Fuchsiella alkaliacetigena gen. nov., sp. nov., an alkaliphilic, lithoautotrophic homoacetogen from a soda lake

Tatyana N. Zhilina,¹ Daria G. Zavarzina,¹ Angela N. Panteleeva,² Georgy A. Osipov,³ Nadezhda A. Kostrikina,¹ Tatyana P. Tourova¹ and Georgy A. Zavarzin¹

Correspondence
Tatyana N. Zhilina
zhilinat@mail.ru

¹Institute of Microbiology, Russian Academy of Sciences, Prospect 60-letiya Oktyabrya 7/2, Moscow 117312, Russia

²Bioengineering Center, Russian Academy of Sciences, Prospect 60-letiya Oktyabrya 7/1, Moscow 117312, Russia

³Bakulev Center for Cardiovascular Surgery, Russian Academy of Medical Sciences, Rublevskoe shosse, Moscow 135, Russia

The first alkaliphilic obligately anaerobic hydrogenotrophic homoacetogenic bacterium, strain Z-7100^T, was isolated from sediments of the soda-depositing soda lake Tanatar III (Altay, Russia). Cells were thin, flexible rods, motile, Gram-negative and spore-forming. The organism was an obligate alkaliphile, growing at pH 8.5 to 10.5, with optimum growth at pH 8.8–9.3, and it grew in soda brines containing 1.9–4.7 M total Na⁺ (optimum at 2.8–3.3 M). It exhibited an obligate dependence upon sodium carbonate but not upon chloride ions with an NaCl range for growth of 0–14 % (w/v) and an optimum at 7.0–8.5 % (w/v). The isolate was mesophilic and grew at temperatures from 25 to 45 °C, with an optimum at 40 °C. An H₂ + CO₂ mixture, ethanol, pyruvate and lactate were utilized with the formation of acetate as the sole metabolic product. Carbohydrates and amino acids did not support growth. The isolate had a respiratory type of metabolism, reducing NO₃⁻, SeO₄²⁻ or anthraquinone-2,6-disulfonate (as electron acceptors with ethanol as an electron donor). It was able to grow chemolithotrophically on H₂ + CO₂ in medium supplemented with a vitamin solution only. The major cellular fatty acids were the saturated fatty acids anteiso-C₁₅, C_{14:0} and C_{16:0} and the aldehydes C₁₆, C₁₄ and anteiso-C₁₅. The DNA G + C content of the isolate was 32.0 mol%. 16S rRNA gene sequence analysis showed that strain Z-7100^T is a member of the order *Halanaerobiales* and represents a new branch within the family *Halobacteroidaceae*, clustering with the type strain of *Selenihalanaerobacter shriftii* (92.9 % gene sequence similarity). On the basis of its physiological characteristics and phylogenetic position, the isolate is considered to represent a novel species in a new genus within the family *Halobacteroidaceae*. The name *Fuchsiella alkaliacetigena* gen. nov., sp. nov. is proposed. The type strain of the type species is Z-7100^T (=DSM 24880^T=VKM B-2667^T).

Soda lakes are the end reservoir of subaerial CO₂ weathering in semi-arid climates. The process is intrinsically terrestrial and might be regarded as an actualistic model for the reconstruction of the Early Proterozoic terrestrial biosphere (Zavarzin, 1993). Knowledge on the alkaliphilic (or more precisely natronophilic) microbiota of these

environments has accumulated mainly over the last two decades (for reviews see Tindall, 1988; Grant *et al.*, 1990; Jones *et al.*, 1998; Zavarzin *et al.*, 1999; Duckworth *et al.*, 1996; Rees *et al.*, 2004; Sorokin & Kuenen, 2005a, b). The major conclusion from our studies is that the alkaliphilic anaerobic microbial community in soda lakes comprises a complete trophic system limited mainly by total mineralization rather than by high alkalinity and pH of about 10 (Zhilina & Zavarzin, 1994; Zavarzin & Zhilina, 2000; Winogradsky Institute of Microbiology, 2007). The hypothesis of the interrelation between salt tolerance and the efficiency of energy metabolism partially explains the cause of such limitation (Oren, 1999).

Abbreviation: AQDS, anthraquinone-2,6-disulfonate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Z-7100^T is HQ828140.

Three supplementary figures are available with the online version of this paper.

