Spirochaeta dissipatitropha sp. nov., an alkaliphilic, obligately anaerobic bacterium, and emended description of the genus *Spirochaeta* Ehrenberg 1835

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A novel obligately anaerobic, mesophilic, alkaliphilic spirochaete, strain ASpC2^T, was isolated from an anaerobic sediment of alkaline, hypersaline Owens Lake in California, USA. The Gram-negative cells are motile, helical in shape and 0.23×8.0–18.0 µm. Growth occurs within the following ranges: 13–41 °C, with optimal growth at 35 °C; 1–3 % (w/v) NaCl, with optimal growth at 2 % (w/v) NaCl; and pH 7.8–10.5, with optimal growth at pH 10.0. The novel isolate is strictly alkaliphilic and requires high concentrations of carbonate ions in the medium. It utilizes some sugars, some organic acids, some amino acids, Casamino acids, yeast extract and peptone. The main end products of glucose fermentation are CO₂ and acetate. Strain ASpC2^T is resistant to kanamycin and rifampicin, but sensitive to ampicillin, chloramphenicol, gentamicin and tetracycline. The DNA G + C content of the new isolate is 43.8 mol%, its genome size is 6×10⁸ Da and the melting temperature of its genomic DNA is 71 °C. DNA–DNA hybridization experiments demonstrated 46 % similarity with the phylogenetically most closely related species, *Spirochaeta asiatica* Z-7591^T. On the basis of physiological and molecular properties, the new isolate belongs taxonomically to a novel species within the genus *Spirochaeta*, for which the name *Spirochaeta dissipatitropha* sp. nov. is proposed (type strain, ASpC2^T=ATCC BAA-1083^T=JCM 12856^T). *S. dissipatitropha* ASpC2^T is the second strain in the genus (after *Spirochaeta smaragdinae* SEBR 4228^T) that is able to use proteolysis products as the sole energy source, and additional tests have shown that other halo-alkaliphilic spirochaetes (*Spirochaeta americana*, *Spirochaeta alkalica* and *Spirochaeta africana*) are also able to grow on yeast extract alone; therefore, an emended description for the genus *Spirochaeta* is given.

Microbiological investigations of a remnant of a vast, meromictic, alkaline, hypersaline soda lake (Owens Lake) in northern California, USA, have resulted in the isolation of several anaerobic isolates. One of them is an alkaliphilic, mesophilic bacterium that represents the recently described genus and species *Anaerovirgula multivorans* (Pikuta *et al.*, 2006a). Another bacterium, free-living spirochaete strain ASpC2^T, was also isolated from

the anaerobic, cellulolytic microbial community of Owens Lake (Pikuta *et al.*, 2005).

According to its definition, the genus *Spirochaeta* is a group of free-living, saccharolytic, obligately or facultatively anaerobic, helical bacteria (Canale-Parola, 1977, 1984, 1992). All species of the genus *Spirochaeta* that have been described match these characteristics. *Spirochaeta isovalerica* is the only species described that, in addition to carbohydrates, is able to ferment L-leucine, L-isoleucine and L-valine, producing isovaleric, 2-methylbutyric and isobutyric acids, respectively, as the metabolic end products. However, it was shown that *S. isovalerica* requires

Abbreviation: T_m, melting temperature.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain ASpC2^T is AY995150.

a fermentable carbohydrate for growth (Harwood & Canale-Parola, 1981a, b, 1983). The conclusion was made that fermentation of amino acids in the absence of glucose does not support measurable growth of *S. isovalerica*, but serves to generate ATP, which is utilized as a source of maintenance energy by the spirochaete when fermentable carbohydrates are not available. Much later, the species *Spirochaeta smaragdinae*, which ferments glycerol, fumarate, peptides and yeast extract in addition to carbohydrates, was described (Magot *et al.*, 1997).

