Desulfonatronum thiodismutans sp. nov., a novel alkaliphilic, sulfate-reducing bacterium capable of lithoautotrophic growth

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A novel alkaliphilic, sulfate-reducing bacterium, strain MLF1^T, was isolated from sediments of soda Mono Lake, California. Gram-negative vibrio-shaped cells were observed, which were 0.6–0.7 × 1.2–2.7 µm in size, motile by a single polar flagellum and occurred singly, in pairs or as short spirilla. Growth was observed at 15–48 °C (optimum, 37 °C), > 1–7 % NaCl, w/v (optimum, 3 %) and pH 8.0–10.0 (optimum, 9.5). The novel isolate is strictly alkaliphilic, requires a high concentration of carbonate in the growth medium and is obligately anaerobic and catalase-negative. As electron donors, strain MLF1^T uses hydrogen, formate and ethanol. Sulfate, sulfite and thiosulfate (but not sulfur or nitrate) can be used as electron acceptors. The novel isolate is a lithoheterotroph and a facultative lithoautotroph that is able to grow on hydrogen without an organic source of carbon. Strain MLF1^T is resistant to kanamycin and gentamicin, but sensitive to chloramphenicol and tetracycline. The DNA G + C content is 63.0 mol% (HPLC). DNA–DNA hybridization with the most closely related species, *Desulfonatronum lacustre* Z-7951^T, exhibited 51 % homology. Also, the genome size (1.6 × 10⁹ Da) and *T_m* value of the genomic DNA (71 ± 2 °C) for strain MLF1^T were significantly different from the genome size (2.1 × 10⁹ Da) and *T_m* value (63 ± 2 °C) for *Desulfonatronum lacustre* Z-7951^T. On the basis of physiological and molecular properties, the isolate was considered to be a novel species of the genus *Desulfonatronum*, for which the name *Desulfonatronum thiodismutans* sp. nov. is proposed (the type strain is MLF1^T = ATCC BAA-395^T = DSM 14708^T).

The sulfate-reducing bacteria present a large spectrum of extremophiles and include psychrophilic, thermophilic, halophilic and alkaliphilic *Archaea* and *Bacteria* (Widdel & Hansen, 1992; Castro *et al.*, 2000; Pikuta & Hoover, 2001). Among this group, there are two genera that contain obligately alkaliphilic species. The first alkaliphilic sulfate-reducer, *Desulfonatronovibrio hydrogenovorans*, was isolated from soda Lake Magadi in equatorial Africa (Zhilina *et al.*, 1997). Later, the distribution of this species was extended to Tuva's soda lakes in Siberia, central Asia (Pikuta *et al.*, 1997) and a novel alkaliphilic species, *Desulfonatronum lacustre*,

was described (Pikuta *et al.*, 1998). Both genera include Gram-negative, non-spore-forming, mesophilic, obligately carbonate-dependent alkaliphiles that are incapable of growth below pH 8.0. The moderately thermophilic and alkaliphilic, spore-forming sulfate-reducer *Desulfotomaculum alkaliphilum* was isolated from manure with neutral pH (Pikuta *et al.*, 2000), indicating that the adaptation of micro-organisms to alkaline environments is not connected with deep genetic changes and that local micro-ecosystems with high pH and temperature could be created in composite substrates, such as soil or manure, by these micro-organisms. All previously described alkaliphilic sulfate-reducers had a lithoheterotrophic metabolism.

In this article we describe a novel alkaliphilic, mesophilic, sulfate-reducing bacterium with the capacity for lithoautotrophic growth.

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The GenBank/EMBL/DBJ accession number for the 16S rDNA sequence of strain MLF1^T is AF373920.

