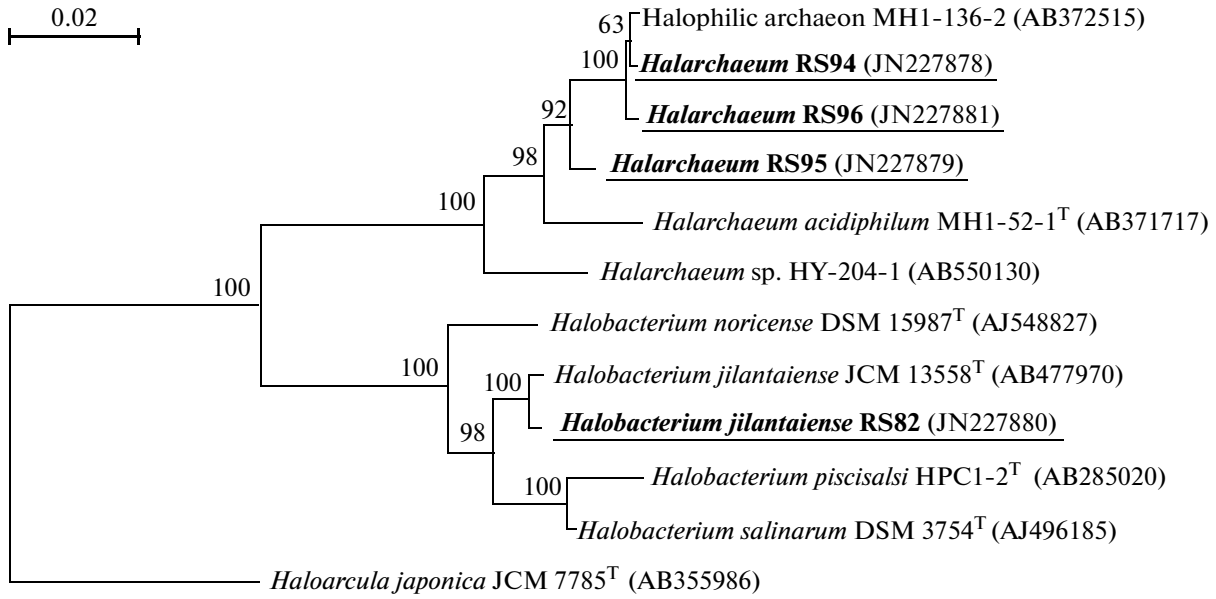

Fig. 3. Phylogenetic position of strains RS82 and RS94–RS96 among members of *Halarchaeum* and *Halobacterium*. The tree was constructed with 1301 nucleotide sequences of the 16S rRNA gene using the neighbor-joining method. The numerals indicate reliability of the branching order determined by bootstrap analysis of 1000 alternative trees. Scale bar is 2 replacements per 100 nucleotides. The sequence of the type strain of *Haloarcula japonica* was used as the outgroup.

$C_{16:0}$ (40), $C_{18:1}$ (20), $C_{18:0}$ (17), $C_{16:1}$ (11), $C_{14:0}$ (5), and $C_{20:0}$ (2).

The DNA G+C content is 65.1–66.4 mol %.

The type strain is *Halarchaeum solikamskense* RS94^T (DNA G+C content, 66.4 mol %).

The organism was isolated from the foamy products of flotation enrichment of potassium minerals, SKRU-3, Silvinit Co., Solikamsk, Russia.

The strain was deposited in the All-Russian Collection of Industrial Microorganisms (=VKPM B-11282^T).

ACKNOWLEDGMENTS

The work was supported by the Molecular and Cell Biology program of the Presidium of the Russian Academy of Sciences no. 22, project no. 01200963684, and the state contract no. 16.552.11.7035 of the Russian Ministry of Education and Science.