In our laboratory, a new spirochaete strain, ASpC2^T, has been isolated from anaerobic sediments of Owens Lake in California, USA. Along with carbohydrates, this new isolate is capable of growth on yeast extract, peptone, Casamino acids, some amino acids and some organic acids. After a detailed study, it was found that the new micro-organism plays the role of a dissimilatory secondary anaerobe in an alkaliphilic cyanobacterial community, and represents a novel species with distinguishing physiological and genetic characteristics. In this article, we present a taxonomic description of this novel spirochaete. We also provide an emended description of the genus *Spirochaeta* Ehrenberg 1835.

Sampling, maintenance, transportation and storage of samples were as described previously (Pikuta *et al.*, 2002, 2006a). The growth medium contained (l⁻¹): NaCl, 10 g; Na₂CO₃, 2.76 g; NaHCO₃, 24.0 g; KCl, 0.2 g; K₂HPO₄, 0.2 g; MgCl₂·6H₂O, 0.1 g; NH₄Cl, 1.0 g; Na₂S·9H₂O, 0.4 g; resazurin, 0.001 g; yeast extract, 0.01 g; 2 ml vitamin solution (Wolin *et al.*, 1963); 1 ml trace mineral solution (Whitman *et al.*, 1982). D-Glucose, D-mannitol or cellulose (filter paper) were used as substrates during purification of the strains. The final pH was adjusted to 9.5. High-purity nitrogen was used for the gas phase. Growth was recorded by measurements of OD₅₉₅ using a spectrophotometer (Genesis 5; Spectronic Instruments) and by microscopy. Unless specified otherwise, the experiments were performed at 35 °C, and substrates, as the sole source of carbon and energy, were added at concentrations of 5 g l⁻¹. To obtain the enrichment culture 0.5 g wet sediment material was injected into Hungate tubes containing growth medium with cellulose and incubated at 35 °C for 4 days. Phase-contrast microscopy of enrichment cultures on growth medium with cellulose indicated good growth of motile, helical cells. A pure culture was obtained by filtration of an enrichment culture through a sterile, 0.4 µm membrane syringe filter, as described by Canale-Parola (1992). Two strains of helical cell cultures were isolated from the mud sample. The method of serial dilutions was applied four times on growth medium with D-mannitol as a substrate without yeast extract. Both strains exhibited indistinguishable characteristics of morphology, metabolism and physiology, and strain ASpC2^T was selected as the type strain of the novel species. Isolate ASpC2^T (=ATCC BAA-1083^T=JCM 12856^T) was obtained in pure culture and was used exclusively in this study.

Growth of colonies was observed after 4 days incubation on an agar medium using the 'roll-tube' method (Hungate, 1969). The colonies were white with veil-like or cotton-ball formations, as a result of growth penetrating through the agar gel. The morphology of colonies growing on the surface of an agar medium with 10 µg rifampicin ml⁻¹ was found to be separate, white, round circles with smooth, regular edges (0.5–2.0 mm in diameter) and a more densely granulated centre, with density diminishing toward the perimeter.

Cells of strain ASpC2^T were helical and highly motile, exhibiting a rotating, undulatory movement typical of spirochaetes. Cells were 0.20–0.25 µm in diameter with a length of 8.0–18.0 µm, and formed regularly shaped spirals. Cells had a Gram-negative reaction and formed spheroplasts at the end of the exponential-growth phase.

All tests to check for dependence on the CO₃²⁻ and Na⁺ ions were carried out as described previously (Hoover *et al.*, 2003; Pikuta *et al.*, 2006a). Analytical procedures, optical microscopy, antibiotic-susceptibility and fatty acid analyses were also done as described previously (Hoover *et al.*, 2003).

The novel isolate was catalase-negative and grew only under strictly anaerobic conditions. It had an obligately chemo-organoheterotrophic fermentative metabolism; it grew on media supplemented by carbohydrates without yeast extract. The doubling time under optimal conditions [35 °C, 2% (w/v) NaCl and pH 10.0] was 5–7 h. Some sugars, organic acids, proteolysis products and amino acids are used as substrates for growth (see species description for details).

The major fatty acid methyl esters were C_{14:0} (20.35%), C_{16:1} *cis*9 (11.03%), C_{16:0} (12.72%), C_{18:0} (5.19%), dimethylacetate C_{16:0} (23.76%), C_{18:0} (2.03%) and summed feature 10 [a mixture of C₁₈ unsaturated fatty acids in both *cis* and *trans* configurations] (12.99%). Aldehydes were represented only by C_{16:0} ALDE (9.24%) (Table 1).

