Fuchsiella ferrireducens sp. nov., a novel haloalkaliphilic, lithoautotrophic homoacetogen capable of iron reduction, and emendation of the description of the genus Fuchsiella

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Two strains of haloalkaliphilic homoacetogenic bacteria capable of iron reduction, Z-7101T and Z-7102, were isolated from soda lake Tanatar III (Altai, Russia). Cells of both strains were flexible, motile, Gram-negative, spore-forming rods. The strains were mesophilic and obligately alkaliphilic: the pH range for growth was 8.5–10.2 (pHopt 9.8). Growth depended on carbonate and chloride ions. The strains were able to grow chemolithoautotrophically on H2 + CO2, producing acetate as the only metabolic product. In medium with carbonates as the only potential electron acceptor, the following substrates were utilized for chemo-organotrophic growth: pyruvate, lactate, ethanol, 1-propanol, ethylene glycol and 1-butanol. Strain Z-7101T was able to reduce nitrate, selenate, thiosulfate and anthraquinone 2,6-disulfonate with ethanol as an electron donor. It was also able to reduce synthesized ferrihydrite to siderite with molecular hydrogen or organic compounds, including acetate and formate, as electron donors. It was able to reduce SO2 with acetate or formate as electron donors. The DNA G + C content of strain Z-7101T was 34.6 mol%. 16S rRNA gene sequence analysis showed that strains Z-7101T and Z-7102 were members of the order Halanaerobiales and family Halobacteroidaceae, clustering with Fuchsiella alkaliacetigena Z-7100T (98.9–98.4 % similarity). DNA–DNA hybridization was 63.0 % between strain Z-7101T and F. alkaliacetigena Z-7100T. Based on morphological and physiological differences from F. alkaliacetigena Z-7100T and the results of phylogenetic analysis and DNA–DNA hybridization, it is proposed to assign strains Z-7101T and Z-7102 (=DSM 26052=VKM B-2790) to the novel species Fuchsiella ferrireducens sp. nov. The type strain is strain Z-7101T (=DSM 26031=VKM B-2766).

The anaerobic chemotrophic community of soda lakes has been investigated intensively since the end of the 20th century. This community represents an autonomous entity with a limited dependence on inputs from the outside (Zavarzin et al., 1999; Zavarzin & Zhilina, 2000; Zavarzin, 2007). Its trophic system is sufficiently complete to close the main biogeochemical cycles. As the sulfur cycle dominates in modern soda lakes, the hydrogen and acetate sink in the anaerobic community is provided mainly by hydrogenotrophic sulfidogens, sometimes in syntrophic associations (Sorokin et al., 2011a, b, c; Zhilina et al., 2005). Extremely natronophilic hydrogenotrophic methanogens and acetogens are usually suppressed by sulfidogens and, until recently, they were not known. Methanocalculus natronophilus (Zhilina et al., 2013) and Fuchsiella alkaliacetigena (Zhilina et al., 2012), isolated from sediments of soda lake Tanatar (Altay Region, Russia), were the first representatives of such micro-organisms. The finding of a possible microbial H2 sink in sulfate-limited terrestrial ecosystems (such ecosystems could have occurred on land in the early Precambrian era) was of special interest, as the iron cycle dominated on the Earth at that period of time (Trendall, 2002; Klein, 2005; Beukes & Gutzmer, 2008; Heimann et al., 2010). According to the hypothesis advanced by Zavarzin (1993), modern soda lakes may...
represent a refuge for relict terrestrial communities of the ancient continents of the early Proterozoic eon. In such communities, where the sulfur cycle was suppressed, alkaliphilic iron reducers could play an important role in the final stage of organic matter decomposition. The iron reduction process occurs widely in soda lakes, and its intensity is independent of alkalinity and total mineralization (Zavarzina et al., 2006). Over the last decade, several species of bacteria able to reduce synthesized ferrihydrite (SF) have been isolated from sediments of soda lakes (Zavarzina et al., 2006, 2013; Zhilina et al., 2009a, b).