Isolation, cultivation and morphology

Black mud sediments ($\text{pH } 9.9 \pm 0.02$) with a strong smell of sulfide were collected anaerobically from under shallow water (temperature, $21.6 \pm 0.1^\circ\text{C}$; salinity, 7%) near the south shore of Mono Lake in California on 15 August 2000. Mud samples were hermetically sealed in sterile glass vessels with screw caps, maintained at 4°C during transportation and stored at 2°C in the Astrobiology Laboratory of the NASA Marshall Space Flight Center. For cultivation of the novel isolate, modified medium was used (Pikuta *et al.*, 1998), which contained (1^{-1}): Na_2SO_4 , 3 g; NaCl , 30 g; Na_2CO_3 , 2.76 g; NaHCO_3 , 24.0 g; KCl , 0.2 g; K_2HPO_4 , 0.2 g; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.1 g; NH_4Cl , 1.0 g; $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$, 0.4 g; resazurin, 0.001 g; yeast extract, 0.2 g; Na-formate, 5.0 g; 2 ml vitamin solution (Whitman *et al.*, 1963); and 1 ml trace mineral solution (Whitman *et al.*, 1982). The final pH was adjusted to 9.5. High-purity nitrogen was used as the gas phase, except in the case of cultivation with hydrogen as the electron donor, for which the gas phase was filled by pure hydrogen. To obtain enrichment cultures, 0.5 g wet sediment material ($\text{pH } 10.0$) was injected into standard Hungate tubes that contained medium and incubated at 35°C for 10–14 days. Pure cultures were obtained by the dilutions method on sulfate-containing medium with hydrogen as the electron donor. Growth of colonies was checked by the 'roll-tube' method on 3% (w/v) agar medium, where carbonates were added separately after autoclaving. Colonies were white-yellowish, lens-shaped in deep agar and 0.5–2.0 mm in diameter with irregular, smooth edges. One colony was chosen for further characterization and designated strain MLF1^T (=ATCC BAA-395^T = DSM 14708^T). Purity of the culture during this study was indicated by the absence of growth on glucose-peptone sulfate-free medium that contained excessive quantities of yeast extract and by microscopic control.

Transmission electron microscopy was carried out by using a JEOL TEM 100 CX II. Negative staining was performed with uranyl acetate. Cells of strain MLF1^T were highly motile and vibrio-shaped, 0.6–0.7 μm in diameter and 1.2–2.7 μm long and had a single polar flagellum (Fig. 1). Cells occurred singly, in pairs or as short spirilla. Multiplication occurred by binary fission. Gram-stained cells of strain MLF1^T exhibited the red colour typical for the reaction with Gram-negative cell walls. Spores were not observed.

Growth characteristics and metabolic properties

Bacterial growth was measured by direct cell count under a phase-contrast microscope (Fisher Micromaster), by measuring sulfide in the growth medium (Trüper & Schlegel, 1964) or by estimating an increase in OD_{510} (Genesis 5; Spectronic Instruments). All experiments were performed at 35°C . Obligate dependence on the CO_3^{2-} ion was detected by using a medium that was described previously (Pikuta *et al.*, 2000). Dependence on Na^+ ions was detected by substitution of Na-containing salts by K-containing salts. Dependence on Cl^- ions was checked by substitution of

Fig. 1. Image of *Desulfonatronum thiodismutans* MLF1^T by transmission electron microscopy, showing single polar attached flagellum. Bar, 0.3 μm .

