Spirochaeta americana sp. nov., a new haloalkaliphilic, obligately anaerobic spirochaete isolated from soda Mono Lake in California

Richard B. Hoover,¹ Elena V. Pikuta,¹ Asim K. Bej,² Damien Marsic,³ William B. Whitman,⁴ Jane Tang⁵ and Paul Krader⁵

Correspondence

Richard B. Hoover
Richard.B.Hoover@NASA.GOV

Elena V. Pikuta
Elena.Pikuta@msfc.nasa.gov

¹NASA/NSSTC, SD-50, 320 Sparkman Dr., Huntsville, AL 35805, USA

²Department of Biology, University of Alabama at Birmingham, Birmingham, AL 35294, USA

³Laboratory for Structural Biology, The University of Alabama in Huntsville, MSB, Huntsville, AL 35899, USA

⁴Department of Microbiology, University of Georgia, Athens, GA 30602-2605, USA

⁵American Type Culture Collection, 10801 University Blvd, Manassas, VA 20110, USA

A novel, obligately anaerobic, mesophilic, haloalkaliphilic spirochaete, strain ASpG1^T, was isolated from sediments of the alkaline, hypersaline Mono Lake in California, USA. Cells of the Gram-negative strain were motile and spirochaete-shaped with sizes of 0.2–0.22 × 8–18 µm. Growth of the strain was observed between 10 and 44 °C (optimum 37 °C), in 2–12 % (w/v) NaCl (optimum 3 % NaCl) and between pH 8 and 10.5 (optimum pH 9.5). The novel strain was strictly alkaliphilic, required high concentrations of carbonates in the medium and was capable of utilizing D-glucose, fructose, maltose, sucrose, starch and D-mannitol. End products of glucose fermentation were H₂, acetate, ethanol and formate. Strain ASpG1^T was resistant to kanamycin and rifampicin, but sensitive to gentamicin, tetracycline and chloramphenicol. The G + C content of its DNA was 58.5 mol%. DNA–DNA hybridization analysis of strain ASpG1^T with its most closely related species, *Spirochaeta alkalica* Z-7491^T, revealed a hybridization value of only 48.7 %. On the basis of its physiological and molecular properties, strain ASpG1^T appears to represent a novel species of the genus *Spirochaeta*, for which the name *Spirochaeta americana* is proposed (type strain ASpG1^T = ATCC BAA-392^T = DSM 14872^T).

The genus *Spirochaeta* contains a group of free-living, saccharolytic, obligately or facultatively anaerobic, helix-shaped bacteria (Canale-Parola, 1977, 1984). Analysis of 16S rRNA gene sequences has shown that the spirochaetes represent a monophyletic phylum within the *Bacteria* (Paster *et al.*, 1991). At the time of writing, the genus *Spirochaeta* contained 13 species of bacteria. These organisms occur in a wide variety of freshwater and marine environments (Canale-Parola, 1992), such as aquatic habitats (*Spirochaeta aurantia*) (Breznak & Canale-Parola, 1975), freshwater mud (*Spirochaeta zuelzeriae*, *Spirochaeta stenostrepta*) (Canale-Parola, 1980), marine mud (*Spirochaeta isovalerica*, *Spirochaeta litoralis*) (Hespell & Canale-Parola, 1970; Harwood & Canale-Parola, 1983), oilfields (*Spirochaeta smaragdinae*) (Magot *et al.*, 1997) and microbial mats (Teal *et al.*, 1996). Some members of the genus *Spirochaeta* are

microbial extremophiles that inhabit environments with extremes of salinity, alkalinity, pressure and/or temperature. The spirochaete extremophiles may harbour important enzymes and proteins and, consequently, are of particular interest to biotechnologists (Bermudes *et al.*, 1987; Barth *et al.*, 1991; Munson *et al.*, 1993).

