INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION

Alain *et al.*, *Int. J. Syst. Evol. Microbiol.* 2021;71:004895

DOI 10.1099/ijsem.0.004895

MICROBIOLOGY SOCIETY

Thermococcus henrietii sp. nov., a novel extreme thermophilic and piezophilic sulfur-reducing archaeon isolated from a deep-sea hydrothermal chimney

Karine Alain[1,*], Erwann Vince[1,2], Damien Courtine[1,2,3], Lois Maignien[1,2], Xiang Zeng[2,4], Zongze Shao[2,4] and Mohamed Jebbar[1,2]

Abstract

A novel extreme thermophilic and piezophilic chemoorganoheterotrophic archaeon, strain EXT12c^T, was isolated from a hydrothermal chimney sample collected at a depth of 2496 m at the East Pacific Rise 9° N. Cells were strictly anaerobic, motile cocci. The strain grew at NaCl concentrations ranging from 1 to 5 % (w/v; optimum, 2.0%), from pH 6.0 to 7.5 (optimum, pH 6.5–7.0), at temperatures between 60 and 95 °C (optimum, 80–85 °C), and at pressures from 0.1 to at least 50 MPa (optimum, 30 MPa). Strain EXT12c^T grew chemoorganoheterotrophically on complex proteinaceous substrates. Its growth was highly stimulated by the presence of elemental sulphur or L-cystine, which were reduced to hydrogen sulfide. The DNA G+C content was 54.58 mol%. Phylogenetic analyses based on 16S rRNA gene sequences and concatenated ribosomal protein sequences showed that strain EXT12c^T falls into the genus *Thermococcus* and is most closely related to *Thermococcus nautili* strain 30-1^T. Overall genome relatedness index analyses (average nucleotide identity scores and *in silico* DNA–DNA hybridizations) showed a sufficient genomic distance between the new genome and the ones of the *Thermococcus* type strains for the delineation of a new species. On the basis of genotypic and phenotypic data, strain EXT12c^T is considered to represent a novel species, for which the name *Thermococcus henrietii* sp. nov. is proposed, with the type strain EXT12c^T (=UBOCC M-2417^T=DSM 111004^T).

Archaea of the order *Thermococcales* are common inhabitants and key players of the hot areas of deep-sea hydrothermal vents [1]. They also populate various high-temperature environments around the world ranging from hot terrestrial hot springs to marine subseafloor sediments and then to subterranean oil reservoirs, and so on [1]. Nevertheless, the vast majority of the *Thermococcales* strains isolated so far, which are formerly described, originate from marine hydrothermal vents. At the time of writing, the order *Thermococcales* (phylum *Euryarchaeota*) encompasses three genera: *Pyrococcus* [2] (six validly published species and two non-validly published ones), *Thermococcus* [3] (33 validly published species and three non-validly published ones) and *Palaeococcus* [4] (three species). With 33 formerly described species, the genus *Thermococcus* is the most represented in public collections of micro-organisms and it is also the one that has been the subject of the largest number of functional studies. *Thermococcus* species are mainly obligate chemoorganoheterotrophs, growing in organic-rich peptide media, and very preferably in the presence of inorganic sulphur compounds. Some species are able to grow by carboxydotrophy (i.e. [5–7]), others have been isolated from oligotrophic or methanogenic-targeting culture media [8, 9], and others have been suggested to convey exoelectrogenic abilities [10]. These archaea often dominate enrichment cultures grown anaerobically at 75–90 °C, on rich media, with deep-sea hydrothermal vent samples. They are therefore ubiquitous and metabolically versatile actors that are very important in the high-temperature zones of hydrothermal ecosystems. In this article, we describe a novel extreme thermophilic organoheterotrophic sulfur-reducer,