The hydrogen sink in this anaerobic chemotrophic community is controlled by hydrogenotrophic sulfate reducers and sometimes by syntrophic associations (Zhilina *et al.*, 1997, 2005a, b; Pikuta *et al.*, 1998, 2003; Sorokin *et al.*, 2008a, b), whereas methanogens, except for methylotrophs, are mostly repressed by high salinity and therefore substituted by acetogens (Zavarzin *et al.*, 1996, 1999; Zavarzin & Zhilina, 2000). Among the 22 genera with more than a hundred acetogenic species listed by Drake *et al.* (2008), representatives of only two genera, namely *Natronincola acetigena* (Zhilina *et al.*, 1996) and *Natronincola histidinovorans* (Zhilina *et al.*, 1998), have been isolated from soda lakes. They are however unable to utilize hydrogen. Finding a possible microbial H_2 sink in terrestrial ecosystems limited by sulfate (such ecosystems could occur on land in the Early Precambrian period) is of special interest. The aim of our study was to search for hydrogenotrophic alkaliphilic bacteria at pH around 10, high alkalinity and salt content close to the precipitation of evaporitic minerals *in situ*. The Tanatar soda lake group is found on the right bank of the Irtysh River (Altay Region, Russia) in cryo-arid steppe and was chosen as a site for these studies. This site has been a classical field for the investigation of alkaliphilic communities since the 1930s (Issatchenko, 1951). Our search resulted in the isolation of the first alkaliphilic autotrophic H_2 -consuming bacterium producing acetate as a sole product at pH 8.5–10.5 and total salt content close to that conditioning sodium carbonates precipitation. The isolate is a novel alkaliphilic representative of the order *Halanaerobiales*. Its physiological properties and phylogenetic position suggest that it represents a novel species in a new genus within family *Halobacteroidaceae*.

Samples of sediments and water from the Tanatar soda lake group (Altay Region, Russia) were collected by V. V. Kevbrin in June 2008. The samples were collected in screw-capped glass vials without headspace and transported at ambient temperature to the laboratory, where they were stored at 4 °C. The pH of water at the Tanatar III sampling site was 10.35 and salinity was 10.8 % as measured by refractometer (Carl Zeiss).

The initial enrichment was obtained on an anoxically prepared selective medium containing ($g\ l^{-1}$): NH_4Cl , 0.5; KH_2PO_4 , 0.2; $MgCl_2 \cdot 6H_2O$, 0.1; KCl , 0.2; yeast extract (Difco), 0.05; Na_2CO_3 , 50.1; $NaHCO_3$, 7.5; $NaCl$, 47.42; trace element solution (Kevbrin & Zavarzin, 1992) 1 ml; $Na_2S \cdot 9H_2O$, 0.5 (pH 10.16). The medium was dispensed into 120 ml glass flasks with screw caps and the headspace (100 ml) was filled with H_2 at atmospheric pressure. Flasks were inoculated with 1 g sediments (from Lake Tanatar III) and incubated at 35 °C. The composition of the mineral medium for cultivation and maintenance was ($g\ l^{-1}$): Na_2CO_3 , 68; $NaHCO_3$, 38; $NaCl$, 60 (pH 9.2); yeast extract (Difco), 0.05 or vitamin solution (Wolin *et al.*, 1963), 10 ml l^{-1} ; $Na_2S \cdot 9H_2O$, or sodium thioglycollate, 0.5; substrates: H_2 (100 % headspace) or ethanol 96 % (w/v) 5 ml l^{-1} (corresponding to 87 mM). No changes were made in the other medium components. Serial dilutions in

selective medium with H_2 in the gas phase were made and the last positive dilution was used in attempts to isolate colonies in roll-tubes as described previously (Zhilina *et al.*, 1998). However, the organism did not form colonies on agar medium. After repeated tenfold dilutions on medium with yeast extract and vitamins, a pure culture of the novel isolate was obtained and designated strain Z-7100^T. Culture purity was controlled by direct microscopy, negative growth on organic-rich media, and by 16S RNA gene sequence analyses.