Here, we describe a novel extremely haloalkaliphilic, lithoautotrophic, homoacetogenic representative of the genus *Fuchsiella* capable of reducing SF during oxidation of acetate and molecular hydrogen in soda-saturating conditions. Samples of sediments from the Tanatar soda lake group (Altay Region, Russia) were collected by V. V. Kevbrin in June 2007. The parameters of the water measured at the sampling site were as follows: pH 10.3, total salinity 20.4–20.0 % (w/v), temperature 27 °C.

The initial enrichment was obtained in an anoxically prepared selective medium containing (unless indicated, in g l⁻¹): NH₄Cl, 0.5; KH₂PO₄, 0.2; MgCl₂ · 6H₂O, 0.1; KCl, 0.2; yeast extract (Difco), 0.05; Na₂CO₃, 100; NaHCO₃, 15; NaCl, 95; trace element solution (Kevbrin & Zavarzin, 1992) 1 ml l⁻¹; Na₂S · 9H₂O, 0.5 (pH 10.2). The medium was dispensed into 120 ml glass flasks with screw caps, and the head space (100 ml) was filled with H₂ at atmospheric pressure. The flasks were inoculated with 1 g sediment sample and incubated at 35 °C.

The composition of the basal medium for cultivation and maintenance was, after growth optimization, as follows (unless indicated, in g l⁻¹): NH₄Cl, 0.5; KH₂PO₄, 0.2; MgCl₂ · 6H₂O, 0.1; Na₂CO₃, 68; NaHCO₃, 38; NaCl, 60; yeast extract (Difco), 0.05, or vitamin solution (Wolin et al., 1963), 10 ml l⁻¹; trace element solution, 1 ml l⁻¹; Na₂S · 9H₂O, 0.5, or sodium thioglycolate, 0.5. After autoclaving, the pH was 9.5. H₂ (100 % of the headspace) or 5 ml H₂ at atmospheric pressure. The flasks were inoculated with 1 g sediment sample and incubated at 35 °C.

Serial dilutions in selective medium with H₂ in the gas phase were made from the investigated samples, and the highest positive dilution was used for isolation of colonies in roll-tubes with basal medium supplemented with ethylene glycol (3 g l⁻¹), NaCl (80 g l⁻¹) and Noble agar (30 g l⁻¹; Difco) and autoclaved in anaerobic tubes (Bellco Glass). A concentrated solution of carbonates was added to the medium before inoculation to a final concentration of 1.2 M Na₂CO₃. After 30 days of incubation at 37 °C, colonies were translucent, spherical with a pinkish to pink lustrous surface and 0.5–1.0 mm in diameter. Single colonies were picked up with glass capillaries and inoculated into liquid medium. The culture purity of two isolates, designated strains Z-7101⁰ and Z-7102, was checked by phase-contrast microscopy, absence of growth on conventional media rich in organic compounds and 16S rRNA gene sequence analysis.

Phase-contrast photomicrographs and cell-size measurements were made by using a Zeiss Saco light microscope (Reichert). Ultrathin sections and electron microscopy of negatively stained whole cells were performed as described previously (Zhilina et al., 1996). Cells of the strains were flexible rods measuring, at the exponential phase of growth, 2–5 μm and sometimes up to 10 μm in length (Fig. 1a). Multiplication was by fission, often unequal; strain Z-7101⁰ sometimes formed mini-cells. The width of the cells depended on the growth stage: at the early exponential phase, it was 0.4–0.6 μm, but later, when cells formed endospores (1.0–1.2 μm in diameter) of a plectridial type (Fig. 1b), the cell width increased to 0.8–1.0 μm (Fig. 1b). At the late-exponential growth phase, the cells lysed, yielding spheroplasts (Fig. 1b). Cells were motile by peritrichously located flagella (Fig. 1c). Ultrathin sections of strain Z-7101T revealed cell walls with outer membranes, typical of Gram-negative bacteria (Fig. 1d).