The DNA G+C content (mol%) was determined as described by Mesbah *et al.* (1989), except that 2 U S1 nuclease (Sigma-Aldrich) was used in place of 1 U P1 nuclease and the nuclease degradation was performed in a 20 mM sodium acetate buffer, pH 5.0. Controls indicated that this modification yielded results equivalent to the original procedure. It was chosen because of the lower cost and higher availability of S1 nuclease. The total G+C content of the purified genomic DNA of strain ASpC2^T was 43.8 ± 0.2 mol% (mean ± SD, n=12). This value is the lowest so far observed within the genus *Spirochaeta*, but is not so different from those of *Spirochaeta caldaria* and *Spirochaeta asiatica* at 45 and 49 mol%, respectively (Pohlschroeder *et al.*, 1994; Zhilina *et al.*, 1996). Thus, the range of DNA G+C contents within the genus *Spirochaeta* is truly large, 44–65 mol%, which is reflected below in the emended description of the genus.

Table 1. Cellular fatty acid contents of strain ASpC2^T and related species

Taxa: 1, *S. dissipatitropha* ASpC2^T; 2, *S. asiatica* ATCC 700261^T (Zhilina *et al.*, 1996); 3, *S. africana* ATCC 700263^T (Zhilina *et al.*, 1996). All three organisms were grown using ATCC 2042 medium (<http://www.atcc.org/Attachments/3203.pdf>), but strain ASpC2^T had only 1% NaCl. Values are percentages (w/w) of total fatty acids. Abbreviations: ALDE, aldehyde; DMA, dimethyl acetate; FAME, fatty acid methyl ester.

Fatty acid	1	2	3
C _{14:0} FAME	20.35	13.79	6.63
C _{14:0} DMA	1.24	2.36	4.99
C _{16:0} ALDE	9.24	6.66	3.83
C _{16:1} <i>cis</i> 9 FAME	11.03	9.64	6.34
C _{16:1} <i>cis</i> 11 FAME	—	1.42	—
C _{16:0} FAME	12.72	12.32	18.95
C _{16:1} <i>cis</i> 9 DMA	—	—	1.06
C _{16:0} DMA	23.76	21.29	15.31
C _{18:1} <i>cis</i> 9 FAME	—	1.22	1.35
Summed feature 10*	12.99	22.40	34.86
C _{18:0} FAME	5.19	6.43	1.76
C _{18:0} DMA	2.03	1.56	—
C _{18:1} <i>cis</i> 11 DMA	—	—	0.80
C _{20:1} <i>cis</i> 13/ <i>trans</i> 11 FAME	—	—	2.37

*Summed feature 10 is C_{18:1}*cis*11/*trans*9/*trans*6 FAME and/or an unknown fatty acid with an equivalent chain length of 17.834.

The melting temperature (T_m) of total genomic DNA for strain ASpC2^T, *S. asiatica* Z-7591^T and *Spirochaeta africana* Z-7692^T was determined by using the procedure described by De Ley *et al.* (1970) and Gillis *et al.* (1970). Thirty micrograms of purified genomic DNA (Ausubel *et al.*, 1987) from each of the three strains was denatured in 1 × SSC buffer (pH 7.2) by increasing the temperature of the sample from 25 to 98 °C (at a rate of 0.7–1.0 °C min⁻¹); OD₂₇₀ measurements were then recorded. In addition, the T_m analysis was confirmed in 1 × SSC buffer (pH 7.0) by using SYBR Green I (Roche Applied Science) double-stranded DNA-binding dye and a Cepheid Smart Cycler instrument (Olson *et al.*, 2007; Panicker *et al.*, 2004). The T_m values (°C) for DNA from each species were recorded from the fluorescent readings obtained with the SYBR Green I dye following 50% dissociation of the DNA with a steady increase in the temperature (at the rate of 0.2 °C s⁻¹). These experiments were conducted in triplicate. The T_m values were as follows (mean ± SD, $n=3$): strain ASpC2^T, 71 ± 0.03 °C; *S. africana* Z-7692^T, 75.4 ± 0.06 °C; *S. asiatica* Z-7591^T, 72.7 ± 0.02 °C.

