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TAXONOMIC DESCRIPTION

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Syntrophotalea acetylenivorans sp. nov., a diazotrophic, acetylenotrophic anaerobe isolated from intertidal sediments

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Abstract

A Gram-stain-negative, strictly anaerobic, non-motile, rod-shaped bacterium, designated SFB93^T, was isolated from the intertidal sediments of South San Francisco Bay, located near Palo Alto, CA, USA. SFB93^T was capable of acetylenotrophic and diazotrophic growth, grew at 22–37 °C, pH 6.3–8.5 and in the presence of 10–45 g l^{-1} NaCl. Phylogenetic analyses based on 16S rRNA gene sequencing showed that SFB93^T represented a member of the genus *Syntrophotalea* with highest 16S rRNA gene sequence similarities to *Syntrophotalea acetylenica* DSM 3246^T (96.6%), *Syntrophotalea carbinolica* DSM 2380^T (96.5%), and *Syntrophotalea venetiana* DSM 2394^T (96.7%). Genome sequencing revealed a genome size of 3.22 Mbp and a DNA G+C content of 53.4%. SFB93^T had low genome-wide average nucleotide identity (81–87.5%) and <70% digital DNA–DNA hybridization value with other members of the genus *Syntrophotalea*. The phylogenetic position of SFB93^T within the family *Syntrophotaleaceae* and as a novel member of the genus *Syntrophotalea* was confirmed via phylogenetic reconstruction based on concatenated alignments of 92 bacterial core genes. On the basis of the results of phenotypic, genotypic and phylogenetic analyses, a novel species, *Syntrophotalea acetylenivorans* sp. nov., is proposed, with SFB93^T (=DSM 106009^T=JCM 33327^T=ATCC TSD-118^T) as the type strain.

INTRODUCTION

Acetylene (C_2H_2) is a well-recognized biogeochemical tool used to probe the input (nitrogen-fixation [1, 2]) or loss (denitrification [1, 3, 4]) components of the nitrogen cycle that occur in assayed environmental samples. In the latter case, the acetylene-block technique prevents the reduction of nitrous oxide (N_2O) to molecular nitrogen (N_2), thereby allowing the rate of accumulation of N_2O to stand as a measure of the process of anaerobic nitrate respiration that evolves N_2 [3, 5]. Experimental reports of the application of the acetylene–block techniques to diverse soils and sediments, in addition to following the kinetics of N_2O, sometimes noted the disappearance of the acetylene with time [6–9]. A culture was established from South San Francisco Bay intertidal sediment which proved capable of using acetylene as its sole carbon and energy source [7, 10]; but eventually this culture was lost without being fully characterized. Both freshwater and estuarine strains of another anaerobic acetylene-degrading cultivar, *Syntrophotalea acetylenica* (formerly *Pelobacter acetylenicus*), were obtained from European muds [11]: DSM 3246^T (freshwater, type strain) and DSM 3247 (estuarine). Recently, these organisms along with aerobic acetylene-degraders were collectively termed acetylenotrophs [12]. *S. acetylenica* and its acetylene hydrating acetylene hydratase (AH) enzyme have been studied extensively [13–20]. In addition there are a number of synthesis reviews on the evolutionary and astrobiological implications of acetylenotrophy and the unusual, tungsten-centred AH enzyme of *S. acetylenica* [10, 21–24]. We note that in 2020 members of the genus *Pelobacter* were reclassified [25], e.g. *P. acetylenicus* is now *Syntrophotalea acetylenica*. To be consistent with references cited herein the original organism names are qualified parenthetically with the new genus name and Table S1 (available in the online

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Keywords: *Syntrophotalea*; acetylenotrophy; acetylene-degrader; acetylenotroph.
Abbreviations: AF, alignment fraction; AH, acetylene hydratase; ALDH, acetaldehyde dismutase; ANI, average nucleotide identity; BRIG, Blast Ring Image Generato; CM, modified bicarbonate medium; DDH, DNA–DNA hybridization; DSMZ, Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen; FAME, fatty acid methyl ester; FID-GC, gas chromatography with flame ionization detection; gANI, genome-wide average nucleotide identity; GSI, gene support index; HGAP, Hierarchical Genome Assembly Process; HSP, high-scoring segment pair; NCBI, National Center for Biotechnology Information; PGAP, Prokaryotic Genome Annotation Pipeline; UBCG, Up-to-Date Bacterial Core Genome.
The GenBank accession numbers for the 16S rRNA gene and genome sequences of strain SFB93^T are JQ085863.1 and NZ_CP015519.1, respectively.
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Two supplementary tables and two supplementary figures are available with the online version of this article.

version of this article) relates the new classifications to the prior names and taxonomy.

In a survey meant to assess the occurrence of acetylenotrophy in nature, activity was once again identified in San Francisco Bay sediment slurries, and a pure culture was established that proved capable of anaerobically growing on acetylene [26]. The culture, strain SFB93^T, was able to interact with dehalo-respiring bacteria (e.g. *Dehalococcoides mccartyii*) by providing H_2 and acetate from acetylene fermentation that the dehalogenators used as electron donor and carbon sources to enable their reduction of chlorinated ethenes to ethylene [27]. Further genomic investigation of SFB93^T identified sequences for two AH-encoding genes, distinguishing SFB93^T from *S. acetylenica* DSM 3246^T and DSM 3247 which hava a single copy of AH in their genomes [28, 29]. The genome of SFB93^T also contained components required for a functional MoFe-nitrogenase (*nif*), and diazotrophic growth upon the acetylene substrate was confirmed experimentally [28, 29].

We now report genomic and phenotypic characterization of SFB93^T and propose the name *Syntrophotalea acetylenivorans*. The genus *Syntrophotalea* was recently named during the reclassification of species in the class *Deltaproteobacteria* into the phylum *Desulfobacterota* [25]. This new genus includes three known species: *S. acetylenica*, *S. venetiana* (formerly *P. venetianus*), and *S. carbinolica* (formerly *P. carbinolicus*) [25], which are all former members of the genus *Pelobacter* [30] (Table S1). The species are anaerobic, Gram-negative rod-shaped bacteria that cannot ferment sugar [30]. The genus name originates from the Greek word *syntrophos* and Latin word *talea* which mean 'living with or nourished with' and 'rod', respectively, which combines to describe a 'rod that feeds together with others' [25]. Here, we analyse the morphology, genome and physiology of SFB93^T within the genus *Syntrophotalea* and the family *Syntrophotaleaceae* (order *Desulfuromonadales*, class *Desulfuromonadia*). We find that SFB93^T is distinct within this group and propose to establish the species name *Syntrophotalea acetylenivorans* sp. nov.