Phase-contrast photomicrographs and cell size measurements were made by using a Zetopan light microscope (Reichert). Ultrathin sections and electron microscopy of negatively stained cells were performed as described previously (Zhilina *et al.*, 1996). Cells of strain Z-7100^T were long, thin flexible rods measuring $0.2\text{--}0.5 \times 10.0\text{--}30.0\ \mu m$. Cells were so flexible that they often folded into hooks or nooses (Fig. 1a). The cells were motile by peritrichous flagella (Fig. 1b). At the late-exponential growth stage, the cells lysed, yielding spheroplasts (see upper arrow in Fig. 1a). Strain Z-7100^T formed round endospores (see lower arrow in Fig. 1a). Ultrathin sections revealed cell walls with outer membranes, typical of Gram-negative bacteria (Fig. 1c).

Strain Z-7100^T was an obligate anaerobe and grew only in anoxically prepared media with reductants such as sodium sulfide, cysteine or thioglycollate. In aerobically prepared medium and medium containing 1.5 or 2.0 % O_2 no growth occurred. However, the cells were catalase-positive as determined by the formation of O_2 bubbles on the cell pellet treated with 3 % (v/v) H_2O_2 solution. Bacterial growth was monitored by direct cell counting under a phase-contrast microscope and by measuring OD_{600} (Spectrophotometer-2100; UNICO). Quantitative measurements by GC showed that acetate was the sole product. Acetate was assayed by GC Crystall 5000.2 (Russia) equipped with 1 m glass column filled with the Carbowax C with 0.3 % Carbowax 20M and 0.1 % H_3PO_4 (Supelco) stationary phase. Hydrogen was measured on the same device equipped with 1 m glass column filled with molecular Sieve SA (Serva).

For substrate utilization tests, the culture was inoculated into mineral medium with yeast extract ($0.05\ g\ l^{-1}$). All organic substrates (peptides, carbohydrates, amino acids, alcohols and organic acids) were added to a final concentration of $3\ g\ l^{-1}$ (w/v) or $3\ g\ l^{-1}$ (v/v) for alcohols as filter-sterilized ($0.2\ \mu m$; Millipore) concentrated solutions. Medium without substrates and yeast extract ($0.05\ g\ l^{-1}$) was used as a control. For growth-positive tests, three subsequent transfers to the same medium were performed. Strain Z-7100^T could only utilize a few substrates. It did not utilize carbohydrates (arabinose, fructose, glucose, galactose, lactose, maltose, mannose, ribose, sucrose, trehalose or xylose). It did not ferment Casamino acids or amino acids used by other homoacetogens (Zhilina *et al.*, 1996, 1998; Zhilina & Zavarzin, 1990; Kevbrin *et al.*, 1995). It was negative for growth on aspartate, glutamate, histidine, ornithine, methanol, n-propanol, iso-propanol, butanol, mannitol, glycerol, ethylene glycol, 2-3-butanediol,

Fig. 1. Morphology of strain Z-7100^T. (a) Cells viewed by phase-contrast microscopy (the upper arrow points to a spheroplast, whereas the lower arrow points to an endospore); bar, 10 µm. (b) Negatively stained flagellated cell; bar, 0.5 µm. (c) Longitudinal section showing the Gram-negative type structure of the cell wall; bar, 0.1 µm.

trimethylamine, monomethylamine, formate, succinate, propionate and *N*-acetylglucosamine. Only ethanol, lactate and pyruvate were used by strain Z-7100^T for chemo-organotrophic growth. In all cases, acetate and CO₂ were the only metabolic products of strain Z-7100^T. Traces of H₂ were recorded as intermediates during chemo-organotrophic growth. The strain could grow chemolithotrophically with H₂ and CO₂ without yeast extract, which could be replaced by a vitamin solution (Wolin *et al.*, 1963). For each 4 mol H₂ consumed it produced 1 mol acetate according to the reaction:

Capacity for anaerobic respiration with various acceptors was tested with ethanol as an electron donor. Growth was

Fig. 2. Growth and acetate production by strain Z-7100^T with hydrogen as an electron donor and CO₂ as an electron acceptor. Growth of strain Z-7100^T (▲), acetate production (■) and hydrogen consumption (●).