Prior studies have shown that some species of the genus *Spirochaeta* are naturally resistant to sulfide concentrations at levels that inhibit the growth of many other microorganisms. The mesophilic halophile *Spirochaeta halophila* was isolated from black mud (smelling strongly of H₂S) of Solar Lake, a high-salinity pond on the Sinai shore of the Gulf of Aqaba (Greenberg & Canale-Parola, 1976). The obligate anaerobe strain GS-2 is a mesophilic barophile that was isolated from sulfide-rich mud samples collected at a depth of 2550 m near deep-sea hydrothermal vents at the Galapagos Rift ocean-floor spreading centre (Harwood *et al.*, 1982). '*Spirochaeta bajacaliforniensis*' (Fracek & Stolz, 1985) is another anaerobic marine spirochaete that was isolated from a microbial mat community in sulfide-rich

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The GenBank accession number for the 16S rDNA sequence of *Spirochaeta americana* ASpG1^T is AF373921.

mud at Laguna Figueroa, Baja California. It was suggested that this might be a strain of *S. litoralis*, since it had almost identical physiological characteristics and a similar DNA G + C content to *S. litoralis* (Canale-Parola, 1992). However, 16S rRNA gene sequence data indicate that '*S. bajacaliforniensis*' and *S. litoralis* do not represent the same species, as a pairwise comparison shows only 83.8% similarity between the two species (data from this study).

Other spirochaete extremophiles include anaerobic, thermophilic spirochaetes from hot springs in New Zealand (Patel *et al.*, 1985), moderately thermophilic *Spirochaeta caldaria* from cyanobacterial mats of freshwater hot springs in Oregon and Utah (Pohlschroeder *et al.*, 1994), and extremely thermophilic *Spirochaeta thermophila* from a marine littoral hydrotherm of Shikotan Island, Russia (Aksenova *et al.*, 1992).

The first alkaliphilic spirochaetes (*Spirochaeta alkalica*, *Spirochaeta africana*, *Spirochaeta asiatica*) were isolated from brine sediments under crusts of Trona [$\text{Na}_2(\text{CO}_3)(\text{HCO}_3) \cdot 2(\text{H}_2\text{O})$] in the alkaline equatorial Lake Magadi in Kenya (Africa) and from sulfide-saturated mud sediments of alkaline Lake Khatyn in Tuva, Siberia (Central Asia) (Zhilina *et al.*, 1996). In this report, we describe a novel, haloalkaliphilic, obligately anaerobic, mesophilic spirochaete isolated from a soda lake in California, USA.

Strain ASpG1^T was isolated from a black mud sample (with a strong odour of H_2S) collected on 15 August 2000 from soda Mono Lake of northern California, USA. The mud sample was collected anaerobically under shallow water (temperature, 21.6 ± 0.1 °C; salinity, 7%; pH 9.9 ± 0.02) near the south shore of Mono Lake. To obtain the enrichment culture, 0.5 g of wet sediment material was injected into standard Hungate tubes containing a glucose medium and incubated at 35 °C for 3–4 days. Phase-contrast microscopy of the enrichment cultures indicated good growth of motile, spirochaete-shaped cells. Pure cultures were obtained by serially diluting the enrichment cultures in a fresh medium and filtering 1 ml of these cultures (from 7 dilutions) through a 0.4 µm sterile membrane syringe filter (Gelman Laboratory) into medium containing D-mannitol, as described previously (Canale-Parola, 1992). Three strains of spirochaetes that exhibited indistinguishable morphological, metabolic and physiological characteristics were isolated; strain ASpG1^T was selected for further characterization. The culture of strain ASpG1^T was judged to be pure because only a single type of colony morphology was observed on the agar surface after growth of the organism, and only one cell type was observed upon microscopic examination during growth of the strain on a variety of substrates.

Strain ASpG1^T was grown in a medium modified from Zhilina *et al.* (1996). The medium contained (l^{-1}) 30 g NaCl, 2.76 g Na_2CO_3 , 24.0 g NaHCO_3 , 0.2 g KCl, 0.2 g K_2HPO_4 , 0.1 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 1.0 g NH_4Cl , 0.4 g $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$, 0.001 g resazurin, 0.5 g yeast extract, 5.0 g D-glucose, 2 ml

vitamin solution (Wolin *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 for the gas phase. Growth was observed by phase-contrast microscopy and by measurements of optical density at 595 nm using a spectrophotometer (Genesis 5, Spectronic Instruments).