ISOLATION AND MORPHOLOGY

SFB93^T was isolated from an estuarine mudflat in Palo Alto, CA, USA, where it (or a close relative) had been obtained from previously but subsequently lost [7, 10]. Photomicrographs of liquid cell suspensions were taken with a Zyla scientific complementary metal oxide semiconductor (CMOS) camera (Andor) through a Eclipse E800 microscope (Nikon). Scanning electron micrographs were made of critical point-dried cell preparations as detailed elsewhere [31] using a Vega3 scanning electron microscope (Tescan). Cells were non-motile, Gram-stain-negative rods occurring singly or in pairs (Fig. 1a). Cell dimensions were 4.0×0.5 microns (Fig. 1b). Acetylene-grown colonies on agar were milky, small convex ovals, 1.0–2.0 mm in diameter.

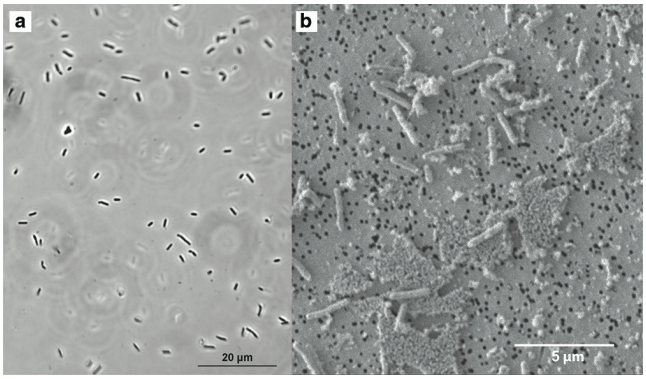

Fig. 1. Photomicrograph (a) and scanning electron micrograph (b) of SFB93^T cells.

PHYLOGENY

Previous phylogenetic reconstructions for SFB93^T had been based on partial (~1300 bp) [26] and genomic 16S rRNA gene sequences [29]. Here, we present a new phylogenetic reconstruction based on nearly full-length 16S rRNA genes. Furthermore, the availability of the SFB93^T genome [28] and those of other members of the class *Desulfuromonadia* allowed us to reconstruct a phylogeny based on a large number of bacterial core genes. To sequence the 16S rRNA gene of SFB93^T, a culture was grown to high density then pelleted by centrifugation. DNA was extracted using the DNeasy blood and tissue kit (Qiagen) and quantified as described by Akob *et al.* 2017 [29]. Purified DNA was shipped to GeneWiz (South Plainfield, NJ, USA) for bidirectional Sanger 16S rRNA gene sequencing. The 16S rRNA gene sequences from SFB93^T and related taxa were aligned using the SINA v1.2.11 aligner [32] according to the global ARB SILVA alignment for rRNA genes [33, 34]. The alignment was downloaded and filtered to remove columns of gaps and discard non-overlapping columns of data. The final gapped alignment (1546 bp) was used to reconstruct a maximum-likelihood tree with RAxML version 8.2.11 [35] in Geneious Prime 2019.2.3 [36] using the GTR GAMMA nucleotide model. The robustness of inferred tree topology was evaluated by 100 bootstrap resamplings.

Phylogenetic reconstruction based on near-full length 16S rRNA gene sequences revealed that SFB93^T falls within the order *Desulfuromonadales* and represents a unique species within the genus *Syntrophotalea* (Fig. 2). The class *Desulfuromonadia* bifurcates into two distinct orders: the *Desulfuromonadales* and *Geobacterales* (supported by >80% bootstrap values). SFB93^T falls clearly into the *Syntrophotaleaceae* clade which only contains species of the genus *Syntrophotalea*. SFB93^T forms a unique branch which is flanked by *S. carbinolica* DSM 2380^T and the cluster containing *S. acetylenica* strains and *S. venetiana* DSM 2394^T. Pairwise comparisons of aligned 16S rRNA genes revealed that SFB93^T shared 96.5, 96.6 and 96.7% sequence identity to *S. carbinolica* DSM 2380^T, *S. acetylenica* DSM 3246^T and *S. venetiana* DSM 2394^T, respectively, which are

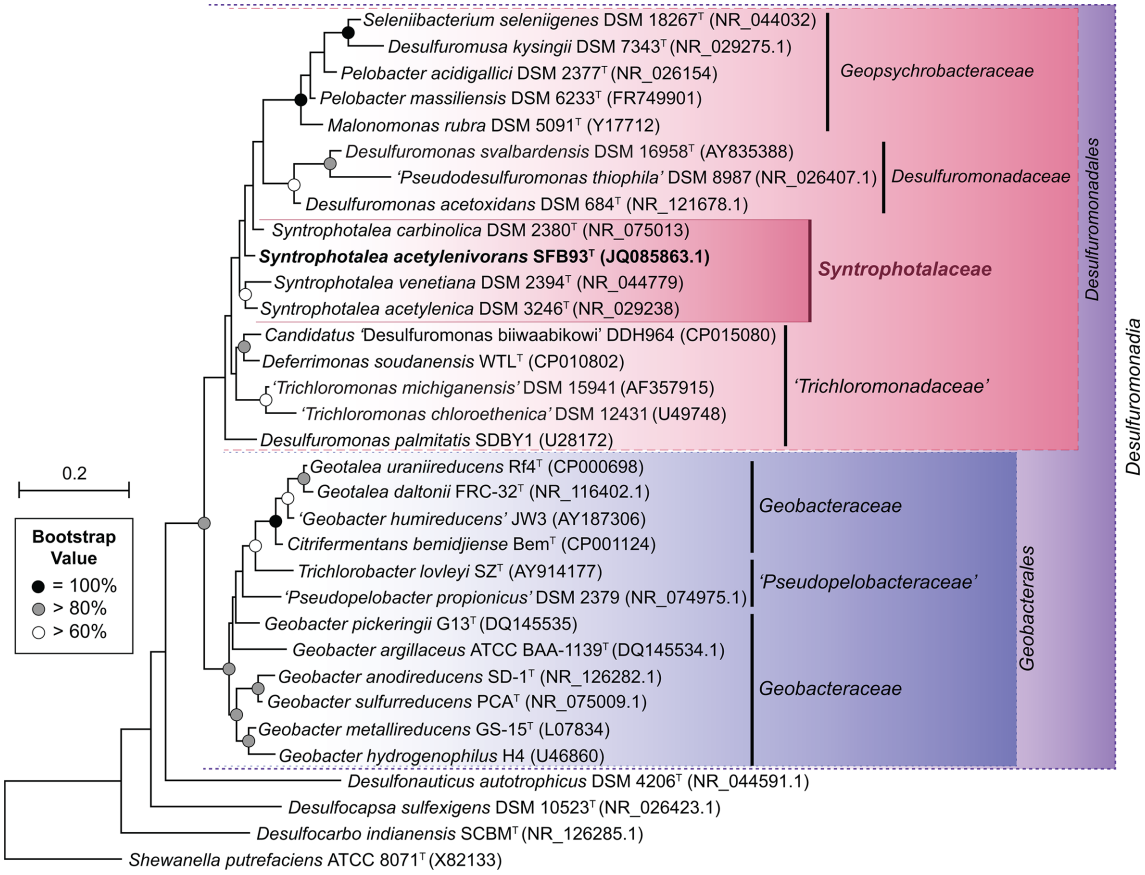

Fig. 2. Phylogenetic reconstruction for SFB93^T based on nearly full-length 16S rRNA gene sequences. Bootstrap values (percentages) are indicated at branching points; Bar, 0.2 substitutions per nucleotide position.