monitored by measuring OD₆₀₀ and the levels of reduction products were determined as follows: by measuring sulfide (Trüper & Schlegel, 1964) for sulfur compounds; and iron (II) ferrozine complex at A₅₆₂ (Lovley & Phillips, 1986) for iron acceptors. Arsenate was detected as described previously (Zavarzina *et al.*, 2009). Selenate and selenite were quantified by ion chromatography with Dionex Ion Pack AS4-ASC 4.6 × 250 mm with 2.04/2.16 mM bicarbonate-carbonate as eluent. Strain Z-7100^T reduced NO₃⁻ (10 and 20 mM), SeO₄²⁻ (5 mM) and AQDS (2 mM). No growth occurred with S₂O₄²⁻ (20 mM), SO₃²⁻ (20 mM), NO₂⁻ (2 mM), iron (III)-EDTA, S⁰ (1 w/v), AsO₄³⁻ (5 mM), SeO₃²⁻ (2.5 mM) or crotonate (10 mM; Sigma). Fumarate (5 mM; Fluka), and SO₄²⁻ (20 mM) did not inhibit growth, but also were not reduced. Only weak reduction of S₂O₃²⁻ was observed. Strain Z-7100^T, with selenate as acceptor and ethanol as donor, completely reduced selenate with formation of an orange-red tint in the medium. The addition of nitrate as an electron acceptor stimulated growth of strain Z-7100^T. Nitrate was reduced to ammonium which was measured by microdiffusion with Nessler reagent. Neither nitrite (Griss reagent after acidification and Merckoquant 1.10020 nitrate-nitrite tests) nor gaseous nitrogen (GC analysis) were produced. Strain Z-7100^T did not grow under 100%, 30% or 15% CO in the gas phase without acceptors and reaction of H₂ formation was not recorded by GC. As inorganic nitrogen sources, the organism used ammonium as well as nitrate and it was incapable of nitrogen fixation. The DNA of strain Z-7100^T was tested for the presence of nitrogen fixation genes by using universal primers targeting the *nifH* gene (Marusina *et al.*, 2001), but the gene was not detected.

Vancomycin, novobiocin and rifampicin at 200 µg l⁻¹ completely inhibited growth of strain Z-7100^T. Streptomycin, bacitracin and benzylpenicillin at the same concentration did not inhibit growth.

The optimal conditions for growth of strain Z-7100^T were tested in carbonate-buffered medium with ethanol (0.5%,

w/v) added as the substrate and NaNO_3 (10 mM) added as electron acceptor. Growth of strain Z-7100^T occurred at 25 to 45 °C, with an optimum at 40 °C (Fig. S1).

The pH optimum was determined at 37 °C in the basal medium with 90 g NaHCO_3 l⁻¹ and 70 g NaCl l⁻¹. Na_2CO_3 was omitted. The pH value was adjusted by titration of the medium with 12 M NaOH or 12 M HCl. Strain Z-7100^T was an obligate alkaliphile. The pH range for growth was 8.5 to 10.5 with an optimum at pH 8.8–9.3. No growth occurred at pH lower than 7.6 or higher than 10.6 (Fig. S2).

The NaCl concentration range suitable for growth was determined in carbonate-buffered basal medium. In situations where the NaCl concentration was below 60 g l⁻¹, the total dissolved solids deficiency state was compensated for by adding an equivalent amount of Na_2SO_4 (e.g. in the variant with 1 g NaCl l⁻¹, 59 g Na_2SO_4 l⁻¹ was added). Strain Z-7100 did not require Cl^- for growth. However, it was halotolerant and could grow within a Cl^- ion concentration range from 0 to 2.5 M with an optimum at 1.2–1.5 M Cl^- .

The dependence of the growth rate upon the total salts content and the salt composition was determined in media containing mixtures of sodium carbonates and chlorides. The basal medium (see above) but with 4.7 M Na^+ (3.58 M Na_2CO_3 , 0.6 M NaHCO_3 and 0.5 M NaCl) and a saturated solution of carbonates containing 1.75 M CO_3^{2-} and 0.6 M HCO_3^- were diluted by the basal medium without sodium salts in the following ratio: 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1. Strain Z-7100^T could grow within the ranges 1.9–4.7 M for Na^+ and 0.94–2.35 M for $\text{CO}_3^{2-} + \text{HCO}_3^-$. Growth rate was maximal within the ranges 2.8–3.3 M for Na^+ and 1.41–1.64 M for $\text{CO}_3^{2-} + \text{HCO}_3^-$ (Fig. S3). No growth occurred when the organism was inoculated into media with TRIS or CAPS buffers as described previously (Zavarzina *et al.*, 2006) in order to determine the dependence of growth upon sodium carbonates. Strain Z-7100^T was an obligate and extreme natronophile.