Cl-containing salts by sulfates. The novel isolate grew only under strictly anaerobic conditions and was catalase-negative (Gerhardt *et al.*, 1981). The isolate was mesophilic and grew at 15 – 45°C , with optimum growth at 37°C ; growth was absent at 10 and 53°C . The novel isolate grew at $\text{pH } 8.0$ – 10.0 ; no growth was detected at pH values of 7.8 or 10.5 . Strain MLF1^T required Na^+ ions (from NaCl) for growth; it did not grow at 0% (w/v) NaCl . The optimum NaCl concentration was 3% (w/v), the range for growth was 1 – 7% (w/v) and growth was absent at 10% (w/v) NaCl . The generation time at optimal conditions [37°C , 3% (w/v) NaCl and $\text{pH } 9.5$] was 20 h. The novel isolate was obligately dependent on Na^+ ions, as it did not grow on medium with potassium salts instead of sodium salts. The absence of growth on serine-buffered medium demonstrated obligate dependence on CO_3^{2-} ions. Strain MLF1^T was not dependent on Cl^- ions, but cell motility was significantly decreased on medium without Cl-containing salts. The novel isolate required sulfates for respiration. End-products of sulfate respiration in the liquid phase were determined by HPLC. Sodium salts (formate, acetate, lactate, pyruvate, propionate and isovalerate) were used as standards. Separation was done on an Aminex HPX-87H column (Bio-Rad), with $5\text{ mM H}_2\text{SO}_4$ as the mobile phase. Gases (hydrogen, carbon dioxide, carbon monoxide and methane) were measured by using a model 3700 gas chromatograph (Varian) equipped with a Porapak Q column and a thermal conductivity detector. Nitrogen was used as the gas carrier. During sulfate respiration with formate, strain MLF1^T excreted only hydrogen sulfide (more than 30 mM) and no organic end-products were detected. CO_2 measurement at high pH was not feasible because of the high carbonate content of the medium.

Strain MLF1^T had a lithoautotrophic type of metabolism. Growth was observed during three consequent inoculations

on hydrogen medium with no organic carbon source. Growth on media without an organic carbon source was slow (10–14 days) with lower optical density ($0.08\text{--}0.1$) and the number of cells was approximately 10^8 ml^{-1} . During lithoautotrophic growth ($\text{H}_2 + \text{CO}_2$), high sulfidogenesis ($10\text{--}15\text{ mM H}_2\text{S}$) was registered. Strain MLF1^T also grew on H_2 media with yeast extract or acetate (2 mM). Organic carbon sources stimulated growth, with more rapid growth occurring on media with yeast extract than with acetate (optical density of growth on acetate was $0.2\text{--}0.25$, and on yeast extract it was $0.3\text{--}0.5$). Strain MLF1^T does not have a fermentative type of metabolism for organic substrates and has a very restricted spectrum of electron donors: only hydrogen, formate and ethanol were utilized. Growth did not occur on acetate, propionate, butyrate, pyruvate, lactate, methanol, glycerol, glycine, cysteine, serine, alanine, glutamate, aspartate, Casamino acids, yeast extract, peptone, bacto-tryptone, betaine, trimethylamine, glucose, fructose, mannose, starch or citrate. Sulfate, sulfite and thiosulfate were utilized as electron acceptors and elemental sulfur inhibited growth. Nitrate did not support growth. The novel isolate was capable of performing dismutation (inorganic fermentation); good growth, with optical density $0.2\text{--}0.25$, was observed on sulfite (5 mM) or thiosulfate (10 mM) with 2 mM acetate. Sulfidogenesis was registered at thiosulfate dismutation ($8\text{ mM H}_2\text{S}$) and at sulfite dismutation ($3\text{--}4\text{ mM H}_2\text{S}$). Sulfate production was measured nephelometrically with BaCl_2 . Sulfate concentration was 8 mM during thiosulfate dismutation and 3.8 mM during sulfite dismutation; this stoichiometry is close to the dismutation data for *Desulfovibrio sulfodismutans* ThAc01^T (Bak & Pfennig, 1987). Growth was inhibited by 5 mM molybdate, which blocks ATP sulfurylase in sulfate-reducing bacteria. Strain MLF1^T was resistant to kanamycin and gentamicin ($250\text{ }\mu\text{g ml}^{-1}$), as growth without morphological changes was observed, and sensitive to tetracycline ($250\text{ }\mu\text{g ml}^{-1}$) and chloramphenicol ($125\text{ }\mu\text{g ml}^{-1}$).