all below the 97% sequence identity criterion for separate species delineation. '*Pseudopelobacter propionicus*' DSM 2380 falls distinctly into the *Geobacterales* clade despite it being initially named *Pelobacter propionicus* DSM 2380^T. This observation has been documented previously [30, 37, 38], indicating that the original genus *Pelobacter* was polyphyletic requiring the reclassification presented by Waite *et al.* 2020 [25].

For genome-based phylogeny, published genome sequences from members of the genus *Syntrophotalea*, other strains of members of the order *Desulfuromonadales*, and more distant relatives were downloaded from GenBank [39]. A list of full names, original and updated taxonomy, and accession numbers for the genome sequences used are given in Table S1. Ninety-two core genes in the genomes were annotated, extracted, and aligned using the programme Up-to-Date Bacterial Core Genome (UBCG) [40]. The resulting concatenated alignment had a total length of 87945 bp. The final tree was reconstructed using RAxML version 8.2.12 [35] within UBCG.

Genome-based phylogeny confirmed that SFB93^T represents a member of the order *Desulfuromonadales* and the family *Syntrophotaleaceae* (Fig. 3). The UBCG tree confirms unique phylogeny of SFB93^T and division of the order into multiple families, which was observed in the 16S rRNA gene phylogeny based on nearly full-length gene sequences (Fig. 2). The closest relative of SFB93^T is *S. carbinolica* DSM 2380^T confirming that SFB93^T represents a species within the genus *Syntrophotalea*; this genus designation is further confirmed via genome analysis as discussed below. These data are consistent with the results of our other analyses in supporting the conclusion that SFB93^T represents a novel species in the genus *Syntrophotalea*.

GENOME FEATURES

The genome of SFB93^T was assembled using the Hierarchical Genome Assembly Process (HGAP; Pacific Biosciences) using long reads generated with a PacBio RSII instrument. Sequencing on a SMRT cell produced 63938 sequence reads with an N_{50} read length of 18803 nucleotides and ~87 fold sequencing coverage. The assembler produced a single circular contig containing 3218469 bp with 53.4% DNA G+C content. Annotation with the Prokaryotic Genome Annotation Pipeline (PGAP) from the National Centre for Biotechnology Information (NCBI) revealed 2915 genes.

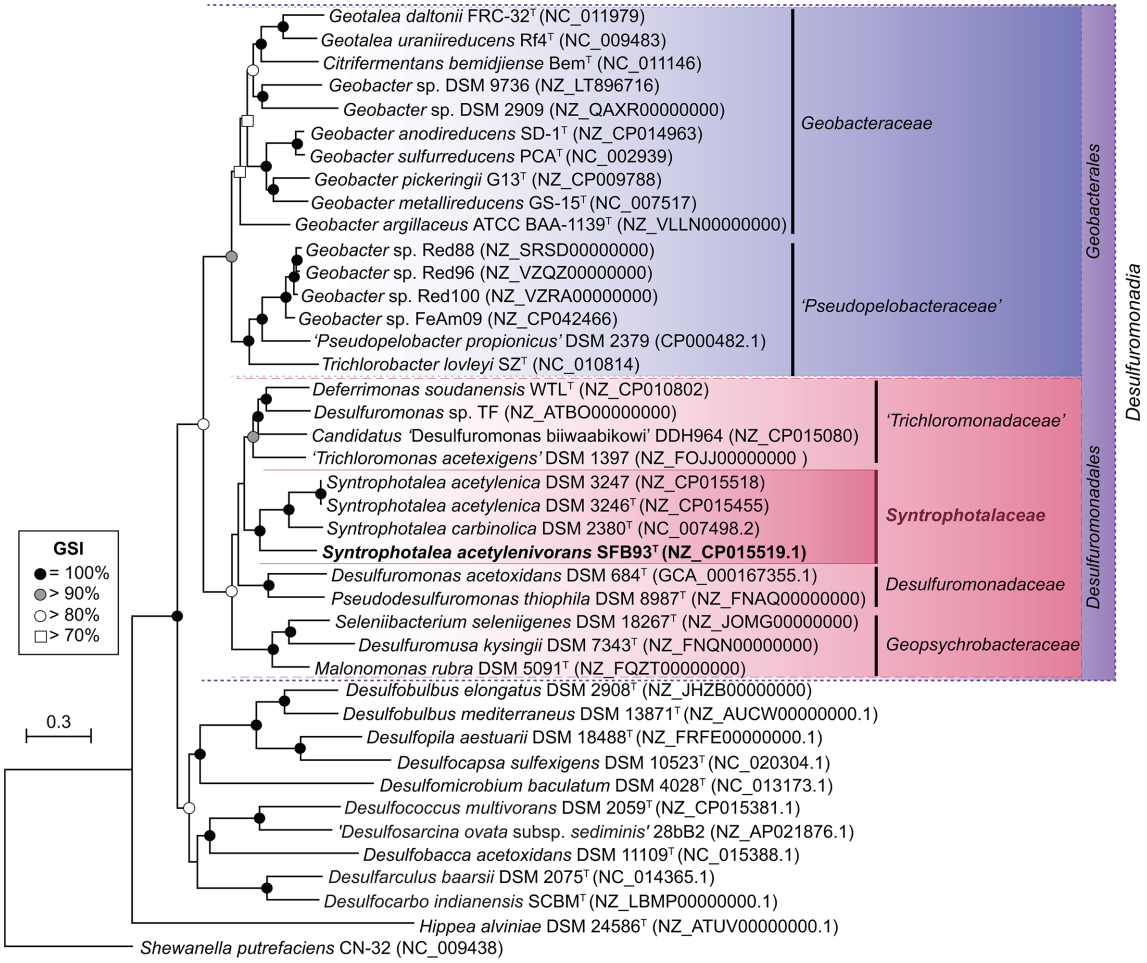

Fig. 3. Phylogenetic analysis of SFB93^T and related taxa based on 92 bacterial core genes. A RAxML [35] phylogenetic tree inferred using UBCG (concatenated alignment of 92 core genes) [57]. Gene support ndices (GSI) are indicated at branching points. Bar, 0.3 changes per nucleotide position. *Shewanella putrefaciens* CN-32 was used as the outgroup.

