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ORIGINAL PAPER

# *Desulfonatronum zhilinae* sp. nov., a novel haloalkaliphilic sulfate-reducing bacterium from soda Lake Alginskoe, Trans-Baikal Region, Russia

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**Abstract** A novel haloalkaliphilic sulfate-reducing bacterium, designated Al915-01$^T$, was isolated from benthic sediments of the Lake Alginskoe, a soda lake located in the Trans-Baikal Region, Russia. Cells of the strain were Gram-stain negative, motile, non-spore-forming vibrion (0.4–0.5 × 1.2–2.3 μm). Strain Al915-01$^T$ grew in the pH range from 8.0 to 10.5 (optimum pH 9.0) and required NaCl for growth (5–100 g $l^{-1}$ NaCl, optimum 40 g $l^{-1}$). The bacterium grew at 10–40 °C (optimally at 36 °C) and used lactate, formate and pyruvate as electron donors in the presence of sulfate. It was able to reduce sulfate, sulfite, thiosulfate and nitrate with lactate as an electron donor. The isolate was able to grow lithoheterotrophically with sulfate and molecular hydrogen if acetate was added as a carbon source. The predominant fatty acids were anteiso$C_{15:0}$, iso$C_{17:1}$, $C_{18:1\omega7}$ and $C_{16:1\omega7}$. The G+C content in the DNA was 58.3 ± 1 mol %. Analysis of the 16S rRNA gene sequence showed that the new bacterium belongs to the genus *Desulfonatronum*. The closest relatives were *Desulfonatronum buryatense* Ki5$^T$ (99.9 % similarity) and *Desulfonatronum lacustre* Z-7951$^T$ (99.2 % similarity). On the basis of the genotypic, phenotypic and phylogenetic characteristics, the isolate is proposed as a representative of a novel species *Desulfonatronum zhilinae* with the type strain Al915-01$^T$ (=VKM B-2744$^T$ = DSM 26338$^T$).

**Keywords** Soda lakes · Sulfate-reducing bacteria · Haloalkaliphilic · Sulfidogenesis · *Desulfonatronum* sp.

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## Introduction

In the eastern region of the Lake Baikal there are numerous shallow lakes that completely dry up during the summer season. These soda lakes are distinguished by alkaline pH and a wide range of salinity: from a low-level salinity up to saturation (Namsaraev and Namsaraev 2007). Studies of low and moderately saline soda lakes have shown presence of microbial communities performing complete biogeochemical cycles of the main biogenic elements (Zavarzin et al. 1999; Sorokin et al. 2011a). However, in Trans-Baikal soda lakes, these biogeochemical processes have not been fully described.

In moderately saline and brackish soda lakes, the final stage of decomposition of organic matter is conducted by sulfate-reducing bacteria (SRB) (Zavarzin et al. 1996; Gorlenko et al. 1999). Since the discovery of the obligately alkaliphilic SRB (Zhilina and Zavarzin 1994), the number of their taxa has increased, and currently four genera within the orders *Desulfovibrionales* and *Desulfobacteriales* have been described (Kuever et al. 2005a, b).

One species of the obligately alkaliphilic and moderately thermophilic SRB also has been described within the genus *Desulfotomaculum*, *D. alkaliphilum* (Pikuta et al. 2000). It is the first spore-forming alkaliphilic SRB with very narrow pH range; its ecology is not affiliated with soda lakes but rather with farming and man-made ecosystems.

The genus *Desulfonatronum* originally described by Pikuta, Zhilina and co-workers (1998) is the most numerous and physiologically diverse. The phylogenetic position (analysis performed by Dr. Fred Rainey) of the genus *Desulfonatronum* proved it to be very distant from all known bacterial species, suggesting the existence of the separate family *Desulfonatronaceae* (Kuever et al. 2005c). All *Desulfonatronum* species were isolated from hyper- and hypo-saline soda lakes from different continents. The type species of the genus, *Desulfonatronum lacustre*, is a litho-heterotrophic low-salinity alkaliphile isolated from athalassic soda lake Khadyn in Tuva, Siberia, Russia (Pikuta et al. 1998). Later, the lithoautotrophic species *D. thiodismutans* with thalassic salinity optimum has been described from Mono Lake in California (Pikuta et al. 2003). The third species of this genus *D. cooperativum* was isolated from an acetate-utilizing enrichment of the Lake Khadyn. This species has a wider pH range of 6.7–10.3 (Zhilina et al. 2005). The next two species, *D. thioautotrophicum* and *D. thiosulfatophilum*, were isolated from lakes of the Kulunda Steppe in the Altai Region of Russia (Sorokin et al. 2011b).