For substrate-utilization tests, the culture was inoculated into basal medium with yeast extract (0.05 g l⁻¹) supplemented with organic substrates (peptides, carbohydrates, amino acids, alcohols or organic acids) added to a final concentration of 3 g l⁻¹ (w/v) or 3 ml l⁻¹ (v/v). Sugars were prepared as stock solutions and filter-sterilized (0.22 μm) prior to injection into the tubes. The medium without substrates (except yeast extract, 0.05 g l⁻¹) was used as the control. Bacterial growth was monitored by direct cell counting under a phase-contrast microscope and by measuring the OD₆₀₀ (Spectrophotometer-2100; UNICO). Hydrogen and metabolic products formed during bacterial growth were assayed with a Crystall 5000.2 gas chromatograph (Chromatek) as described previously (Zhilina et al., 2012). For the growth-positive variants, three consecutive transfers to the same medium were performed. In medium with carbonates as the only potential electron acceptor, strain Z-7101T could utilize only a few substrates for organotrophic growth: ethanol, 1-propanol, 1-butanol, ethylene glycol, lactate and pyruvate. Strain Z-7101T did not utilize carbohydrates (arabinose, fructose, glucose, galactose, lactose, maltose, mannose, ribose, sucrose, trehalose or xylose), yeast extract, Casamino acids or histidine, glutamate, aspartate or ornithine, used by some haloalkaliphilic acetogens (Zhilina et al., 1996, 1998). Methanol, 2-propanol, mannitol, glycerol, 2,3-butanediol, betaine, trimethylamine, monomethylamine, formate, succinate, propionate and N-acetylglucosamine were not utilized. In medium with carbonates as the only potential electron acceptor, acetate was the sole metabolic product when ethanol, lactate or pyruvate was added as the electron donor. On ethylene glycol, in addition to acetate, propionate (1.3 mM) and isobutyrate (2.2 mM) were formed as minor products; on 1-propanol, the minor product was propionate (1.7 mM). 1-Butanol was fermented to propionate (14.8 mM), acetate (2.6 mM) and butyrate (3.9 mM). The strain could grow chemolithoautotrophically with H₂ and CO₂ in the presence of vitamin solution, producing acetate.
stoichiometrically according to the equation $4\text{H}_2 + 2\text{CO}_2 \rightarrow \text{CH}_3\text{COOH} + 2\text{H}_2\text{O}$.

For determination of the key enzymes of the acetyl-CoA pathway (Wood & Ljungdahl, 1991), strain Z-7101$^\text{T}$ was grown with H$_2$ as an electron donor. To obtain cell-free extracts, the cells were precipitated by centrifugation and resuspended in 50 mM anaerobic HEPES buffer (pH 8.0) that contained (mM): EDTA, 0.1; sodium formate, 10; sodium thioglycolate, 25. The suspension was sonicated anaerobically in a CPX130PB ultrasonic disintegrator (Cole-Palmer) twice for 0.5 min at 0.4 mA. Intact cells and cell debris were then sedimented by centrifugation for 40 min at 4000 $g$. Enzyme activities were determined in the supernatant by measuring the reduction of benzyl viologen under anaerobic conditions on a Cary 100Bio spectrophotometer (Varian) at 600 nm. The reaction mixtures were prepared in 50 mM Tris/HCl (pH 7.5) and additionally contained the following: (i) for the CO dehydrogenase assay, 2.5 mM benzyl viologen, 2 mM dithionite, 3.5 mM dithiothreitol; the mixture was purged with CO; (ii) for the hydrogenase assay, 10 mM benzyl viologen, 2 mM dithionite, 3.5 mM dithiothreitol; the mixture was purged with H$_2$; (iii) for the formate dehydrogenase assay, 50 mM formate, 10 mM benzyl viologen, 2 mM dithionite, 3.5 mM dithiothreitol; the mixture was purged with N$_2$; (iv) for the pyruvate:ferredoxin oxidoreductase assay, 50 mM sodium