Full genome information and statistics were reported in Sutton *et al.* 2017 [28].

We used the Genome-to-Genome Distance Calculator 2.1 (available at http://ggdc.dsmz.de/ggdc.php#) to compute the *in silico* genomic distance between pairs of microbes. Briefly, the method calculates a digital DNA to DNA hybridization value (DDH) by using BLAST [41] to identify regions of similarity between microbial genomes and computes a distance matrix based on similarities between these high-scoring segment pairs (HSPs) [42]. The resulting DDH metric is closely related to the established wet-lab technique of estimating species similarity through hybridization of a pair of strains. Additionally, we used the Kostas lab web portal (http://enve-omics.ce.gatech.edu/ani/) to calculate the average nucleotide identity (ANI) between pairs of microbes [43]. The ANI minimum length of aligned regions was 700 bp and greater than 70% identity was required for alignment. The alignment fraction (AF) was calculated by dividing the lengths of aligned genes by the summed length of all genes in the genome, and the genome-wide average nucleotide identity (gANI) was the average percentage identity calculated across the aligned genes (Table 1). To validate these ANI results we also calculated OrthoANI values, ANI in orthologous regions, with the OAT software [44]. A Blast Ring Image Generator (BRIG) plot [45] was reconstructed comparing the full genome sequences of *S. acetylenica* DSM 3246^T, *S. carbinolica* DSM 2380^T, *P. propionicus* DSM 2379^T, and '*Candidatus* Desulfuromonas biiwaabikowi' DDH964 to the genome of strain SFB93^T.

Digital DDH analysis utilizing full genome sequences indicated that hybridization between two strains of the same species, *S. acetylenica* DSM 3246^T and DSM 3247, occurs at 98.5%, whereas DDH between strain SFB93^T and DSM 3246^T occurs at 21.5% (Table 1). DDH for strain SFB93^T and *S. carbinolica*, the closest relative on the basis of the results of phylogenetic analysis, was only 19.4%. The AF was 0.029 and the gANI between SFB93^T and *S. acetylenica* DSM 3246^T and between SFB93^T and *S. carbinolica* DSM 2380^T was 87.5 and 83.3%, respectively (Table 1). The DDH and gANI values are similar to the

Table 1. Genome similarities between *Syntrophotalea acetylenivorans* strain SFB93^T and members of the class *Desulfuromonadia* based on genome-to-genome digital DNA–DNA hybridization (DDH) and genome average nucleotide score (gANI)

DDH is the *in silico* DNA–DNA hybridization value in percent (typically,<70% defines distinct species). AF is the alignment fraction (AF = lengths of aligned genes (<70% similarity) / summed length of genes in genome 1) and gANI is the genome average nucleotide identity [gANI = summed (percentage identity * alignment length) / length of aligned genes; typically,<95% defines distinct species].

Query genome	Reference genome	DDH (%)	Confidence Interval (%)	Intergenomic Distance	Probability DDH ≥70%	Aligned Bases	AF	gANI (%)
S. acetylenivorans SFB93^T	*P. propionicus* DSM 2379^T	22.3	[20–25]	0.197	0	7942	0.003	81.0
S. acetylenivorans SFB93^T	*S. carbinolica* DSM 2380^T	19.4	[17–22]	0.226	0	85223	0.031	83.3
S. acetylenica DSM 3246^T	*P. propionicus* DSM 2379^T	22.0	[20–25]	0.199	0	14390	0.005	81.9
S. acetylenica DSM 3246^T	*S. carbinolica* DSM 2380^T	20.6	[18–23]	0.213	0	396870	0.142	83.7
S. acetylenica DSM 3246^T	SFB93^T	21.5	[19–24]	0.204	0	81510	0.029	87.5
S. acetylenica DSM 3247	*S. acetylenica* DSM 3246^T	98.5	[98–99]	0.003	97.6	3176364	1.00	99.5
S. acetylenica DSM 3247	*P. propionicus* DSM 2379^T	21.6	[19–24]	0.203	0	14391	0.005	81.9
S. acetylenica DSM 3247	*S. carbinolica* DSM 2380^T	20.5	[18–23]	0.214	0	396876	0.143	83.7
S. acetylenica DSM 3247	SFB93^T	21.3	[19–24]	0.206	0	81538	0.029	87.5
S. carbinolica DSM 2380^T	*P. propionicus* DSM 2379^T	21.2	[19–24]	0.207	0	12262	0.004	81.1

expected values reported between closely related species within the genus *Syntrophotalea*. Sequence identity between SFB93^T and the other species in the class *Desulfuromonadia* was low and there were few large alignable genomic regions (Fig. S1). The notable exceptions with high sequence identity were the 16S rRNA gene region which was 89.3–97.4% identical between SFB93^T and the other species of the genus *Syntrophotalea*. OrthoANI values were high when comparing the two isolates of *S. acetylenica* (DSM 3246^T and DSM 3247) and dropped to <76% when comparing across species of the orders *Desulfuromonadales* or *Geobacterales* (Fig. 4, Table S2). SFB93^T had OrthoANI values of 66.0–70.7% across these comparisons, indicating genomic divergence from other members of the class *Desulfuromonadia*. These genome characteristics further support the phylogenetic analysis that places SFB93^T as a novel species within the family *Syntrophotaleaceae* and genus *Syntrophotalea*.

Another region in the genomes that had high sequence identity between SFB93^T and the strains of *S. acetylenica* was the region containing the *ahy* gene, which codes for acetylene hydratase. This region was 90.6–92.4% identical between SFB93^T and that of the two strains of *S. acetylenica*; these three strains are the only known anaerobic acetylenotrophs [12]. No other genomes of members of the genus *Syntrophotalea* contained genes with homology to the *ahy* gene. The genome of SFB93^T contained two copies of the *ahy* gene that were 96.9% identical to each other. The two copies were 88.4 and 90.4% identical to the single copy *ahy* amino acid sequence of *S. acetylenica* DSM 3246^T, respectively.