The morphology and size range of cells of strain ASpG1^T were determined by phase-contrast microscopy, fluorescent microscopy and field emission scanning electron microscopy. Cells of strain ASpG1^T were 0.20–0.22 µm in diameter, 8–18 µm in length and had regular-shaped spirals (Fig. 1a). They also stained Gram-negative. In the final stage of exponential growth, living cells, spherical bodies (sphaeroplasts) and conglomerates of dead cells of strain ASpG1^T were imaged using a Leitz Diaplan Epifluorescent microscope and the BacLight (Molecular Probes) live versus dead fluorescent stain (Fig. 1b). Cells of strain ASpG1^T were highly motile and exhibited a rotating undulatory movement typical of spirochaetes. Hitachi S-4000 field emission scanning electron microscope images of strain ASpG1^T cells that had been destroyed by osmotic shock showed a flagellum released from the periplasmic space (Fig. 1c).

Growth of strain ASpG1^T was observed after 3–4 days incubation on agar medium using the 'roll-tube' method (Hungate, 1969). Confluent growth was observed, and the mass was white with veil-like, irregular formations, which were a result of migration of the bacteria through the agar gel. Individual colonies were observed when strain ASpG1^T was grown on an agar medium containing 10 µg rifampicin ml^{-1} . These colonies were white and round with smooth, regular edges (0.5–2.0 mm in diameter). Individual colonies were densely granulated at their centres, with the granulation diminishing towards the perimeter of the colonies (1–5 dilutions). In deep agar (6–8 dilutions), colonies of strain ASpG1^T were 'cotton-ball-like', with an irregular spherical shape and a higher density toward their centres.

Strain ASpG1^T grew between 10 and 44 °C, with optimum growth at 37 °C; no growth was observed at 5 or 48 °C. Growth of the strain at 37 °C occurred between pH 7.8 and 10.5; no growth was detected at pH 7.0 or 11.0. Strain ASpG1^T required NaCl for growth, but no growth was observed in 1% (w/v) or 15% (w/v) NaCl; the optimum concentration of NaCl for growth was 3% (w/v), and the range over which growth was observed was 2–12% (w/v) NaCl. The novel isolate required sodium ions for growth and did not grow on a medium with K_2CO_3 instead of Na_2CO_3 . Similarly, when the carbonate buffer was replaced with serine, cells of strain ASpG1^T did not grow. Thus, carbonate ions were also required for growth. Growth of strain ASpG1^T was not dependent on chloride ions. Growth of the strain was observed after three sequential transfers in a medium in which the chloride salts were replaced with sulfate salts, but the cells became straight and lost their motility. The doubling time of the organism at optimal conditions [37 °C, 3% (w/v) NaCl, pH 9.5] was 5 h.

Fig. 1. Morphology of *Spirochaeta americana* ASpG1^T. (a) Phase-contrast image of a cell with a sphaeroplast in the centre. Bar, 10 µm. (b) BacLight live versus dead fluorescent stain of *S. americana* ASpG1^T culture incubated for 3 days with rifampicin (250 µg ml⁻¹) showing conglomerates of dead cells (red), living cells (green) and spherical bodies (sphaeroplasts). Bar, 10 µm. (c) Field emission scanning electron microscope image of a cell with its membrane destroyed by osmotic shock to show flagellum released from the periplasmic space. Bar, 2 µm.