REFERENCES

1. Grant, W.D., Kamekura, M., McGenity, T.J., and Ventosa, A., Order I. *Halobacteriales*, in *Bergey's Manual of Systematic Bacteriology, 2nd ed.*, Boone, D.R., Castenholz, R.W., and Garritty, G.M., Eds., New York: Springer, 2001, vol. 1, pp. 294–334.
2. Wainø, M., Tindall, B.J., and Ingvorsen, K., *Halorhabdus uthahensis* gen. nov., sp. nov., an Aerobic, Extremely Halophilic Member of the *Archaeae* from Great Salt Lake, Utah, *Int. J. Syst. Evol. Microbiol.*, 2000, vol. 50, pp. 183–190.
3. Oren, A., Elevi, R., Watanabe, S., Ihara, K., and Corcelli, A., *Halomicrobium mukohataei* gen. nov., comb. nov., and Emended Description of *Halomicrobium mukohataei, Int. J. Syst. Evol. Microbiol.*, 2002, vol. 52, pp. 1831–1835.
4. Hezayen, F.F., Tindall, B.J., Steinbüchel, A., and Rehm, B.H.A., Characterization of a Novel Halophilic Archaeon, *Halobiforma haloterrestris* gen. nov., sp. nov., and Transfer *Natronobacterium nitratireducens* to *Halobiforma nitratireducens* comb. nov., *Int. J. Syst. Evol. Microbiol.*, 2002, vol. 52, pp. 2271–2280.
5. Vreeland, R.H., Straight, S., Krammes, J., Dougherty, K., Rosenzweig, W.D., and Kamekura, M., *Halosimplex carlsbadense* gen. nov., sp. nov., a Unique Halophilic Archaeon, with Three 16S rRNA Genes, that Grows Only in Defined Medium with Glycerol and Acetate or Pyruvate, *Extremophiles*, 2002, vol. 6, pp. 445–452.
6. Itoh, T., Yamaguchi, T., Zhou, P., and Takashina, T., *Natronolimnobius baerhuensis* gen. nov., sp. nov. and *Natronolimnobius innermongolicus* sp. nov., Novel Haloalkaliphilic Archaea Isolated from Soda Lakes in Inner Mongolia, China, *Extremophiles*, 2005, vol. 9, pp. 111–116.
7. Xue, Y., Fan, H., Ventosa, A., Grant, W.D., Jones, B.E., Cowan, D.A., and Ma, Y., *Halalkalicoccus tibetensis* gen. nov., sp. nov., Representing a Novel Genus of Haloalkaliphilic Archaea, *Int. J. Syst. Evol. Microbiol.*, 2005, vol. 55, pp. 2501–2505.
8. Castillo, A.M., Gutiérrez, M.C., Kamekura, M., Ma, Y., Cowan, D.A., Jones, B.E., Grant, W.D., and Ventosa, A., *Halovivax asiaticus* gen nov., sp. nov., a Novel Extremely, Halophilic Archaeon Isolated from Inner Mongolia, China, *Int. J. Syst. Evol. Microbiol.*, 2006, vol. 56, pp. 765–770.