PHYSIOLOGY AND CHEMOTAXONOMY

Culture conditions and strain characterization

The estuarine 'ABW' medium of Culbertson [7] supplemented with a trace elements solution [46] was employed in the enrichment and isolation process. Details have been given previously [26], briefly the process consisted of sub-sampling anoxic sediment slurries that had repeatedly consumed acetylene and transferring them into Balch tubes until a stable enrichment was achieved. Cultures were purified by decimal dilution of the enrichment, followed by streaking the highest positive dilution onto agar bottle plates [47] that contained a N_2 atmosphere to which acetylene was added. Monitoring of the bottle's gas phase was achieved by sampling through a butyl rubber stopper followed by gas chromatography with flame ionization detection (FID-GC), as described previously [26]. Isolated colonies from plates showing continuous acetylene consumption were picked and inoculated into anoxic sterile Balch tubes containing ABW medium [7] and were sealed under N_2. To eliminate precipitates in the growth medium, a modified bicarbonate medium (CM) adapted from one described previously [48] was developed that supported growth without precipitates [29]. The gas phase was provided with acetylene via aseptic syringe injection. Consumption of acetylene was taken as putative evidence for a pure culture, designated strain SFB93^T.

Growth tests for pH, temperature and salinity optima, electron donor usage and terminal electron acceptor usage were conducted using anaerobic CM media in Balch tubes, and growth was determined by measuring turbidity (OD_{680}). Substrates tested for fermentative growth were added at 10 mM, or in the case of polyethylene glycol, as 200, 6000, and

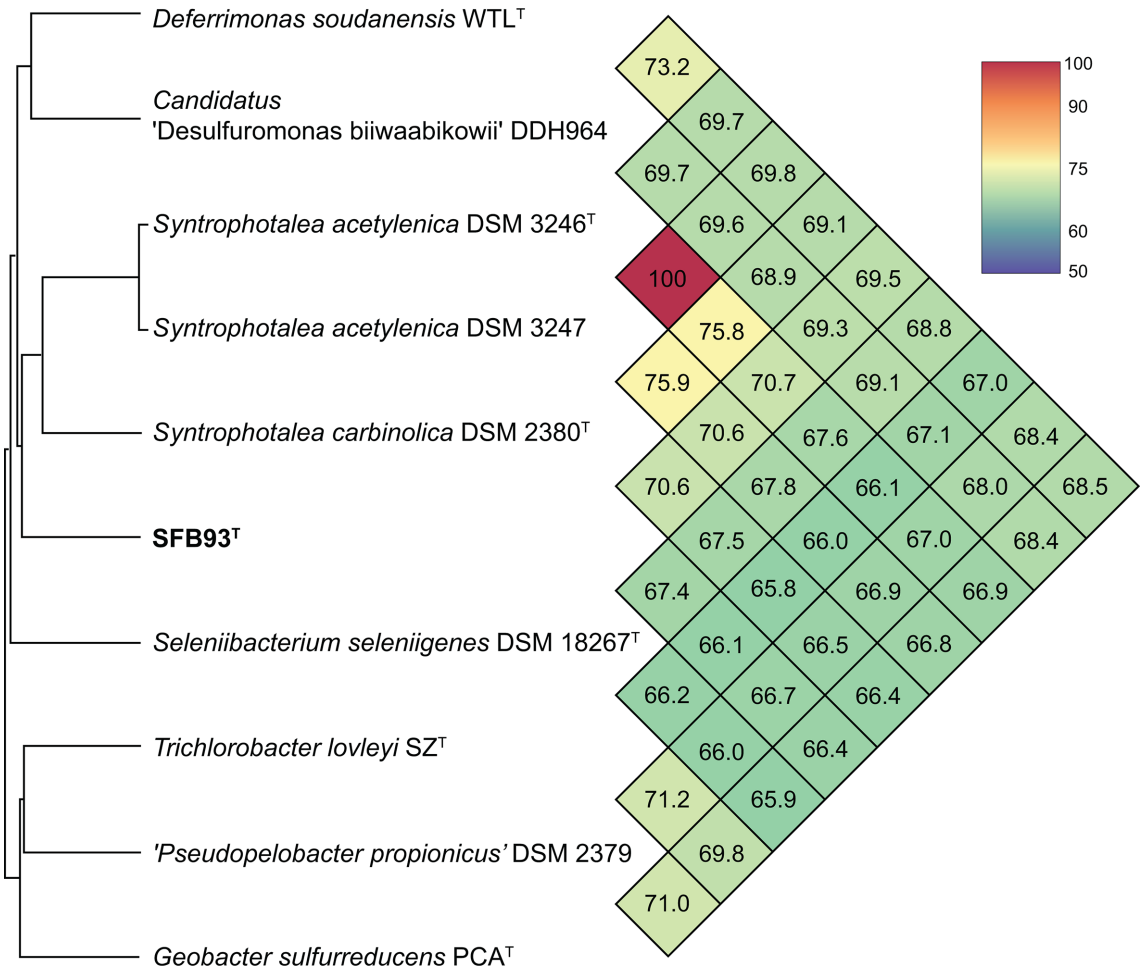

Fig. 4. OrthoANI values between SFB93^T and related species of the class *Desulfuromonadia*. Values are also presented in Table S2; full names, taxonomy, and accession numbers of genome sequences are presented in Table S1.

20000 g l^{-1} concentrations. Respiratory growth was tested on several electron acceptors using 5 mM acetate as the electron donor with 5 mM Fe(III)-EDTA, 1 mM As(V), 5 mM thiosulfate, 5 mM nitrate, and S(0) was added (~3 mM) as a colloidal suspension [49].

SFB93^T exhibited physiological ranges for growth that were consistent with the conditions of the estuarine, intertidal environment from which it was isolated (Fig. S2a–c). SFB93^T grew across a broad temperature range (5–42 °C) with optimal growth from 22 to 37 °C (Table 2). The pH optimum for SFB93^T was 6.3–8.5, but it could grow at up to pH 9. This pH range for growth is broader than reported for other closely related species of the genus *Syntrophotalea* and class *Desulfuromonadia* (Table 2). SFB93^T grew across a broad range of salinity (5–50 g l^{-1} NaCl) with optimal growth from 10 to 45 g l^{-1} NaCl (Table 2), which is consistent with the fluctuating salinity conditions in estuarine environments.