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**Fig. 1.** Morphology of strain Z-7101$^\text{T}$. (a, b) Cells under phase-contrast microscope. Bar, 10 $\mu$m. (c) Negatively stained flagellated cell. Bar, 0.5 $\mu$m. (d) Longitudinal section showing that the cell-wall structure is of the Gram-negative type. Bar, 1 $\mu$m.
pyruvate, 0.2 mM CoA, 25 mM dithiothreitol, 1 mM benzyl viologen; the mixture was purged with N₂. For all assays, the volume of the reaction mixture was 1.0 ml. High activities of these enzymes were found in cell-free extracts of strain Z-7101ᵀ (Table S1, available in the online Supplementary Material), which agrees with acetate synthesis in this organism via the reductive acetyl-CoA pathway.

Strain Z-7101ᵀ could grow (three consecutive transfers) with N₂ (100 % in the gas phase) as the only nitrogen source. However, tests for the presence of nitrogen-fixation genes performed by using universal primers targeting the nifH gene (Marusina et al., 2001) were unsuccessful.

The capacity for anaerobic respiration with various acceptors was tested with ethanol (0.5 %, w/v) as an electron donor. Growth was monitored by measuring the OD₆₀₀ and the levels of reduction products. In the case of sulfur compounds added as acceptors, sulfide was measured by the methylene blue method, as described previously (Zhilina et al., 2012). In the case of iron compounds, the concentration of the Fe(II)–ferrozine complex was determined from the A₅₆₂ as described previously (Zhilina et al., 2012). Arsenate was determined as described by Zavarzina et al. (2009). Selenate and selenite were quantified by ion chromatography as described previously (Zhilina et al., 2012). Strain Z-7101ᵀ reduced nitrate (10, 20 mM), anthraquinone 2,6-disulfonate (AQDS) (2 mM) and selenate (5 mM). No growth occurred with sulfate (20 mM), dithionite (20 mM), sulfite (20 mM), S₀ (1 g l⁻¹), nitrite (2 mM), ferric EDTA, selenite (2.5 mM), arsenate (5 mM), fumarate (Fluka; 5 mM) or crotonate (Fluka; 5 mM). Only weak reduction of thiosulfate was observed (5 mmol sulfide l⁻¹ was formed).

The capacity for iron respiration was tested with SF [containing 50 mmol Fe(III) l⁻¹ and prepared as described by Zavarzina et al. (2006)] as an electron acceptor. All organic substrates that were utilized by strain Z-7101ᵀ, and also 20 mM acetate, 40 mM formate and molecular hydrogen, were tested as electron donors. Strain Z-7101ᵀ reduced SF in all cases. With acetate, it reduced SF to siderite, detected by Mössbauer spectroscopy, according to the equation \( \text{CH}_3\text{COO}^- + 8\text{Fe}^{3+} \rightarrow \text{CO}_2 + 7\text{H}^+ + 8\text{Fe}^{2+} \) (Fig. 2).

Strain Z-7101ᵀ could also reduce SF chemolithoautotrophically with molecular hydrogen as an electron donor. During growth, the production of acetate was recorded (Fig. 3). As strain Z-7101ᵀ was an obligate natronophile, we could not omit carbonate from the medium. This means that, in fact, there were two available electron acceptors in the medium: Fe(III) and CO₂. The reduction of Fe(III) and production of acetate (hydrogenotrophic acetogenesis) started simultaneously (Fig. 3). The final amounts of molecular hydrogen utilized, acetate produced, and Fe(III) reduced corresponded to the following overall reaction of hydrogenotrophic homoacetogenesis and hydrogenotrophic iron reduction:

\[ 5\text{H}_2 + 2\text{CO}_2 + 2\text{Fe}^{3+} \rightarrow \text{CH}_3\text{COOH} + 2\text{Fe}^{2+} + 2\text{H}_2\text{O} + 2\text{H}^+ \]  (Fig. 3).

With acetate or formate as electron donors, strain Z-7101ᵀ could also reduce S₀, with the production of 12.7 and 11.6 mmol sulfide l⁻¹, respectively.

