Desulfuribacillus stibiiarsenatis sp. nov., an obligately anaerobic, dissimilatory antimonate- and arsenate-reducing bacterium isolated from anoxic sediments, and emended description of the genus Desulfuribacillus

Christopher A. Abin¹ and James T. Hollibaugh²*  

Abstract

A novel anaerobic, Gram-stain-negative, endospore-forming bacterium, designated strain MLFW-2ᵀ, was isolated from anoxic sediments collected from the drainage area of a geothermal spring near Mono Lake, CA, USA. Optimal growth was achieved at 34 °C and pH 8.25–8.50 in medium containing 0.75 % (w/v) NaCl. Catalase, but not oxidase, was produced. Strain MLFW-2ᵀ was an obligate anaerobe capable of respiring with nitrate, nitrite, DMSO, arsenate, antimonate, selenate and selenite as terminal electron acceptors. Lactate, pyruvate, formate and H₂ could serve as electron donors to support growth. The isolate was incapable of fermentation. The predominant fatty acids were C₁₆:₀, C₁₆:₁ω₉c, C₁₆:₁ω₇c, C₁₈:₁ω₉c and C₁₈:₁ω₇c. The major polar lipids were phosphatidylglycerol and phosphatidylethanolamine. The only isoprenoid quinone detected was menaquinone 7 (MK-7). The DNA G+C content was 38.2 mol%. Phylogenetic analysis based on the 16S rRNA gene sequence demonstrated that strain MLFW-2ᵀ was a member of the order Bacillales and was most closely related to Desulfuribacillus alkaliiarsenatis AHT28ᵀ (93.9 % similarity). On the basis of phenotypic and phylogenomic evidence, strain MLFW-2ᵀ represents a novel species of the genus Desulfuribacillus, for which the name Desulfuribacillus stibiiarsenatis sp. nov. is proposed. The type strain is MLFW-2ᵀ (=DSM 28709ᵀ=JCM 30866ᵀ). An emended description of the genus Desulfuribacillus is also provided.

Antimony (Sb) is a trace element widely distributed throughout the environment as a result of both natural processes and anthropogenic activities. The biogeochemistry of Sb has long been an understudied topic, even though it is toxic and its use dates back several thousand years [1, 2]. Under oxic and anoxic conditions, thermodynamics predict that Sb should occur primarily as oxyanions of antimonate [Sb(V)] and antimonite [Sb(III)], respectively [3]. In spite of these predictions, significant concentrations of thermodynamically unstable species have been measured in the environment and biological activity has been invoked as a possible source [4].

A number of aerobic and anaerobic Sb(III)-oxidizing bacteria have already been isolated [5–10]. All of the Sb(III)-oxidizers identified thus far belong to the Proteobacteria or Actinobacteria, with the Gammaproteobacteria accounting for 49 % of strains [11]. With respect to the reductive side of the cycle, the first unequivocal evidence for microbial Sb(V) reduction came from experiments with anoxic sediments collected from an abandoned Sb mining site [12]. Subsequently, a chemolithoautotrophic microbial consortium dominated by Rhizobium spp. was shown to reduce Sb(V) using H₂ as the electron donor [13]. Currently, only two Sb (V)-reducing bacteria exist in pure culture [14, 15]. One of these isolates, strain MLFW-2ᵀ, was capable of respiring Sb (V) using lactate as the electron donor. Reduction of Sb(V) by strain MLFW-2ᵀ was accompanied by the precipitation of Sb(III) as a mixture of cubic and orthorhombic microcrystals of antimony trioxide (Sb₂O₃). Here we describe the morphological, physiological and chemotaxonomic characteristics of strain MLFW-2ᵀ and demonstrate that it represents a novel species of the genus Desulfuribacillus.

