Thermococcus camini sp. nov., a hyperthermophilic and piezophilic archaeon isolated from a deep-sea hydrothermal vent at the Mid-Atlantic Ridge

Damien Courtine, Erwann Vince, Loïs Maignien, Xavier Philippon, Nicolas Gayet, Zongze Shao, Karine Alain

To cite this version:

Damien Courtine, Erwann Vince, Loïs Maignien, Xavier Philippon, Nicolas Gayet, et al.. Thermococcus camini sp. nov., a hyperthermophilic and piezophilic archaeon isolated from a deep-sea hydrothermal vent at the Mid-Atlantic Ridge. International Journal of Systematic and Evolutionary Microbiology, 2021, 71 (7), 10.1099/ijsem.0.004853 . hal-03367146

HAL Id: hal-03367146
https://hal.science/hal-03367146
Submitted on 6 Oct 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
1 Thermococcus camini sp. nov., a hyperthermophilic and piezophilic archaeon isolated from a deep-sea hydrothermal vent at the Mid-Atlantic Ridge

2 Damien Courtine,1,2,3 Erwann Vince,1,2 Loïs Maignien,1,2 Xavier Philippon,1,2 Nicolas Gayet,4
3 Zongze Shao,5,2 & Karine Alain1,2

5

6 1Univ Brest, CNRS, Ifremer, Laboratoire de Microbiologie des Environnements Extrêmes LM2E, IUEM, Rue Dumont d’Urville, F-29280 Plouzané, France
7 2IRP 1211 MicrobSea, Sino-French Laboratory of Deep-Sea Microbiology, LM2E (Plouzané, France)-
8 KLAMBR (Xiamen, China)
9 3Aix Marseille Univ, CNRS, INSERM, CIML, Turing Centre for Living Systems, Marseille, France –
10 Current address
11 4Ifremer, EEP, F-29280 Plouzané, France
12 5Key Laboratory of Marine Genetic Resources, Third Institute of Oceanography, Ministry of Natural
13 Resources, Xiamen 361005, China
14
15 Correspondence Karine Alain Karine.Alain@univ-brest.fr
16
17 Subject: NEW TAXA Archaea
18 Running title: Thermococcus camini sp. nov.
19
20 Abbreviations: ANI, Average Nucleotide Identity; CAPSO, N-cyclohexyl-3-aminopropanesulfonic
21 acid; DMSO, Dimethylsulfoxide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid;
22 HOMOPIPES, Homopiperazine-1,4-bis(2-ethanesulfonic acid); ICSP, International Committee on Sys-
23 tematics of Prokaryotes; MAR, Mid-Atlantic Ridge; MES, 2-(N-Morpholino)EthaneSulfonic acid;
24 PIPES, Piperazine-N,N’-bis(2-ethanesulfonic acid); TAPS, N-[Tris(hydroxymethyl)methyl]-3-
25 aminopropanesulfonic acid, [(2-Hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]-1-propanesulfonic acid;
26 UBOCC University of Brest (UBO) Culture Collection.
The DDBJ/GenBank/ENA accession number for the genome sequence of *Thermococcus camini* Iri35c<sup>T</sup> sp. nov. is LR881183.1. The complete 16S rRNA gene sequence of *T. camini* sp. nov. strain Iri35c<sup>T</sup> is available at GenBank/EMBL/DDBJ/PIR under accession no. MT921160. Two supplementary figures and one supplementary table are available with the online version of this paper.