The optimal conditions for growth of strain Z-7101ᵀ were determined in basal medium with ethanol (0.5 %, w/v) added as the substrate and 10 mM NaNO₃ added as the electron acceptor. Strain Z-7101ᵀ was an obligate anaerobe. No growth occurred under a gas phase containing 2 % O₂ or even under an anaerobic gas phase in aerobically prepared medium. The catalase test was positive. Growth of strain Z-7101ᵀ occurred at 25–45 °C, with an optimum at 30–37 °C. The pH optimum was determined at 35 °C in the basal medium with 90 g NaHCO₃ l⁻¹ and 70 g NaCl l⁻¹ and without Na₂CO₃, as described previously (Zhilina et al., 2012). Strain Z-7101ᵀ was an extreme alkaliphile, showing growth at pH 8.2–10.2 and an optimum at pH 9.8. The NaCl concentration range suitable for growth was determined in carbonate-buffered basal medium as

![Fig. 2. Growth of strain Z-7101ᵀ with acetate as an electron donor and SF as an electron acceptor.](http://ijs.sgmjournals.org) 2435

![Fig. 3. Growth and acetate production by strain Z-7100ᵀ with hydrogen as an electron donor and SF as an electron acceptor.](http://ijs.sgmjournals.org) 2435
described previously (Zhilina et al., 2012). Strain Z-7101T was an obligate haloalkaliphile. It required Cl\(^{-}\) for growth, and grew within a Cl\(^{-}\) concentration range of 0.2–2.7 M with an optimum at 1.2 M Cl\(^{-}\) in the presence of 1.7 M Na\(^{+}\) from total carbonates. The dependence of the growth rate on the total salt content and salt composition was determined in media containing mixtures of sodium carbonates and chlorides as described previously (Zhilina et al., 2012). Strain Z-7101T was an obligate and extreme natronophile. No growth occurred in carbonate-free medium, when carbonates were replaced by equimolar amounts of NaCl or Na\(_2\)SO\(_4\) in the medium with 50 mM TABS buffer (pH 9.0). The strain could grow within the range 1.4–4.2 M Na\(^{+}\) and total carbonate concentrations of 0.7–2.1 M (CO\(_2\)\(^{3-}\) + HCO\(_3\)\(^{-}\)). The growth rate was maximal at 3.3–3.8 M Na\(^{+}\) and 1.6–1.9 M CO\(_2\)\(^{3-}\) + HCO\(_3\)\(^{-}\).

Sensitivity to antibiotics (100 \(\mu\)g L\(^{-1}\)) was tested on basal medium with ethanol (0.5 %, w/v). No growth was observed with chloramphenicol, metronidazole, erythromycin, clindamycin or rifampicin, active against Gram-positive bacteria, or with bacitracin, novobiocin, cycloserine, neomycin, polymyxin B, tetracycline or ampicillin. Streptomycin, kanamycin, penicillin, gentamicin and vancomycin did not inhibit growth.

For fatty acid analysis, cells of strain Z-7101T were grown in basal medium with ethanol (0.5 %, w/v), added as the substrate, at pH 9.5, 2.75 M Na\(^{+}\) and 35 °C, and harvested at the late-exponential growth phase. Fatty acid extraction and analysis were carried out by GC-MS as described previously (Zhilina et al., 2012). Each compound was identified from its mass spectrum and NIST mass spectral database library search. The fatty acid profile of strain Z-7101T proved to be dominated by the saturated fatty acids anteiso-C\(_{15:0}\) (22.7 %), C\(_{14:0}\) (6.1 %) and C\(_{16:0}\) (6.0 %) and the aldehydes C\(_{16:0}\) (17.2 %), anteiso-C\(_{15:0}\) (10.9 %) and C\(_{16:1\text{a}}\) \(\text{c9a}\) (7.8 %) (Table S2).

