Potential Applications of *Spirochaeta americana* for Hydrogen Production

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Science for Human Security: Biohydrogen Production and Use
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INTRODUCTION

In response to the Climate and Energy Crisis the *"U.S. National Clean Hydrogen Strategy and Roadmap"* stresses the importance of developing Zero- and Low-Carbon Hydrogen to a "sustainable and equitable clean energy future."

(Bipartisan Infrastructure Law(BIL) provides $9.5 Billion for Clean Hydrogen Development; Inflation Reduction Act (IRA) provides additional incentives and production tax credits.)

2021 *Hydrogen Energy Earthshot* to Stimulate Private Sector Investments.

These Developments and Stimulation from WAAS led us to again consider the possible role of the haloalkaliphile *Spirochaeta americana* isolated from Mono Lake for the anaerobic fermentative production of Zero-Carbon Biohydrogen.

Biohydrogen Production

1939: Gaffon[1] reports molecular hydrogen released from water by photosynthetic activity of green algae *Scenedesmus obliquus.*

1993: Taguchi[2] reports isolation of hydrogen-producing bacterium from termites

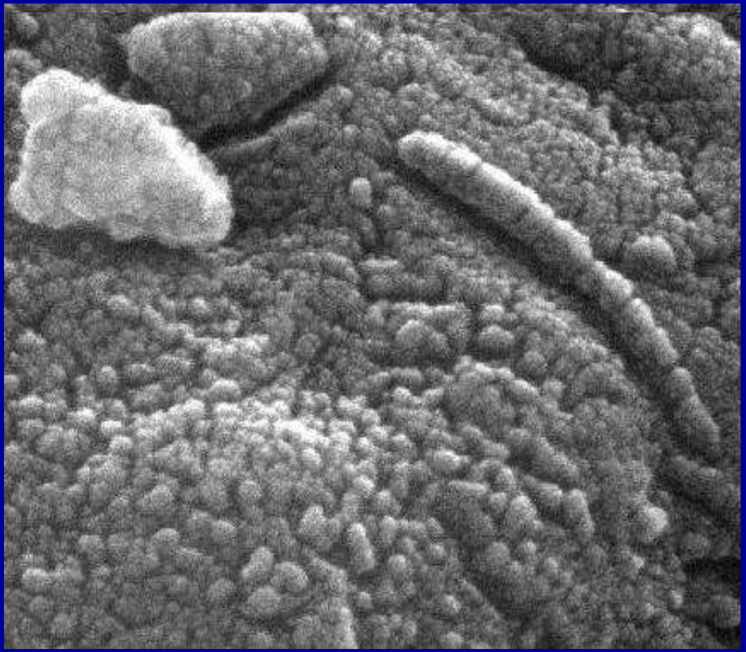
2002: Siebert *et al*. [3] clone and sequence hydrogenase genes (hydA1 and hydA2) from the green algae *Chlamydomonas reinhardtii*. Hydrogen from algae high efficiency - may suffer from inhibition by O_2 produced during photosynthesis.

2003: Hydrogen shown to be primary end product of fermentative metabolism of sugars by novel anaerobe from Mono Lake.[4] NASA/MSFC funds research on hydrogen production by *Spirochaeta amaricana* for potential application for providing continual supply of hydrogen for fuel cells on Space Station and long duration space flights for human explorations of the Moon & Mars. [5]

1. Gaffron, H., (1939) *Reduction of carbon dioxide with molecular hydrogen in green algae.* Nature 143, 204-205,
2. Taguchi, J. D. et al., (1993) *Isolation of a hydrogen-producing bacterium, Clostridium beijerinckii, strain AM21B, from termites.* Can. J. Microbiol. 39, 726-730,
3. Siebert, M., et al. (2002) *Molecular engineering of algal H2 production.* Proc. U.S. DOE Hydrogen Program Review, NREL/CP-601-32405, 1-10.
4. Hoover, R. B., Pikuta, E. V. et al. (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.
5. Pikuta, E. V. and Hoover, R. B. (2004) Potential application of anaerobic extremophiles for hydrogen production. Proc. SPIE, Vol. 5555, 203-214.