**Abstract:** A coccoid-shaped, strictly anaerobic, hyperthermophilic and piezophilic organoheterotrophic archaeon, strain Iri35c<sup>T</sup>, was isolated from a hydrothermal chimney rock sample collected at a depth of 2,300 m at the Mid-Atlantic Ridge (Rainbow vent field). Cells of strain Iri35c<sup>T</sup> grew at NaCl concentrations ranging from 1 to 5% (w/v) (optimum 2.0%), from pH 5.0 to 9.0 (optimum 7.0-7.5), at temperatures between 50 and 90 °C (optimum 75-80 °C) and at pressures from 0.1 to at least 50 MPa (optimum: 10-30 MPa). The novel isolate grew on complex organic substrates, such as yeast extract, tryptone, peptone or beef extract, preferentially in the presence of elemental sulfur or L-cystine; however, these molecules were not necessary for growth. Its genomic DNA G+C content was 54.63%. The genome has been annotated and the metabolic predictions are in accordance with the metabolic characteristics of the strain and of *Thermococcales* in general. Phylogenetic analyses based on 16S rRNA gene sequences and concatenated ribosomal protein sequences showed that strain Iri35c<sup>T</sup> belongs to the genus *Thermococcus*, and is closer to the species *T. celericrescens* and *T. siculi*. Average nucleotide identity scores and *in silico* DNA-DNA hybridization values between the genome of strain Iri35c<sup>T</sup> and the genomes of the type species of the genus *Thermococcus* were below the species delineation threshold. Therefore, and considering the phenotypic data presented, strain
Iri35c is suggested to represent a novel species, for which the name *Thermococcus camini* sp. nov. is proposed, with the type strain Iri35c (=UBOCC-M-2026 =DSM 111003).

Contrary to many orders within the *Archaea*, the order *Thermococcales* has led to the isolation of numerous cultured representatives that have been the subject of physiological and genomic studies. *Thermococcales* populate a variety of high-temperature natural ecosystems (deep-sea and shallow-marine hydrothermal vents, terrestrial hot springs, oil reservoirs, solfataric systems, etc.), the most important of which are marine hydrothermal vents, from which the largest number of isolates originate (Godfroy *et al.*, in press). Among the 3 genera (*Thermococcus*, *Pyrococcus* and *Palaeococcus*) of the order *Thermococcales* (Bertoldo & Antranikian, 2006), it is the genus *Thermococcus* that has led to the isolation of the largest number of species, with 33 species (with names validly recognized by the ICSP, the International Committee on Systematics of Prokaryotes) recorded to date. Physiological studies of these species have shown that this genus is composed of hyperthermophilic anaerobic taxa developing mainly through chemoorganoheterotrophy by coupling the oxidation of peptides or sugars to the reduction of elemental sulfur and protons (Bertoldo & Antranikian, 2006). This genus is also described to contain carboxydotrophic species (i.e. Sokolova *et al.*, 2004; Kozhevnikova *et al.*, 2016).

Thanks to the different genetic tools available to work on models of *Thermococcus* species, such as *Thermococcus kodakarensis* or *Thermococcus barophilus* for example, many functional studies have been carried out and have allowed significant advances in our knowledge of...
their metabolism, genomic maintenance mechanisms and biological adaptations, instrumental in advancing our understanding of the biology of the Thermococcales and of the Archaea in general (e.g. Thiel et al., 2014; Atomi & Reeve, 2019). In addition to being important and ubiquitous players in the hot areas of the hydrothermal ecosystems, Thermococcus species are also of particular interest for learning more about the cellular processes at the limits of life, as this genus contains extremophilic and polyextremophilic organisms, adapted to one or more extreme physical or chemical conditions of their natural habitat. In this way, all taxa of the genus Thermococcus are hyperthermophilic, capable for some of them to divide up to a maximal temperature of 100°C (e.g. T. eurythermalis, T. kodakarensis, T. peptonophilus) (González et al., 1995; Atomi et al., 2004; Zhao et al., 2015), tolerating in addition high doses of gamma radiation for some of them (e.g. T. gammatolerans resist up to 30 kGy of γ-radiation) (Jolivet et al., 2003), and with better growth under high hydrostatic pressure (e.g. T. barophilus, T. piezophilus; T. piezophilus holds the current record of pressure range for growth, growing from atmospheric pressure to 130 MPa) (Marteinsson et al., 1999; Dalmasso et al., 2016) or under alkaline pH conditions for others (e.g. T. alcaliphilus) (Keller et al., 1995).

In this article, we describe a novel hyperthermophilic organoheterotrophic sulfur-reducer, strain Iri35cT, isolated from a hydrothermal rock sample from the Rainbow vent field, at the Mid-Atlantic Ridge. Genotypic and phenotypic characteristics meet the standard nomenclatural criteria to delineate a novel species. We propose to name this new species Thermococcus camini.
A chimney rock sample was collected at a depth of 2,300 m from a hydrothermal vent at the Rainbow vent field (36°13’N, 33°54’W), at the Mid-Atlantic Ridge, in June 2001, during the IRIS oceanographic cruise. Onboard, the sample was preserved in a sealed sterile anoxic vial and stored at 4 °C, since the objective was to cultivate (hyper)thermophiles from this sample.