All strains of the genus *Desulfonatronum* obligately require $Na^+$, $Cl^-$, and $HCO_3^-$ ions and therefore they all are obligate alkaliphiles. The species described as *D. buryatense* (Ryzhmanova et al. 2013) was isolated from alkaline brackish Lake Solenoe in Buryatia, Russia; this species is able to reduce Fe(III). The last species described was *D. alkalitolerans* (Sorokin et al. 2013). It was isolated from the microbial consortia of a bioreactor in the Netherlands, used to remove $H_2S$ from biogas under microaerophilic conditions.

In this article, we describe a novel alkaliphilic SRB capable of nitrate reduction.

## Methods

### Site and source of isolation

The shallow Lake Alginskoe (53°37′N, 109°38′E) is a brackish eutrophic lake with sulfate–sodium–bicarbonate type of water. The measured concentration of sulfate ions in the water reached 25.7 g $l^{-1}$, the total concentration of sodium and potassium ions was 15.7 g $l^{-1}$, carbonate and bicarbonate ions were determined to be in the concentration range of 3.0–4.8 g $l^{-1}$, respectively, and the chlorine ion content was low (0.03 g $l^{-1}$). The water samples had a pH of 9.6 and a salinity of 45 g $l^{-1}$. Sediment samples were collected from the lake during the 2005 expedition.

### Media and cultivation conditions

Enrichment and isolation were performed using anaerobic techniques (Hungate 1969). The basal growth medium (MI) contain (g $l^{-1}$): $Na_2CO_3$, 1.2; $NaHCO_3$, 1.85; NaCl, 15; $K_2HPO_4$, 0.5; $MgCl_2{\cdot}6H2O$, 0.1; $Na_2SO_4$, 3.0; $Na_2S{\cdot}9H_2O$, 0.5; yeast extract (Difco), 0.2; Pfennig's trace element solution (Pfennig 1965), 1 ml. $MgCl_2{\cdot}6H_2O$, $Na_2SO_4$ and $Na_2S{\cdot}9H_2O$ were added to the medium after autoclaving (Sorokin et al. 2008). The final pH was adjusted to 9.6. A mixture of substrates, sodium lactate and sodium acetate, at the final total concentration of 20 mM, served as a carbon source. High-purity nitrogen was used as the gas phase. To obtain enrichment cultures, a 1.0-g wet sediment material was injected into standard Hungate tubes with MI medium. The tubes were incubated at 30 °C for 14 days. A pure culture was obtained by the dilution method in the sulfate-containing medium MI with lactate as an electron donor. Culture purity was assessed by observing the uniform cell types with the help of phase-contrast microscopy and by the absence of growth in the MI medium with 1 g glucose and 1 g peptone. The pure culture was maintained at 36 °C in modified MI medium (with 40 g $l^{-1}$ NaCl and 20 mM lactate). Growth of colonies was checked by the 'roll-tube' method using 2 % (w/v) agar medium, where carbonates were added separately after autoclaving. Reference strains *Desulfonatronum buryatense* Ki5$^T$, VKM B-2477$^T$ and *Desulfonatronum lacustre* Z-7951$^T$, DSM 10312$^T$ were grown in SA medium (Ryzhmanova et al. 2013) and DSMZ 813 medium, respectively.