INTRODUCTION

1996-Dr. David McKay reports evidence for nanofossils in 3.2 Gya Mars meteorite ALH84001

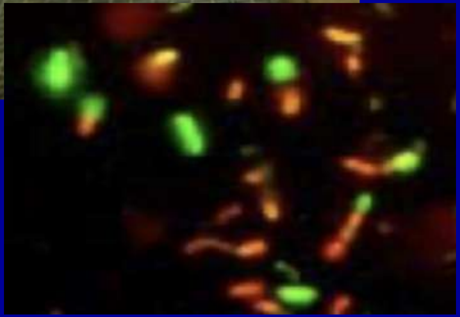


1997-NASA/MSFC Astrobiology group formed to study Microbial Extremophiles and Microfossils in Meteorites

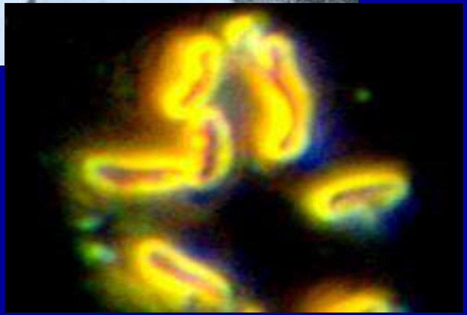
NASA Astrobiology Expeditions

Search for life in Earth's Most Hostile Environments

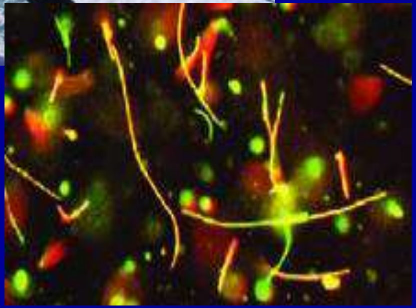
Permafrost and Ice of Siberia, Alaska & Antarctica as Analogs for Polar Ice Caps of Mars and Icy Moons



Carnobacterium pleistocenium



Sanguibacter gelidistatuarii



Williamwhitmania taraxaci

Microbial Life in Endorheic Basins

Life in closed hypersaline alkaline lakes/evaporates as terrestrial analog for life in endorheic volcanic basins/impact craters of Mars


Vestatitas Borealis


Korolev Crater

Volcanic/Impact craters on Mars


Owens Lake

Spirochaeta dissipatitropha
Anaerovigula multivorans

Spirochaeta americana
Tindallia californiensis
Desulfonatronum thiodismutans

Image of Mono Lake in Long Valley Caldera by Landsat 7

Volcanic Paoha Island with a hot (~90 °C), sulfurous, alkaline springs emerged 350 years ago in center of endorheic, soda Mono Lake in northern California

Sampling of Mono Lake

Spectacular Tufa (CaO; CaO_2) and Ikaite ($CaCO_3 \cdot 6H_20$) columns on south shore of soda Mono Lake in California

August 15, 2000: Black mud with strong H_2S odor sampled anaerobically from south shore under shallow water with Temp. 21.6 ^{o}C; Salinity 7%; pH 9.9