Fatty acids

Fatty acid methyl esters were extracted from fresh biomass grown for 5 days and identified by using the Microbial Identification system (MIDI) Moore Library of anaerobic bacteria. The major fatty acid methyl esters were (%): $\text{C}_{14:0}$ (16.08); $\text{C}_{16:1}$ (21.23); $\text{C}_{18:0}$ (17.91); and a mixture of C_{18} unsaturated fatty acids in *cis*- and *trans*-configurations (19.80). In lower amounts were (%): $\text{C}_{16:0}$ (11.54); $\text{C}_{18:1}$ (10.61); and $\text{C}_{18:2}$ (2.84).

DNA analysis

The G + C content of the genomic DNA of strain MLF1^T was measured by the HPLC method as described previously (Mesbah *et al.*, 1989). An Alltima C_{18} column [$250 \times 4.6\text{ mm}$, $5\text{ }\mu\text{m}$ particle size (Alltech)] and 8% (v/v) methanol were used in this study. The results reported are the mean of three determinations for each of two degradations of the DNA. The G + C content of the genomic

DNA of strain MLF1^T was $63.0 \pm 0.1\%$ (mean $\pm$ standard deviation, $n=6$).

16S rRNA gene sequence analysis

Isolation of genomic DNA, amplification of the 16S rRNA gene and sequence determination were performed as described previously (Hoover *et al.*, 2003). The quality of the consensus sequence was confirmed with data from eight different clones. The sequence of strain MLF1^T was aligned with closely related sequences found in GenBank after a BLAST search (Altschul *et al.*, 1990) by using the PileUp program from the GCG Wisconsin package. Pairwise distances were computed with MEGA version 2.0 (Kumar *et al.*, 2001) by using the Jukes–Cantor (1969) model. An unrooted phylogenetic tree was constructed with the MEGA program by using the neighbour-joining method (Saitou & Nei, 1987). A sequence that covered 1481 nt of the 16S rRNA gene of strain MLF1^T was obtained, corresponding to positions 28–1515 of the *Escherichia coli* 16S rRNA gene sequence. The G + C content of this sequence was 56.58% . The sequence was compared with all sequences presently available in GenBank and appeared to be highly homologous to sequences from sulfate-reducing bacteria that belong to the δ -branch of the *Proteobacteria*. The phylogenetic tree, based on 1258 common nucleotide positions, shows that the closest relationship of strain MLF1^T is to the genus *Desulfonatronum*, which is located between two large clusters that represent the genera *Desulfovibrio* and *Desulfomicrobium* (Fig. 2). The highest degree of relatedness of strain MLF1^T was observed with *Desulfonatronum lacustre* Z-7951^T. Pairwise comparison of the complete 16S rRNA gene sequences of strain MLF1^T and *Desulfonatronum lacustre* Z-7951^T showed only 97.6% similarity over an alignment of 1359 nt.

DNA melting temperatures

Melting temperatures (T_m) of total genomic DNA from strain MLF1^T and *Desulfonatronum lacustre* Z-7951^T were determined by procedures described previously (De Ley *et al.*, 1970; Gillis *et al.*, 1970). Purified genomic DNA ($200\text{ }\mu\text{g}$) from strain MLF1^T and *Desulfonatronum lacustre* Z-7951^T was sonicated to generate DNA fragments of $500\text{--}700\text{ bp}$. Any residual RNA and single-stranded DNA was removed by treatment with RNase A and S1 nuclease, respectively (Ausubel *et al.*, 1987). Concentration and purity of the DNA were determined from A_{260} readings and the ratio between A_{260} and A_{280} readings, by using a Shimadzu UV-160 spectrophotometer. DNA ($80\text{ }\mu\text{g}$) from each of these micro-organisms was then denatured in $1 \times \text{SSC}$ buffer (pH 7.0) by increasing the temperature of the sample from 26 to $100\text{ }^\circ\text{C}$ (at a rate of $1\text{ }^\circ\text{C min}^{-1}$) and A_{260} was recorded. The experiment was conducted in triplicate. The melting temperature (T_m) was determined by calculating the temperature at which the hyperchromicity reached half of the value obtained after complete melting. The T_m value of the genomic DNA of strain MLF1^T was $71 \pm 2\text{ }^\circ\text{C}$ (mean $\pm$

Fig. 2. Phylogenetic tree showing the position of *Desulfonatronum thiodismutans* MLF1^T within the radiation of sulfate-reducing bacteria that belong to the δ -subclass of the *Proteobacteria*. The position of the root was determined by including *E. coli* as the outgroup. Bar, 2 substitutions per 100 nucleotides.

standard deviation, $n=3$), whereas it was 63 ± 2 °C for *Desulfonatronum lacustre* Z-7951^T.