Strain MLFW-2ᵀ was isolated from arsenic-rich anoxic sediments collected from the drainage area of a geothermal spring adjacent to Mono Lake, CA, USA (37° 56’ 28.7” N 119° 1’ 22.4” W) [14]. A lactate-oxidizing, Sb(V)-reducing

Author affiliations: ¹Department of Microbiology, University of Georgia, Athens, GA 30602, USA; ²Department of Marine Sciences, University of Georgia, Athens, GA 30602, USA.

Correspondence: James T. Hollibaugh, aquadoc@uga.edu

Keywords: anaerobic respiration; antimony; arsenic; Mono Lake.

Abbreviations: AAI, average amino acid identity; ANI, average nucleotide identity; POCP, per cent of conserved proteins.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and the draft genome of strain MLFW-2ᵀ are KF387535 and MJAT00000000, respectively.

Four supplementary figures and one supplementary table are available with the online Supplementary Material.
enrichment culture was established under anoxic conditions in a basal salts medium (BSM-1; Table S1, available in the online Supplementary Material) supplemented with 1 mM lactate and 2 mM Sb(V). A pure culture of strain MLFW-2\textsuperscript{T} was obtained on agar media as previously described [14].

Cell morphology during growth on Sb(V) was examined using a Leica DM RXA optical light microscope (Leica Microsystems), Zeiss 1450EP scanning electron microscope (Carl Zeiss) and Tecnai F20 transmission electron microscope (FEI). Cells of strain MLFW-2\textsuperscript{T} were motile and consisted of curved rods typically 0.3–0.5 µm wide and 2.0–11.0 µm long (Fig. 1a). During the stationary phase of growth, cells formed ellipsoidal endospores in terminal, swollen sporangia (Fig. S1). The Gram stain and KOH string test were performed on mid-exponential phase cells as previously described [16, 17]. Strain MLFW-2\textsuperscript{T} stained Gram-negative and cell lysis occurred following incubation in 3 % KOH. However, no outer membrane was apparent in ultrathin sections (Fig. 1b). It should be noted that strain MLFW-2\textsuperscript{T} was erroneously described as Gram-positive by Abin and Hollibaugh [14].

Genomic DNA was extracted from a cell pellet using the PureLink Genomic DNA Mini Kit (Invitrogen). A fragment of the 16S rRNA gene was amplified by PCR with universal bacterial primers 27F (5\textsuperscript{\prime}–AGAGTTTGATCCTGGCTCAG-3\textsuperscript{\prime}) and 1492R (5\textsuperscript{\prime}–GGTTACCTTGTGTAAGACCT–3\textsuperscript{\prime}) [18]. The amplicons were sequenced directly using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and an Applied Biosystems 3730xl DNA Analyzer. The forward and reverse primers listed above were used for sequencing from both directions. The consensus 16S rRNA gene sequence of strain MLFW-2\textsuperscript{T} was compared with reference sequences in GenBank and the Ribosomal Database Project Release 11.4 using the BLAST N [19] and RDP Classifier [20] algorithms, respectively. Sequence data from strain MLFW-2\textsuperscript{T} and its closest relatives were aligned using MUSCLE [21]. The MEGA6 software package [22] was used to generate phylogenetic trees using the neighbour-joining [23], maximum-parsimony [24] and maximum-likelihood [25] algorithms with Jukes–Cantor evolutionary distances [26]. Tree topologies were evaluated by bootstrap analysis [27] with 1000 replicates.