The novel isolate was catalase-negative (method of Gerhardt *et al.*, 1981) and only grew under strictly anaerobic conditions. It had a fermentative type of metabolism and was an obligate chemoheterotroph. Growth of strain ASpG1^T on organic substrates was determined in the presence of 0.2 g yeast extract l⁻¹, as no growth of the organism was observed in the absence of yeast extract. Strain ASpG1^T grew on D-glucose, fructose, maltose, sucrose and D-mannitol. No growth was observed with acetate, lactate, pyruvate, propionate, butyrate, citrate, formate, methanol, ethanol, glycerol, peptone, yeast extract, Casamino acids, betaine or trimethylamine. Although yeast extract was required for growth of strain ASpG1^T, it could not support growth when used as the sole substrate. The end products of fermentation in liquid phase were determined by HPLC. Separation was done on an Aminex HPX-87H (Bio-Rad) column with 5 mM H₂SO₄ as the mobile phase. Gases were measured using a gas chromatograph 3700 (Varian) equipped with a Porapak Q column and a TCD detector (nitrogen was the carrier gas). During growth of strain ASpG1^T on glucose, H₂ was the main end product of fermentation, and formate, acetate and ethanol were minor end products. CO₂ production could not be measured due to the high carbonate level in the medium. The susceptibility of strain ASpG1^T to kanamycin, gentamicin, tetracycline, rifampicin (all at 250 µg ml⁻¹) and chloramphenicol (125 µg ml⁻¹) was tested. Strain ASpG1^T was resistant to kanamycin and rifampicin, but sensitive to gentamicin, tetracycline and chloramphenicol.

Fatty acid methyl esters (FAMES) were extracted from fresh biomass and identified following the procedure recommended for the Microbial Identification System (MIDI, Sherlock Microbial Identification System Version 4.0, MIS Operating Manual March 2001). Cells of strain ASpG1^T were harvested from a broth culture grown in the modified medium described above and incubated at 37 °C for 48 h. Extract preparation included sonication, methylation, organic extraction (transferring FAMES from an aqueous to an organic phase) and a base wash. Then, the cell extracts were subjected to GC analysis (MIDI). The major FAMES of strain ASpG1^T were C_{14:0} (11.94%), C_{16:0} (14.42%), dimethyl acetate C_{16:0} (12.55%), C_{18:1} (9.75%) and a mixture of C₁₈ unsaturated fatty acids in both the *cis* and *trans* configurations (31.58%). Aldehydes were present in lower amounts: C_{16:0} (4.69%), C_{16:1} (5.79%), C_{18:0} (5.21%) and C_{20:1} (2.63%).

The G + C content of the genomic DNA of strain ASpG1^T was determined by the HPLC method (Mesbah *et al.*, 1989), except that an Alltima C18 column [250 × 4.6 mm, 5 µm particle size (Alltech)] and 8% (v/v) methanol were used. The result reported is the mean of two determinations for each of two degradations of the DNA. The G + C content of the genomic DNA of strain ASpG1^T was 58.5 ± 0.2 mol% (mean ± SD, *n* = 4).

Genomic DNA was isolated through phenol/chloroform extraction followed by ethanol precipitation (Ausubel *et al.*, 1987). The 16S rRNA gene of strain ASpG1^T was selectively

amplified by PCR using primers designed by us: 5'-AGAGTTTGATCCTGGCTCAG-3' (forward, *Escherichia coli* positions 8–27); 5'-TACGGCTACCTTGTTACGAC-3' (reverse, *E. coli* positions 1512–1493). PCR was performed with 30 pmol of each primer in a total volume of 50 µl, using 1.2 U *Pfu* polymerase (Promega) in the provided buffer, 10 nmol of each dNTP and 10 % DMSO. The thermal cycling profile used was 5 min at 95 °C (initial denaturation), followed by 10 cycles of 2 min at 95 °C (denaturation), 45 s at 56 °C (annealing) and 3 min at 73 °C (extension), then 20 additional cycles with annealing at 50 °C (other parameters were the same as for the first 10 cycles). The final extension step was for 15 min at 73 °C. The amplified fragment was extracted from a 1.5 % agarose gel using the Qiaquick extraction kit (Qiagen), then subcloned using the Zero Blunt TOPO PCR Cloning kit (Invitrogen). Three clones were sequenced in both directions using the dye terminator AmpliTaq FS cycle sequencing kit (Applied Biosystems), with both vector-specific primers (T3 and T7) and the following primers (designed by us), which were specific to internal regions of the 16S rRNA gene: 5'-CGGAATTACTGGGCGTAAAG-3' (*E. coli* positions 555–574); 5'-GTGCTGCATGGCTGTCGTC-3' (*E. coli* positions 1047–1065); 5'-GGGTTGCGCTCGTTGCGGG-3' (*E. coli* positions 1113–1095); 5'-GCGCTCGCTTTACGCCCAAT-3' (*E. coli* positions 581–562). The 16S rRNA gene sequence of strain ASpG1^T was aligned with those of 12 closely related sequences found in GenBank after a BLAST search (Altschul *et al.*, 1990). The alignment was done using the PILEUP program from the Genetic Computer Group (GCG) package. Pairwise distances were computed with MEGA version 2.0 (Kumar *et al.*, 2001) using the Jukes–Cantor model (Jukes & Cantor, 1969). An unrooted phylogenetic tree was constructed with the same MEGA program using the neighbour-joining method (Saitou & Nei, 1987).