Once in the lab, enrichment cultures followed by three series of dilutions-to-extinction were performed at 85°C, in reduced TRM medium (pH 6.8), containing 5 g.L⁻¹ elemental sulfur, as described elsewhere (Zeng et al., 2009). A collection of pure strains was then deposited in the UBOCC collection (https://ent.univ-brest.fr/lm2e/home/#/), at –80 °C with 5% (v/v) DMSO.

The strain Iri35cᵀ described here bears the accession number UBOCC-M-2026ᵀ. The purity of this isolate was confirmed routinely by microscopic examination, and by sequencing of its genome.

The genomic DNA of Iri35cᵀ was extracted using a phenol-chloroform procedure. The TruSeq DNA PCR-free kit (Illumina, USA) was then used to prepare paired-end sequencing libraries with an average insert size of 550 nt, before the genome was sequenced using the Illumina’s MiSeq technology (2 × 300 bp paired-reads, V3 chemistry), at the Marine Biological Laboratory (Woods Hole, MA, USA). Paired-end reads were filtered with the Python package illumina-utils using the command “iu-filter-quality-minoche” and default parameters (Minoche et al.,
The de novo assembly of the genome was carried out using CLC Genomics Workbench v8.5.1 (https://www.qiagenbioinformatics.com/products/clc-genomics-workbench). A total of 482,309 read pairs of 300bp were used for the genome assembly, representing a mean coverage of about 120×. The assembled genome was analyzed and annotated with the MicroScope Microbial Genome Annotation and Analysis Platform (MaGe) (https://mage.genoscope.cns.fr/microscope/home/index.php) using KEGG and BioCyc databases (Vallenet et al., 2020). It consists of one circular chromosome of 2,022,529 base pairs in size, and has a G+C content of 54.63%. CheckM estimated the genome to be 100% complete based on the presence of default single-copy marker genes (four markers were missing) and without any contamination. The genome consists of 2,204 encoding protein sequences, 46 tRNA genes, a single 16S-23S rRNA operon, 2 5S rRNA, and 14 miscellaneous RNA genes. This genome is available in DDBJ/ENA/GenBank under the accession number LR881183.1 (BioProject: PRJEB40155).

Pairwise 16S rRNA gene sequence similarity was determined using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; (Kim et al., 2012)). 16S rRNA gene sequences were aligned using MAFFT v7.427 (parameters -maxiterate 1000 -localpair) (Katoh and Stanley, 2013), and the alignment was trimmed with BMGE v1.12 (default parameters) (Criscuolo and Gribaldo, 2010). Then, PhyML v. 3.3.20190909 was used to build the tree thanks to the web-server http://www.atgc-montpellier.fr/phylml/ (Guindon et al., 2010). The evolutionary model was
selected with the SMS algorithm (Lefort et al., 2017) and the branch support was computed with the aLRT SH-like method. The tree was visualized with iToL (Letunic and Bork, 2019) and rooted between the Thermococcus and Pyrococcus genera. Since the 16S rRNA gene sequence is highly conserved and therefore not very discriminating between Thermococcales, a phylogenetic tree based on ribosomal proteins was also constructed. This phylogenomic tree was based on the concatenation of 49 ribosomal proteins shared by all genomes. Each protein was aligned and trimmed separately with MAFFT (parameters -globalpair -maxiterate 1000) and BMGE (default parameters), respectively. Then, each alignment block was concatenated into a single alignment that was submitted to PhyML. 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) was calculated using two methods: OrthoANIu from the EzBioCloud web server (https://www.ezbiocloud.net/tools/ani) (Yoon et al., 2017); and ANIb values by JSpeciesWS Online Service (Richter et al., 2016; http://jspecies.ribohost.com/jspeciesws/#analyse). These ANI scores were calculated between the genome of strain Iri35cT and the genomes of the 4 closest type species whose genomes are available: T. celeriacrescens TS2T (NZ_LLYW0000000.1), T. siculi RG20T (NZ_CP015102.1), T. clefientes CL1T (NC_018015.1) and T. pacificus P-4T (NZ_CP015102.1). Digital DNA-DNA hybridization (dDDH) scores were also determined by
the genome-to-genome distance calculator (GGDC 2.1), using the formula 2 (Meier-Kolthoff et al., 2013).