A nearly full-length 16S rRNA gene sequence (1446 nt) was obtained from strain MLFW-2\textsuperscript{T}. Taxonomic analysis by the RDP Classifier revealed that strain MLFW-2\textsuperscript{T} was a member of the order Bacillales, although it did not cluster with any established families at a 90 % confidence threshold. The closest phylogenetic relative of strain MLFW-2\textsuperscript{T} was Desulfuribacillus alkaliarsenatis AHT28\textsuperscript{T}, with a sequence similarity of 93.9 %. D. alkaliarsenatis AHT28\textsuperscript{T} was a Gram-positive, obligately anaerobic, dissimilatory sulfur- and arsenate-reducing haloalkaliphile isolated from soda lake sediments collected in the Kulunda Steppe, Altai, Russia [28]. Strain MLFW-2\textsuperscript{T} only shared a maximum of 90.6 % 16S rRNA gene sequence similarity with other type strains of the Bacillales. The 16S rRNA gene was also related to uncultured bacterial clones retrieved from an anaerobic aquifer (GenBank accession number KC166752; similarity value 94.7 %), an alkaline, hypersaline lake (DQ206424 and DQ206425; 92.8 and 92.7 %, respectively), a municipal compost pile (FN667347; 92.7 %) and a landfill leachate sediment (HQ183747; 91.6 %). Phylogenetic trees depicting the evolutionary relationship between strain MLFW-2\textsuperscript{T} and the most closely related type strains of the Bacillales are presented in Figs 2, S2 and S3. Strain MLFW-2\textsuperscript{T} consistently shared a branching node with D. alkaliarsenatis AHT28\textsuperscript{T} regardless of the treeing algorithm used. In each case, the node was supported by a bootstrap value of 100 %.

All growth experiments designed to characterize the physiological properties of strain MLFW-2\textsuperscript{T} were carried out in duplicate at 30 °C under an atmosphere of 5 % H\textsubscript{2}+95 % N\textsubscript{2}, except where noted. Tests for the use of electron donors and acceptors were performed in BSM-1. Growth was assessed by monitoring cell density using acridine orange staining and epifluorescence microscopy [29]. For a positive result, growth had to reach a level of at least two-fold that of the negative control and had to maintain that threshold for three consecutive transfers.

The range of terminal electron acceptors used by strain MLFW-2\textsuperscript{T} was investigated with 10 mM lactate as the electron donor. The following electron acceptors were tested, all at a concentration of 5 mM (unless stated otherwise): nitrate, nitrite (2 mM), amorphous Fe(III) oxyhydroxide (5 g l\textsuperscript{–1}), Fe(III) citrate, colloidal Mn\textsubscript{O}\textsubscript{2} (5 g l\textsuperscript{–1}), sulfate,
sulfite (2 mM), tetrathionate, thiosulfate, elemental sulfur (5 g l⁻¹), arsenate, selenate, selenite, chromate, vanadate, molybdate, fumarate, DMSO, and trimethylamine-N-oxide (TMAO). All electron acceptors were added from anoxic, filter-sterilized stock solutions. The ability to grow aerobically (~21 % O₂) was tested in a 200 ml Erlenmeyer flask open to the atmosphere. The capacity for microaerophilic incubation. An amount of dissolved selenium present at the start of the incubation.

The ability of strain MLFW-2T to grow using a range of electron donors was tested with 5 mM arsenate as the electron acceptor (data not shown). Strain MLFW-2T reduced nitrate and nitrite to ammonium. Selenate and selenite were reduced to a red allotrope of elemental selenium. Nitrate and selenate reduction were sequential processes involving the transient accumulation of nitrite and selenite, respectively, in the culture medium. No growth was observed in the presence of O₂, indicating that strain MLFW-2T was an obligate anaerobe. *D. alkaliarsenatis* AHT28T was also a strict anaerobe capable of respiring arsenate, although elemental sulfur and thiosulfate could support growth as well. Another key difference between the two strains lies in the inability of *D. alkaliarsenatis* AHT28T to grow using nitrate, nitrite, selenate, selenite and DMSO as terminal electron acceptors. The growth rate was highest when arsenate was provided as the electron acceptor (data not shown). Strain MLFW-2T reduced nitrate and nitrite to ammonium. Selenate and selenite were reduced to a red allotope of elemental selenium. Nitrate and selenate reduction were sequential processes involving the transient accumulation of nitrite and selenite, respectively, in the culture medium. No growth was observed in the presence of O₂, indicating that strain MLFW-2T was an obligate anaerobe. Another key difference between the two strains lies in the inability of *D. alkaliarsenatis* AHT28T to grow using nitrate, nitrite, selenate, selenite and DMSO as electron acceptors [28].

