INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

RESEARCH ARTICLE

L'Haridon *et al.*, *Int. J. Syst. Evol. Microbiol.* 2023;73:005646

DOI 10.1099/ijsem.0.005646

MICROBIOLOGY SOCIETY

Methanocaldococcus lauensis sp. nov., a novel deep-sea hydrothermal vent hyperthermophilic methanogen

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Abstract

Three hyperthermophilic methanogens, designated strain SG7^T, strain SG1 and strain SLH, were isolated from the ABE and Tu'i Malila deep-sea hydrothermal vent fields along the Eastern Lau Spreading Center. Phylogenetic analysis based on 16S rRNA gene sequence indicated that strains SG7^T, SG1 and SLH were affiliated with the genus *Methanocaldococcus* within the family *Methanocaldococcaceae*, order *Methanococcales*. They shared 95.5–99.48 % 16S rRNA gene sequence similarity to other *Methanocaldococcus* species and were most closely related to *Methanocaldococcus bathoardescens*. Cells of strains SG7^T, SG1 and SLH were cocci, with a diameter of 1.0–2.2 µm. The three strains grew between 45 and 93 °C (optimum, 80–85 °C), at pH 5.0–7.1 (optimum pH 6.2) and with 10–50 g l^{-1} NaCl (optimum 20–25 g l^{-1}). Genome analysis revealed the presence of a 5.1 kbp plasmid in strain SG7^T. Based on the results of average nucleotide identity and digital DNA–DNA hybridization analyses, we propose that strains SG1 and SG7^T are representatives of a novel species, for which the name *Methanocaldococcus lauensis* sp. nov. is proposed; the type strain is SG7^T (=DSM 109608^T=JCM 39049^T).

DATA SUMMARY

Supplementary information for this manuscript is available at: https://doi.org/10.6084/m9.figshare.20768005 [1]

INTRODUCTION

The order *Methanococcales* [2] (class *Methanococci*, phylum *Euryarchaeota*), based on Bergey's taxonomy system of Archaea and Bacteria, is divided into two families, the *Methanococcaceae* [3] and the *Methanocaldococcaceae* [4]. The *Methanococcaceae* encompassed the genera *Methanococcus* [5], *Methanofervidicoccus* [6] and *Methanothermococcus* [7]. The *Methanocaldococcaceae* is composed of the genera *Methanotorris* [8] and *Methanocaldococcus* [9] Recently, the Genome Taxonomy Database (GTDB, release 202) proposed changes to the taxonomic structure of this order, including reclassifying the *Methanotorris* within the family *Methanococcaceae*, placing the entire order within the newly designated phylum *Methanobacteriota*, and placing the species *Methanocaldococcus infernus* and *Methanocaldococcus villosus* [10, 11] in a separate genus. The family *Methanocaldococcaceae* is composed of hyperthermophilic, anaerobic, salt requiring strains that produce methane from CO_2 reduction using H_2 as electron donor.

Methanocaldococcus species have been isolated from submarine hydrothermal vents around the world. The species *Methanocaldococcus jannaschii*, *Methanocaldococcus fervens*, *Methanocaldococcus vulcanius* and *Methanocaldococcus bathoardescens* were isolated from the East Pacific Rise [12–14], *Methanocaldococcus indicus* from the Central Indian Ocean Ridge [15], *Methanocaldococcus villosus* from the Kolbeinsey Ridge [16], and *Methanocaldococcus infernus* from the Mid-Atlantic Ridge [17]. Although a 16S rRNA gene microbial diversity survey also detected *Methanocaldococcus* at deep-sea vents along the Eastern Lau Spreading Center (ELSC) [18], prior to this study no strain has been isolated from these deep-sea hydrothermal areas.

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Keywords: deep-sea hydrothermal vent; *Euryarchaeota*; *Methanocaldococcaceae*; *Methanococcales*; *Methanococci*; *Methanocaldococcus*.
The GenBank/EMBL/DDBJ 16S rRNA gene sequence accession numbers for strains SG1, SG7^T and SLH are MK602360.1, MK602649.1 and OL415192.1 respectively. The genome sequences of strains SG1 and SG7^T for this study have been deposited in GenBank/EMBL/DDBJ under accession numbers GCA_902827205.1 and GCA_902827225.1, respectively.
One supplementary figures and seven supplementary tables are available with the online version of this article.