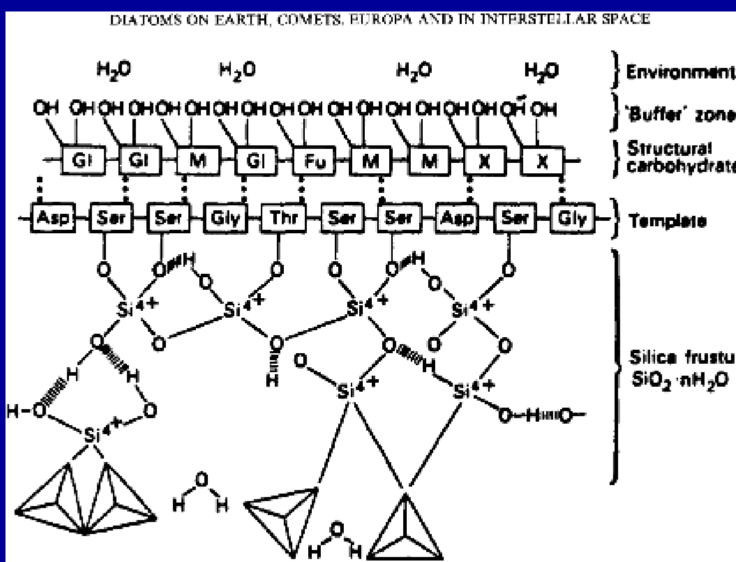
Primary Producers of Mono Lake

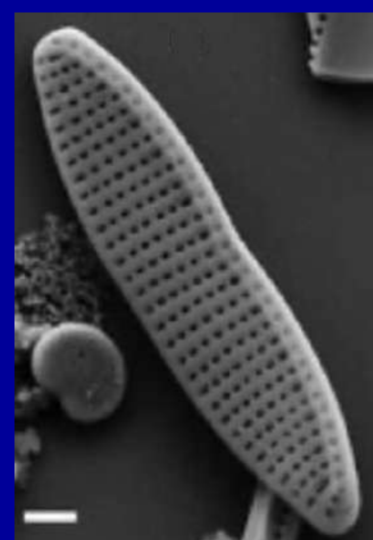
Small Pennate Diatoms and Picocyanobacteria

Mean relative abundances and distribution of diatom taxa in Mono Lake samples[a]

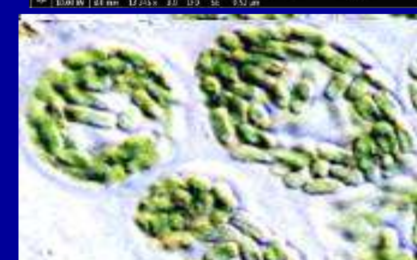
	BPTS					
	1 m		5 m		10 m	
	Sediment	Rock	Sediment	Rock	Sediment	Rock
Navicula crucialis (O. Müll.)	42.8	0.2	65.1	27.4	55.5	30.6
Nitzschia frustulum (Kütz.) Grun.	25.3	62.9	30.4	60.1	34.2	20.4
N. latens Hust.	31.9	36.9	3.4	10.4	2.7	7.2
N. monoensis sp. nov.	P	P	0.9	1.0	4.1	11.3
N. reimerii sp. nov.	P		P	0.2		0.2

KOCIOLEK, J. P & HERBS, D.B. (1992) Taxonomy and Distribution of Benthic Diatoms from Mono Lake, California, U.S.A.'(1992) Trans. Amer. Micros. Soc. 111, 338-355.(





Nitzschia frustulum



Cyanobium sp.

Extremophiles of Mono Lake

2001: NASA/NSSTC Dr. Elena V. Pikuta obtains enrichment cultures & isolates 3 novel strains:

MLF1^{T} - Anaerobic, alkaliphilic, magnetotactic, sulfate reducing lithoheterotroph capable of growth on hydrogen without organic source of carbon

ASpG1^{T} - Obligately anaerobic, haloalkaliphilic, sugar-lytic, hydrogen-producing spirochaete.

APO^{T} – Obligately anaerobic, extremely haloalkaliphilic, spore-forming acetogen

Extremophiles of Mono Lake

International Journal of Systematic and Evolutionary Microbiology (2003), 53, 815–821 DOI 10.1099/ijs.0.02535-0

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

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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 H_2, 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}).