Author affiliations: [1]Univ Brest, CNRS, Ifremer, Laboratoire de Microbiologie des Environnements Extrêmes LM2E, IUEM, Rue Dumont d'Urville, F-29280 Plouzané, France; [2]IRP 1211 MicrobSea, Sino-French Laboratory of Deep-Sea Microbiology, LM2E (Plouzané, France)-KLAMBR, Xiamen, PR China; [3]Aix Marseille Univ, CNRS, INSERM, CIML, Turing Centre for Living Systems, Marseille, France; [4]Key Laboratory of Marine Genetic Resources, Third Institute of Oceanography, Ministry of Natural Resources, Xiamen 361005, PR China.
***Correspondence:** Karine Alain, karine.alain@univ-brest.fr
Keywords: hydrothermal; thermophile; *Thermococcus*; piezophile.
Abbreviations: ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridization; EPR, East Pacific Rise.
The DDBJ(/GenBank/ENA) accession number for the genome sequence of strain EXT12c^T sp. nov. is LT900021. The complete 16S rRNA gene sequence of strain Ext12c^T is available at GenBank/EMBL/DDBJ/PIR under accession no. MT731286.
One supplementary table and two supplementary figures are available with the online version of this article.

strain EXT12c^T, isolated from a hydrothermal sulfide sample from the East Pacific Rise. Genomic and phenotypic studies indicate that it represents a novel species of the genus *Thermoccocus*.

An outer part of a deep-sea sulfide chimney was collected at a depth of 2496 m from a hydrothermal vent at the East Pacific Rise 9° N (9° 50′ 40.2″ N, 104° 17′ 37.798″ W) in October 2001, during the EXTREME oceanographic cruise. Onboard, the sample was preserved anaerobically in a sealed sterile vial and stored at 4 °C. Once in the lab, enrichment cultures were performed at 85 °C in reduced TRM *(Thermococcales* Rich Medium) medium (pH 6.8) containing 5 g l^{-1} elemental sulphur, as described elsewhere [11]. A collection of pure strains was isolated by repeated streaking on plates (solidified with 8 g l^{-1} Gelrite gellan gum), and then deposited in the UBOCC collection (https://ent.univ-brest.fr/lm2e/home/#/), at –80 °C with 5 % (v/v) DMSO. Strain EXT12c^T described here bears the accession number UBOCC-M-2417^T. The purity of this isolate was confirmed routinely by microscopic examination, and by sequencing of its genome.

The genome of strain EXT12c^T was sequenced using Illumina MiSeq technology (2×300 bp paired-reads, V3 chemistry), as detailed elsewhere [12]. It was analysed and annotated with the MicroScope Microbial Genome Annotation and Analysis platform (https://mage.genoscope.cns.fr/microscope/home/index.php) using the KEGG and BioCyc databases [13]. It consists of a single chromosome of 2155760 base pairs in size (with an average coverage of 350×) and has a G+C content of 54.58 mol%. The genome consists of 2375 protein-encoding sequences, 46 tRNA genes, a single 16 S-23S rRNA operon, two 5S rRNA and 18 miscellaneous RNA genes. This genome is available in DDBJ/ENA/GenBank under the accession number LT900021.

Pairwise 16S rRNA gene sequence similarity was determined using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/ [14]). The 16S rRNA gene sequences were aligned using MAFFT version 7.427 (parameters -maxiterate 1000 -localpair) [15], and the alignment was trimmed with BMGE version 1.12 (default parameters) [16]. Then, PhyML version 3.3.20190909 was used to build the tree using the web server at www.atgc-montpellier.fr/phyml/ [17]. The evolutionary model was selected with the SMS algorithm [18] and the branch support was computed with the aLRT SH-like method. The tree was visualized with iToL [19] and rooted between the genera *Thermococcus* and *Pyrococcus* (Fig. S1). Since the genes encoding the 16S rRNA gene sequences are highly conserved and therefore not very discriminating between *Thermoccocales*, a phylogenetic tree based on ribosomal proteins was also calculated. This phylogenomic tree was based on the concatenation of 49 ribosomal proteins shared by all genomes in the tree [proteins associated with the large ribosomal subunit: L1, L2, L3, L4, L6, L7AE, L11, P1 (=L12P), L13, L15, L15E, L18, L18A, L18E, L19E, L21E, L22, L24, L29, L30, L30E, L31E, L32E, L37AE, L37E, L39E, L40E, L44E; proteins associated with the small ribosomal subunit: S2, S3, S3AE, S4, S4E, S5, S6E, S7, S8, S8E, S9, S10, S12, S13, S15, S17, S17E, S19, S19E, S27E, S28E]. Each protein was aligned and trimmed separately, MAFFT (parameters -globalpair -maxiterate 1000) and BMGE (default parameters), respectively. Then, each alignment block was concatenated into a single alignment and submitted to PhyML to build the tree. The evolutionary model was selected with SMS and the branch support was computed with the aLTR SH-like method. The tree was visualized with iToL and rooted between the *Thermococcus* and *Pyrococcus* branches. Average nucleotide identity scores (ANI; OrthoANIu values) were calculated using the ANI calculator tool provided by the EzBioCloud web server (www.ezbiocloud.net/tools/ani) on genomes of strain EXT12c^T with those of all type strains of the genus *Thermococcus* with 16S rRNA gene sequences with more than 98.7% identity to that of strain EXT12c^T (Tables 1 and S1, available in the online version of this article) [20]. Estimated *in silico* DNA–DNA hybridization (DDH) values were also determined using the Genome-to-Genome Distance Calculator (GGDC 2.1), using formula 2 [21] (Tables 1 and S1).