The spreading axis of the Lau back-arc basin is composed of the ELSC and includes the Valu Fa Ridge (VFR). There are at least six vent fields (north to south) located along the 397 km ridge segment of the ELSC: Kilo Moana, Tow Cam, Tahi Moana, ABE, Tu'i Malila, and Mariner. The Tu'i Malila vent field is approximately 140 km south of ABE on the VFR and the two vent fields are on different spreading segments [19]. The relatively low abundance of methanogen genomes (*Methanocaldococcaceae* and *Methanococcaceae*) observed in metagenome libraries at all ELSC vent fields further supports the importance of H_2 concentrations in structuring the archaeal communities of deep-sea hydrothermal environments [20–25]. Here we report the description of a novel hyperthermophilic hydrogenotrophic *Methanocaldococcus* species and expand our knowledge of the presence of this genus in the deep-sea hydrothermal environments of the southwestern Pacific.

ISOLATION AND ECOLOGY

Deep-sea hydrothermal deposits from the ABE vent field (20° 45.8′ S, 176° 11.5′ W; 2104–2163 m) were collected by the ROV *Jason II* in April 2015 during the expedition RR1507, and deposits from the Tu'i Malila vent field (21° 59.35′ S, 176° 34.06′ W, 1839–1928 m) were collected during the RR1507 expedition and the ChuBacArc expedition by the ROV *Victor* in June 2019. Chimney samples were processed as described previously [15, 26]. Initial enrichments were performed shipboard in a culture medium that contained the following (per 1 l distilled water): 25 g NaCl, 3 g $MgCl_2{\cdot}2H_2O$, 4 g Na_2SO_4, 0.5 g KCl, 0.25 g NH_4Cl, 0.2 g KH_2PO_4, 0.15 g $CaCl_2.2H_2O$, 0.5 g Difco yeast extract, 1 ml trace element mixture [27], 0.2 mg sodium tungstate, 50 mg sodium selenate, 1 ml vitamin mixture [27], 1 ml thiamine solution [27], 0.05 mg vitamin B12, 1 ml growth-stimulating factors [28] and 1 mg resazurin. Prior to autoclaving, the pH of the medium was adjusted to pH 6.5 using 5 M HCl. After autoclaving, the pH of the medium was readjusted to pH 6.3 at room temperature under H_2/CO_2 (80:20; 200 kPa) as the gas phase. Each sulphide slurry sample (0.5 ml) was inoculated into 10 ml medium and the serum tubes were incubated at 80 °C for 2–3 days or until turbidity was noted. When growth occurred, it consisted of coccoid cells that fluoresced intense green at 420 nm under UV light. Pure cultures were obtained by streaking subcultures onto solidified medium with 0.8% gellan gum (Phytagel, Sigma-Aldrich) incubated in a modified agar flask (Bellco) under H_2/CO_2 (80:20, 150 kPa) at 80 °C. After 3–5 days of incubation, visible mustard-coloured colonies with a diameter of 1–3 mm were picked and subcultured. After the third transfer on solid medium, a single colony from each subculture was selected, and the colonies were designated as strains SG7^T, SG1 and SLH. Strain SG7^T was isolated from an active chimney sample from the ABE vent field (20°46′ S, 176° 11′ W) at 2130 m, and strains SG1 and SLH were isolated from active chimney samples from the Tu'i Malila vent field at 1872 m depth (21° 59′ S, 176° 34′ W) and 1891 m depth (21° 11′ S, 176° 20′ W), respectively.

The purity of these isolates was confirmed by microscopy, by sequencing of the 16S rRNA gene and by inoculating the same medium complemented with tryptone (0.5%) under N_2/CO_2 (80:20; 100 KPa). Stock cultures of the isolates were stored in culture medium at 4 °C. For long-term storage, pure cultures were stored at −80 °C in the same medium containing 5% (w/v) DMSO [29]. Only strains SG1 and SG7^T are described in detail here.