A sequence covering 1467 nt of the 16S rRNA gene of strain ASpG1^T was obtained, corresponding to positions 28–1551 of the *E. coli* 16S rDNA sequence. The G+C content of this sequence was 55.96 mol%. The sequence was compared with all sequences presently available in the GenBank database and appeared to be highly similar to sequences from the spirochaete group of organisms. A dendrogram showing the phylogenetic relationship of strain

ASpG1^T to other spirochaetes, based on 1223 common nucleotide positions, was constructed (Fig. 2). Strain ASpG1^T appears to belong to the alkaliphilic–halophilic cluster of spirochaetes, with similarity values ranging between 88.4 and 99.3 % (distances based on 1223 nt positions). The highest similarity was observed with *S. alkalica* Z-7491^T. A pairwise alignment of the 16S rDNA sequence of strain ASpG1^T with the complete available sequence of the *S. alkalica* 16S rRNA gene showed 99.2 % identity (1446 nt) with 1457 nt positions compared.

Since the phylogenetic analysis showed a high level of sequence similarity between strain ASpG1^T and *S. alkalica* Z-7491^T, additional analyses were carried out to measure the DNA melting temperatures, genome sizes and DNA–DNA hybridization values for the two strains. Melting temperatures (T_m) of total genomic DNA from *S. alkalica* and strain ASpG1^T were determined by following the procedures described by De Ley *et al.* (1970) and Gillis *et al.* (1970). The T_m value for the genomic DNA of strain ASpG1^T was 68 ± 2 °C (mean $\pm$ SD, $n=3$), whereas it was 74 ± 2 °C (mean $\pm$ SD, $n=3$) for *S. alkalica* Z-7491^T. The genome size of strain ASpG1^T was 2.98×10^9 Da, as calculated using the equation described by Gillis *et al.* (1970) and the reported genome size of *S. alkalica* Z-7491^T (Zhilina *et al.*, 1996). A comparison of the genome size of strain ASpG1^T with those of previously reported halo-alkaliphilic spirochaetes is provided in Table 1.

To determine the percentage homology of genomic DNA between *S. alkalica* Z-7491^T and strain ASpG1^T, DNA–DNA hybridization was performed followed by DNA reassociation kinetics as described by De Ley *et al.* (1970) and Johnson (1985). Genomic DNA from both of these microorganisms was sonicated using a Sonic Dismembrator 50 (Fisher Scientific) to generate DNA fragments of between 400 and 800 bp in size. Residual RNA or single-stranded DNA was removed by using RNase I (Sigma) or S1 nuclease (US Biochemical) (Ausubel *et al.*, 1987), respectively. The concentration and purity of the DNA were determined at 260 nm and a ratio of 260/280 nm, respectively, by using a Shimadzu UV-160 spectrophotometer. Initial reassociation kinetics were determined by linear regression analysis. DNA–DNA hybridization established 48.7 % homology between the genomes of strain ASpG1^T and *S. alkalica* Z-7491^T based on the reassociation kinetics and the

Fig. 2. Unrooted phylogenetic tree, constructed from 16S rRNA gene sequences, showing the position of strain ASpG1^T within the spirochaetes. Sequence accession numbers are shown in parentheses; culture collection codes are given, if known. Bar, 2 substitutions per 100 nucleotides.