Genomic DNA was extracted according to a previously described procedure (Boulygina et al., 2002). PCR of the 16S rRNA genes and sequencing of PCR products were performed using universal 16S rRNA gene primers (Lane, 1991). PCR fragments were prepared for sequencing and cloning using the standard Wizard PCRPreps and pGEMT Easy Systems protocols (Promega). Direct sequencing of the nearly complete 16S rRNA gene of Z-7101T revealed an ambiguous region beginning at position 184 (Escherichia coli numbering). Therefore, we performed cloning in the pGEM-T vector of 16S rRNA gene PCR products obtained from genomic DNA of strain Z-7101T. This was followed by ABI3730 sequencing of 50 independent clones. The resulting sequences were aligned using the CLUSTAL W (Thompson et al., 1994) algorithm implemented in the BioEdit software package version 7.0.9.0 (Hall, 1999). Detailed analysis revealed the presence of three different gene variants (with approximately equal representation) among the sequenced clones. The maximal difference between gene copies was 0.7 %. The 16S rRNA gene sequences obtained from both strains, Z-7101T and Z-7102, 1499 nt long (positions 4–1506 in the E. coli numbering), were compared with all GenBank entries by BLAST search (http://www.ncbi.nlm.nih.gov/blast), which showed that the closest relative (98.9 % sequence similarity with Z-7101T and 98.4 % with Z-7102) was Fuchsiella alkaliacetigena Z-7100T (Zhilina et al., 2012), a representative of the family Halobacteroidaceae of the order Halanaerobiales (Rainey et al., 1995). The sequence similarity with other members of this group varied from 82.3 to 93.3 %. A phylogenetic tree was reconstructed using the MEGA 5.0 software package (Tamura et al., 2011). Evolutionary distances were calculated by using the Tamura–Nei model (Tamura & Nei, 1993), and the branching order was determined by the maximum-likelihood method. The tree (Fig. 4) is a consensus of 500 replicate trees. The percentages of trees in which associated taxa clustered together are shown next to the branches. The initial tree(s) for the heuristic search was obtained automatically by applying neighbour-joining and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach, and then selecting the topology with superior log-likelihood value. The rate variation among sites was explored in the MOLPHY software program (Tamura et al., 2011). Evolutionary distances were calculated by the maximum-likelihood method. The tree (Fig. 4) is a consensus of 500 replicate trees. The analysis involved 21 nucleotide sequences. All positions with less than 70 % site coverage were eliminated. That is, fewer than 30 % alignment gaps, missing data and ambiguous bases were allowed at any position.

The G+C content of the genomic DNA was determined from thermal denaturation/reassociation curves as described previously (Zhilina et al., 2012). DNA–DNA hybridization was determined by the optical reassociation method using a Cary 100 Bio spectrophotometer (Varian) at a rate of 0.5 °C min\(^{-1}\). The G+C contents of the genomic DNA of strains Z-7101T and Z-7102 were 34.6 and 34.7 mol%, respectively. DNA–DNA hybridization between strains Z-7101T and Z-7102 was 96 %, indicating that the strains belonged to the same species. DNA–DNA hybridization was 63 % between strain Z-7101T and F. alkaliacetigena Z-7100T and 68 % between strain Z-7102 and F. alkaliacetigena Z-7100T.

The new isolates are alkaliphilic, homoacetogenic bacteria and, to the best of our knowledge, are the first isolated alkaliphilic, hydrogenotrophic, homoacetogenic bacteria capable of dissipatory iron reduction and sulfur respiration. Phenotypic distinctions between strain Z-7101T and its closest phylogenetic relative F. alkaliacetigena Z-7100T are presented in Table 1. First of all, these two organisms are rather different morphologically: cells of strain Z-7101 were shorter, wider and less flexible than cells of F. alkaliacetigena Z-7100T and had straight, not tapered, ends. The sporulation type and spore size are also different. Cells of strain Z-7101T became swollen before spore formation, whereas cells of F. alkaliacetigena Z-7100T did not change in diameter, and their spores corresponded to the cell width (0.5 μm) and were half the size of spores.
of strain Z-7101T. Metabolic distinctions of strain Z-7101T were its ability to utilize two- and three-carbon alcohols with different numbers of hydroxyl groups, such as 1-propanol, 1-butanol and ethylene glycol, and its ability to oxidize formate with SF or S0 as an electron acceptors. The capacity of strain Z-7101T was qualitatively close to that of F. alkaliacetigena Z-7100T, but it differed in the quantitative ratios of fatty acids (see Table S2). In contrast to the halotolerant F. alkaliacetigena Z-7100T, strain Z-7101T was an obligate halophile and needed NaCl in the medium.