In addition to growth on acetylene, SFB93^T could utilize acetaldehyde and 1,2-propanediol (Table 2). Utilization of 1,2-propandiol was only possible in the presence of acetate. Acetaldehyde and 1,2-propanediol utilization is also a characteristic of the close relatives *S. acetylenica* (strains DSM 3246^T and DSM 3247) and *S. venetiana* DSM 2394^T. The inability of SFB93^T to grow on acetoin distinguishes it phenotypically from its close relatives *S. acetylenica* (strains DSM 3246^T and DSM 3247), *S. venetiana* DSM 2394^T and *S. carbinolica* DSM 2380^T and distantly related '*P. propionicus*' DSM 2379 (Table 2). SFB93^T further differed from other species of the genus *Syntrophotalea* by its inability to grow on polyethylene glycols, glycerol or pyruvate. SFB93^T, similarly to both *S. acetylenica* (strains DSM 3246^T and DSM 3247) and *S. venetiana* DSM 2394^T, is a strictly anaerobic, fermentative organism, with no clear affinity for dissimilatory respiration on other electron acceptors such as sulfur, thiosulfate, sulfate, selenate, nitrate or Fe(III) (Table 2).

Fatty acid methyl ester (FAME) analysis of cell membrane constituents of SFB93^T was performed using a Sherlock Microbial Identification System (MIS) (MIDI). As no fatty acid data were available in the literature for other species of

Table 2. Phenotypic characteristics of SFB93^T and related species of the order *Desulfuromonadales*

Strains: 1, SFB93^T (*Syntrophotalea acetylenivorans;* data from this study); 2, *Syntrophotalea acetylenica* DSM 3246^T [11]; 3, *Syntrophotalea venetiana* DSM 2394^T [58]; 4, *Syntrophotalea carbinolica* DSM 2380^T [59]; 5, '*Pseudopelobacter propionicus*' DSM 2379 [59]; and 6, *Pelobacter acidigallici* DSM 2377^T [60];. +, Positive; –, negative; ND, no data.

Characteristic	1	2	3	4	5	6
Width (μm)	0.5	0.6–0.8	0.5–1.0	0.5–0.7	0.5–0.7	0.5–0.8
Length (μm)	4.0	1.5–4.0	2.5	1.2–3.0	1.2–6.0	1.5–3.5
DNA G+C content (%)	53.4	57.1	52.2	52.3	57.4	51.8
pH optimum	6.3–8.5	6.5–7.5	7.0–7.5	6.5–7.2	7.0–8.0	6.5–7
Temperature optimum (°C)	22–37	28–34	33	35–40	33	35
Salinity optimum (g l^{-1})	10–45	ND	ND	ND	ND	ND
Substrates metabolized						
Galactose	–	ND	ND	ND	ND	ND
Glucose	–	–	–	ND	ND	ND
Sucrose	–	ND	ND	ND	ND	ND
Glycine	–	ND	–	ND	ND	ND
Fumarate	–	ND	–	ND	ND	ND
H_2 +5 mM acetate	–	ND	ND	ND	ND	ND
Acetoin	–	+	+	+	+	–
1,2-Butanediol	–	ND	ND	ND	ND	ND
Polyethylene glycols	– *	–	+	–	ND	–
1,2-Propanediol	+†	+†	+†	–	ND	–
Acetylene	+	+	–	–	ND	–
Acetaldehyde	+	+	+	ND	ND	ND
Pyruvate	–	–	–	–	+	–
Glycerol	–	+†	+†	–	ND	–
Electron acceptor usage						
Nitrate	–	–	–	ND	ND	ND
Iron (III)	–	ND	ND	ND	ND	ND
Selenate	–	ND	ND	ND	ND	ND
Thiosulfate	–	–	–	ND	ND	ND
Sulfur	–	–	–	ND	ND	ND
Sulfate	–	–	–	ND	ND	ND
Nitrogen fixation	+	–‡	ND	ND§	ND	ND

*Strain SFB93 was tested for growth with PEG200, PEG6000 and PEG20000.
†Growth is possible only in the presence of small amounts of acetate for cell carbon synthesis.
‡*S. acetylenica* DSM 3246^T contains the genes for a MoFe-nitrogenase but nitrogen fixation was not demonstrated in culture [29].
§The genome of *S. carbinolica* DSM 2380^T contains a full-suite of compulsory genes for a MoFe-nitrogenase; However, the strain was not tested for nitrogen fixation.
||
¶Growth is only possible in the presence of a hydrogen-scavenging anaerobe.
**Growth only possible in the presence of 10 mM acetate.

the genus *Syntrophotalea*, cellular fatty acids were analysed for type strains of three species of the genus *Syntrophotalea* (DSM 3246^T, DSM 2380^T, and DSM 2394^T) at the Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany). The fatty acid composition of SFB93^T significantly differed from those of the other species of the genus *Syntrophotalea* (Table 3). The dominant fatty acids in SFB93^T were iso-$C_{15:0}$ 3-OH (13.7%), anteiso-$C_{15:0}$ (15.1%), iso-$C_{15:0}$ (18.0%), and $C_{16:0}$ (22.5%). In contrast, the fatty acid $C_{16:1}\omega7c$ dominated the composition of the other strains (26.0–38.6%) while comprising only 1.66% of the fatty acids of SFB93^T.

Acetylenotrophic metabolism

Determination of a growth curve for SFB93^T was conducted in 160 ml serum bottles containing 50 ml CM medium under a 110 ml headspace of N_2 to which 2.0 ml of acetylene was added. All experimental conditions were run with triplicate cultures. Headspace acetylene disappearance and the appearance of H_2 were detected via gas chromatography, acetaldehyde and ethanol were quantified by FID-GC following direct on-column injections, and acetate was quantified by high performance liquid chromatography on filtered aqueous samples [26]. Cell counts were achieved by acridine orange direct counts [50].

Cells completely consumed acetylene after ~12 h incubation, which resulted in a 17.5-fold increase in cells to 1.8×10^7 cells ml^{-1} by 24 h (Fig. 5a). In comparison, inoculated samples in medium without added acetylene remained at the 1.0×10^6 cells ml^{-1} level throughout [51]. Coincident with acetylene consumption was the production of H_2 (30 µmoles) along with a small quantity of acetaldehyde, as well as higher concentrations of ethanol and acetate (Fig. 5b). These results are entirely consistent with the initial descriptions of acetylenotrophic metabolism by strains of *S. acetylenica* by which acetylene is hydrated by acetylene hydratase (AH) to acetaldehyde, followed by acetaldehyde dismutation to ethanol, acetate and H_2 [11]. It also conforms to the biochemical pattern observed for the original isolate from San Francisco Bay, indicating that it too was a *Syntrophotalea*-like acetylenotroph [10]. It is notable that not all the observed incidents of anaerobic acetylenotrophs proceed by this route, with the case in point of the freshwater mixed association of strain SV7 that produces primarily acetate and formate with only traces of ethanol and acetaldehyde [26]. Moreover, strain SV7 does not contain any organisms belonging to the genus *Syntrophotalea*, nor could the AH gene be amplified, indicating a somewhat different mode of acetylenotrophic metabolism. Nonetheless, the key end-products of acetylene metabolism – H_2 and acetate serve as the basis for syntrophic interactions with terminal electron accepting microorganisms, be they sulfate-reducers [10], methanogens [13, 14, 26], or dehalorespirers [27].