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Paul Krader

Tindallia californiensis sp. nov., a new anaerobic, haloalkaliphilic, spore-forming acetogen isolated from Mono Lake in California

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Abstract A novel extremely haloalkaliphilic, strictly anaerobic, acetogenic bacterium strain APO was isolated from sediments of the athalassic, meromictic, alkaline Mono Lake in California. The Gram-positive, spore-forming, slightly curved rods with sizes 0.55–0.7×1.7–3.0 µm were motile by a single laterally attached flagellum. Strain APO was mesophilic (range 10–48 °C, optimum of 37 °C); halophilic (NaCl range 1–20% (w/v) with optimum of 3–5% (w/v), and alkaliphilic (pH range 8.0–10.5, optimum 9.5). The novel isolate required sodium ions in the medium. Strain APO was an organotroph with a fermentative type of metabolism and used the substrates peptone, bacto-tryptone, casamino acid, yeast extract, L-serine, L-lysine, L-histidine, L-arginine, and pyruvate. The new isolate performed the Stickland reaction with the following amino acid pairs: proline + alanine, glycine + alanine, and tryptophan + valine. The main end product of growth was acetate. High activity of CO dehydrogenase and hydrogenase indicated the presence of a homoacetogenic, non-cycling acetyl-CoA pathway. Strain APO was resistant to kanamycin but sensitive to chloramphenicol, tetracycline, and gentamycin. The G+C content of the genomic DNA was 44.4 mol% (by HPLC method). The sequence of the 16S rRNA gene of strain APO possessed 98.2% similarity with the sequence from *Tindallia magadiensis* Z-7934, but the DNA-DNA hybridization value between these organisms was only 55%. On the basis of these physiological and molecular properties, strain APO is proposed to be a novel species of the genus *Tindallia* with the name *Tindallia californiensis* sp. nov., (type strain APO = ATCC BAA-393 = DSM 14871).

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

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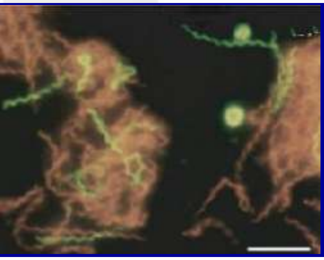
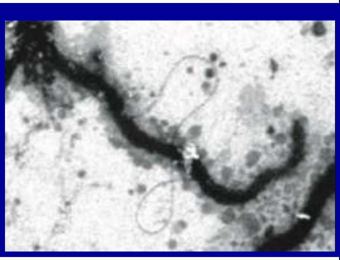
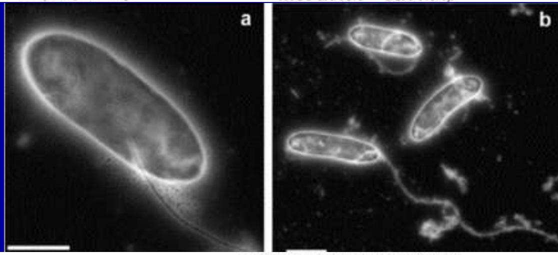
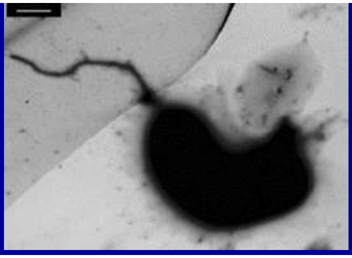
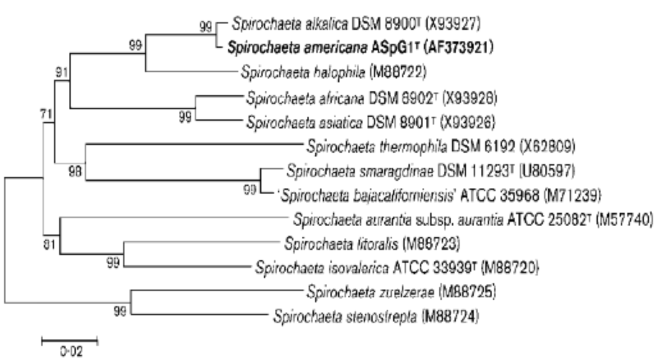
A novel alkaliphilic, sulfate-reducing bacterium, strain MLF1^{T}, was isolated from sediments of soda Mono Lake, California. Gram-negative vibrio-shaped cells were observed, which were 0·6–0·7 × 1·2–2·7 µm in size, motile by a single polar flagellum and occurred singly, in pairs or as short spirilla. 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 × 10^9 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 × 10^9 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}).