16S RRNA GENE PHYLOGENY

Genomic DNA was isolated and the 16S rRNA genes were amplified and sequenced as previously described in [15, 15]. For comparison, 16S rRNA genes were obtained from the genomes of strains SG1 and SG7^T. A full-length 16S rRNA gene was also reconstructed from metagenomic reads from Tu'i Malila (described below), using the SPAdes version 3.14.0 assembler [30] in phyloFlash version 3.3b2 [31]. This reconstructed 16S rRNA gene (T11.Pfspades_18, GenBank/EMBL/DDBJ accession OL740032.1) showed 100 % sequence similarity to two small (108 bp) 16S rRNA gene fragments found in a *Methanocaldococcus* metagenome-assembled genome (MAG) also recovered from Tu'i Malila, T11_73 (GenBank/EMBL/DDBJ accession JACCLX000000000; described below). EzBioCloud [32] was used to identify the closest relatives of each isolate and to perform pairwise 16S rRNA gene sequence similarity comparisons. For phylogenetic placement, the 16S rRNA genes were aligned using MAFFT version 7.450 [33] in Geneious version 10.2.6 (www.geneious.com), and positions with >50% sequence variability were removed. RAxML version 8.2.12 [34] was used to perform maximum-likelihood phylogenetic analysis with 1000 rapid bootstrap inferences, and the tree was visualized using the Interactive Tree of Life version 6 [35].

The 16S rRNA gene sequences recovered from SG7^T and SG1 were identical, albeit slightly different in length (1328 and 1342 bp, respectively). Two identical full-length (1481 bp) 16S rRNA genes were found in the SG1 genome, which were nearly identical to the SG1 PCR-generated sequence except for a single base deletion near the 5′ end. Although the SG7^T genome harboured a 16S rRNA gene sequence identical to those in the SG1 genome, it also encoded a divergent 16S rRNA gene with several deletions and Single Nucleotide Plymorphisms (SNPs), and a single insertion compared to the well-supported gene that matched the SG1 sequences. To provide the most accurate data, the pairwise alignments were performed using the identical, well-supported 16S rRNA genes from the strain SG1 and SG7^T genomes.

Strain SG7^T and SG1 16S rRNA genes showed very high similarity to *M. bathoardescens* (98.61%, Table S1, available in the online version of this article), *M. jannaschii* (98.58%), *M. fervens* (98.57%) and *Methanocaldococcus* sp. FS406-22 (98.47%), and much

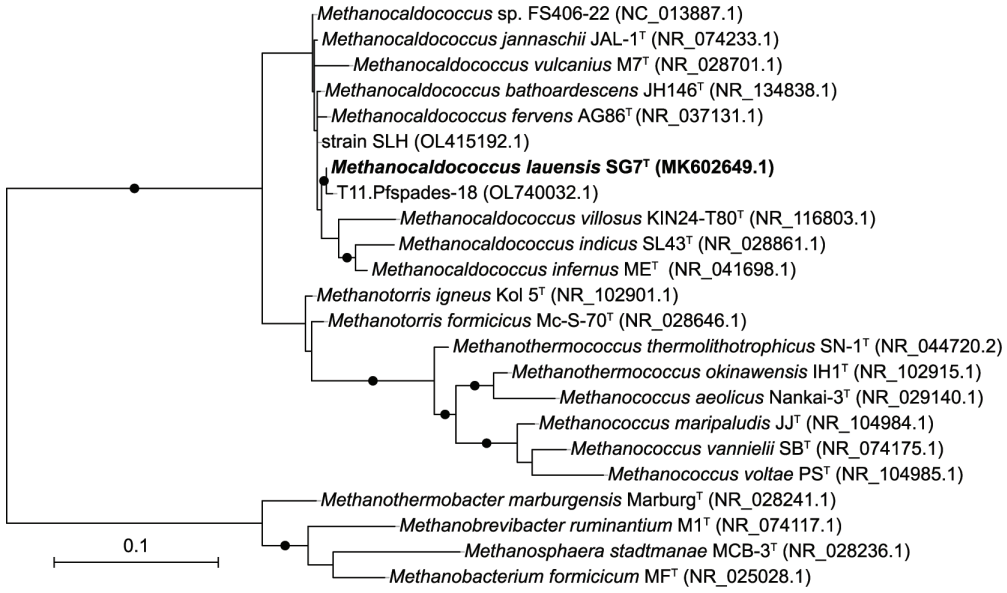

Fig. 1. Maximum-likelihood 16S rRNA gene tree showing the positions of strains SG7^T and SLH in the *Methanocaldococcus* (1272 nt). Accession numbers for GenBank/EMBL/DDBJ are shown in parentheses, and bootstrap support of 80–100% is indicated with filled circles. The scale bar represents 0.1 substitutions per nucleotide.