### Effects of pH, temperature, and NaCl

Kinetic parameters of growth were determined in sulfate-containing medium MI with 20 mM lactate at different temperatures (4, 10, 20, 23, 30, 36, 40 and 47 °C), pH values (7.0, 8.0, 8.5, 9.0, 9.3, 10.0, and 10.5), and NaCl concentrations (0, 5, 10, 40, 60, 100, 150 and 200 g $l^{-1}$). To study the pH dependence, the following buffer solutions were used: pH 7.0 (50 ml 0.1 M $KH_2PO_4$, 29.1 ml 0.1 M NaOH; water to 100 ml); pH 8.0 and 8.5 (7.5 g $l^{-1}$KCl, 6.2 g $l^{-1}$ $H_3BO_3$, 3.9 ml 0.1 M NaOH and 7.5 g $l^{-1}$ KCl, 6.2 g $l^{-1}$ $H_3BO_3$, 10.1 ml 0.1 M NaOH, respectively); pH 9.0 (12.5 g $l^{-1}$ $NaHCO_3$, 2.0 g $l^{-1}$ $Na_2CO_3$); pH 9.3 (1.85 g $l^{-1}$ $NaHCO_3$, 1.2 g $l^{-1}$ $Na_2CO_3$); pH 10.0 (2.76 g $l^{-1}$ $NaHCO_3$, 1.84 g $l^{-1}$$Na_2CO_3$); pH 10.5 (1.5 g $l^{-1}$ $Na_2CO_3$). Sterile buffer solutions were added to the medium before inoculation. The dependence of growth on NaCl content was determined on the medium MII of the following composition (g $l^{-1}$): $K_2CO_3$, 1.2; $KHCO_3$, 1.85; $K_2HPO_4$, 0.5; $MgSO_4{\cdot}7H_2O$, 0.1; $(NH_4)_2SO_4$, 3.0; yeast extract (Difco), 0.2; Pfenning's trace element solution (Pfennig 1965), 1 ml. The was adjusted to pH 9.6 with 10 % KOH. NaCl at concentrations from 0 to 200 g $l^{-1}$ was added separately to each vial with the medium before inoculation. To examine the need of $Na^+$ for growth, NaCl was substituted by KCl (15 g $l^{-1}$). The dependence on $Cl^-$ was investigated

on medium MI, without NaCl. The effect of carbonates was determined by replacing them with the equimolar amounts of $Na_2SO_4$ and maintaining the pH with 50 mM 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS). All the tests were done in triplicate and confirmed by growth with two subsequent transfers.

### Growth measurements

Growth was assessed by measuring the changes in $OD_{560}$, by direct cell counting under a phase-contrast microscope, and by hydrogen sulfide production.

### Microscopy methods

Morphology and ultrathin structure were examined using phase-contrast microscopy (Olympus BX41) at 1300× magnification and a Jeol JEM-100C electron microscope (Japan). Preparations for electron microscopy were contrasted with 0.2 % aqueous uranyl acetate solution. Ultrathin sections were prepared from cells collected by centrifugation, fixed according to Ryter et al. (1958), dehydrated, and embedded in Epon; an LKB ultramicrotome (Sweden) was used. The sections were placed on Formvar-coated copper grids and contrasted according to Reynolds (1963).

Gram staining was determined by the standard protocol (Smibert and Krieg 1994).

### Electron donors and acceptor utilization

To study possible sources of energy, electron donors were tested with 20 mM sulfate as a terminal electron acceptor, and electron acceptors were tested with 20 mM sodium lactate as an electron donor. To test different electron donors, sodium lactate was replaced with volatile fatty acids, amino acids, sugars, alcohols [final concentration, 0.12 % (w/v)]. To search for possible electron acceptors, $Na_2SO_4$ was replaced with $Na_2SO_3$, 10 mM; $NaNO_3$, 10 mM; $Na_2S_2O_3{\cdot}5H_2O$, 20 mM; $S^o$ 2 g $l^{-1}$; dimethyl sulfoxide (DMSO), 2 ml $l^{-1}$. $Fe^{3+}$ (90 mmol $l^{-1}$) was added as amorphous iron (III) oxide, prepared by titration of acidic $FeCl_3$ solution with 10 % (w/v) NaOH to pH 7.0 (Lovley et al. 1993). All the tests were in triplicate and confirmed by two transfers.

### Analytical methods

Products of lactate oxidation in the culture medium were assayed with an HPLC system (Knauer, Germany). The analytical column was Inertsil ODS-3 (5 μm, 250 × 4.6 mm; Dr. Maisch GmbHs Germany). Chromatography was carried out in 20 mM $H_3PO_4$ at 210 nm, at a temperature of 35 °C and a pressure of 130 bar, resulting in an eluent flow rate of 1.0 ml per min. The products were identified using standard solutions of acids 1 g $l^{-1}$ (Sigma-Aldrich, USA) according to a retention time. Acetate concentration in the samples was calculated from height and peak area using EuroChom software, v. 3.05 P5 (Knauer GmbH, Germany).

Sulfide was measured by the Pachmayr method (Cline 1969). Nitrite was analyzed according to Griess-Romijn van Eck (1966). Reduction of Fe(III) was determined as described by Lovley and Phillips (1986).