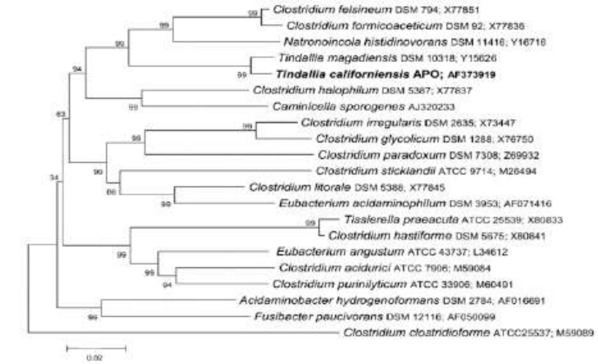


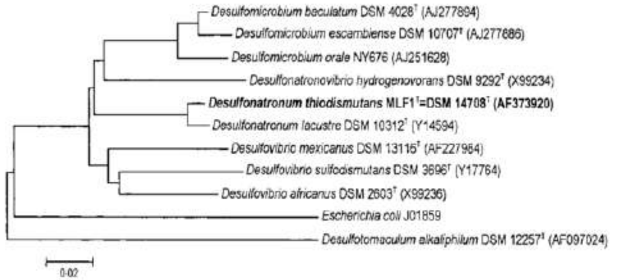




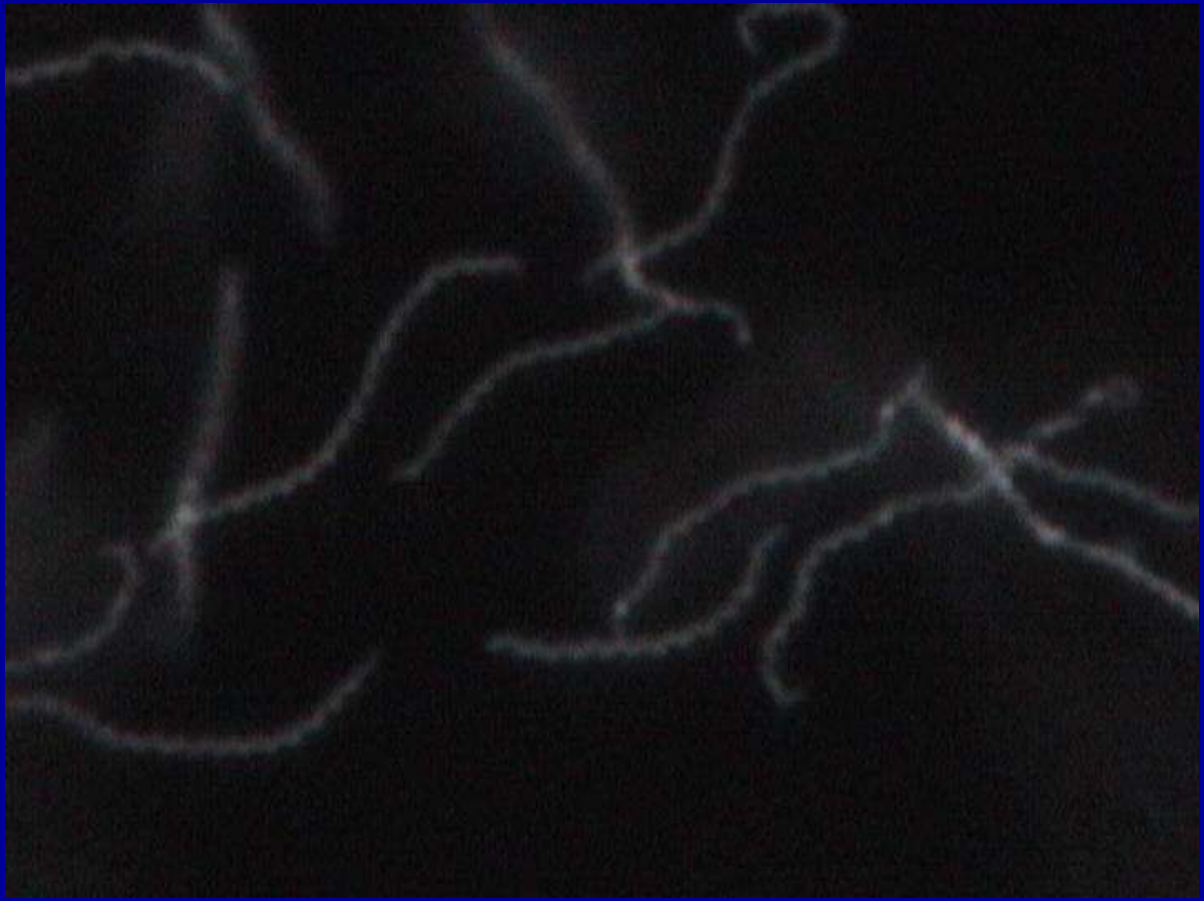








Spirochaeta americana ASpG1^T



Dr. Elena Pikuta obtained enrichment cultures & isolated novel strain ASpG1^T; an obligately anaerobic, mesophilic, haloalkaliphilic, chemoheterotrophic, sugar-lytic, gram negative, motile helical spirochaete with single flagellum in periplasmic space. Since *Spirochaeta americana* requires carbonate & sodium ions and exhibits no growth at pH 7 it is therefore free-living, non-pathogenic.

Spirochaeta americana ASpG1^T

Characteristic	1	2	3	4	5	6
	S. dissipatitropha ASpC2^T	*S. asiatica* Z-7591^T	*S. americana* ASpG1^T	*S. alkalica* Z-7491^T	*S. africana* Z-7692^T	*S. halophila* RS-1^T
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.

S. americana yielded ~80-90% hydrogen - more than all other species

Alkalispirochaeta americana ASpG1^{T}

2016 *Spirochaeta americana* ASpG1^{T} reclassified in new genus *Alkalispirochaeta*