It is relevant that acetylene is a potent inhibitor of a number of microbial processes [52, 53], and its inhibition of many metalloenzymes [54] probably poses a challenge to the cultivation of acetylenotrophs directly upon acetylene. We observed better initial growth of SFB93^T with low provided concentrations of acetylene (e.g.~0.1 ml per 15 ml headspace) which can be ramped up over time as growth is noted (e.g.~1.0 ml). Results of previous work have also indicated that SFB93^T was capable of growth coupled to nitrogen fixation using a MoFe-nitrogenase; it is noted that *S. acetylenica* strains DSM 3246^T and DSM 3247 contained the genes for a MoFe-nitrogenase but nitrogen fixation was not observed [29].

PROPOSAL OF *SYNTROPHOTALEA ACETYLENIVORANS* SP. NOV

Strain SFB93^T can be discriminated from other members of the genus *Syntrophotalea* by genomic and physiological characteristics. The 16S rRNA gene sequence of SFB93^T meets the criterion of ≤97% sequence identity for separate species delineation compared with closely related species of the genus *Syntrophotalea* (96.5–96.7%). This result was reinforced strongly by the digital DDH estimates, which identified only 21.5% and 19.4% similarity between SFB93^T and *S. acetylenica* DSM 3246^T and between SFB93^T and *S. carbinolica* DSM 2380^T, respectively. The gANI between *S. acetylenica* DSM 3246^T and *S. carbinolica* DSM 2380^T to SFB93^T was 87.5 and 83.3%, respectively, within alignable blocks but <3% of the genomes aligned at a threshold of >70% similarity. Within orthologous regions the ANI dropped to 60–70% (Fig. 4).

These statistics indicate that although some genomic regions are highly conserved between *S. acetylenica* DSM 3246^T and SFB93^T the overall picture is one of high genomic differentiation within the genus *Syntrophotalea*. Conserved genomic regions may reflect functional constraints on gene sequences and metabolic processes. For example, SFB93^T was able to grow at the expense of acetylene, producing hydrogen, acetaldehyde, ethanol and acetate in the process (Fig. 5a, b) as occurs in *S. acetylenica* strains via the enzymes acetylene hydratase (AH) and acetaldehyde dismutase (ALDH) [11]. However, the genome of SFB93^T revealed two *ahy* gene copies while both strains of *S. acetylenica* contain only one [29]. It is not currently known which environmental factors trigger the expression of either or both of these SFB93^T *ahy* genes, or whether the AH themselves have different affinities for acetylene; these could be subjects for future investigations. Even less is known about the acetaldehyde dismutases of these acetylenotrophic strains of species of the genus *Syntrophotalea*. It is notable that both strains of *S. acetylenica,* DSM 3246^T and DSM 3247, as well as SFB93^T contain all of the relevant genes required for a functional nitrogenase (*nifHDKEN*), but to date diazotrophy has only been shown to be active in SFB93^T [29].

SFB93^T also differs phenotypically in substrate affinities from strains of *S. acetylenica* and *S. venetiana* DSM 2394^T (Table 2). Members of the genus *Syntrophotalea* are generally fermentative, although *S. carbinolica* DSM 2380^T can also achieve respiratory growth linked to Fe(III)

Table 3. Fatty acid composition (percentages) of SFB93^T and related strains of species of the genus *Syntrophotalea*

Strains: 1, SFB93^T (*S. acetylenivorans*); 2, *S. acetylenica* DSM 3246^T; 3, *S. carbinolica* DSM 2380^T; 4, *S. venetiana* DSM 2394^T. ND=not detected. All data generated in this study.

Fatty acid type		1	2	3	4
Saturated straight-chain:	$C_{12:0}$	0.3	0.6	0.4	0.2
	$C_{14:0}$	4.5	17.1	24.3	19.9
	$C_{15:0}$	0.3	0.1	0.3	0.2
	$C_{16:0}$	22.5	10.0	21.2	16.6
	$C_{18:0}$	0.4	0.1	2.0	0.2
Unsaturated straight-chain:	$C_{16:1}\omega9c$	0.2	0.6	N.D.	5.3
	$C_{16:1}\omega7c$	1.7	34.2	26.0	38.6
	$C_{16:1}\omega7c$ DMA	0.6	ND	ND	ND
	$C_{16:1}\omega5c$	ND	0.2	0.4	ND
	$C_{16:1}\omega7t$	ND	ND	3.8	ND
	$C_{18:1}\omega7c$	ND	0.4	0.2	0.6
	$C_{18:1}\omega9c$	ND	ND	nd	0.3
Unsaturated	iso-$C_{15:1}\omega7c$	ND	1.1	0.3	0.2
	anteisoiso-$C_{15:1}\omega7c$	ND	0.3	ND	ND
	anteiso-$C_{15:0}$ 3-OH	ND	0.5	0.2	ND
	iso-$C_{17:1}\omega9c$	ND	ND	0.6	1.7
	anteiso-$C_{17:1}\omega9c$	ND	1.0	0.3	0.2
Straight-chain hydroxy:	$C_{14:0}$ 3-OH	ND	6.3	6.3	5.9
	$C_{14:1}$ 3-OH	1.5	ND	ND	ND
	$C_{16:1}$ 3-OH	1.8	ND	ND	ND
	$C_{16:1}$DMA	0.4	ND	ND	ND
	$C_{16:0}$ 3-OH	ND	1.9	1.0	0.8
Saturated branched-chain:	iso-$C_{13:0}$	2.6	3.1	1.6	0.2
	anteiso-$C_{13:0}$	0.3	0.4	ND	ND
	iso-$C_{14:0}$	0.2	1.8	0.7	0.6
	iso-$C_{15:0}$	18.0	4.1	5.4	2.8
	anteiso-$C_{15:0}$	15.1	10.4	3.7	3.7
	iso-$C_{16:0}$	1.5	0.5	ND	0.6
	iso-$C_{17:0}$	5.4	0.2	0.1	ND
	anteiso-$C_{17:0}$	2.9	0.2	ND	ND
Branched-chain hydroxy:	iso-$C_{13:0}$ 3-OH	0.1	ND	ND	ND
	iso-$C_{15:0}$DMA	0.4	ND	ND	ND
	anteiso-$C_{15:0}$DMA	0.6	ND	ND	ND
	iso-$C_{15:0}$ 3-OH	13.7	0.7	ND	0.3
	anteiso-$C_{15:0}$ 3-OH	3.8	0.5	0.2	ND
	iso-$C_{17:0}$ 3-OH	0.6	ND	ND	ND
	anteiso-$C_{17:0}$ 3-OH	0.7	ND	ND	ND