lower similarity compared to *M. vulcanius, M. infernus*, *M. indicus* and *M. villosus* (95.5%–97.21%). Based on EzBioCloud alignments [32], strain SLH showed slightly higher sequence similarity to *M. bathoardescens* (99.48%) and *Methanocaldococcus* sp. FS406-22 (99.33%) than to strain SG7^T (99.19%). The reconstructed 16S rRNA gene from the Tu'i Malila metagenomic reads also showed 99.53% sequence similarity to strains SG7^T and SG1. The pattern seen in 16S rRNA gene similarity is reflected in the phylogenetic tree, where SG7^T clusters with *M. fervens*, *M. bathoardescens*, *M. jannaschii* and *Methanocaldococcus* sp. FS406-22, with *M. vulcanius* on a lineage within this group, and *M. infernus*, *M. indicus* and *M. villosus* forming a separate cluster (Fig. 1).

GENOME FEATURES

Sequencing and assembly of the strain SG1 and SG7^T genomes were carried out at the Genoscope laboratory (Evry, France). Genome sequencing was performed using a combination of Illumina technology and Oxford Nanopore technology (ONT). For Illumina sequencing, 250 ng DNA was sonicated to a 100–1000 bp size range using the E220 Covaris Focused-Ultrasonicator (Covaris, Inc.). The fragments were end-repaired, then 3′-adenylated and NEXTflex HT Barcodes (Bio Scientific Corporation) were added using NEBNext DNA modules products (New England Biolabs). After two consecutive cleanups with 1×AMPure XP (Beckman Coulter), the ligated product was amplified by 12 PCR cycles using the Kapa Hifi Hotstart NGS library amplification kit (Kapa Biosystems), followed by purification with 0.6×AMPure XP. After library profile analysis conducted by an Agilent 2100 Bioanalyzer (Agilent Technologies) and qPCR quantification (MxPro, Agilent Technologies), the library was sequenced on an Illumina MiSeq with a MiSeq Reagent Kit version 2 (2×250 bp; Illumina Inc.). A total of 7.05×10^5 and 7.03×10^5 paired-end reads were obtained for SG1 and SG7^T, respectively. The Illumina reads were trimmed by removing low-quality nucleotides (Q<20), sequencing adaptors and primer sequences using fastx_clean (Fastx_clean software, www.genoscope.cns.fr/fastxtend), an internal software based on the FASTX library (FASTX-Toolkit, http://hannonlab.cshl.edu/fastx_toolkit/index.html). Reads shorter than 30 nucleotides after trimming were also discarded. For Nanopore sequencing, library preparation was done with 1 µg of the same input DNA following the 1D Native barcoding genomic DNA protocol with EXP-NBD104 and SQK-LSK109 (ONT). The library was sequenced using a Nanopore R9.4.1 flow cell (ONT) and the MinION device with the MinKNOW version 3.3.2 and Guppy version 2.3.5 software following recommendations of ONT. A total of 153308 reads were obtained with an N50 of 7.8 Kbp for SG1 and 178019 reads with an N50 of 7.5 Kbp for SG7^T. Hybrid assemblies were launched with Unicycler version 0.4.6 (default options). The final SG1 assembly resulted in a single chromosome 1.48 Mbp in length with a G+C content of 29.08mol%. A single chromosome (1.53 Mbp) and a plasmid (5.1 kbp) were recovered for SG7^T with a G+C content of 29.05 and 29.3mol%, respectively. Genome features were predicted on the MicroScope platform (https://mage.genoscope.cns.fr/microscope, Table S2), and annotations were added to the SG7^T genome using the Prokaryotic Genome Annotation Pipeline [36]. CRISPR arrays were predicted for the SG1 genome using the CRISPRCas++ online tool [37]. Reference genomes and plasmids were downloaded from NCBI GenBank, and additional annotation was performed on all genomes using GhostKoala [38] and the Archaeal Clusters of

Orthologous Genes (arCOG) database (E-value cutoff of 1×10^{-5}) [39] (Tables S3 and 4). Conserved Domain Database searches [40] were used to provide additional annotation validation when required.