T. Sravanthi and others

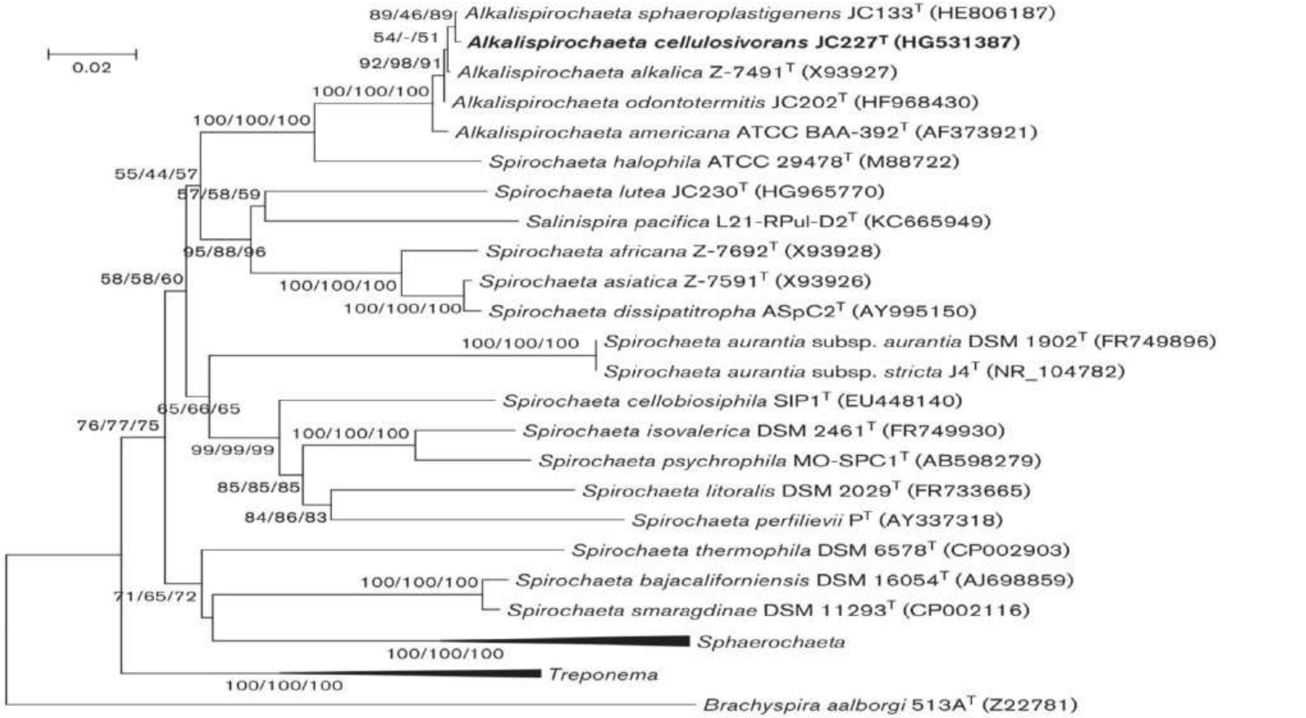


Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the relationship of strain JC227^{T} with the most closely related members of the genus *Spirochaeta*. The tree was reconstructed by the NJ method using the MEGA6 software and rooted by using *Brachyspira aalborgi* 513A^{T} as the outgroup. Numbers at nodes represent bootstrap values (based on 1000 resamplings) and bootstrap percentages refer to NJ/ML/ME analysis. GenBank accession numbers are shown in parentheses. Bar, 2 substitutions per 100 nucleotide positions. The collapsed groups represent the following taxa: *Sphaerochaeta coccoides* DSM 17374^{T} (GenBank accession no. CP002659), *Sphaerochaeta associata* GLS2^{T} (JN944166), *Sphaerochaeta globosa* DSM 22777^{T} (AF357916) and *Sphaerochaeta pleomorpha* DSM 22778^{T} (AF357917) (*Sphaerochaeta*) and *Treponema stenostrepta* DSM 2028^{T} (FR733664), *Treponema caldaria* DSM 7334^{T} (CP002868) and *Treponema zuelzerae* DSM 1903^{T} (FR749929) (*Treponema*).

Sravanthi, T., *et al*. (2016) *Alkalispirochaeta cellulosivorans* gen. nov,, sp. nov., a cellulose-hydrolyzing, alkaliphilic halotolerant bacterium isolated from the gut os a wood-eating cockroach (*Cryptocercus punctulatus*) and reclassification of four species of *Spirochaeta* as new combinations within *Alkalispirochaeta* gen. nov.… IJSEM 66, 1612-1619

Potential Applications of *Spirochaeta americana* for Hydrogen Production

CONCLUSIONS

Heat value (~142 MJ/kg) of Hydrogen is > 2.7X times petroleum fuels. Main H_2 production method today is steam reforming of fossil fuels but contribution to Climate Change is not sustainable.

Clean Hydrogen is main metabolic product of fermentation of sugars by non-pathogenic, anaerobic *Spirochaeta americana*.

Further research needs to be conducted into developing low-cost as feedstocks such as sugars from alkaliphilic benthic diatoms, picocyanobacteria or carbohydrate-rich organic wastes and scaling the technology to Industrial Levels.