### Lipid analysis

Cellular fatty acids were determined using strain Al915-$01^T$, *D. buryatense* $Ki5^T$ and *D. lacustre* $Z\text{-}7951^T$ cells grown at optimal temperature and pH in medium MII and harvested during the late exponential growth phase. Comparative analysis of fatty acid methyl esters performed using the Sherlock MIS (MIDI Inc. Delaware, USA) system was carried out by Dr Y. Osipov in Bakulev Centre of Cardiovascular Surgery, Moscow, Russia. Lipids were extracted from the cell biomass (3–5 mg dry cells) by acid methanolysis. Fatty acid methyl esters and other lipid components were extracted twice with 200 μl of hexane. The extract was dried, treated with 20 μl of *N*,*O*-*bis*(trimethylsilyl)trifluoroacetamide at 80 °C for 15 min to form trimethylsilyl esters of hydroxy acids. A 2-μl sample of the reaction mixture was analyzed in the automatic mode. Substances ambiguously determined by their retention times in the Sherlock MIS system were identified on an AG-5973 gas chromatograph–mass spectrometer system (Agilent Technologies, USA). Separation was done on a capillary column (25 m × 0.25 mm) covered with the HP-5ms Hewlett-Packard chemically bound methyl silicon immobile phase (layer thickness, 0.2 μm). Chromatography was conducted in the temperature programming mode from 130 up to 320 °C at a rate of 5°/min. The data were processed using the instruments' standard software.

### DNA base composition and DNA–DNA hybridization

DNA was isolated from cell biomass according to Marmur (1961). DNA–DNA hybridization (four replications) was performed as described by De Ley et al. (1970) and modified by Huß et al. (1983) using a PyeUnicam SP 1800 spectrophotometer equipped with a thermoprogrammer and hermetically sealed thermocuvettes. The standard deviations of the hybridization experiments were between 5.5 and 9.0 %.

### Phylogenetic analysis

For determination of the 16S rRNA gene sequence, genomic DNA was isolated by standard methods (Sambrook et al. 1989). The 16S rRNA gene was amplified

using universal primers, 11F and 1492R (Lane 1991). The PCR product was purified using a Wizard PCR Preps DNA Purification System. The sequencing reactions were performed using a CEQ Dye Terminator Cycle Sequencing kit according to the protocols provided by the manufacturer and analyzed in a Beckman Coulter CEQ 2000 XL automatic DNA sequencer. The NCBI GenBank BLAST utility (Altschul et al. 1997; Benson et al. 1998) was used to reveal the closest relatives of strain Al915-01$^T$. The Neighbor-Joining (NJ) tree was constructed according to the Jukes–Cantor model (Jukes and Cantor 1969), and this tree was used as the base for the consensus tree. For the construction of the minimum evolution (ME) tree, the Jukes–Cantor substitution model was also used, while the maximum composite likelihood (MCL) tree was created with the MCL model. The percentages of replicate trees where associated taxa formed the same clusters were calculated from 1000 replicates using the bootstrap test (Felsenstein 1985). The nucleotide sequence of strain Al915-01$^T$ was deposited in the GenBank under accession number JX984981.

## Results

### Enrichments and isolation

In the bottom sediment sampled for the study, the number of the sulfate-reducers in media with lactate, formate or acetate reached $10^5$, $10^4$ and $10^6$ cells per gram, respectively. Our attempts to obtain an SRB pure culture capable to oxidize acetate in alkaline conditions failed despite the highest number of SRB observed in the enrichment culture with acetate as an electron donor. The isolation procedure using lactate as a growth substrate was successful.

After tenfold serial dilutions, the cultures were transferred to agar containing medium in Hungate tubes. The colonies appearing on the solid medium surface after 4–5 weeks were shiny, white, smooth, convex, even-edged and 1–2 mm in diameter. After serial transfer of the single colonies from the solid to liquid medium, a pure culture of SRB called strain Al915-01$^T$ was isolated.

### Cell morphology

Cells of strain Al915-01$^T$ were highly motile and vibrio-shaped, 0.4–0.5 μm in diameter and 1.2–2.7 μm long (Fig. 1a, b). Cells occurred singly, in pairs or as short spirilla (up to 4 cells in the chain). Multiplication occurred by binary fission. Gram-stained cells of the strain exhibited the red color typical for the reaction with Gram-negative cell walls. Spores were not observed.