To compare the isolate genomes with metagenomic data, a metagenome was also sequenced from a hydrothermal sulphide deposit obtained from Tu'i Malila during the RR1507 expedition (sample J2-819-7-R2; BioSample accession SAMN10217879). Sampling, DNA extraction, metagenomic sequencing, read trimming, assembly and binning were performed as previously described [41]. A single *Methanocaldococcus* MAG (T11_73) was identified using a phylogenetic tree reconstructed from 16 conserved ribosomal proteins as described previously [42] (data not shown). The MAG was curated iteratively, first using Emerging Self Organizing Mapping [43] with the tetramerFreq package (https://github.com/tetramerFreqs). Genes were then assigned a putative taxonomy using GhostKoala [38], and contigs with genes exclusively linked to divergent taxonomic groups were removed. The final T11_73 MAG (GenBank accession JACCLX000000000) was assessed for completeness and contamination using CheckM version 1.0.7 [44]. Genomic features were predicted using PGAP, and additional gene annotation was performed as described above.

For whole-genome comparison, average nucleotide identity (ANI) analysis was performed with the ANI/AAI Matrix Tool (http://enve-omics.ce.gatech.edu/g-matrix/). Digital DNA–DNA hybridization (dDDH) was also performed using the Genome-to-Genome Distance Calculator [45]. GTDB-Tk version 1.5.0, database release 202 [46] was used to assign taxonomy with the classify workflow. A multiple sequence alignment based on 122 archaeal proteins was also generated in GTDB-tk, and an unrooted phylogenetic tree with Shimodaira–Hasegawa (SH) support values was inferred with FastTree version 2.1.10 (www.microbesonline.org/fasttree/) via the GTDB-tk infer program (parameter–gamma). A high degree of similarity was observed between the 16S rRNA gene sequences from SG1 and SG7^T and the described type species belonging to the genus *Methanocaldococcus* (98.61% compared to *M. bathoardescens*). However, ANI firmly suggests that strains SG7^T and SG1 (and the T11_73 MAG) are a novel species within the genus *Methanocaldococcus*, with ANI values between 81–82 % compared to *M. bathoardescens*, *M. fervens*, *M. jannaschii* and *Methanocaldococcus* sp. FS406-22 [47] (Table S5). These values are far below the ANI value of 95–96% generally accepted as a boundary for species delineation [48]. dDDH scores were also well below the DDH threshold level for species demarcation (70%), with values below 30 % between the genomes of strains SG7^T, SG1 and the genome of *M. bathoardescens* [49]. These results based on standard genomes relatedness indices provide evidence that strains SG7^T and SG1 represent a new genomic species [50]. However, GTDB-tk analysis placed SG7^T, SG1 and T11_73 in a novel genus in the *Methanocaldococcaceae* based on relative evolutionary distance values [46]. Although the three genomes form a small cluster with high branch support in the concatenated protein tree (Fig. S1, File S1), genomic and phenotypic features of strains SG7^T and SG1 are similar to the other species of the genus *Methanocaldococcus* namely in G+C content, genome size, and in temperature, pH and salt growth ranges. We propose to describe strains SG7^T and SG1 as a new species in the *Methanocaldococcus*. SG7^T and SG1 differ in terms of genome size, the presence of a plasmid in strain SG7 ^{T}, optimal temperature and salinity and pH range.

The genomes of strains SG1 and SG7^T are similar in size (1.48–1.53 Mbp) and in the range (~1.25–1.76 Mbp) to other *Methanocaldococcus* species (Table 1, Table S2). Both genomes contain seven ribosomal RNA genes (two 16S, two 23S and three 5S rRNA genes) whereas all other *Methanocaldococcus* species to date only have six (two of each type, Table S2). The plasmid harboured by strain SG7^T is very small (5.1 kbp) compared to most other *Methanocaldococcus* plasmids and only contains two predicted open reading frames (Table S4), which encode for an uncharacterized membrane protein (arCOG13135) and a protein with OB-fold-like and PCI domains (arCOG02261). The only plasmid similar in size is found in *M. vulcanius* (4.7 kbp), which also harbours a second, much larger plasmid (10.7 kbp). Plasmids in the other cultivated *Methanocaldococcus* spp. (*M. fervens*, *M. jannaschii* and *Methanocaldococcus* sp. FS406-22) range from 12 to 58 kbp.