Based on 16S rRNA gene and concatenated ribosomal protein phylogenetic analyses, the novel isolate was affiliated with the order *Thermococcales*, in the archaeal domain, falling into the genus *Thermococcus* (Figs 1 and S1). Strain EXT12c^T had the highest 16S rRNA gene sequence similarity of 99.9% to *Thermococcus nautili* 30-1^T, followed by *Thermococcus gammatolerans* EJ3^T with 99.7%. It also had more than 98.7 % 16S rRNA gene sequence similarity to the sequences of 14 other type species of this genus (Table S1). The OrthoANIu values between the genome of strain EXT12c^T and the genomes of its 10 closest relatives were all below 91.5%, which is far below the standard ANI criterion for species identity (95–96 %) [22] (Tables 1 and S1). The digital DDH (dDDH) scores between the genome of strain EXT12c^T and those of its 10 closest relatives whose genomes are sequenced were all between 20.30 and 43.60%, well below the threshold level (70%) for a new species delineation [23] (Tables 1 and S1). Strain EXT12c^T therefore meets currently accepted criteria for describing a new genomic species [24].

Morphological characteristics of strain EXT12c^T were observed by using light microscopy (Olympus BX60 and CX40) and scanning electron microscopy (FEI Quanta 200) (Fig. S2). Cells were irregular motile cocci (1.60±0.32 µm in diameter, n=30) that generally occurred singly.

Unless noted otherwise, physiological tests were carried out anaerobically (N_2 headspace) in modified Ravot medium, at 80 °C (pH 7.0, atmospheric pressure), in duplicate (+negative controls), using elemental sulphur as an external electron acceptor, as described elsewhere [9]. Growth tests were generally carried out as described previously [9]. Cells were routinely counted by direct cell counting by using a modified Thoma chamber (depth 10 µm), and checked by flow cytometry (CyFlowSpace, Sysmex Partec). Cells were fixed with 2.5 % (v/v) glutaraldehyde (Sigma) and stored at –80 °C, before counting by the two methods described above. Determination of the temperature range for growth was carried out at 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 and 100 °C. The isolate was

Table 1. Characteristics differentiating strain EXT12c^T from closest species of the genus *Thermococcus*

Strains: 1, EXT12c^T (data from this study); 2, *T. nautili* 30-1^T [27]; 3, *T. gammatolerans* EJ3^T [28]; 4, *T. guaymasensis* DSM 1113^T [29]; 5, *T. eurythermalis* A501^T [30]. Characteristics are scored as: +, positive; –, negative; v, variable; S, Stimulatory; R, required.