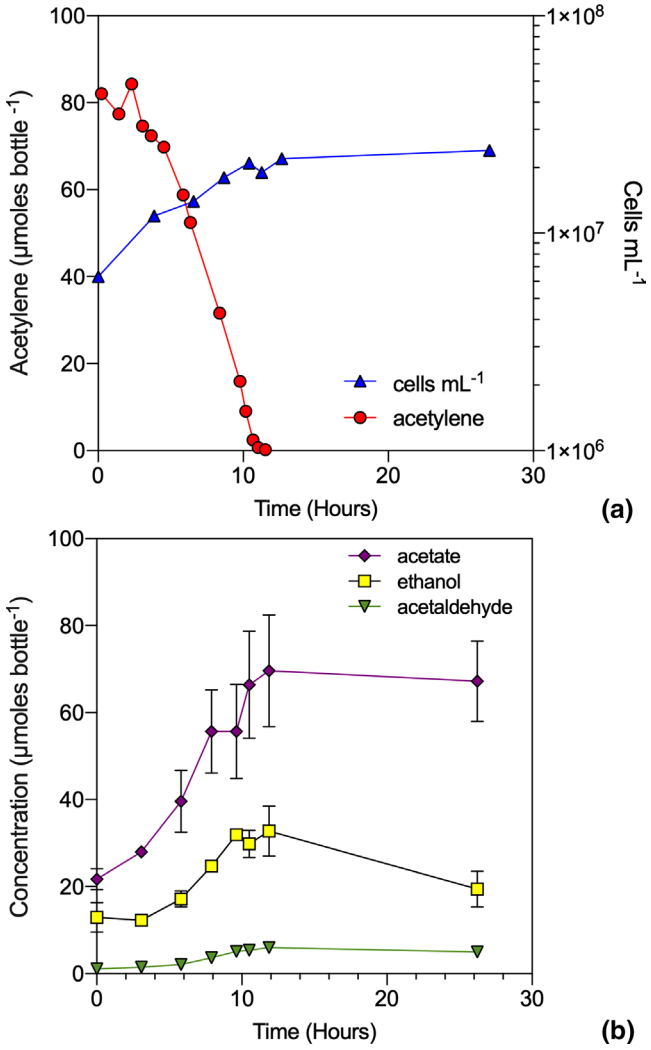

Fig. 5. Growth of SFB93^T on acetylene showing (a) acetylene consumption and cell growth and (b) formation of acetaldehyde, ethanol and acetate. Symbols represent the mean of three cultures and error bars indicate ±1 standard deviation. Carry over values for cells and metabolic products from the 5% inoculum volume were subtracted out from the experimental cultures by using an inoculated medium that lacked the acetylene substrate. All raw data are available from Baesman *et al.* 2019 [51].

or S(0) [55]. SFB93^T, however, did not display growth on any of these three potential electron acceptors (Table 2). Fermentation of acetylene by strains of *S. acetylenica* as well as SFB93^T produces electron donors and carbon sources (H_2 and acetate) for interactions with terminal electron accepting bacteria, such as sulfate-reducers and methanogens [10, 13, 26, 56] and with dehalorespirers like *Dehalococcoides mccartyi* [27]. The microbial ecology of strains of species of the genus *Syntrophotalea*, in general, is still far from clear but it seems that they can be involved in important biogeochemical processes like nitrogen fixation, degradation of environmental contaminants (e.g. trichloroethylene), and in certain cases, the reduction of exogenous electron acceptors (e.g. Fe, Se, and S). Acetylene is abundant in the hydrocarbon-rich atmospheres and emanations of the icy planet(oid)s of the outer Solar System and may therefore represent a food source for putative anaerobes dwelling in the habitable aqueous worlds under their icy surfaces. Hence, acetylenotrophy and the isolation of novel acetylenotrophs are appropriate subjects ripe for more concerted future endeavours [21, 29], perhaps even in the search for signs of extraterrestrial life on Saturn's moons, like Enceladus and Titan [20].

DESCRIPTION OF *SYNTROPHOTALEA ACETYLENIVORANS* SP. NOV

Syntrophotalea acetylenivorans (a.ce.ty.le.ni.vo'rans. N.L. neut. n. *acetylenum* acetylene; L. pres. part. *vorans* eating; N.L. part. adj. *acetylenivorans* eating acetylene).

Gram-stain-negative, strictly anaerobic, non-motile rods that measure 4.0×0.5 microns (Fig. 1). Colonies formed on agar plates during growth on acetylene are milky, slightly irregular, convex ovals, 1.0–2.0 mm in diameter. Optimum growth ranges for temperature, pH and salinity were 22–37 °C, 6.3–8.5 and 10–45 g l^{-1}, respectively. Growth on acetylene forms acetaldehyde as an intermediate, ethanol and acetate as end-products, with hydrogen evolved from oxidation of ethanol. Unable to use glycerol, pyruvate, galactose, glucose, sucrose, glycine, fumarate, acetoin, polyethylene glycols, or 1,2-butanediol, but can grow on 1,2-propanediol if acetate is included in the medium. Unable to grow by respiration of nitrate, Fe(III), selenate, thiosulfate, sulfur or sulfate as electron acceptors. A complete array of diazotrophic genes are aligned in the genome of the type strain, and nitrogen-fixation and N_2-dependent growth was experimentally demonstrated.

The type strain, SFB93^T (=DSM 106009^T=JCM 33327^T) was isolated from intertidal estuarine sediment of South San Francisco Bay located in in the Palo Alto boat harbour (Palo Alto Lat/Long: 37°27.3′ N; 122°6.48′ W). The genomic DNA G+C content is 53.4%. The GenBank accession numbers are JQ085863.1 for the nearly full-length 16S rRNA gene and NZ_CP015519.1 for the genome.

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Author contributions
S. M. B. and R. S. O., isolated SFB93^T, conceived the project and performed all experiments. D. M. A. sequenced the isolate, J. M. S.,

assembled the genome sequence, J. M. S. and D. M. A., completed the phylogenetic analyses and J. M. S. and J. L. F., characterized the genome. S. M. B., R. S. O., D. M. A., J. M. S. and J. L. F., wrote the manuscript.

Conflicts of interest

Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government. The authors declare no competing financial interest and no conflicts of interest.

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