The capacity of F. alkaliacetigena Z-7100T to reduce Fe(III) had been tested previously with soluble EDTA complex, and the results of the test were negative (Zhilina et al., 2012). In this work, we tested this capacity with SF and found that F. alkaliacetigena Z-7100T also exhibited a slight ability to reduce SF. Thus, to the best of our knowledge, representatives of the genus Fuchsiella are the first known alkaliphilic homoacetogenic bacteria able to reduce SF in the course of lithoautotrophic growth with H2. Utilization of acetate as an electron donor is a newly discovered metabolic function of homoacetogens. In the presence of electron acceptors such as Fe(III) or S0, representatives of the genus Fuchsiella possibly oxidized acetyl-CoA to two CO2 molecules via a reversed (oxidative) acetyl-CoA pathway, as some sulfate-reducing bacteria do using sulfate as electron acceptor (Fuchs, 1994). It is only the second report of the ability to respire acetate during anaerobic growth with sulfur in soda-saturated conditions, after Natroniella sulfidigena (Sorokin et al., 2011b).

The ability to reduce iron is quite unexpected in alkaliphilic acetogens isolated from sediments of a modern soda lake, where sulfidogenesis predominates. This finding supports the hypothesis that iron reduction is one of the most ancient types of microbial respiration (Vargas et al., 1998). If so, this ability may be a rudimentary function retained in various metabolic groups of prokaryotes from the early Precambrian – the period of time when the iron cycle dominated on the Earth. It is also unusual that both our strains preferred insoluble SF and did not reduce Fe(III) in complexes with EDTA or citrate. This can be explained by the extremely low solubility of iron in soda-depositing lakes. In such environments, iron-containing minerals (oxides, hydroxides and silicates)

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**Fig. 4.** Phylogenetic analysis by the maximum-likelihood method of 16S rRNA gene sequences of strains Z-7101T and Z-7102 and other representatives of the family Halobacteroidaceae. Sequences determined in this study are in bold. Bar, 2 substitutions per 100 nucleotide positions.
are the most environmentally relevant forms of iron. The ability of homoacetogenic bacteria to oxidize acetate in the course of dissimilatory iron reduction can be realized in the anaerobic microbial community if the production of acetate exceeds its sink. In modern environments, acetate is easily consumed by many aerobic alkaliphiles, but it could accumulate during decomposition of organic matter in ancient anoxic ecosystems.

According to the recent recommendations (Tindall et al., 2010), the similarity between the 16S rRNA gene sequences of strains Z-7101T and Z-7102 and F. alkaliacetigena Z-7100T is not sufficiently low to propose the novel strains as representing a novel species. At the same time, it is recommended to refrain from species descriptions based on data of phylogenetic analysis only, and to take into account phenotypic characters. Taking into account the data of DNA–DNA hybridization of strain Z-7101T with F. alkaliacetigena Z-7100T (63 % hybridization) and the observed morphological, physiological and biochemical distinctions between them (Table 1), it may be concluded that strains Z-7101T and Z-7102 belong to a novel species of the genus Fuchsiella within the family Halobacteroidaceae. We propose strain Z-7101T as the type strain of a novel species of the genus Fuchsiella, which we name Fuchsiella ferrireducens sp. nov. Our results also necessitate an emended description of the genus Fuchsiella.