Characteristics	1	2	3	4	5
Geographical origin	Hydrothermal chimney, East-Pacific Rise 9° N	Hydrothermal chimney, East-Pacific Rise 13° N	Hydrothermal chimney, Guaymas Basin	Hydrothermal sediment, Guaymas Basin	Hydrothermal chimney, Guaymas Basin
Depth (m)	2496	2633	2616	2000	2006
Growth optimum:*					
Temperature (°C)	80–85	87.5	88	88	85
pH	6.5–7.0	7.0	6.0	7.2	7.0
NaCl (%)	2	2	2	3	2.5
Doubling time (min)†	103	64	95	15	48
Sulphur requirement†	S	R	R	S	R
Growth on:†					
Maltose	–	–	–	+	–
Pyruvate	+ (weak)	+	–	+	–
DNA G+C content (mol%)*	54.58	54	51.3	46	53.5
16S rRNA gene sequence similarity	100	99.93	99.66	99.40	99.38
OrthoANIu (%)	100	91.54	82.84	80.63	82.30
dDDH (%)	100	43.60	29.40	25.50	25.50

*Data from the literature.
†Data obtained for the five strains under same experimental laboratory conditions.

hyperthermophilic and grew between 60 and 95 °C with an optimum at 80–85 °C. Salt tolerance was tested at 80 °C with various concentrations of NaCl (0, 1, 2, 3, 4, 5, 6, 7 and 8%, w/v). Strain EXT12c^T required NaCl for growth and grew at NaCl concentrations between 1 and 5% (optimum: 2%). The pH range for growth was tested from pH 3.0 to 10.0 (initial pH at 20 °C) with increments to one unit near the limits of the pH range, and with increments of 0.5 unit around the optimum. For this experiment, we used the following buffers (each at 20 mM, Sigma-Aldrich): for pH 3.0, no buffer; for pH 4.0 and 5.0, HOMOPIPES buffer; for pH 5.5 and 6.0, MES buffer; for pH 6.5 and 7.0, PIPES buffer; for pH 7.5 and 8.0, HEPES buffer; for pH 9.0, 9.5 and 10.0, CAPSO buffer. Growth of the isolate was observed in a narrow pH range, between pH 6.0 and 7.5, with an optimum around 6.5–7.0. The pressure range for growth of strain EXT12c^T was tested in high-pressure high-temperature reactors (Top Industrie), at 0.1, 10, 20, 30, 40 and 50 MPa, as described previously [25]. The novel isolate was piezophilic, growing from atmospheric pressure (0.1 MPa) to at least 50 MPa, and showed optimal growth under 30 MPa. Under optimal growth conditions (80 °C, pH 6.8, 2% NaCl, 5 g l^{-1} S° and 30 MPa), the doubling time of the novel isolate was 103 min.

Utilization of various individual substrates for growth was tested in a basal medium supplemented with 0.05% (w/v) yeast extract as growth factor, and without this growth factor, as described previously [9]. The following substrates were tested, at the final concentrations shown in brackets: tryptone (0.5% w/v), peptone (0.5% w/v), yeast extract (0.5% w/v), beef extract (0.5% w/v), casamino acids (0.4% w/v), casein (0.5% w/v), formate (20 mM), acetate (20 mM), pyruvate (20 mM), fumarate (20 mM), propionate (20 mM), succinate (20 mM), maltose (20 mM), fructose (20 mM), lactose (20 mM), ribose (20 mM), galactose (20 mM), glucose (20 mM), chitin (0.2% w/v) and starch (0.2% w/v). Unsupplemented media were used as negative controls for all conditions. To examine the ability of the strain to grow in the absence of elemental sulphur, cells were cultivated in modified Ravot medium without sulphur. Alternative electron acceptors were also tested in a sulfur-depleted medium, under a gas phase of N_2 (100%, 150 kPa): L-cystine (5 g l^{-1}), polysulfides (0.5 mM), thiosulfate (20 mM), sulphate (20 mM), sulfite (5 mM), nitrate (20 mM), nitrite (5 mM) and dioxygen (0.5, 5, 20 % v/v). Growth was monitored over 3 days of incubation. The results were considered positive when growth was still observed after two successive subcultures (1/100th subculturing) on the test medium. All tests were performed in duplicate and growth was confirmed after microscopic observation. Hydrogen sulfide production was tested as described previously [26]. Cations and anions produced from peptone and yeast extract