### Growth characteristics

The isolate grew optimally at a temperature of 36 °C. Growth and sulfidogenesis of the strain were not observed below 10 °C and above 40 °C. Strain Al915-01$^T$ is an obligate alkaliphile growing within the pH range 8.0–10.5, with an optimum at pH 9.0 (Fig. 2a). The isolate grew at NaCl concentrations of 5–100 g $l^{-1}$, with the optimum growth at 40 g NaCl $l^{-1}$ (Fig. 2b). Strain Al915-01$^T$ required $Na^+$. Chloride ions were not obligatory components of the medium for the strain. No growth was observed when carbonates were replaced by sodium sulfate at the optimal sodium concentration.

### Electron donors and electron acceptors

Strain Al915-01$^T$ used lactate, formate, and pyruvate as electron donors in the presence of sulfate, but it did not oxidize succinate, malate, fumarate, oxalate, propionate, butyrate, benzoate, glucose, fructose, serine and ethanol. Cell doubling time ($t_d$) of strain in the exponential phase of

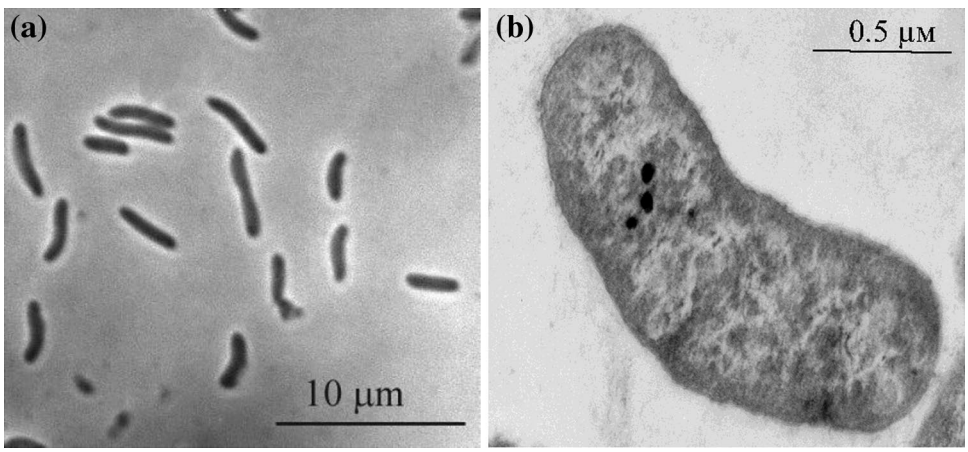


**Fig. 1** Morphology of strain Al915-01$^T$ cells grown with lactate: **a** phase-contrast micrograph, **b** scanning electron micrograph