**Table 1. Characteristics that differentiate strain Z-7101T from F. alkaliacetigena Z-7100T**

Data for F. alkaliacetigena Z-7100T were taken from Zhilina et al. (2012).

<table>
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<th>Characteristic</th>
<th>Strain Z-7101T</th>
<th>F. alkaliacetigena Z-7100T</th>
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<td></td>
</tr>
<tr>
<td>Width</td>
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<tr>
<td>Length</td>
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<td>Type of sporulation</td>
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<td>Spore size (µm)</td>
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<td>pH for growth</td>
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<tr>
<td>Range</td>
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<td>8.5–10.5</td>
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<tr>
<td>Optimum</td>
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<td>Total Na⁺ concentration for growth (M)</td>
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<tr>
<td>Range</td>
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<td>1.9–4.7</td>
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<tr>
<td>Optimum</td>
<td>3.3–3.8</td>
<td>2.8–3.3</td>
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<tr>
<td>NaCl concentration for growth (M)</td>
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<td>Optimum</td>
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<td>1-Propanol</td>
<td>+</td>
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<tr>
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<td>1-Butanol</td>
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<td>Sensitivity to vancomycin</td>
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<tr>
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</table>

**Emended description of the genus Fuchsiella**

The characteristics of the genus are as described by Zhilina et al. (2012) with the following addition. Members of the genus are capable of dissimilatory iron and sulfur reduction.

**Description of Fuchsiella ferrireducens sp. nov.**

_Fuchsiella ferrireducens_ [fer‘ri.re.du‘cens. L. n. ferrum iron; L. part. adj. _reducens_ (from L. v. _reducere_) leading back, bringing back; in chemistry, converting to a different oxidation state, reducing; N.L. part. adj. _ferrireducens_ iron-reducing (converting iron to a reduced oxidation state)].

Cells are flexible rods, 0.4–0.6 × 5.0–15.0 µm, motile by means of peritrichously located flagella. Sporulating cells are 0.8–1.0 µm wide. In the late-exponential growth phase, the cells lyse, yielding spheroplasts. Spore-forming; spores are round and terminal. The cell-wall structure is of the Gram-negative type.

Obligate anaerobe. In the absence of external electron acceptors other than carbonates, can grow chemolithoautotrophically with H₂ as an electron donor or, chemo-organotrophically, on ethanol, 1-propanol, 1-butanol, lactate, pyruvate or ethylene glycol. Acetate is the main metabolic product. Vitamin solution can substitute for yeast extract. Capable of respiratory metabolism and can reduce nitrate, selenate and AQDS as electron acceptors with ethanol as an
electron donor. Oxidizes acetate or formate during sulfur reduction. Dissimilatory iron reducer, able to use synthesized ferrihydrite as an electron acceptor with acetate, ethanol, 1-butanol, lactate, pyruvate, ethylene glycol, formate or molecular hydrogen as electron donors. Obligately alkaliphilic, with a pH range for growth of 8.5–10.7 and optimum growth at pH 9.8. Extremely natronophilic. Carboxylates are required for growth. The Na⁺ concentration range for growth is 1.4–4.2 M (0.7–2.1 M carbonates), with an optimum of 3.3–3.8 M Na⁺ (1.6–1.9 M carbonates). Halophilic: the Cl⁻ concentration range for growth is 0.2–2.7 M with an optimum at 1.2 M Cl⁻. Mesophilic: the growth temperature range is 25–45 °C with an optimum at 30–37 °C. The dominant cellular fatty acids are anteiso-C₁₅:₀, C₁₄:₀ and C₁₆:₀. The major aldehydes are C₁₆:₀, anteiso-C₁₅:₀ and C₁₆:₁₀7a.

The type strain, Z-7101T (＝DSM 2603¹T＝VKM B-2766⁷T), and strain Z-7102 (＝DSM 2605²２＝VKM B-2790) were isolated from collectors of soda lake Tanatar III (Altay, Russia). The G+C content of genomic DNA of the type strain is 34.6 ± 0.1 mol%.

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