Phylogenetic analyses of the 16S rRNA gene sequences and concatenated ribosomal proteins both confirmed that the new isolate branched within the archaeal genus *Thermococcus* (Fig. 1 and Supplementary Fig. 1). The most closely related species of strain Iri35cT were *Thermococcus celericrescens* TS2T (99.66% 16S rRNA gene sequence similarity), *T. siculi* RG-20T (99.26%), *T. barossii* SHCK-94T (98.79%), *T. thioreducens* DSM 14981T (98.79%), *T. hydrothermalis* AL662T (98.75%), *T. cleftensis* CL1T (98.59%) and *T. pacificus* P-4T (98.05%).

Due to the high degree of similarity between the sequences of 16S rRNA genes, overall genomes relatedness indices were calculated. The genomes of strain Iri35cT and of its closest relatives shared OrthoANIu values ranging from 79.45% to 88.20% (Table 1 and Supplementary Table 1), and ANIb values between 78.68% and 87.31% (Table 1). These values are far below the ANI value of 95-96% generally accepted as a boundary for species delineation (Richter & Rossello-Mora, 2009). Digital DNA-DNA hybridization scores were also well below the DDH threshold level for species demarcation (70%), with values from 22.20% to 35.10% between the genome of strain Iri35cT and the genomes of its closest neighbors, respectively (Table 1 and Supplementary Table 1)(Wayne et al., 1987). These results based on standard genomes relatedness indexes provide evidence that strain Iri35cT represents a new genomic species (Rosselló-Móra and Whitman, 2019).
Morphological characteristics of strain Iri35\(^T\) were determined by light microscopy (Olympus BX60 and CX40) and scanning electron microscopy (FEI Quanta 200) (Supplementary Fig. 2). Cells were motile cocci that occurred generally singly and divided by constriction (Supplementary Fig. 2). Under optimal growth conditions and in the mid-exponential phase of growth, cells occurred as irregular cocci of 0.8-1.7 \(\mu\)m in diameter (mean 1.1 \(\pm\) 0.2 \(\mu\)m, \(n=31\)).

Unless stated otherwise, physiological assays were carried out anaerobically (N\(_2\) headspace) in modified Ravot medium, at 80°C, in duplicates, in the presence of elemental sulfur, as described elsewhere (Dalmasso et al., 2016). Growth tests were generally carried out as described previously (Dalmasso et al., 2016). Cells were routinely counted by direct cell counting using a modified Thoma chamber (depth 10 \(\mu\)m), and checked by flow cytometry (CyFlowSpace, Sysmex Partec, GmbH, Görlitz, Germany). 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 hyperthermophilic and grew between 50 and 90 °C with an optimum at 75-80 °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 Iri35\(^T\) 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 pH 10.0 (initial pH at 20 °C) with increments to 1 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 5.5-6.5, MES buffer; for pH 7.0, PIPES buffer; for pH 7.5-8.0, HEPES buffer; for pH 8.5, TAPS buffer; for pH 9.0 and 10.0, CAPSO buffer. Growth of strain \textit{Iri35c}\textsuperscript{T} was observed from pH 5.0 to pH 9.0, with an optimum around 7.0-7.5. The pressure range for growth of strain \textit{Iri35c}\textsuperscript{T} was tested into high-pressure high-temperature reactors (Top Industrie, Vaux-le-Pénil, France), at 0.1, 10, 20, 30, 40 and 50 MPa, as described previously (Alain \textit{et al}., 2002). The novel isolate was piezophilic, growing from atmospheric pressure (0.1 MPa), to at least 50 MPa, and showed optimal growth at 10-30 MPa. Under optimal growth conditions (80°C, pH 7.0, 2% NaCl, 5 g.L\textsuperscript{-1} S\textsuperscript{2}, and 20 MPa), the doubling time of the novel isolate was 80 minutes.