For fatty acid analysis, cells of strain Z-7100^T were grown on mineral medium with ethanol as the substrate at pH 9.5, 2.75 M Na^+ and 35 °C and harvested in the late exponential growth phase. Fatty acid extraction of micro-organisms from dry samples (5 mg) was carried out by whole biomass acid methanolysis in 0.4 ml 1.2 M HCl in methanol by heating to 80 °C for 1 h. Resulting fatty acid methyl esters were extracted twice with 0.2 ml hexane and processed on an Agilent Technologies AT-5850/5973 GC-MS system. The fatty acid profile of strain Z-7100^T demonstrated that the dominant saturated fatty acids were $\text{C}_{14:0}$ (11.39%), anteiso- C_{15} (14.02%) and $\text{C}_{16:0}$ (6.42%), which constituted about 31.83% of the total fatty acids. The major aldehydes were C_{16} (14.24%), anteiso- C_{15} (6.85%) and C_{14} (7.83%), which constituted about 28.92% of the total fatty acids (Table 1). Strain Z-7100^T was unique in that it possessed an extraordinary diversity

Table 1. Cellular fatty acid content of strain Z-7100^T

Values are percentage (w/w) of total fatty acids. Major fatty acids are shown in bold. Fatty acids are formatted as follows. For a17:1a, 17 is the number of carbon atoms; the number after the colon denotes the number of double bonds; a or i at the beginning indicates anteiso or iso methyl-branching; a after the numbers indicates aldehyde (e.g. a17:1a represents anteiso-heptadecenoic aldehyde; 16:1 ether represents 1-hexadecenyl ether; i16:1 ether represents iso-1-hexadecenyl ether).

Fatty acid	Strain Z-7100 ^T
13:0	0.57
i14	3.32
14:1 ω 5	1.06
14:1 ω 3	1.19
14:0	11.39
12:0 3-OH	0.20
i14a	0.81
14a	7.83
i15	1.81
a15	14.02
a13:0 3-OH	0.20
i16:1 ether	3.74
15:0	2.91
i15a	1.07
a15a	6.85
16:1 ether	3.61
15a	4.23
i16	0.84
16:1 ω 7	1.87
16:0	6.42
i16a	1.24
16:1 ω 9a	0.85
16:1 ω 7a	3.63
16:1 ω 5a	0.24
16a	14.24
i17	0.20
a17	0.37
i17:1a	0.60
a17:1a	0.31
i17a	0.28
a17a	0.69
17:1a	0.20
17a	0.28
18:1 ω 9	0.26
18:1 ω 7	0.25
18:0	1.13
18:1a ω 9	0.54
18:1a ω 7	0.19
18a	0.58
Σ	100

of fatty acids. The identified fatty acids and aldehydes comprise 39 straight and branched species, including hydroxy acids $\text{C}_{12:0}$ 3-OH and anteiso- $\text{C}_{13:0}$ 3-OH species. The lipid profile alone would suggest that strain Z-7100^T represents a novel taxon.

Fig. 3. Neighbour-joining tree with Jukes–Cantor distances based on 16S rRNA gene sequences, showing the position of strain Z-7100^T in relation to the closest relatives within the family *Halobacteroidaceae*. The tree was rooted with the 16S rRNA gene sequence of the type strain of *Bacillus subtilis* as the outgroup. Numbers at nodes are bootstrap values in percentages based on 1000 replicates; only values greater than 70 % are shown. Bar, 5 nt substitutions per 100 nt.

The G+C content of the DNA was determined by the thermal denaturation/reassociation technique using a Unicam SP 1800 spectrophotometer (UK) at a rate of $0.5\text{ }^{\circ}\text{C min}^{-1}$ and calculated according to Owen *et al.* (1969). The genomic DNA G+C content of strain Z-7100^T was $32.0 \pm 0.1\text{ mol}\%$.