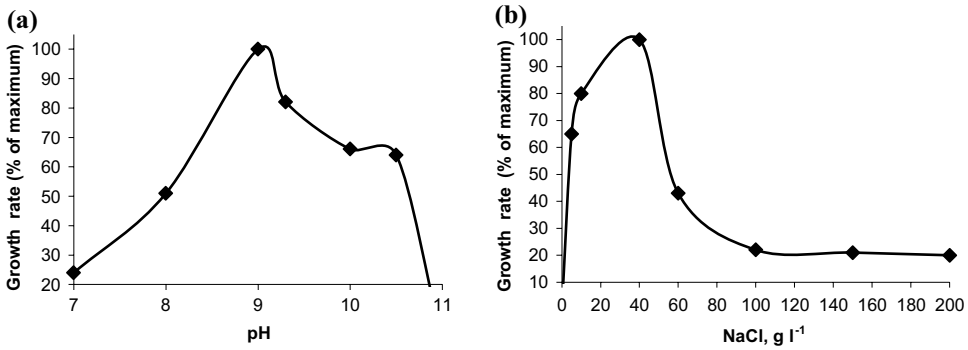


**Fig. 2** pH (**a**) and NaCl (**b**) effect on the growth of strain Al915-01$^T$

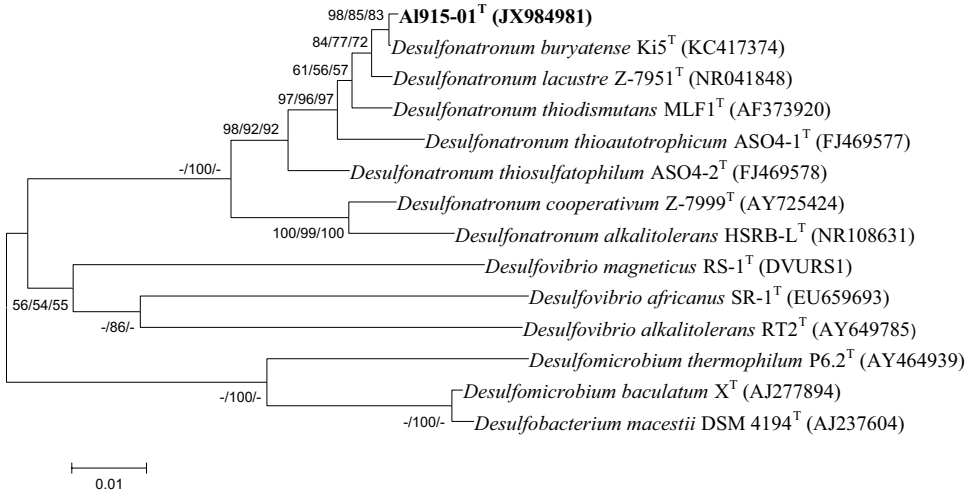


**Fig. 3** Phylogenetic consensus tree based on 16S rRNA gene sequences showing the position of strain Al915-01$^T$ within SRB of *Deltaproteobacteria.* Bootstrap values (>50 %) based on 1000 replicates are shown at branch nodes. *Bar* 0.01 substitutions per nucleotide position

growth was minimal (72 h) in the medium MI with lactate and sulfate.

The strain was able to grow lithoheterotrophically with sulfate using molecular hydrogen as electron donors in the presence of acetate as a carbon source as confirmed by growth with two subsequent transfers. During 14 days of incubation of strain Al915-01$^T$ in acetate- and sulfate-containing medium the number of cells increased from $5.6 \times 10^5$ to $4.4 \times 10^6$, accompanied by the formation from 1–2.5 mM hydrogen sulfide. However, no oxidation of acetate was detected. Besides sulfate the isolate reduced thiosulfate and sulfite with lactate as an electron donor. After 2 weeks of incubation strain Al915-01$^T$ formed 5.4 and 4.9 mM of hydrogen sulfide with thiosulfate and sulfite, respectively.

Nitrate was reduced to nitrite as determined by the Griess reaction after 1 week of cultivation with lactate as an electron donor. On day 14, a full recovery of nitrite was observed. At the same time, an increase in the cell number from $3.5 \times 10^6$ to $1.2 \times 10^7$ was observed. This ability of the strain Al915-01$^T$ was maintained for three passages. $S^o$, $Fe(OH)_3$ and DMSO were not used as an electron acceptors for growth.

## Phylogenetic analysis

Analysis of the 16S rRNA gene sequence (1501 bp) of strain Al915-01$^T$ showed that it had the highest sequence similarity (99.9 %) to *D. buryatense* Ki5$^T$ and *D. lacustre* Z-7951$^T$ (99.2 %). The consensus phylogenetic tree indicated that the novel bacterium was affiliated with the *Desulfonatronum* genus within family *Desulfonatronaceae* (Fig. 3).

## Lipid analysis

Lipid profile of strain Al915-01$^T$ was significantly different from the fatty acids composition of its closest relatives *D. buryatense* Ki5$^T$ and *D. lacustre* Z-7951$^T$ (Table 1). Unsaturated fatty acids $C_{16:1\omega7}$, is-$C_{17:1}$ and $C_{18:1\omega7}$ were found in large quantities (12.1, 16.9 and 12.7 %, respectively). We detected saturated anteiso-$C_{15:0}$ (15.3 %), which is not characteristic for the species of *Desulfonatronum* genus (Table 1). However, $C_{14:0}$ found in all the species of the genus (*D. buryatense* Ki5$^T$-11.2 %; *D. lacustre* Z-7951$^T$-14.6 %; *D. thiodismutans* MLF1$^T$-16.1 %; *D. thoisulfatophilum* ASO4-2$^T$-9.1 %; *D. thioautotrophicum* ASO4-1$^T$-6.9 %) was just 1.4 % in the novel strain.