Table 1. Comparison of morphological and physiological characteristics of strain ASpG1^T with those of other members of the genus *Spirochaeta*

Species: 1, *S. americana* ASpG1^T (this study); 2, *S. alkalica* Z-7491^T (Zhilina *et al.*, 1996); 3, *S. africana* Z-7492^T (Zhilina *et al.*, 1996); 4, *S. asiatica* Z-7591^T (Zhilina *et al.*, 1996); 5, *S. halophila* RS1^T (Greenberg & Canale-Parola, 1976). ND, Not determined; +, substrate used/positive; −, substrate not used/negative; OA, obligate anaerobe; FA, facultative anaerobe. All strains utilized D-glucose, maltose, sucrose and starch as substrates. All strains produced acetate and ethanol from glucose.

Characteristic	1	2	3	4	5
Sizes (μm)	0.20–0.22 × 8–18	0.4–0.5 × 9–18	0.25–0.3 × 15–30	0.2–0.25 × 15–22	0.4 × 15–30
Optimum (range) pH	9.5 (8.0–10.5)	8.7–9.6 (8.4–10.7)	8.8–9.75 (8.1–10.7)	8.4–9.4 (7.9–9.7)	7.5
Optimum (range) NaCl (%)	3 (2–12)	5 (3–10)	5–7 (3–10)	3–6 (2–8)	3–5 (0.3–7.3)
Optimum (range) temperature (°C)	37 (10–45)	33–37 (15–44)	30–37 (15–47)	33–37 (20–43)	35–40
Substrate:					
Fructose	+	+	+	−	+
D-Mannitol	+	−	−	+	ND
Product of glucose fermentation:					
H ₂	+	+	+	−	+
Formate	+	ND	ND	ND	−
Lactate	−	+	+	+	+
Relationship to O ₂	OA	FA	FA	OA	FA
DNA G + C content (mol%)	58.5	57.1	56.1	49.2	62.0
Genome size (× 10 ⁹ Da)	2.98	2.70	2.50	2.10	ND

equation described by Gillis *et al.* (1970). All statistical analyses were performed using Microsoft Excel.

Strain ASpG1^T was isolated from Mono Lake, an ancient (~750 000 years old), continental, athalassic, sulfurous, hypersaline (~80 g NaCl l^{−1}), alkaline (pH 9.4–10.5) soda lake in California. Mono Lake is a closed basin with high evaporation rates that is in close proximity to active volcanoes (Tierney, 1997). Dominant ions of Mono Lake water (pH 9.7) are sodium (1400 mM), carbonate (400 mM) and sulfate (130 mM) (Oremland & Miller, 1993). The salinity of Mono Lake varies with the water level and has ranged from ~50 to ~100 g salt l^{−1} (when Mono Lake was at its lowest level, in 1982).

The volcanic island Paoha, with a hot (~90 °C) alkaline spring, emerged only 350 years ago in the middle of Mono Lake. In the winter months, along the south shoreline, metastable minerals such as gaylussite [CaNa₂(CO₃)₂·5(H₂O)] and ikaite (CaCO₃·6H₂O) precipitate abundantly (Bischoff *et al.*, 1991, 1993; Council & Bennett, 1993). In warmer months, these minerals transform into tufa (anhydrous CaCO₃), and tufa columns grow where calcium-rich spring water mixes with alkaline lake water. The microbial community of Mono Lake exhibits a complete cycle of trophic chains: the primary producers are phototrophs, such as cyanobacteria, diatoms and other algae, and the primary micro-organisms responsible for the destruction of organic matter under anaerobic conditions include species of *Spirochaeta* (strain ASpG1^T) and *Tindallia* (novel strain APO), some cellulolytic bacteria and secondary anaerobes, such as *Desulfonatronum* sp. (novel sulfate-reducer strain MLF1), using the end products of primary anaerobes.