All the genes involved in Wolfe cycle of methanogenesis [51] and Wood–Ljungdahl CO_2 fixation are present in both genomes and the T11_73 MAG (Tables S6 and S7). Like their more distant relatives *M. villosus* and *M. infernus*, strains SG1 and SG7^T and the T11_73 MAG each contain a single copy of the *mcrA* gene. In contrast, the more closely related *M. bathoardescens*, *M. jannaschii, M. vulcanius* and *Methanocaldococcus* sp. FS406-22 each encode two copies of the *mcrA* gene, while *M. fervens* encodes for a second copy that is predicted to be a pseudogene. Despite the lack of observed motility, both the SG1 and SG7^T genomes encode for a full suite of archaell genes (*arlB*,*C*,*D/E*,*F*,*G*,*H*,*I*,*J*,*K*). Thus, it is likely that *M. lauensis* is motile under different growth conditions. A proteomics study suggested that expression of archaella in *M. jannaschii* was suppressed under excess of H_2 conditions [52], although a later study reported a more nuanced picture involving differential expression of various archaellar genes under varying H_2 levels, and low expression of two archaellar genes under conditions of limiting ammonium [53]. Thus, it appears that expression of archaella in this lineage is likely regulated by environmental factors such as low H_2, as reported in the hydrothermal vent fluids of the ELSC [54].

PHYSIOLOGY AND CHEMOTAXONOMY

Cell morphology and motility were examined by phase-contrast microscopy and transmission electron microscopy (JEM 100 CX II, JEOL). Transmission electron microscopy observations were performed directly on cells deposited on the grids. The cells are regular cocci with a diameter of 1–2.2 µm, non-motile, and the presence of archaella was not detected under TEM observations

Table 1. Diagnostic and descriptive features of the eight described species of *Methanocaldococcus*

Strains: 1, SG7^T (data from our study); 2, SG1 (data from our study); 3, SLH (data from our study); 4, *M. bathoardescens*; 5, *M. vulcanius*; 6, *M. jannaschii*; 7, *M. fervens*; 8, *M. villosus*; 9, *M. infernus*; 10, *M. indicus*. Data were obtained from Jones *et al*. [12], Jeanthon *et al*. [13, 17], L'Haridon *et al*. [15], Bellack *et al*. [16] and Stewart *et al*. [14]. ND, Not determined.

Feature	1	2	3	4	5	6	7	8	9	10
Latitude of isolation source	20° 46′ S	21° 59′ S	21° 11′ S	45° 55′ N	12° 48′ N	20° 50′ N	27° 00′ N	67° 05′ N	14° 45′ N	25° 19′ S
Longitude	176° 11' W	176° 34′ W	176° 20′ W	129° 59′ W	103° 56′ W	109° 06′ W	111° 24'' W	18° 42′ W	44° 59′ W	70° 02′ E
Origin	Lau	Lau	Lau	Juan de Fuca	EPR	EPR	Guaymas	Kolbeinsey	MAR	CIR
Depth (m)	2130	1872	1891	1520	2600	2600	2003	103	3000	2420
Morphology	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci
Diameter (µm)	1–2	1.3–2.2	1.0–2.2	1–2	1–3	Up to 1.5	1–2	1–2	1–3	1–3
Motility	–	–	–	+	+	+	+	+	+	+
Temperature for growth:										
Range (°C)	45–93	45–90	ND	58–90	49–89	50–85	48–92	55–90	55–91	50–86
Optimum (°C)	85	80	ND	82	80	85	85	80	85	85
pH for growth:										
Range	5–6.7	5.15–7.14	ND	4.5–9	5.25–7	5.2–7	5.5–7.6	5.5–7	5.25–7	5.5–6.7
Optimum	6.15	6.2	ND	7	6.5	6.5	6	6.5	6.5	6.5
NaCl concentration for growth :										
Range (g l^{-1})	10–50	10–50	ND	16–74	6.25–56.25	5.8–58	5–50	5–55	12.5–56.25	15–50
Optimum (g l^{-1})	25	20	ND	29	25	29.22	30	25	25	30
Genome accession number	GCA_902827225.1	GCA_902827205.1	LR792634.1	NZ_CP009149.1	NC_013407.1	NC_000909.1	NC_013156.1	NZ_AQUK00000000.1	NC_014122.1	ND
Genome size (Mbp)	1.53	1.48	ND	1.61	1.75	1.66	1.49	1.25	1.33	nd
Plasmid(s)	1	0	0	0	2	2	1	0	0	ND
Coding proteins	1539	1585	ND	1619	1694	1723	1528	1348	1443	nd
G+C content (mol%)	29.05	29.08	ND	30.8	31.5	31.4	32.9	29.5	33.0	30.7*
rRNA	7	7	ND	6	6	6	6	6	6	ND
CRISPR	10	9	ND	11	17	15	7	8	14	ND
Phage	–	–	ND	ND	+	+	+	ND	ND	ND