The ability of strain MLFW-2T to grow using a range of electron donors was tested with 5 mM arsenate as the electron acceptor under an N₂ atmosphere. The following substrates were tested, all at a concentration of 10 mM (unless stated otherwise): acetate, pyruvate, formate (±2 mM acetate), fumarate, malate, succinate, maleate, oxalate, ascorbate, citrate, tartrate, glycolate, propionate, d-glucose, ...
D-galactose, D-fructose, ethanol, methanol, glycerol, D-sorbitol, L-glycine, L-glutamate, L-serine and 100 % (v/v) H₂ (±2 mM acetate). In addition to lactate, strain MLFW-2T was able to grow using formate, pyruvate and H₂ as electron donors. No fermentative growth on these substrates was observed when arsenate was omitted from the medium. Growth on formate and H₂ was only possible when acetate was provided as a carbon source, indicating that strain MLFW-2T was incapable of autotrophic growth.

For all optimum growth experiments, lactate and arsenate were used as the electron donor and acceptor, respectively. The influence of salinity on the growth rate of strain MLFW-2T was assessed in BSM-2 (Table S1). The final NaCl concentration (w/v) of the medium was adjusted to 0, 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 %. The pH tolerance was determined in BSM-3 and BSM-4 (Table S1) from pH 7 to 10 (at intervals of 0.25 pH units). Buffers comprised of NaH₂PO₄/Na₂HPO₄ (BSM-3; pH 7.0–8.0) and NaHCO₃/Na₂CO₃ (BSM-4; pH 8.25–10.0) were added to a final concentration of 50 mM to maintain a stable pH. The temperature tolerance was tested in BSM-5 (Table S1) at 10, 15, 20, 25, 30, 32, 34, 36, 38, 40, 42 and 43 °C. Optimal growth of strain MLFW-2T occurred at 34 °C (range 10–43 °C), pH 8.25–8.50 (range 7.0–10.0) and 0.75 % (w/v) NaCl (range 0–5.0 %).

Oxidase activity was tested using XeroStrips (Biorex Labs). Catalase activity was assessed by checking for the production of gas bubbles after the addition of a drop of 3 % (v/v) H₂O₂ to a smeared pellet of freshly cultured cells. Strain MLFW-2T was catalase-positive and oxidase-negative. This is in slight contrast to D. alkaliarsenatis AHT28T, which was both catalase- and oxidase-negative [28].

For chemotaxonomic analyses, cells were cultivated in BSM-5 at 34 °C using lactate and arsenate as the electron donor and acceptor, respectively. Cellular fatty acids were analysed as the methyl ester derivatives prepared from 20 mg of wet cellular biomass. GC analysis of fatty acid methyl esters was performed by Microbial ID, Inc. using the Sherlock Microbial Identification System. Only saturated, unsaturated and branched fatty acids were detected in the fatty acid profile of strain MLFW-2T (Table 1). The dominant fatty acids were C₁₈:₁ω7c (35.4 %), C₁₆:₀ (21.7 %), C₁₆:₁ω9c (14.1 %), C₁₆:₁ω7c (13.9 %) and C₁₈:₁ω9c (7.3 %). The same major components were detected in D. alkaliarsenatis AHT28T, although C₁₈:₁ω7c and C₁₆:₁ω9 were only accounted for 20.7 and 66.6 % of the total cellular fatty acids, respectively, while C₁₆:₁ω7c was present in higher abundance [28]. Both strains lacked the C₁₅ fatty acids normally prominent in members of the phylum Firmicutes [36].