DNA–DNA hybridization

To determine the homology of genomic DNA between strain MLF1^T and *Desulfonatronum lacustre* Z-7951^T, DNA–DNA hybridization was performed by DNA reassociation kinetics as described previously (De Ley *et al.*, 1970; Johnson, 1985). Purified, sonicated genomic DNA (80 µg) from each of these micro-organisms was added to 4 × SSC buffer (pH 7.0) and 25 % deionized formamide. DNA was denatured by raising the temperature to 100 °C and then cooled to 5 °C above their respective melting temperatures. Then the temperature was rapidly lowered (1.5 min) to the reassociation temperature and A_{270} was recorded at 5 s intervals for a total of 20 min. The initial reassociation kinetics were determined by linear regression analysis. The homology of the DNA from these two micro-organisms was calculated by using the equation described by De Ley *et al.* (1970). All statistical analyses were performed using Microsoft Excel software. DNA–DNA hybridization established 51 % homology between the genomes of strain MLF1^T and *Desulfonatronum lacustre* Z-7951^T.

Genome size

The genome sizes of strain MLF1^T and *Desulfonatronum lacustre* Z-7951^T were determined by using DNA reassociation kinetics, following the equation described by Gillis *et al.* (1970). The genome size for strain MLF1^T was 1.6×10^9 Da, whereas that for *Desulfonatronum lacustre* Z-7951^T was 2.1×10^9 Da.

DISCUSSION

The presence of alkaliphilic sulfate-reducers in continental athalassic soda lakes on the African, Eurasian and American continents demonstrates the wide geographical distribution of these related organisms. The ancient nature of alkalic soda lakes could provide insights into the origin of bacterial diversity and distribution on planet Earth (Zavarzin *et al.*, 1999). In previous work, a study of anaerobic bacterial communities was performed on samples of equatorial soda Magadi Lake (Kenya) and central Asian Khadyn alkalic lake (Siberia). Our investigation dealt with the unique ecosystem

of an ancient soda lake in California, which also has an athalassic nature. The novel isolate MLF1^T is a typical secondary anaerobe, the main function of which in the bacterial anaerobic community is the consumption of hydrogen and low-molecular-mass end-products of primary anaerobes. Two novel primary anaerobes, ASpG1^T and APO^T, were also isolated from the same sample. Detailed study of these new isolates showed that they belonged to novel species: *Spirochaeta americana* sp. nov. (Hoover *et al.*, 2003) and ‘*Tindallia californiensis*’ sp. nov. (Pikuta *et al.*, 2003), respectively. In both cases, the closest relationships (with <1 % difference) were with the species *Spirochaeta alkalica* and *Tindallia magadiensis*, respectively, isolated from the alkaline anaerobic community of soda Lake Magadi (Zhilina *et al.*, 1996; Kevbrin *et al.*, 1998, 1999). It is interesting to note that the sulfate-reducer *Desulfonatronovibrio hydrogenovorans* Z-7935^T, which was also isolated from Lake Magadi in Kenya (Zhilina *et al.*, 1997), exhibited 10 % difference in 16S rDNA sequence from strain MLF1^T. The most closely related species to strain MLF1^T was *Desulfonatronum lacustre* Z-7951^T, a sulfate-reducer from the alkaline anaerobic bacterial community of Khadyn Lake, central Asia. Notwithstanding the fact that strains MLF1^T and Z-7951^T have the same morphology and catabolism (an identical list of electron donors and electron acceptors), these strains have different physiologies and anabolisms: American strain MLF1^T is an alkaliphile with dependence on NaCl and an autotrophic type of anabolism, but Asian strain Z-7951^T is a lithoheterotroph that does not need NaCl in the growth medium. Also, analyses of DNA G+C content showed a significant difference between strain MLF1^T and Z-7951^T (>6 mol%). DNA–DNA hybridization of genomic DNA between strain MLF1^T and *Desulfonatronum lacustre* Z-7951^T exhibited 51 % homology. The melting temperature (T_m) of the genomic DNA of strain MLF1^T was 8 °C higher than that of *Desulfonatronum lacustre* Z-7951^T. Finally, there was a significant difference in the genome size between strain MLF1^T and the closely related species *Desulfonatronum lacustre* Z-7951^T. Comparison of these DNA data of strain MLF1^T with those of the most closely related species, *Desulfonatronum lacustre* Z-7951^T, indicates that there are sufficient differences for strain MLF1^T to be considered as a separate species. In Table 1, comparative characteristics for all known alkaliphilic sulfate-reducers are provided.