9. Castillo, A.M., Gutiérrez, M.C., Kamekura, M., Ma, Y., Cowan, D.A., Jones, B.E., Grant, W.D., and Ventosa, A., *Halostagnicola larsenii* gen. nov., sp. nov., an Extremely Halophilic Archaeon from a Saline Lake in Inner Mongolia, China, *Int. J. Syst. Evol. Microbiol.*, 2006, vol. 56, pp. 1515–1517.
10. Savege, K.N., Krumholz, L.R., Oren, A., and Elshahed, M.S., *Haladaptatus paucihalophilus* gen. nov., sp. nov. a Halophilic Archaeon Isolated from a Low-Salt, Sulfide-Rich Spring, *Int. J. Syst. Evol. Microbiol.*, 2007, vol. 57, pp. 19–24.
11. Burns, D.G., Janssen, P.H., Itoh, T., Kamekura, M., Li, Z., Jensen, G., Rodriguez-Valera, F., Bolhius, H., and Dyall-Smith, M.L., *Haloquadratum walsbyi* gen. nov., sp. nov., the Square Haloarchaeon of Walsby, Isolated from Saltern Crystallizers in Australia and Spain, *Int. J. Syst. Evol. Microbiol.*, 2007, vol. 57, pp. 387–392.
12. Bardavid, R.E., Mana, L., and Oren, A., *Haloplanus natans* gen. nov., sp. nov. an Extremely Halophilic, Gas-Vacuolate Archaeon Isolated from Dead Sea–Red Sea Water Mixtures in Experimental Outdoor Ponds, *Int. J. Syst. Evol. Microbiol.*, 2007, vol. 57, pp. 780–783.
13. Gutiérrez, M.C., Castillo, A.M., Kamekura, M., Xue, Y., Ma, Y., Cowan, D.A., Jones, B.E., Grant, W.D., and Ventosa, A., *Halopiger xanaduensis* gen nov., sp. nov., an Extremely Halophilic Archaeon Isolated from Saline Lake Shangmatala in Inner Mongolia, China, *Int. J. Syst. Evol. Microbiol.*, 2007, vol. 57, pp. 1402–1407.
14. Savege, K.N., Krumholz, L.R., Oren, A., and Elshahed, M.S., *Halosarcina palluda* gen. nov., sp. nov., Halophilic Archaeon from a Low-Salt, Sulfide-Rich Sping, *Int. J. Syst. Evol. Microbiol.*, 2008, vol. 58, pp. 856–860.
15. Minegishi, H., Echigo, A., Nagaoka, S., Kamekura, M., and Usami, R., *Halarchaeum acidiphilum* gen. nov., sp. nov., a Moderately Acidiphilic Halarchaeon Isolated from Commercial Solar Salt, *Int. J. Syst. Evol. Microbiol.*, 2010, vol. 60, pp. 2513–2516.
16. Gruber, C., Legat, A., Pfaffenhuemer, M., Radax, C., Weidler, G., Busse, H.-J., and Stan-Lotter, H., *Halobacterium noricense* sp. nov., an Archaeal Isolate from a Bore Core of Alpine Permian Salt Deposit, Classification of *Halobacterium* sp. NRC-1 as a Strain of *H. salinarum* and Emended Description of *H. salinarum, Extremophiles*, 2004, vol. 8, pp. 431–439.
17. Yang, Y., Cui, H.-L., Zhou, P.-J., and Liu, S.-J., *Halobacterium jilantaiense* sp. nov., a Halophilic Archaeon Isolated from a Saline Lake in Inner Mongolia, China, *Int. J. Syst. Evol. Microbiol.*, 2006, vol. 56, pp. 2353–2355.
18. Yachai, M., Tanasupawat, S., Itoh, T., Benjakul, S., Visessanguan, W., and Valyasevi, R., *Halobacterium piscisalsi* sp. nov., from Fermented Fish (*Pla-Ra*) in Thailand, *Int. J. Syst. Evol. Microbiol.*, 2008, vol. 58, pp. 2136–2140.
19. Saralov, A.I., Kuznetsov, B.B., Reutskikh, E.M., Baslerov, R.V., Panteleeva, A.R., and Suzina, N.E., *Arhodomonas recens* sp. nov., a Halophilic Alkane-Utilizing Hydrogen-Oxidizing Bacterium from the Brines of Flotation Enrichment of Potassium Minerals, *Mikrobiologiya*, 2012, vol. 81, no. 5, pp. 582–587.
20. Kolganova, T.V., Kuznetsov, B.B., and Turova, T.P., Designing and Testing Oligonucleotide Primers for Amplification and Sequencing of Archaeal 16S rRNA Genes, *Microbiology*, 2002, vol. 71, no. 2, pp. 243–246.
21. Kushwaha, S.C., Kramer, J.K.G., and Kates, M., Isolation and Characterization of C_{50} Carotenoid Pigments and Other Polar Isoprenoids from *Halobacterium cutirubrum, Biochim. Biophys. Acta*, 1975, vol. 398, pp. 303–318.
22. Takaichi, S., Characterization of Carotenes in a Combination of a C_{18} HPLC Column with Isocratic Elution and Absorption Spectra with a Photodiode-Array Detector, *Photosynth. Res.*, 2000, vol. 65, pp. 93–99.
23. Pugh, E.L., Wassef, M.K., and Kates, M., Inhibition of Fatty Acid Synthetase in *Halobacterium cutirubrum* and *Escherichia coli* by High Salt Concentration, *Can. J. Biochem.*, 1971, vol. 49, no. 8, pp. 953–958.
24. Pugh, E.L. and Kates, M., Acylation of Proteins of the Archaebacteria *Halobacterium cutirubrum* and *Methanobacterium thermoautotrophicum, Biochim. Biophys. Acta. Biomembranes,* 1994, vol. 1196, no. 1, pp. 38–44.