**Table 1** Fatty acids composition of strain Al915-01$^T$ and its closest phylogenetic neighbors

| Fatty acids | CFA composition (%) | | |
|---|---|---|---|
| | Al915-01$^T$ | *D. buryatense* Ki5$^T$ | *D. lacustre* Z-7951$^T$ |
| $C_{14:0}$ | 1.4 | **11.2** | **14.6** |
| Iso-$C_{15:1}$ | 1.6 | 2.3 | 0.9 |
| Iso-$C_{15:0}$ | 7.5 | 9.5 | 4.7 |
| Anteiso-$C_{15:0}$ | **15.3** | **14.1** | 3.0 |
| $C_{15:0}$ | 0.4 | 0.5 | 1.0 |
| Iso-$C_{16:1}$ | 0.7 | 3.5 | 1.2 |
| Anteiso-$C_{16:1}$ | 2.7 | – | – |
| $C_{16:1\omega7}$ | **12.1** | 6.0 | **22.9** |
| $C_{16:0}$ | 6.4 | 3.1 | 7.7 |
| Iso-$C_{17:1}$ | **16.9** | 7.8 | 4.5 |
| Anteiso-$C_{17:1}$ | 6.2 | – | 0.6 |
| Iso-$C_{17:0}$ | 2.0 | – | 0.8 |
| $C_{17:0}$ | 1.3 | 0.3 | 1.0 |
| $C_{18:1\omega9}$ | 2.2 | 3.1 | 9.5 |
| $C_{18:1\omega7}$ | **12.7** | 7.1 | 10.1 |
| $C_{18:0}$ | 8.1 | 7.9 | **10.7** |

Major components are indicated in bold. Values are percentages of total fatty acids

– Not detected

### DNA base composition and DNA–DNA hybridization

The DNA G+C content of the isolate was 58.3 ± 1 mol % and the value was within the limits defined for the genus *Desulfonatronum* (48.8–59.1 mol %).

DNA–DNA hybridization values between *D. buryatense* Ki5$^T$ and *D. lacustre* Z-7951$^T$ with strain Al915-01$^T$ were 55 and 40 %, respectively; while DNA–DNA hybridization value between *D. lacustre* Z-7951$^T$ and *D. buryatense* Ki5$^T$ was 53 %.

## Discussion

Previously, microbiological studies have shown that the microbial community of the bottom sediments of Lake Alginskoe includes alkaliphilic bacteria that play an important role in the oxidation of organic compounds (Abidueva et al. 2011). We attempted to detect a sulfate-reducing bacterium that performs the terminal stage of this process, and successfully identified a representative of the *Desulfonatronum* genus.

On the basis of the 16S rRNA gene sequence analysis and morphological and physiological characteristics, strain Al915-01$^T$ is related to the genus *Desulfonatronum*. In contrast to *D. buryatense* Ki5$^T$ and *D. lacustre* Z-7951$^T$, the strain was unable to use ethanol as a sole carbon source. Unlike *D. lacustre* Z-7951$^T$ and strain Al915-01$^T$, *D. buryatense* Ki5$^T$ reduced $Fe(OH)_3$ and $S^o$, (Table 2). The fatty acid composition of the cell membrane in the isolate also differed significantly from that of closely related species of the genus *Desulfonatronum.* DNA–DNA hybridization level of strain Al915-01$^T$ with *D. lacustre* DSM 10312$^T$ indicates that the strains belong to different species.

It has been known that denitrification in soda lakes is carried out by highly salt-tolerant alkalophilic representatives of the genus *Halomonas* (Boltyanskaya 2007; Shapovalova et al. 2009) and by several anaerobic lithotrophs, for example, by representatives of *Thioalkalivibrio* genus or *Alkalilimnicola–Alkalispirillum* group (Sorokin et al. 2001, 2006; Hoeft et al. 2007). At the same time, a growing number of studies have demonstrated the possibility of electron acceptance by sulfate-reducing bacteria from nitrate. Nitrate is reduced via nitrite to ammonia by few strains belonging mainly to the genus *Desulfovibrio* (Dalsgaard and Bak 1994; Hubert and Voordouw 2007; Barton and Fauque 2009). Thus, the question still remains whether or not dissimilatory ammonification competes with denitrification in soda lakes (Sorokin et al. 2014).

In addition to sulfur-containing electron acceptors used by all described species of the genus, strain Al915-01$^T$ also reduces nitrate with lactate as an electron donor. This property was observed among species of the genus *Desulfonatronum* for the first time. It may increase the survival of this bacterium in more oxidized conditions.

On the basis of the genotypic, phenotypic and phylogenetic characteristics, the isolate is proposed to be a representative of a novel species *Desulfonatronum zhilinae* with the type strain Al915-01$^T$ (=VKM B-2744$^T$ =DSM 26338$^T$).