On the basis of phenotypic and genotypic characteristics

Table 1. Characteristics that distinguish *Desulfonatronum thiodismutans* MLF1^T from other alkaliphilic sulfate-reducing bacteria

Species: 1, *Desulfonatronum thiodismutans* MLF1^T (data from this study); 2, *Desulfonatronum lacustre* Z-7951^T [data from Pikuta *et al.* (1998)]; 3, *Desulfonatronovibrio hydrogenovorans* Z-7935^T [data from Zhilina *et al.* (1997)]; 4, *Desulfotomaculum alkaliphilum* S1^T [data from Pikuta *et al.* (2000)]. +, Positive; −, negative; (+), slow growth; ND, not determined. These four species are all capable of using H₂ + acetate and formate + acetate as electron donors, and sulfate, sulfite and thiosulfate (but not sulfur) as electron acceptors.

Characteristic	1	2	3	4
Cell shape	Vibrioid	Vibrioid	Vibrioid	Curved rod
Flagella	+	+	+	−
Spores	−	−	−	+
Size (μm)	0.6–0.7 × 1.2–2.7	0.7–0.9 × 2.5–3.0	0.4–0.5 × 1.5–2.0	0.6–0.7 × 3.0–3.5
pH range (optimum)	8.0–10.0 (9.5)	8.0–10.1 (9.3–9.5)	8.0–10.2 (9.5–9.7)	7.8–9.2 (8.7)
NaCl range (%) (optimum)	> 1–7 (3)	0–10 (0)	1.0–12.0 (3)	0–5.0 (0.1)
Temperature range (°C) (optimum)	15–48 (37)	22–45 (40)	22–45 (37)	37–58 (53)
DNA G + C content (mol%)	63.1	57.3	48.6	40.9
Electron donors (with SO ₄ ^{2−}):				
H ₂ + CO ₂	(+)	−	−	−
Lactate	−	−	−	+
Pyruvate	−	−	−	+
Ethanol	+	+	−	+
Genome size (Da)	1.6 × 10 ⁹	2.1 × 10 ⁹	2.1 × 10 ⁹	ND
Melting temperature (T _m) (°C)	71 ± 2	63 ± 2	74.5	ND

(Gram-negative cell wall, sulfate type of respiration, 16S rDNA sequence and DNA–DNA hybridization), strain MLF1^T was identified as a novel species of the genus *Desulfonatronum*. The name *Desulfonatronum thiodismutans* sp. nov. is suggested for this organism, in accordance with the capability of this bacterium to perform dismutation.

Description of *Desulfonatronum thiodismutans* sp. nov.

Desulfonatronum thiodismutans (thi.o.dis'mu.tans. Gr. *thios* sulfur; N.L. part. adj. *dismutans* dismutating, splitting; N.L. part. adj. *thiodismutans* sulfur-dismutating, pertaining to the ability of the organism to dismutate thiosulfate and sulfite).