Table 1. Cellular fatty acid content of strain MLFW-2T and D. alkaliarsenatis AHT28T

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Strain MLFW-2T</th>
<th>D. alkaliarsenatis AHT28T</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₁₆:₀</td>
<td>21.7</td>
<td>24.6</td>
</tr>
<tr>
<td>C₁₆:₀ ALDE</td>
<td>–</td>
<td>2.8</td>
</tr>
<tr>
<td>C₁₆:₁ω9</td>
<td>14.1*</td>
<td>6.6</td>
</tr>
<tr>
<td>C₁₆:₁ω9 ALDE</td>
<td>–</td>
<td>2.3</td>
</tr>
<tr>
<td>C₁₆:₁ω7 ALDE</td>
<td>–</td>
<td>1.3</td>
</tr>
<tr>
<td>C₁₆:₁ω7c</td>
<td>13.9</td>
<td>20.0</td>
</tr>
<tr>
<td>C₁₆:₁ω5</td>
<td>1.3*</td>
<td>3.5</td>
</tr>
<tr>
<td>iso-C₁₇:₀</td>
<td>–</td>
<td>1.1</td>
</tr>
<tr>
<td>C₁₈:₀</td>
<td>1.2</td>
<td>1.4</td>
</tr>
<tr>
<td>C₁₈:₁ω9</td>
<td>7.3*</td>
<td>6.0</td>
</tr>
<tr>
<td>C₁₈:₁ω9 ALDE</td>
<td>–</td>
<td>1.0</td>
</tr>
<tr>
<td>C₁₈:₁ω7 ALDE</td>
<td>–</td>
<td>4.1</td>
</tr>
<tr>
<td>C₁₈:₁ω7c</td>
<td>35.4</td>
<td>20.7</td>
</tr>
<tr>
<td>C₁₈:₁ω5</td>
<td>1.5*</td>
<td>1.4</td>
</tr>
</tbody>
</table>

*D only the cis stereoisomer was detected.

Polar lipids and respiratory lipoquinones were analysed by the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). The polar lipid profile of strain MLFW-2T was dominated by phosphatidylglycerol and phosphatidylethanolamine (Fig. S4). Moderate amounts of two unknown phospholipids (PL1 and PL2) and two unknown polar lipids (L2 and L3) were also present, as well as minor amounts of other unknown polar lipids (L1, L4–L7). The only respiratory lipoquinone detected was menaquinone-7 (MK-7).

To test the sensitivity of strain MLFW-2T to various antibiotics, cells were grown to the late exponential phase in BSM-5 and inoculated into a series of Hungate tubes containing fresh BSM-5 supplemented with ampicillin, kanamycin, rifampicin, chloramphenicol, erythromycin, gentamicin, streptomycin or nalidixic acid at concentrations of 5, 10, 25, 50, 75 and 100 µg ml⁻¹. The tubes were prepped and scored for positive growth after an incubation period of 96 h at 34 °C. Strain MLFW-2T was only able to grow in the presence of 5 µg chloramphenicol ml⁻¹, 5–25 µg kanamycin ml⁻¹ and 5–75 µg nalidixic acid ml⁻¹.

The genome of strain MLFW-2T was sequenced using the Illumina Miseq platform with 250 bp paired-end reads. Genomic DNA from strain MLFW-2T was sheared using a Covaris E220 Evolution Focused-ultrasonicator to generate an average fragment size of 696 bp. Paired-end DNA libraries were prepared using the TruSeq DNA LT Sample Prep Kit (Illumina) and indexed with TruSeq DNA LT single index adapters (Illumina). The library was sequenced for 500 cycles using a MiSeq Reagent Kit v2 (Illumina). The reads were randomly subsampled to an approximate 85-fold median coverage with seqtk version 1.0-r63 (https://github.com/lh3/seqtk). Read cleaning and de novo assembly was performed using the A5-Miseq pipeline [37]. Genome annotation was performed using
the RAST server [38]. Genome completeness was assessed using AMPHORA2 [39].