DNA was isolated by a modified Birnboim–Doly alkaline extraction procedure (Birnboim & Doly, 1979) and according to the Promega Wizard technology. A universal primer system (Lane, 1991) was used to amplify 16S rRNA gene fragments. The amplification products were sequenced by the Sanger method (Sanger *et al.*, 1977) on an ABI PRISM 3730 automatic sequencer (Applied Biosystems) using the Big Dye Terminator v.3.1 kit (Applied Biosystems) according to the manufacturer's instructions. Sequencing was performed in both directions using both external and internal primers. The obtained 16S rRNA sequence of strain Z-7100^T (1486 nt from positions 25 to 1487 in *Escherichia coli* numbering) was compared with all GenBank entries by a BLAST search (<http://www.ncbi.nlm.nih.gov/BLAST>), which showed that the closest relatives to the strain were representatives of the family *Halobacteroidaceae* of the order *Halanaerobiales* (Rainey *et al.*, 1995; Oren, 2000). These sequences were retrieved and aligned with the sequence of strain Z-7100^T using the CLUSTAL W (Thompson *et al.*, 1994) algorithm implicated in the BioEdit v.7.0.9.0 software package (Hall, 1999). Phylogenetic trees were reconstructed by using the TREECON software package (Van de Peer & De Wachter, 1994). Evolutionary distances were calculated by using the Jukes–Cantor algorithm, and the branching order was determined via the neighbour-joining algorithm. The tree in Fig. 3 is a consensus of 1000 replicate trees.

Among species with validly published names, the highest 16S rRNA gene sequence similarity level (92.9 %) was with

the type strain of the type species of the genus *Selenihalanaerobacter*, namely *Selenihalanaerobacter shriftii* DSSe-1^T, which was isolated from the Dead Sea (Switzer Blum *et al.*, 2001). Sequence similarity with other members of this group was in the range 85.9–89.7 %. Sequence similarities with other members of the family *Halobacteroidaceae* were no more than 92.9 %, which corresponds to an intergeneric level within the family of 85.4–95.0 %. Thus, it could be concluded that strain Z-7100^T forms a new line at the genus level within the radiation of the family *Halobacteroidaceae*.

The novel isolate is a novel alkaliphilic homoacetogenic bacterium in the family *Halobacteroidaceae* and the first alkaliphilic hydrogenotrophic homoacetogenic bacterium. Strain Z-7100^T could grow anaerobically by respiratory metabolism and reduced selenate and nitrate, as observed in *Selenihalanaerobacter shriftii*, or could grow lithotrophically with $\text{H}_2 + \text{CO}_2$ like *Acetohalobium arabaticum* (Zhilina & Zavarzin, 1990; Kevbrin *et al.*, 1995). Phenotypic differences between strain Z-7100^T and its closest phylogenetic relative *S. shriftii* were significant enough to indicate that the isolate belongs to a new genus. *S. shriftii* is a neutrophilic moderate halophile, which oxidizes glucose and glycerol to acetate + CO_2 with selenate and other electron acceptors, while Z-7100^T is an alkaliphilic halotolerant natronophile, which can grow lithoautotrophically with molecular hydrogen and does not utilize carbohydrates. Morphologically, strain Z-7100^T is clearly distinct from *S. shriftii*, the cells of which are non-motile, non-spore-forming straight rods. *Natroniella acetigena* (Zhilina *et al.*, 1996) is rather close to strain Z-7100^T phenotypically: it is a natronophilic, homoacetogen able to grow with ethanol, lactate or pyruvate. However it is Cl^- -dependent and does not utilize H_2 but can use glutamate and propanol. Morphologically, the organisms were clearly distinct and the 16S rRNA sequence similarity

Table 2. Characteristics that differentiate strain Z-7100^T from homoacetogenic representatives of family Halobacteroidaceae

Strains: 1, Z-7100^T; 2, *Selenihalanaerobacter shriftii* DSSe-1^T (Switzer Blum *et al.*, 2001); 3, *Natroniella acetigena* Z-7937^T (Zhilina *et al.*, 1996); 4, *Acetohalobium arabaticum* Z-7288^T (Zhilina & Zavarzin, 1990; Kevbrin *et al.*, 1995). NR, Not reported.