*HPLC method.
SNP, Single Nucleotide Ploymorphism.

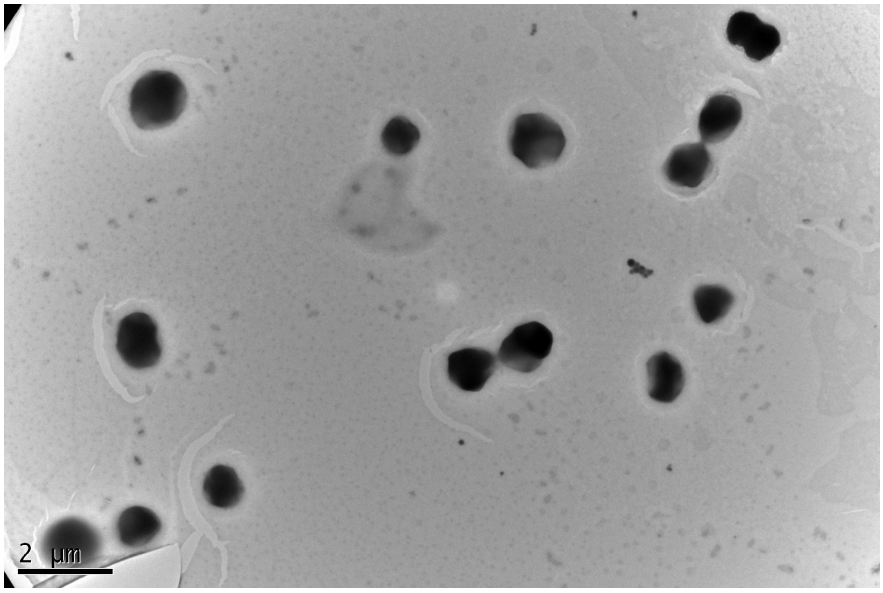

Fig. 2. Electron microspoe image of strain SG7^T.

(Fig. 2). The cells stained Gram-negative and occurred singly or in pairs. As reported for *Methanococcales*, cells lysed rapidly in SDS (0.01%) and in distilled water [55].

All physiological characterizations (Table 1) were done at 80 °C, pH 6.3 and with 20 g l^{-1} NaCl in the medium described above. Growth was determined by direct cell counting using a flow cytometer (CyFlow Space, Partec). The temperature range for growth was determined between 30 and 95 °C (with 5 °C increments). The effect of NaCl on growth was determined between 0 and 8% (w/v) NaCl (with increments of 0.5%). The pH range for growth was examined in the medium with a carbonate buffer. The effects of pH and NaCl concentration were determined at the optimal temperature for growth. Under these conditions, strains SG7^T and SG1 grew between 45 and 93 °C, with optimum growth at around 80–85 °C; no growth was detected at or below 40 and at or above 94 °C. Growth was observed between pH 5 and 7.1, with optimum growth around pH 6.2. No growth was observed at or below pH 4.8 or below and at or above 7.2. Growth occurred in NaCl concentrations ranging from 10 to 50 g l^{-1}, with optimum growth at 20–25 g l^{-1}. No growth was observed with 5 or 60 g l^{-1} NaCl.