Motile, vibrio-shaped cells with a single polar flagellum, 0.6–0.7 × 1.2–2.7 μm in size. Gram-negative. Non-spore-forming. Haloalkaliphilic: pH range for growth is 8.0–10.0, with optimum growth at pH 9.5. Growth is obligately dependent on Na⁺ and CO₃^{2−} ions. Range of NaCl for growth is > 1–7 % (w/v); optimum growth occurs at 3 % (w/v) NaCl. Mesophilic: temperature range for growth is 15–48 °C; optimum growth occurs at 37 °C. Cells can be stored frozen in liquid medium. Strictly anaerobic and catalase-negative. Does not grow without sulfate, which is used as an electron acceptor with H₂S as the end product. Sulfite and thiosulfate are alternative electron acceptors, but sulfur and nitrate are not. Elemental sulfur and molybdate inhibit growth. Lithoheterotrophic, facultatively lithoautotrophic (slow growth on H₂ + CO₂) and uses H₂, formate and ethanol as electron donors. A supplement of vitamins is required and yeast extract stimulates growth. Capable of receiving energy by the dismutation of

thiosulfate or sulfite (with 2 mM sodium acetate and carbonate as carbon sources). Resistant to kanamycin and gentamicin, but sensitive to tetracycline and chloramphenicol. Genomic DNA G + C content of the type strain is 63.0 mol% (HPLC). Major fatty acid methyl esters are C_{14:0}, C_{16:1}, C_{18:0} and a mixture of C₁₈ unsaturated fatty acids.

The type strain is MLF1^T (= DSM 14708^T = ATCC BAA-395^T). Isolated from mud sediments of alkaline, hypersaline, soda Mono Lake in California, North America.

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REFERENCES

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.
- Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (editors) (1987). *Current Protocols in Molecular Biology*, pp. 2.10–2.11. New York: Wiley.
- Bak, F. & Pfennig, N. (1987). Chemolithotrophic growth of *Desulfovibrio sulfodismutans* sp. nov. by disproportionation of inorganic sulfur compounds. *Arch Microbiol* **147**, 184–189.
- Castro, H. F., Williams, N. H. & Ogram, A. (2000). Phylogeny of sulfate-reducing bacteria. *FEMS Microbiol Ecol* **31**, 1–9.

- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Gerhardt, P., Murray, R. G. E., Costilow, R. N., Nester, E. W., Wood, W. A., Krieg, N. R. & Phillips, G. B. (editors) (1981). *Manual of Methods for General Bacteriology*. Washington, DC: American Society for Microbiology.
- Gillis, M., De Ley, J. & De Cleene, M. (1970). The determination of molecular weight of bacterial genome DNA from renaturation rates. *Eur J Biochem* **12**, 143–153.
- Hoover, R. B., Pikuta, E. V., Bej, A. K., Marsic, D., Whitman, W. B., Tang, J. & Krader, P. (2003). *Spirochaeta americana* sp. nov., a new haloalkaliphilic, obligately anaerobic spirochaete isolated from soda Mono Lake in California. *Int J Syst Evol Microbiol* **53**, 815–821.
- Johnson, J. L. (1985). DNA reassociation and RNA hybridization of bacterial nucleic acids. *Methods Microbiol* **18**, 33–74.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kevbrin, V. V., Zhilina, T. N., Rainey, F. A. & Zavarzin, G. A. (1998). *Tindallia magadii* gen. nov., sp. nov.: an alkaliphilic anaerobic ammonifier from soda lake deposits. *Curr Microbiol* **37**, 94–100.
- Kevbrin, V. V., Zhilina, T. N., Rainey, F. A. & Zavarzin, G. A. (1999). *Tindallia magadiensis* gen. nov., sp. nov. In *Validation of the Publication of New Names and New Combinations Previously Effectively Published Outside the IJSB*, List no. 68. *Int J Syst Bacteriol* **49**, 1–3.
- Kumar, S., Tamura, K., Jakobsen, I. B. & Nei, M. (2001). MEGA2: molecular evolutionary genetic analysis software. *Bioinformatics* **17**, 1244–1245.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Pikuta, E. V. & Hoover, R. B. (2001). Sulfate- and sulfur-reducing bacteria as terrestrial analogs for microbial life on Jupiter's satellite Io. In *Instruments, Methods, and Missions for Astrobiology IV*, Proceedings of SPIE Conference, vol. 4495, pp. 232–254. Edited by R. B. Hoover, G. V. Levin, R. R. Paepe & A. Y. Rozanov. San Diego: SPIE.
- Pikuta, E. V., Lysenko, A. M. & Zhilina, T. N. (1997). Distribution of *Desulfonatovibrio hydrogenovorans* in soda lakes of Tuva. *Mikrobiologiya* **66**, 262–268 (in Russian).
- Pikuta, E. V., Zhilina, T. N., Zavarzin, G. A., Kostrikina, N. A., Osipov, G. A. & Rainey, F. A. (1998). *Desulfonatovibrio lacustre* sp. nov.: a new alkaliphilic sulfate-reducing bacterium utilizing ethanol. *Mikrobiologiya* **67**, 123–131 (in Russian).
- Pikuta, E., Lysenko, A., Suzina, N., Osipov, G., Kuznetsov, B., Tourova, T., Akimenko, V. & Laurinavichius, K. (2000). *Desulfotomaculum alkaliphilum* sp. nov., a new alkaliphilic, moderately thermophilic, sulfate-reducing bacterium. *Int J Syst Evol Microbiol* **50**, 25–33.
- Pikuta, E. V., Hoover, R. B., Bej, A. K., Marsic, D., Detkova, E. N., Whitman, W. B. & Krader, P. (2003). *Tindallia californiensis* sp. nov., a new anaerobic, haloalkaliphilic, spore-forming acetogen isolated from Mono Lake in California. *Extremophiles Online First*, <http://dx.doi.org/10.1007/s00792-003-0326-7>.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Trüper, H. G. & Schlegel, H. G. (1964). Sulfur metabolism in *Thiorhodaceae*. I. quantitative measurements on growing cells of *Chromatium okenii*. *Antonie van Leeuwenhoek* **30**, 225–238.
- Whitman, W. B., Ankwarda, E. & Wolfe, R. S. (1982). Nutrition and carbon metabolism of *Methanococcus voltae*. *J Bacteriol* **149**, 852–863.
- Widdel, F. & Hansen, T. A. (1992). The dissimilatory sulfate-reducing bacteria. In *The Prokaryotes*, 2nd edn, vol. 1, pp. 583–624. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer-Verlag.
- Wolin, E. A., Wolin, M. J. & Wolfe, R. S. (1963). Formation of methane by bacterial extracts. *J Biol Chem* **238**, 2882–2886.
- Zavarzin, G. A., Zhilina, T. N. & Kevbrin, V. V. (1999). The alkaliphilic microbial community and its functional diversity. *Mikrobiologiya* **68**, 579–599 (in Russian).
- Zhilina, T. N., Zavarzin, G. A., Rainey, F., Kevbrin, V. V., Kostrikina, N. A. & Lysenko, A. M. (1996). *Spirochaeta alkalica* sp. nov., *Spirochaeta africana* sp. nov., and *Spirochaeta asiatica* sp. nov., alkaliphilic anaerobes from the continental soda lakes in Central Asia and the East African Rift. *Int J Syst Bacteriol* **46**, 305–312.
- Zhilina, T. N., Zavarzin, G. A., Rainey, F. A., Pikuta, E. N., Osipov, G. A. & Kostrikina, N. A. (1997). *Desulfonatovibrio hydrogenovorans* gen. nov., sp. nov., an alkaliphilic, sulfate-reducing bacterium. *Int J Syst Bacteriol* **47**, 144–149.