Characteristic	1	2	3	4
Cell size (µm)				
Width	0.2–0.3	0.6	1.0–1.2	0.7–1.0
Length	10.0–30.0	2.0–6.0	6.0–15.0	2.0–5.0
Motility	+	–	+	+
Flexibility	+	–	–	–
Spore formation	+	–	+	+
Growth temperature (°C)				
Range	25–45	16–42	26–42	NR–47
Optimum	40	38	37	38–40
Growth pH				
Range	8.5–10.5	5.3–8.9	8.1–10.7	5.8–8.4
Optimum	8.8–9.3	7.2	9.7–10.0	7.4–8.0
Na ⁺ total tolerance (M)				
Range	1.9–4.7	1.7–4.1	1.7–4.4	1.7–4.3
Optimum	2.8–3.3	3.6	2.1–2.7	2.7–3.1
NaCl tolerance (%)				
Range	0–14	10–24	10–25	10–26
Optimum	7.0–8.5	21	15–18	12–18
Dependence on carbonates	+	–	+	–
Utilization of:				
Glucose	–	+	–	–
Glutamate	–	–	+	+
n-Propanol	–	–	+	–
Glycerol	–	+	–	–
Ethanol	+	–	+	–
Lactate	+	–	+	+
Pyruvate	+	–	+	+
Ethanol	+	–	–	+
Growth with H ₂ + CO ₂	+	–	–	+
DNA G + C content (mol%)	32.0	31.2	31.9	33.6
Isolation source	Soda Lake Tanatar III, Altay	Dead Sea	Soda Lake Magadi, Kenya	Lake Sivash, Crimea

*With electron acceptors.

level between these organisms was low (87.7%). The features differentiating strain Z-7100^T and its closest phenotypic and phylogenetic relatives among the homoacetogens are summarized in Table 2. On the basis of its physiological properties and phylogenetic analysis, we propose that strain Z-7100^T represents a novel species in a new genus, for which the name *Fuchsiella alkaliacetigena* gen. nov., sp. nov. is proposed.

Description of *Fuchsiella* gen. nov.

Fuchsiella [Fuch.si.el'la. N.L. fem. dim. n. *Fuchsiella* named in honour of Professor Georg Fuchs (Freiburg, Germany), who made a most serious contribution to our understanding of multiple pathways of CO₂ assimilation by micro-organisms].

Gram-negative, spore-forming rods, motile by peritrichous flagella. Obligatory anaerobic. Obligately alkaliphilic and

natronophilic. Member of the order *Halanaerobiales*, family *Halobacteroidaceae* performing homoacetogenic metabolism of a restricted number of compounds. Able to grow chemolithotrophically with H₂ + CO₂. Few organic compounds are metabolized with external electron acceptors. Found in soda lakes. The type species is *Fuchsiella alkaliacetigena*.

Description of *Fuchsiella alkaliacetigena* sp. nov.

Fuchsiella alkaliacetigena [al.ka.li.a.ce.ti'ge.na. N.L. n. *alkali* soda ash; N.L. n. *acidum aceticum* acetic acid; L. suff. -genus -a -um (from L. v. *gigno*) producing; N.L. fem. adj. *alkaliacetigena* producing acetic acid in soda ash].

Cells are thin, long, flexible rods, 0.2–0.5 × 10.0–30.0 µm in size, motile by means of peritrichously located flagella. Cells are so flexible that they often turn into nooses. In the

late exponential growth phase, the cells lyse, yielding sphe-roplasts. Spore-forming; spores are round and terminal. The cell wall has a Gram-negative structure.

Obligate anaerobe. Can grow lithoautotrophically on $H_2 + CO_2$ or chemo-organotrophically on ethanol, lactate or pyruvate. Vitamin solution can be used as a substitute for yeast extract. Acetate is the sole metabolic product. Capable of respiratory metabolism and can reduce NO_3^- , SeO_4^{2-} or AQDS as electron acceptors with ethanol as an electron donor. The dominant cellular fatty acids are $C_{14:0}$, anteiso- C_{15} and $C_{16:0}$. The major aldehydes are C_{16} , anteiso- C_{15} and C_{14} . Obligately alkaliphilic with a pH range for growth of 8.5–10.7 and an optimum at pH 8.8–9.3. Extremely natrono-philic. Carbonates are required for growth. The Na^+ concentration range for growth is 1.9–4.7 M (0.94–2.35 M carbonates) with an optimum at 2.8–3.3 M (1.41–1.64 M carbonates). Halotolerant: the NaCl concentration range suitable for growth is 0–14 % (w/v) with an optimum at 7.0–8.5 % NaCl (w/v). Mesophilic: the growth temperature range is from 25 to 45 °C, with an optimum at 40 °C. Vancomycin, novobiocin and rifampicin completely inhibit growth.

The type strain, Z-7100^T (=DSM 24880^T=VKM B-2667^T), was isolated from bottom sediments of soda lake Tanatar III of Kulunda steppe (Altay, Russia). The genomic DNA G + C content of the type strain is 32.0 ± 0.1 mol%.

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