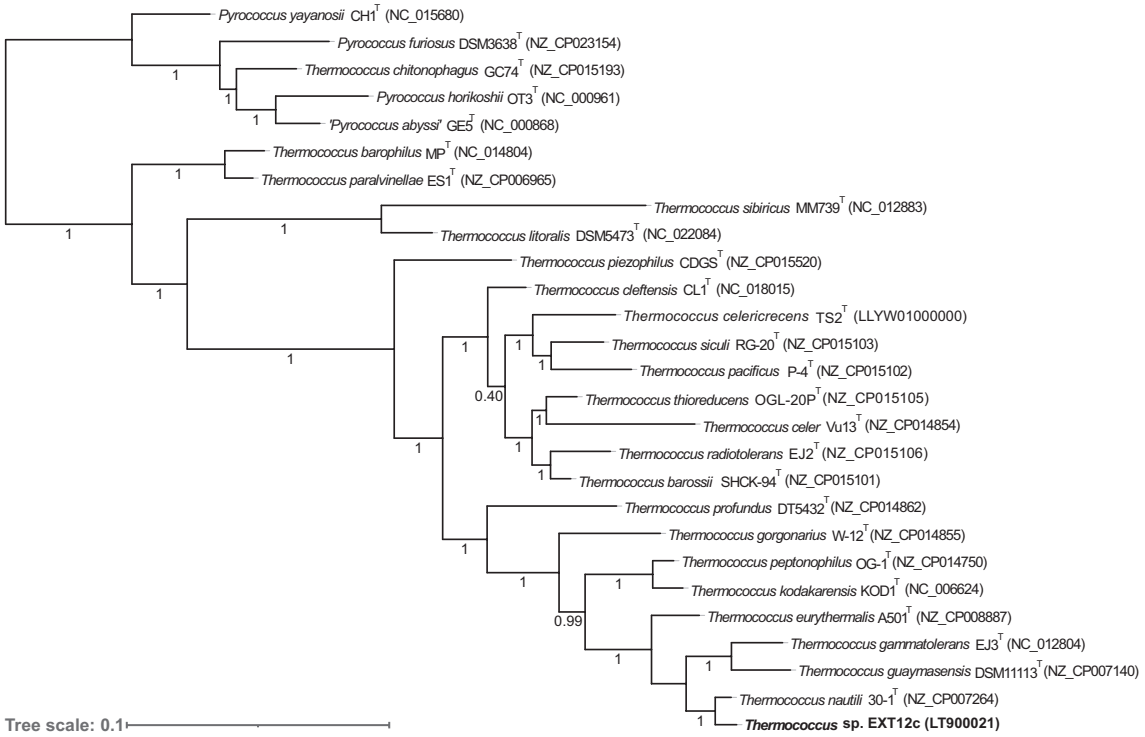

Fig. 1. PhyML phylogenomic tree showing the phylogenetic positions of strain EXT12c^T and representatives of some other related taxa, based on 40 concatenated ribosomal protein sequences. *Pyrococcus* species were used as an outgroup. Branch supports, computed with the aLTR SH-like method, are shown at branch nodes. Bar, 0.01 nucleotide substitution rate (K_{nuc}) units.

fermentation were identified by ionic chromatography on a Dionex ICS-900 ion chromatography system oupled with a CERS 500 4 mm suppressor and a DS5 conductivity detector (40 °C) and fitted with an RFC-10 reagent-free controller, an ASDV autosampler and an IonPac CS16 column maintained at 60 °C in a UltiMate 3000 Thermostated Column Compartment (Thermo Scientific).

Significant growth was observed under strict anaerobic conditions, on complex proteinaceous substrates such as yeast extract, peptone, tryptone and beef extract as carbon and energy sources, and in the presence of elemental sulphur. Cations and anions produced by the fermentation of peptone and yeast extract included formate, acetate, propionate, isobutyrate, succinate or malate, isovalerate, ammonia and hydrogen sulfide. The strain was also able to grow on these substrates by fermentation in the absence of sulfur-containing compounds, but in that case, growth was very poor. Indeed, growth was very clearly stimulated by L-cystine and elemental sulphur, which were both reduced to hydrogen sulfide. Sulphate, thiosulfate, sulfite, polysulfides, nitrate, nitrite and oxygen (aerobic to microaerophilic conditions) were not used by the cells as terminal electron acceptors. In the presence of elemental sulphur, slow and weak growth was also observed on the few following substrates: casamino acids, casein and pyruvate (with 0.05% yeast extract, v/v). No growth was observed in the presence of the other substrates tested. Growth by carboxydotrophy was not tested as the gene encoding the carbon monoxide dehydrogenase CooF, one central protein in the *Thermococcales*'s carbon monoxide metabolism, was absent from the genome.