On the basis of its phenotypic characteristics (e.g. cell morphology, Gram-negative cell wall and fermentation of sugars), strain ASpG1^T was identified as a member of the genus *Spirochaeta*. Among the alkaliphilic spirochaete species, strain ASpG1^T is the only haloalkaliphile capable of growth in a medium containing 12 % NaCl and with a pH of 9.5. Strain ASpG1^T was also shown to be a member of the genus *Spirochaeta* by a molecular phylogenetic analysis. The significant differences between the morphological and physiological characteristics of strain ASpG1^T and its closest phylogenetic relatives of the genus *Spirochaeta* are shown in Table 1. Strain ASpG1^T is notably different from its nearest phylogenetic neighbour, *S. alkalica* Z-7491^T, in both its cell morphology and size; both strains also exhibit differences in important physiological and metabolic characteristics, such as the ability to use D-mannitol, production of lactate from glucose fermentation and their relationship to oxygen (strain ASpG1^T is an obligate anaerobe, whereas *S. alkalica* Z-7491^T is a facultative anaerobe). In addition, strain ASpG1^T does not exhibit visible pigmentation (its biomass is white), but the biomass of *S. alkalica* cells is orange. Furthermore, cells of strain ASpG1^T are regular-shaped spirals, but *S. alkalica* Z-7491^T cells are irregular-shaped coils. Fatty acid analysis demonstrates the absence of branched-chain fatty acids in strain ASpG1^T, as was also observed for *S. alkalica*, *S. africana*, *S. asiatica*, *S. halophila* and *S. litoralis*. DNA–DNA hybridization of genomic DNA between strain ASpG1^T and *S. alkalica* Z-7491^T exhibited 48.7 % homology. The *T_m* value for the genomic DNA of *S. alkalica* Z-7491^T was more than 5 °C higher than the *T_m* value for the genomic DNA of strain ASpG1^T. Finally, the genome size of strain ASpG1^T has been determined to be higher than that of *S. alkalica* Z-7491^T, and the G + C

content of strain ASpG1^T is 1.4 mol% higher than that of *S. alkalica* Z-7491^T.

A comparison of the phenotypic and genotypic data for strain ASpG1^T with those of *S. alkalica* Z-7491^T indicates that there are sufficient differences between the two strains for strain ASpG1^T to be considered a separate species of the genus *Spirochaeta*. Based upon the taxonomic criteria set forth by Wayne *et al.* (1987), the DNA reassociation value of 48.7% between strain ASpG1^T and *S. alkalica* Z-7491^T supports this assertion. Two of the alkaliphilic species of the genus *Spirochaeta* were named after the continent from which they were collected (*S. africana* Z-7692^T from soda Lake Magadi in Central Africa and *S. asiatica* Z-7591^T from Lake Khatyn of Central Asia; Zhilina *et al.*, 1996). In accordance with this convention, the name *Spirochaeta americana* is proposed for strain ASpG1^T, which represents the first haloalkaliphilic spirochaete isolated from the American continents.

Description of *Spirochaeta americana* sp. nov.

Spirochaeta americana (a.mer.i.can'a. N.L. fem. adj. *americana* of American continent, isolated from soda Mono Lake, California, USA).

Cells are motile and helix-shaped. Flagellum present in periplasmic space. Gram-negative. Cells have regular, unstable primary coils. Sphaeroplasts are formed at the end of the growth phase. Haloalkaliphile that cannot grow at pH 7.0. Growth is dependent upon the presence of carbonate and sodium ions in the medium. No growth occurs below 2% (w/v) NaCl or above 12% (w/v) NaCl. Mesophilic. Cells can be stored frozen in a liquid medium. Strictly anaerobic, catalase-negative chemoheterotroph with fermentative type of metabolism. Preferred substrates are D-glucose, fructose, maltose, sucrose, starch and D-mannitol. Requires vitamins and yeast extract for growth. Primary end product of glucose fermentation is H₂; the minor end products in the stationary phase of growth are acetate, formate, ethanol and CO₂. Resistant to kanamycin and rifampicin, but sensitive to gentamicin, tetracycline and chloramphenicol. Other characteristics of the species can be found in Table 1.

The type strain of *Spirochaeta americana* is ASpG1^T (=ATCC BAA-392^T=DSM 14872^T). The *T*_m of its genomic DNA is 68±2 °C. Isolated from mud sediments of the alkaline, hypersaline, meromictic, soda Mono Lake in northern California, USA.

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