The relatedness of genomic DNA between strain ASpC2^T and *S. africana* Z-7692^T and between strain ASpC2^T and *S. asiatica* Z-7591^T was determined by DNA–DNA hybridization using the DNA-reassociation kinetics method described by De Ley *et al.* (1970) and Johnson (1985).

Purified, sonicated genomic DNA (75 µg) from each strain was added to 4 × SSC buffer (pH 7.2) and 25% deionized formamide. The DNA was denatured by increasing the temperature in increments of 1 °C min⁻¹ to 5 °C above the respective T_m values by using a Perkin Elmer UV/vis Lambda II spectrophotometer with a PTP-1 Peltier temperature programmer. The temperature was then lowered rapidly (over approx. 1.5 min) to the reassociation temperature and the A_{270} was recorded at 15 s intervals for a total of 15 min. The initial reassociation kinetics were determined by linear-regression analysis. The experiment was conducted in triplicate. The percentage relatedness of the DNA was calculated by using the equation described by De Ley *et al.* (1970). All statistical analyses were performed by using Microsoft Excel software. The results showed DNA hybridization values of 58 ± 0.8% (mean ± SD, $n=3$) between the genomes of strain ASpC2^T and *S. africana* Z-7692^T, and of 46 ± 1.5% (mean ± SD, $n=3$) between the genomes of strain ASpC2^T and *S. asiatica* Z-7591^T.

The genome size of strain ASpC2^T was determined by using the reported genome size of *S. asiatica* Z-7591^T (2.1 × 10⁹ Da; Zhilina *et al.*, 1996), DNA reassociation rates for strain ASpC2^T and *S. asiatica* Z-7591^T and the equation described by Gillis *et al.* (1970). The calculated genome size of strain ASpC2^T was 6 × 10⁸ Da.

Genomic DNA was isolated by a standard phenol/chloroform extraction, followed by ethanol precipitation (Sambrook *et al.*, 1989). The 16S rRNA gene was amplified as described previously (Hoover *et al.*, 2003), except for the enzyme, which was Rainbow DNA polymerase (Extremozyme), and the fact that no DMSO was used in the reactions. The PCR product was purified by using a GeneClean Turbo kit (Qbiogene) and sequenced as described previously (Hoover *et al.*, 2003). The 16S rRNA gene sequence of strain ASpC2^T was aligned with those of other members of the genus *Spirochaeta*, after a BLAST search (Altschul *et al.*, 1990) indicated closest similarity to members of this genus. Sequence alignment, pairwise-distance computation and phylogenetic-tree construction were performed as described previously (Pikuta *et al.*, 2006b).

A sequence covering 1459 nt of the 16S rRNA gene of strain ASpC2^T was obtained, corresponding to positions 27–1492 of the *Escherichia coli* 16S rRNA gene sequence. The sequence was compared with all sequences available in GenBank and appeared to be highly similar to sequences from the spirochaete group. A phylogenetic dendrogram showing the relationship of strain ASpC2^T to other spirochaetes, based on 1332 common nucleotide positions, was constructed (Fig. 1). The only species that were not included were *Spirochaeta plicatilis* (Ehrenberg, 1835), for which no nucleotide sequence is available, and *Spirochaeta thermophila* (Aksenova *et al.*, 1992), which is not among the closest relatives of strain ASpC2^T and for which the significantly shorter available sequence would have reduced the accuracy of the tree topology. Strain ASpC2^T appears to belong to the alkaliphilic/halophilic cluster of spirochaetes,

Fig. 1. Unrooted phylogenetic tree based on 16S rRNA gene sequences showing the position of strain ASpC2^T among members of the genus *Spirochaeta*, constructed by using the neighbour-joining method. Numbers at nodes are bootstrap percentages (1000 replicates). GenBank accession numbers are shown in parentheses. Bar, two substitutions per 100 nt.

with sequence similarities of between 87.4 and 99.5% (computed from the 1332 nucleotide positions used to construct the tree). The highest similarity was observed with *S. asiatica*.