Metabolic predictions from the genome confirmed these *in vitro* growth results and demonstrated that the strain has the genetic potential to degrade peptides and amino acids. Indeed, its genome codes for peptidases/proteases and for the two key enzymes of amino acid catabolism [the alanine aminotransferase (AlaAT; locus tag TEXT12C_1080) and the glutamate dehydrogenase (GDH; TEXT12C_0879)]. In addition, it contains six complete pathways of amino acid degradation (alanine, arginine, aspartate, asparagine, glutamine, glycine) according to MicroCyc. Concerning the catabolism of sugars, this strain's genome contains the complete Embden–Meyerhof–Parnas pathway (including genes of the ADP-dependent glucokinase GlkA (locus tag TEXT12C_1056), an ADP-specific phosphofructo-kinase (TEXT12C_2105) and a glyceraldehyde-3-phosphate:ferredoxin oxidoreductase (TEXT12C_0008)), typically found within this archaeal order, and contains complete pathways for D-mannose and glycerol degradation. While the genome of strain Ext12c^T has genes coding for several enzymes of the chitin degradation pathway (namely, chitinases TEXT12C_2122–2123; diacetylchitobiose deacetylase TEXT12C_0385; exo-beta-D-glucosaminidase TEXT12C_0375; GlkA; glucosamine-6-phosphate deaminase TEXT12C_0376), we have not demonstrated this property in our experimental conditions. Furthermore, we could

not find any alpha-amylase sequence in the genome, which could explain the fact that we showed that the strain does not degrade starch. Moreover, the genome also encodes different types of hydrogenases involved in energy conservation classically found in *Thermococcales*, such as the membrane-bound hydrogenase Mbh (TEXT12C_0230–0246), the sulfidogenic hydrogenase Mbs (TEXT12C_0689–0694) and the formate hydrogenlyase (TEXT12C_1011–1027).

In summary, strain EXT12c^T is a strict anaerobic archaeon growing chemoorganoheterotrophically on complex proteinaceous substrates and which requires the presence of elemental sulphur or L-cystine to produce a high biomass in culture, like the vast majority of *Thermococcales*.

Antibiotic resistance of the strain was tested, in duplicates, in the presence of a variety of antibiotics (chloramphenicol, kanamycin, rifampicin and tetracycline), provided at four concentrations (10, 25, 50 and 100 μg ml^{-1}). When the antibiotic was diluted in ethanol (chloramphenicol) or DMSO (rifampicin), the same volume of solvent was added to control cultures. Strain EXT12c^T was sensitive to rifampicin at 50 μg ml^{-1} and resistant to the other three antibiotics, at the concentrations tested.

The phylogenetic, phenotypic and physiological data shown in this article support the view that strain EXT12c^T should be assigned to the genus *Thermococcus*. However, there are a few phenotypic differences, detailed in Table 1, between the novel isolate and its closely related species. Strain EXT12c^T is distinguishable from *T. nautili* by a lower optimal growth temperature and an ability to grow weakly by fermentation, in the absence of sulphur. It is able to grow weakly on pyruvate, while *T. gammatolerans* and *T. eurythermalis* are not. Therefore, from the genotypic and physiological evidence, we propose to assign strain EXT12c^T as the type strain of a novel species, for which the name *Thermococcus henrietii* sp. nov. is proposed.

DESCRIPTION OF *THERMOCOCCUS HENRIETII* SP. NOV.

Thermococcus henrietii (hen.ri.e'ti.i N.L. gen. n. *henrietii* of Henriet, in honour of Professor Jean-Pierre Henriet, in recognition of his important contribution to the exploration of the seabed).