Under the optimal conditions for growth, the doubling time of SG7^T was 25–30 min. The three strains are strictly anaerobic autotrophs, and H_2 and CO_2 served as the only substrates for growth. No growth was observed on acetate (10 mM), formate (10 mM), methanol (15 mM), monomethylamine (10 mM), yeast extract (2 g l^{-1}) or tryptone (2 g l^{-1}) with N_2/CO_2 (80 : 20; 200 kPa) or H_2 (100 %; 200 kPa) headspace. Growth was inhibited in the presence of low levels of oxygen. In the presence of H_2 and CO_2, methane production paralleled growth. When supplemented individually, selenate, tungstate, yeast extract and acetate stimulated the growth rate. When sulphur (5 g l^{-1}) was added to the sulphate-free medium in the presence of CO_2 and H_2, growth occurred and H_2S was produced. No dissimilatory reduction of cystine (5 g l^{-1}), sulphate or thiosulfate (20 mM) was observed. Sensitivity to rifampicin supplemented at 50, 100 and 200 µg ml^{-1} was tested in the culture medium at 80 °C. Strain SG7^T was only resistant to 50 µg ml^{-1} of rifampicin, but strain SG1 was sensitive to all concentrations.

PROTOLOGUE

It is evident from the results of the phylogenetic analysis based on the 16S rRNA gene, the concatenated protein tree, and ANI and DDH measurements that strains SG7 and SG1 represent a novel species that are placed in the genus *Methanocaldococcus*. Growth characteristics are in full congruence with other members of the genus, such as cocci shape, hyperthermophilic, anaerobic, salt requiring, and producing methane from CO_2 and H_2.

DESCRIPTION OF *METHANOCALDOCOCCUS LAUENSIS* SP. NOV.

Methanocaldococcus lauensis (*lau.en'sis*. N.L. masc. adj. *lauensis* of or pertaining to Lau, referring to the deep-sea vents in the Lau basin in the south-western Pacific Ocean, from which the type strain was isolated).

Cells are cocci (1.0–2.2 µm diameter) and occur singly and in pairs. Cells lyse rapidly in SDS (0.01%) and in distilled water. Mustard-coloured round colonies about 1–3 mm in diameter form on solid medium after 3–5 days. Growth occurs at between 45 and 93 °C, with an optimum at 80–85 °C, between pH 5 and 7.1, with the optimum at around pH 6.2, and with between 10 and 50 g l^{-1} NaCl, with an optimum of 20–25 g l^{-1}. Obligately anaerobic. Chemolithotrophic. Uses H_2 and CO_2 as energy and carbon sources to produce methane. Growth is stimulated by selenate, tungstate, yeast extract and acetate. Sulphur is reduced to H_2S in the presence of CO_2 and H_2. Strain SG7^T is resistant to 50 µg ml^{-1} rifampicin, while strain SG1 is sensitive.

The type strain, SG7^T (=DSM 109608^T=JCM 39049^T), was isolated from an active chimney sample from the ABE vent field located in the Lau basin in the south-western Pacific Ocean. Strain SG1 (=DSM 109607=JCM 39048) is another strain of the species.

The DNA G+C content of strains SG7^T and SG1 are 29.05 and 29.08 mol%, respectively.

Funding information
This study was funded by the European Union program MaCuMBA (grant agreement 311975) to S.L.'H., by the US-National Science Foundation (OCE1235432 and OCE 1558795) to A.-L.R. and by the Project EVOMOBIL-ERC Grant Agreement no. 340 440 to P.F. Research was supported by UBO, CNRS and Ifremer. The LABGeM (CEA/Genoscope and CNRS UMR8030), the France Génomique and French Bioinformatics Institute national infrastructures (funded as part of Investissement d'Avenir program managed by Agence Nationale pour la Recherche, contracts ANR-10-INBS-09 and ANR-11-INBS-0013) are acknowledged for support within the MicroScope annotation platform.

Acknowledgements
We thank the captain and crew members of the *RV Thomas Thompson*, the *ROV Jason* team, the captain and crew members of the *RV Atalante* and the *ROV Victor* team for assistance in collecting samples. We thank Didier Jolivet and Stéphane Hourdez for the organisation of the ChuBacArc expedition. We thank Patrick Forterre and Valérie Barbe for sequencing facilities at the Genoscope.

Author contribution
S.L.H.: conceptualization, investigation, methodology, supervision, validation, writing – original draft, writing – review and editing. S.G.: investigation, methodology. E.St.J.: investigation, methodology, software, validation, writing – review and editing. S.F.: investigation, methodology, software, writing – review and editing. A.L.R.: resources, funding acquisition, supervision, writing – review and editing.

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

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