Strain ASpC2^T was isolated from wet mud sediments beneath the red crust of a small remnant of the almost-dry Owens Lake on the eastern side of the Sierra Nevada mountains in east-central California, USA. Sedimentary deposits of this ancient lake have provided a palaeoclimatological record extending back approximately 425 000 years. Owens Lake overflowed many times between 52 500 and 12 500 years ago (Benson *et al.*, 1996). At the end of the last ice age (approx. 11 000 years ago), meltwater from the Pleistocene glaciers filled it to a depth exceeding 70 m in places and the area of the lake was >500 km². During the silver boom of the 1870s, Owens Lake was over 10 m deep but, in the early 1900s, the Owens River, which fed the lake, was diverted into the Owens Valley aqueduct to provide water for Los Angeles. By 1926, the water had been almost entirely drained from the lake. Deprived of a replenishing supply, the water in the Owens Lake basin evaporated, leaving a vast hypersaline, hyperalkaline playa with the evaporite minerals trona, burkeite and halite.

The new isolate performs the function of a secondary anaerobe and behaves as a dissimilatory micro-organism (as it utilizes the dissipation products of polymers) within the anaerobic, alkaliphilic microbial community.

On the basis of phenotypic and genotypic characteristics (shape and movement of cells, Gram-negative cell wall, fermentation of sugars, resistance to rifampicin and phylogenetic data), strain ASpC2^T was identified as a member of the genus *Spirochaeta*. Among *Spirochaeta* species, strain ASpC2^T was found to be only the second after *S. smaragdinae* DSM 11293^T (Magot *et al.*, 1997) capable of growth on proteolysis products, but the new isolate is also capable of growth on certain amino acids, with weak cell-mass yield (one to three cells per field on third transfer). Additional tests for the ability to utilize proteolysis products were conducted for all halo-alkaliphilic *Spirochaeta* species (*S. asiatica*, *S. americana*, *S. africana* and *S. halophila*). The results of these tests showed that *S. americana*, *S. alkalica* and *S. africana* were also able

to grow with yeast extract as a sole source of carbon and energy (three transfers). Results also showed that none of the halo-alkaliphilic spirochaetes tested was able to grow on Casamino acids or peptone.

An interesting feature of this novel bacterium is its ability to utilize L- and D-ribose and L- and D-arabinose. This is the second species from Owens Lake found to have the ability to utilize sugars of alternative enantiomers. However, the other species, *A. multivorans* SCA^T, is unable to grow on D-arabinose. Subsequent investigations of enzymes and plasmids of these interesting organisms may provide additional information regarding these abilities.

Differences between the morphological, physiological and genetic characteristics of strain ASpC2^T and closely related (by phylogenetic analysis) species of the genus *Spirochaeta* are shown in Table 2. Strain ASpC2^T is notably different from its phylogenetically nearest neighbour, *S. asiatica* Z-7591^T, in both pH and salinity characteristics and in substrates utilized. Strain ASpC2^T and *S. africana* Z-7692^T are also different in salinity and substrates. Compared with *S. alkalica* Z-7491^T, the new isolate also exhibits differences in cell size, salinity, substrates and metabolic end products. Furthermore, the new isolate is an obligate anaerobe, whereas *S. alkalica* Z-7491^T is a facultative anaerobe. The new spirochaete differs from *S. americana* ASpG1^T in salinity characteristics, substrate utilization and metabolic end products (*S. americana* ASpG1^T produces hydrogen as a major end product, but strain ASpC2^T produces hydrogen only in trace quantities). The new spirochaete differs from *S. halophila* RS-1^T in cell size, pH and salinity characteristics. Their relationship with oxygen is also different. It must be pointed out that the new isolate has the most alkaline optimum pH for growth among all halo-alkaliphilic spirochaetes. The genomic DNA G + C content of the new spirochaete is significantly different from the others (Table 2). Fatty acid analysis demonstrates the absence of branched-chain fatty acids for new isolate ASpG1^T, as was also observed for *S. alkalica*, *S. africana*, *S. asiatica*, *S. halophila* and *Spirochaeta litoralis*.