Cells are irregular motile cocci (diameter: 1.60±0.32 μm). Obligately anaerobic. Growth is observed at temperatures between 60 and 95 °C (optimum 80–85 °C), at NaCl concentrations from 1–5% (optimum, 2%) and at pH from pH 6 to 7.5 (optimum, pH 6.5–7.0). Optimal piezophilic growth at under 30 MPa. Elemental sulphur or L-cystine are not essential for growth but definitely stimulate it. Does not use sulphate, thiosulfate, sulfite, polysulfides, nitrate, nitrite or oxygen (0.5, 5, 20% v/v) as electron acceptors. Chemoorganoheterotrophic growth occurs on complex proteinaceous substrates (yeast extract, peptone, tryptone, beef extract). Poor growth on casamino acids, casein and pyruvate.

The type strain, EXT12c^T (=UBOCC M-2417^T=DSM 111004^T), was isolated from a deep-sea sulfide sample collected at a depth of 2496 m from a hydrothermal chimney at the East Pacific Rise 9° N. The genomic DNA G+C content of the type strain is 54.58 mol%.

Funding information
This work was supported by the MERLIN Abyss progamme to K.A, the 'Laboratoire d'Excellence' LabexMER Axis 3 programmes ENDIV and CULTIVENT (ANR-10-LABX-1) to K.A., and Sino-French IRP 1211 MicrobSea to K.A.

Acknowledgements
The authors thank the co-chiefs, crew and shipboard scientific party of the oceanographic expedition EXTREME. We are grateful to Xavier Philippon and Erwan Roussel for ionic chromatography analyses, and to Nicolas Gayet for scanning electron microscopy. We thank Phil Oger for providing us with the type strains of the closest species for physiological comparisons. We acknowledge Hilary G. Morrison who did the sequencing.

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

References

1. Godfroy A, François D, Hartunians J, Moalic Y, Alain K. *The Microbiology of Deep-Sea. Physiology, Metabolism and Ecology of Thermophiles from Deep-sea Vents*. Springer International,
2. Fiala G, Stetter KO. *Pyrococcus furiosus* sp. nov. represents a novel genus of marine heterotrophic archaebacteria growing optimally at 100 °C. *Arch Microbiol* 1986;145:56–61.
3. Zillig W, Holz I, Janekovic D, Schäfer W, Reiter WD. The archaebacterium *Thermococcus celer* represents, a novel genus within the thermophilic branch of the archaebacteria. *Syst Appl Microbiol* 1983;4:88–94.
4. Takai K, Sugai A, Itoh T, Horikoshi K. *Palaeococcus ferrophilus* gen. nov., sp. nov., a barophilic, hyperthermophilic archaeon from a deep-sea hydrothermal vent chimney. *Int J Syst Evol Microbiol* 2000;50:489–500.
5. Kozhevnikova DA, Taranov EA, Lebedinsky AV, Bonch-Osmolovskaya EA, Sokolova TG. Hydrogenogenic and sulfidogenic growth of Thermococcus archaea on carbon monoxide and formate. *Microbiology* 2016;85:400–410.
6. Sokolova TG, Jeanthon C, Kostrikina NA, Chernyh NA, Lebedinsky A, *et al*. The first evidence of anaerobic CO oxidation coupled with H2 production by a hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent. *Extremophiles* 2004;8:317–323.
7. Vannier P, Marteinsson VT, Fridjonsson OH, Oger P, Jebbar M. Complete genome sequence of the hyperthermophilic, piezophilic, heterotrophic, and carboxydotrophic archaeon *Thermococcus barophilus* MP. *J Bacteriol* 2011;193:1481–1482.
8. Byrne N, Lesongeur F, Bienvenu N, Geslin C, Alain K, *et al*. Effect of variation of environmental conditions on the microbial communities of deep-sea vent chimneys, cultured in a bioreactor. *Extremophiles* 2009;13:595–608.
9. Dalmasso C, Oger P, Selva G, Courtine D, L'Haridon S, *et al*. *Thermococcus piezophilus* sp. nov., a novel hyperthermophilic and piezophilic archaeon with a broad pressure range for growth, isolated from a deepest hydrothermal vent at the Mid-Cayman Rise. *Syst Appl Microbiol* 2016;39:440–444.
10. Pillot G, Frouin E, Pasero E, Godfroy A, Combet-Blanc Y, *et al*. Specific enrichment of hyperthermophilic electroactive Archaea from deep-sea hydrothermal vent on electrically conductive support. *Bioresour Technol* 2018;259:304–311.
11. Zeng X, Birrien JL, Fouquet Y, Cherkashov G, Jebbar M, *et al*. *Pyrococcus* CH1, an obligate piezophilic hyperthermophile: extending the upper pressure–temperature limits for life. *ISME J* 2009;3:873–876.