The genome assembly yielded 40 contigs, with maximum and N₅₀ contig sizes of 400 702 and 233 716 bp, respectively. The total length of the draft genome was 3 119 699 bp with a G+C content of 38.2 %. Genome annotation revealed 3028 coding sequences and 63 tRNA genes. The draft genome was nearly complete, as it contained all 31 phylogenetic marker genes essential in bacteria. The size and G+C content of the draft genome were very similar to the values of 3 106 435 bp and 37.5 %, respectively, reported for the draft genome of *D. alkaliarsenatis* AHT28ᵀ [40]. It should be noted that the thermal denaturation method yielded a G+C content of 39.1 % for the genome of *D. alkaliarsenatis* AHT28ᵀ in the original description of the type strain [28].

To further refine the phylogenetic relationship between strain MLFW-2ᵀ and *D. alkaliarsenatis* AHT28ᵀ, the average nucleotide identity (ANI), average amino acid identity (AAI) and percent of conserved proteins (POCP) between both strains were determined. For species delineation, a POCP of ≥50 % has been proposed as a robust genomic parameter for delimiting the prokaryotic genus boundary [43]. The ANI and AAI were calculated using EzGenome (www.ezbiocloud.net/ezgenome/ani) and the Kostas Lab AAI Calculator (http://enve-omics.ce.gatech.edu/aai/), respectively. The POCP analysis was performed as described by Qin *et al.* [43]. The ANI, AAI and POCP values of strain MLFW-2ᵀ with *D. alkaliarsenatis* AHT28ᵀ were 69.07, 65.43 and 66.58 %, respectively. These values suggest that the novel isolate and *D. alkaliarsenatis* AHT28ᵀ represent two distinct species that belong to the same genus.

On the basis of the morphological, physiological, chemotaxonomic and phylogenetic data presented here, strain MLFW-2ᵀ merits recognition as a novel species of the genus *Desulfuribacillus*, for which the name *Desulfuribacillus stibiiarsenatis* sp. nov. is proposed. While strain MLFW-2ᵀ shares many phenotypic traits with the only other existing member of the genus *Desulfuribacillus*, *D. alkaliarsenatis* AHT28ᵀ, it is possible to distinguish between them using the characteristics shown in Table 2.

**EMENDED DESCRIPTION OF THE GENUS DESULFURIBACILLUS**

Cells are motile, curved rods that stain Gram-positive or Gram-negative and produce terminal endospores. Oxidase-negative. Mesophilic. Obligately anaerobic with an exclusively respiratory catabolism. Hydrogen, formate, lactate and pyruvate can serve as electron donors. Arsenate can serve as an electron acceptor. Incapable of chemoheterotrophic growth. The major cellular fatty acids are C₁₆:0, C₁₆:1ω9, C₁₆:1ω7c, C₁₈:1ω9 and C₁₈:1ω7c. The major respiratory lipoquinone is MK-7. The DNA G+C content is 37.5–38.2 mol%. The type species is *Desulfuribacillus alkaliarsenatis*.

**DESCRIPTION OF DESULFURIBACILLUS STIBIIARSENATIS SP. NOV.**

*Desulfuribacillus stibiiarsenatis* [sti.bi.i.ar.se.na’tis. L. n. stibium antimony; N.L. n. arsenas -atis arsenate; N.L. gen. n. stibiiarsenatis of antimony (and) arsenate, referring to the ability of the strain to use antimonate and arsenate as electron acceptors].