DNA–DNA hybridization of genomic DNA between strain ASpC2^T and the phylogenetically most closely related species, *S. asiatica* Z-7591^T and *S. africana* Z-7692^T,

Table 2. Comparative characteristics of strain ASpC2^T and related species of the genus *Spirochaeta*

Taxa: 1, *S. dissipatitroph*a ASpC2^T (data from the present work); 2, *S. asiatica* Z-7591^T (Zhilina *et al.*, 1996); 3, *S. americana* ASpG1^T (Hoover *et al.*, 2003); 4, *S. alkalica* Z-7491^T (Zhilina *et al.*, 1996); 5, *S. africana* Z-7692^T (Zhilina *et al.*, 1996); 6, *S. halophila* RS-1^T (Greenberg & Canale-Parola, 1976). All species use D-glucose, D-maltose, sucrose, starch and D-trehalose as substrates for growth. All species produce CO₂, acetate and ethanol as products of glucose fermentation. ND, Not determined; OA, obligate anaerobe; FA, facultative anaerobe; +/–, substrate used/not used; (+), weak growth.

Characteristic	1	2	3	4	5	6
Size (µm)	0.23 × 8.0–18.0	0.2–0.25 × 15–22	0.22 × 8.0–15	0.4–0.5 × 9–18	0.25–0.3 × 15–30	0.4 × 15–30
Growth conditions:						
optimum (range)						
pH	10.0 (7.8–10.5)	8.4–9.4 (7.9–9.7)	9.5 (8.0–10.5)	8.7–9.6 (8.4–10.7)	8.8–9.75 (8.1–10.7)	7.5
NaCl (%)	2 (1–3)	3–6 (2–8)	3 (2–12)	5 (3–10)	5–7 (3–10)	3–5 (0.3–7.3)
Temperature (°C)	35 (13–41)	33–37 (20–43)	37 (10–45)	33–37 (15–44)	30–37 (15–47)	35–40
Substrates:						
D-Fructose	+	–	+	(+)	+	+
D-Mannitol	+	+	+	–	–	+
Lactose	–	–	+	+	+	+
D-Arabinose	+	(+)	+	+	–	+
L-Arabinose	+	+	+	+	–	+
D-Mannose	–	+	+	–	+	+
D-Ribose	+	–	+	+	–	+
Yeast extract	+	–	+	+	+	–
Products of glucose fermentation:						
H ₂	+	–	+	+	+	+
Formate	ND	ND	+	ND	ND	–
Lactate	ND	+	–	+	+	+
Relationship to O ₂	OA	OA	OA	FA	FA	FA
DNA G + C content (mol%)	43.8	49.2	58.5	57.1	56.1	62.0
Genome size (Da)	6 × 10 ⁸	2.1 × 10 ⁹	2.98 × 10 ⁹	2.7 × 10 ⁹	2.5 × 10 ⁹	ND

*Data from the present work.

exhibited 46 and 58% homology, respectively, allowing separation of the new isolate into a novel species. The *T_m* value of genomic DNA of the new isolate was >4 °C higher than that of *S. africana* Z-7692^T, and it was 1.7 °C different from that of *S. asiatica* Z-7591^T. Lastly, the genome size of strain ASpC2^T has been determined to be 6 × 10⁸ Da, which is smaller than those reported for the most closely related spirochaetes, *S. africana* (2.5 × 10⁹ Da) and *S. asiatica* (2.1 × 10⁹ Da) (Zhilina *et al.*, 1996).

A comparison of the phenotypic and genotypic characteristics of ASpC2^T with those of the most closely related species indicates that there are sufficient differences for strain ASpC2^T to be considered a separate species, based upon the taxonomic criteria proposed by Stackebrandt *et al.* (2002). The name *Spirochaeta dissipatitroph*a sp. nov. is proposed for the novel species, with the type strain ASpC2^T (=ATCC BAA-1083^T=JCM 12856^T).