12. Courtine D, Alain K, Georges M, Bienvenu N, Morrison HG, *et al*. Complete genome sequence of hyperthermophilic archaeon *Thermococcus* sp. EXT12c, isolated from the East Pacific Rise 9°N. *Genome Announc* 2017;5:e01385-17.
13. Vallenet D, Calteau A, Dubois M, Amours P, Bazin A, *et al*. Microscope: An integrated platform for the annotation and exploration of microbial gene functions through genomic, pangenomic and metabolic comparative analysis. *Nucleic Acids Res* 2020;48:D579–D589.
14. Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, *et al*. Introducing EzTaxone: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* 2012;62:716–721.
15. Katoh K, Standley DM. MAFFT Multiple Sequence Alignment Software Version 7 Improvements in Performance and Usability. *Mol Biol Evol* 2013;30:772–780.
16. Criscuolo A, Gribaldo S. BMGE (Block Mapping and Gathering with Entropy): a new software for selection of phylogenetic informative regions from multiple sequence alignments. *BMC Evol Biol* 2010;10:210.
17. Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, *et al*. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of Phyml 3.0. *Syst Biol* 2010;59:307–321.
18. Lefort V, Longueville J-E, Gascuel O. SMS: Smart Model Selection in PhyML. *Mol Biol Evol* 2017;34:2422–2424.
19. Letunic I, Bork P. Interactive Tree of Life (iTOL) v4: Recent updates and new developments. *Nucleic Acids Res* 2019;47:W256–W259.
20. Yoon SH, Ha SM, Lim JM, Kwon SJ, Chun J. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie van Leeuwenhoek* 2017;110:1281–1286.
21. Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
22. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci U S A* 2009;106:19126–19131.
23. Wayne LG, Stackebrandt E, Kandler O, Colwell RR, Krichevsky M, *et al*. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* 1987;37:463–464.
24. Rosselló-Móra R, Whitman WB. Dialogue on the nomenclature and classification of prokaryotes. *Syst Appl Microbiol* 2019;42:5–14.
25. Alain K, Marteinsson VT, Miroshnichenko M, Bonch-Osmolovskaya E, Prieur D, *et al*. *Marinitoga piezophila*, sp. nov., a rod-shaped thermopiezophilic bacterium isolated under high hydrostatic pressure from a deep-sea hydrothermal vent. *Int J Syst Evol Microbiol* 2002;52:1331–1339.
26. Cord-Ruwisch R. A quick method for the determination of dissolved and precipitated sulfides in cultures of sulfate-reducing bacteria. *J Microbiol Methods* 1985;4:33–36.
27. Gorlas A, Croce O, Oberto J, Gauliard E, Forterre P, *et al*. *Thermococcus nautili* sp. nov., a hyperthermophilic archaeon isolated from a hydrothermal deep-sea vent. *Int J Syst Evol Microbiol* 2014;64:1802–1810.
28. Jolivet E, L'Haridon S, Corre E, Forterre P, Prieur D. *Thermococcus gammatolerans* sp. nov., a hyperthermophilic archaeon from a deep-sea hydrothermal vent that resists ionizing radiation. *Int J Syst Evol Microbiol* 2003;53:847–851.
29. Canganella F, Jones WJ, Gambacorta A, Antranikian G. *Thermococcus guaymasensis* sp. nov. and *Thermococcus aggregans* sp. nov., two novel thermophilic archaea isolated from the Guaymas Basin hydrothermal vent site. *Int J Syst Bacteriol* 1998;48:1181–1185.
30. Zhao W, Zeng X, Xiao X. *Thermococcus eurythermalis* sp. nov., a conditional piezophilic, hyperthermophilic archaeon with a wide temperature range for growth, isolated from an oil-immersed chimney in the Guaymas Basin. *Int J Syst Evol Microbiol* 2015;65:30–35.