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain MLFW-2ᵀ</th>
<th><em>D. alkaliarsenatis</em> AHT28ᵀ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation source</td>
<td>Anoxic sediments</td>
<td>Soda lake sediments</td>
</tr>
<tr>
<td>Cell width (µm)</td>
<td>0.3–0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Cell length (µm)</td>
<td>2.0–11.0</td>
<td>2.0–7.0</td>
</tr>
<tr>
<td>Gram stain</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Endospore shape</td>
<td>Ellipsoidal</td>
<td>Round or ellipsoidal</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Temperature (℃)</td>
<td>Range (optimum) 10–43 (34)</td>
<td>ND – 43 (35)</td>
</tr>
<tr>
<td>pH range (optimum)</td>
<td>7.0–10.0 (8.25–8.50)</td>
<td>8.5–10.6 (10.2)</td>
</tr>
<tr>
<td>NaCl concentration (%)</td>
<td>Range (optimum) 0–5.0 (0.75)</td>
<td>1.2–14.6 (3.5–4.7)</td>
</tr>
<tr>
<td>Electron acceptors</td>
<td>Nitrite</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Nitrate</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Elemental sulfur</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Thiosulfate</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Antimonate</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Selenite</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Selenate</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>DMSO</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Major polar lipids*</td>
<td>PG, PE</td>
</tr>
<tr>
<td></td>
<td>DNA G+C content (mol %) †</td>
<td>38.2</td>
</tr>
</tbody>
</table>

*PG, phosphatidylglycerol; PE, phosphatidylethanolamine.
†Values derived from draft genomes.
In addition to the characteristics listed in the genus description above, cells are approximately 0.3–0.5×2.0–11.0 μm in size and produce ellipsoidal endospores. Gram-stain-negative and lysis occurs upon treatment with 3% KOH. No outer membrane is present. Growth is observed at 10–43 °C (optimum 34 °C), at pH 7.0–10.0 (optimum 8.25–8.50) and with 0–5% (w/v) NaCl (optimum 0.75%). Catalase-positive. Nitrate, nitrite, DMSO, antimonite, selenate and selenite can serve as terminal electron acceptors for anaerobic respiration. Sulfate, sulfite, tetrathionate, thiosulfate, elemental sulfur, chromate, vanadate, amorphous Fe(III) oxyhydroxide, Fe(III) citrate, colloidal MnO₂, molybdate, fumarate and TMAO cannot be used as electron acceptors. Growth on formate and H₂ as electron donors requires supplementation with acetate as a carbon source. Acetate, fumarate, malate, succinate, maleate, oxalate, ascorbate, citrate, tautrate, glycolate, propionate, D-glucose, D-galactose, D-fructose, ethanol, methanol, glycerol, D-sorbitol, L-glutamic, L-glutamate and L-serine cannot serve as electron donors.

The type strain, MLFW-2T (=DSM 28709T=ICM 30866T), was isolated from anoxic sediments collected from the drainage area of a geothermal spring adjacent to the southern shore of Mono Lake, CA, USA (37°56′28.7′′ N 119°1′22.4′′ W). The DNA G+C content of the type strain is 38.2 mol%.

Funding information
This work was supported by National Science Foundation (NSF) grants EAR-09-22771 and DGE-0903734. Additional funding was provided by a Minority PhD Program (MPHD) scholarship from the Alfred P. Sloan Foundation.

Acknowledgements
We thank the editor and three anonymous reviewers for constructive comments that helped to improve the manuscript. We also thank Dr John Shields for assistance with electron microscopy. DNA library preparation and Illumina sequencing was performed by the Georgia Genomics Facility at the University of Georgia in Athens, GA, USA.

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
All prevailing local, national, and international regulations and conventions, and normal scientific ethical practices, have been respected in conducting this research and publishing the results.

References


40. Abin CA, Hollibaugh JT. Draft genome sequence of the type strain *Desulfuribacillus alkaliarsenatis* AHT28, an obligately anaerobic, sulfidogenic bacterium isolated from Russian soda lake sediments. * Genome Announc* 2016;4:e01244-16.


---

**Five reasons to publish your next article with a Microbiology Society journal**

1. The Microbiology Society is a not-for-profit organization.
2. We offer fast and rigorous peer review – average time to first decision is 4–6 weeks.
3. Our journals have a global readership with subscriptions held in research institutions around the world.
4. 80% of our authors rate our submission process as ‘excellent’ or ‘very good’.
5. Your article will be published on an interactive journal platform with advanced metrics.

*Find out more and submit your article at microbiologyresearch.org.*