Our study of *S. dissipatitroph*a ASpC2^T and all halo-alkaliphilic species of the genus *Spirochaeta* has shown that the chemotaxonomic markers in the genus description are too narrowly delimited concerning substrate utilization. *S.*

*dissipatitroph*a ASpC2^T, as well as *S. smaragdinae* SEBR 4228^T, are not strictly saccharolytic species and can also utilize peptone and Casamino acids, some organic acids and, in the case of the new isolate, some amino acids. Furthermore, our studies have shown that *S. americana* ASpG1^T, *S. alkalica* Z-7491^T and *S. africana* Z-7692^T are also able to grow on yeast extract as a sole source of carbon and energy. Based on these distinguishing chemotaxonomic markers, an emended description of the genus *Spirochaeta* Ehrenberg 1835 is also given. The lower boundary for the range of genomic DNA G + C contents is also modified within the emended description.

Description of *Spirochaeta dissipatitroph*a sp. nov.

*Spirochaeta dissipatitroph*a [dis.si.pa.ti.tro'pha. L. part. adj. *dissipatus* dissipated, dispersed; N.L. fem. adj. *troph*a (from Gr. adj. *trophikos*) nursing, tending; N.L. fem. adj. *dissipatitroph*a tending to dissipated products, pertaining to the ability to utilize dissipated products after primary anaerobes].

Cells are motile and helical, with sizes of 0.23×8.0 – 18.0 μm . Cells stain Gram-negative and have regular, unstable primary coils. Spheroplasts are formed at the end of the growth period. Alkaliphilic; cannot grow at pH 7.0. pH range for growth is 7.8–10.5, with optimum growth at pH 10.0. Growth is obligately dependent upon carbonate and sodium ions. Requires NaCl for growth: no growth occurs at ≤ 0.5 or $\geq 3\%$ (w/v) NaCl; optimum growth was observed at 2% (w/v) NaCl. Mesophilic; the temperature range for growth is 13–41 °C, with an optimum at 35 °C. Cells can be stored frozen in liquid medium. Strictly anaerobic, catalase-negative, chemo-organoheterotrophic and dissimilatory with fermentative metabolism. D-Glucose, D-fructose, D-maltose, D-arabinose, L-arabinose, D-trehalose, D-ribose, L-ribose, starch, sucrose, D-mannitol, propionate, citrate, peptone, yeast extract, Casamino acids, L-arginine, L-isoleucine, L-methionine, L-threonine, L-histidine, L-alanine, L-aspartic acid, L-tryptophan and L-valine are used as substrates for growth. No growth occurs with the following substrates: acetate, lactate, pyruvate, butyrate, formate, methanol, ethanol, glycerol, betaine, chitin, pectin, L-glucose, L-fucose, L-mannose, D-mannose, lactose, glycine, L-serine, L-cysteine, L-cystine, L-phenylalanine, L-tyrosine, L-lysine, *trans*-4-hydroxy L-proline, L-glutamic acid, L-glutamine, L-asparagine, L-leucine, L-proline and trimethylamine. Growth on microcrystalline cellulose and filter paper occurs only on the first transfer. Major end products of glucose fermentation are CO_2 and acetate; hydrogen and ethanol are minor trace products. Resistant to kanamycin and rifampicin, but sensitive to ampicillin, gentamicin, tetracycline and chloramphenicol. For the type strain, the genomic DNA G+C content is 43.8 mol% (by HPLC), the T_m of the genomic DNA is 71 °C and the genome size is 6×10^8 Da.

The type strain, ASpC2^T (=ATCC BAA-1083^T=JCM 12856^T), was isolated from anaerobic mud sediments of the alkaline, hypersaline remnant of Owens Lake in east-central California, USA.

Emended description of the genus *Spirochaeta* Ehrenberg 1835

The description of the genus is as that given by Canale-Parola (1984), with the following modifications. Some species are able to grow without sugars by using, as alternative substrates, some amino acids and/or proteolysis products, such as yeast extract, peptone and Casamino acids. Genomic DNA G+C content is 44–65 mol%.

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