### Description of *Desulfonatronum zhilinae* sp. nov.

(zhi.li'nae. N.L. gen. n. *zhilinae*, of Zhilina; named after Russian microbiologist Tatjana Zhilina, who pioneered the study of alkaliphilic sulfate-reducing bacteria in natural ecosystems).

Cells are Gram-stain negative motile vibrios (0.4–0.5 × 1.2–2.3 μm). Oxidizes lactate, formate and pyruvate in the presence of sulfate. Able to grow with sulfate and $H_2$ in the presence of acetate as a C-source. Does not oxidize: acetate, succinate, malate, fumarate, oxalate, propionate, butyrate, benzoate, glucose, fructose, serine and ethanol. Uses sulfate, thiosulfate, sulfite and nitrate as electron acceptors, but does not use DMSO, $S^o$ and $Fe(OH)_3$. Grows at pH from 8.0 up to 10.5 (optimum pH 9.0) and at NaCl content of 5–100 g $l^{-1}$ (optimum 40 g $l^{-1}$). Mesophilic, with an optimum growth temperature of 36 °C. Dominating fatty acids (>10 %) were anteiso-$C_{15:0}$, iso-$C_{17:1}$, $C_{18:1\omega7}$, $C_{16:1\omega7}$.

**Table 2** Differential characteristics of strain Al915-01$^T$ and type strains of *Desulfonatronum* species

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Cell size (μm) | 0.4–0.5 × 1.2–2.3 | 0.5–0.7 × 2.0–2.5 | 0.7–0.9 × 2.5–3.0 | 0.6–0.7 × 1.2–2.7 | 0.4–0.5 × 1.0–2.5 | 0.5–0.6 × 2.0–4.0 | 0.4–0.5 × 1.5–4.0 | 0.5–0.6 × 1.5–2.5 |
| Temperature range (°C) | 10–40 | 20–40 | 22–45 | 15–48 | 15–40 | | | |
| Optimum | 36 | 34 | 40 | 37 | 35–38 | Maximum 40 | | Maximum 42 |
| pH range | 8.0–10.5 | 7.5–10.5 | 8.0–10.1 | 8.0–10.5 | 6.7–10.3 | 8.3–10.5 | 8.0–10.4 | 7.5–9.7 |
| Optimum | 9.0 | 9.4 | 9.3–9.5 | 9.5 | 8.0–9.0 | 9.3-10.0 | 9.5 | 8.5–9.0 |
| NaCl range (g $l^{-1}$) | 5–100 | 2–40 | 0–100 | 10–70 | 10–80 | 5–130 | 5–76 | 5–100 |
| Optimum | 40 | 5 | 0 | 30 | 5–15 | 23–35 | 17 | 17–35 |
| Electron donors with sulfate | | | | | | | | |
| Lactate | + | +[a] | +[a] | − | + | + | + | + |
| Pyruvate | + | +[a] | −[a] | − | − | + | + | + |
| Ethanol | − | +[a] | +[a] | + | − | + | + | − |
| Electron acceptors with lactate | | | | | | | | |
| $Fe(OH)_3$ | − | +[a] | −[a] | nd | nd | nd | nd | nd |
| $S^o$ | − | +[a] | −[a] | − | − | − | − | − |
| Nitrate | + | −[a] | −[a] | − | − | nd | nd | nd |
| G+C (mol %) | 58.3 ± 1 | 48.8[a] | 57.3 ± 1[a] | 59.1 ± 0.1 | 56.5 ± 1 | 57.6 | 57.0 | 55.8 |

Taxa: 1, Strain Al915-01$^T$ ; 2, *D. buryatense* Ki5$^T$ (Ryzhmanova et al. 2013); 3, *D. lacustre* Z-7951$^T$ (Pikuta et al. 1998); 4, *D. thiodismutans* MLF1$^T$ (Pikuta et al. 2003); 5, *D. cooperativum* Z-7999$^T$ (Zhilina et al. 2005); 6, *D. thioautotrophicum* ASO4-1$^T$ (Sorokin et al. 2011); 7, *D. thiosulfatophilum* ASO4-2$^T$ (Sorokin et al. 2011); 8, *D. alkalitolerans* HSRB-L$^T$ (Sorokin et al. 2013)

*nd* no data

[a] Obtained in our study

The type strain Al915-01$^T$ (=VKM B-2744$^T$ = DSM 26338$^T$) was isolated from the bottom sediments of the soda lake Alginskoe, Buryatiya, Russia. The G+C content in the DNA of type strain is 58.3 ± 1 mol %.

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