Utilization of various individual substrates for growth was tested in a basal medium supplemented with 0.05 % (w/v) yeast extract (YE) as growth factor, and without this growth factor, as described previously (Dalmasso \textit{et al}., 2016). 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) and glucose (20 mM). Positive controls were performed for all tests, and unsupplemented media were used as negative controls. To examine the ability of the strain to grow in the absence of elemental sulfur, cells were cultivated in modified Ravot medium with-
out sulfur. Alternative electron acceptors were also tested in a sulfur-depleted medium, under a gas phase of N₂ (100%, 150 kPa): L-cystine (5 g L⁻¹), polysulfides (0.5 mM), thiosulfate (20 mM), sulfate (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 2 successive subcultures (1/100th subculturing), on the test medium. All tests were performed in duplicates and growth was confirmed after microscopic observation. Hydrogen sulfide production was monitored with a colorimetric test as described previously (Cord-Ruwisch, 1985). Gas concentrations in the headspace phase were determined using a modified INFICON/MicroGC FUSION Gas Analyser (INFICON, Basel, Switzerland) fitted with a pressure gauge (CTE8005AY0, Sensortechnics GmbH) and two conductivity detectors. Separation was performed using two columns: molecular sieve 10 m column and argon as a carrier gas; and a RT-Q12 m using helium as a carrier gas. Cations and anions produced from peptone and yeast extract fermentation were identified by ionic chromatography on a Dionex ICS-900 Ion Chromatography System (Dionex, Camberley UK) coupled with a CERS 500 4 mm suppressor and a DS5 conductivity detector (40°C) and fitted with a 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, Waltham, MA, USA).

In the presence of elemental sulfur and under strict anaerobic conditions, complex carbon sources such as yeast extract, peptone, tryptone and beef extract supported fast and significant
growth. Cations and anions produced by the fermentation of peptone and yeast extract included
formate, acetate, propionate, isobutyrate, succinate or malate, isovalerate, thiosulfate, ammo-
nia, carbon dioxide and hydrogen sulfide. The presence of all or part of these organic acids has
already been reported as products of amino acid catabolism in other *Thermococcus* species (*T.
gorgonarius, T. alcaliphilus, T. piezophilus...*) (Keller et al., 1995; Miroshnichenko et al.,
1998; Dalmasso et al., 2016). The production of ammonium, already reported in *T. alcaliphilus*
for example, probably results from the transamination or oxidation of amino acids (Keller et
al., 1995). In the absence of elemental sulfur, the strain produced hydrogen by fermentation of
peptone and yeast extract. Under our experimental conditions, no obvious growth was ob-
served with the other carbon sources tested, with the exception of maltose which slightly en-
hanced growth. Poor growth was observed on peptone and yeast extract under pure fermenta-
tion conditions, in the absence of sulfur species. Although not necessary for growth, L-cystine
and elemental sulfur clearly stimulated the growth of the strain, and were both reduced to hy-
drogen sulfide. None of the other sulfur species tested (sulfate, thiosulfate, sulfite,
polysulfides) had an effect on growth. Nitrate, nitrite and oxygen (aerobic to microaerophilic
conditions) were not used by the cells as terminal electron acceptors. 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. Similarly, growth on chitin and starch has not been tested because the degra-
dation pathways of these compounds are incomplete based on the MetaCyc database.
The annotation of the Iri35c\textsuperscript{T} genome confirmed that the strain has the genetic potential to grow organoheterotrophically from peptides, amino acids and carbohydrates. Concerning the catabolism of peptides and amino acids, the genome encodes several proteases and two central enzymes involved in the oxidation of amino acids into their respective organic acids: the alanine aminotransferase (AlaAT; TIRI35C\_0605) and the glutamate dehydrogenase (GDH; TIRI35C\_0530). It has been proposed that these two enzymes, AlaAT and GDH, may act in a coordinated manner to maintain the redox balance in Thermococcales metabolism and to form an electron sink, by modulating the transformation of pyruvate towards acetate or alanine; alanine could be accumulated as an end-product under high H\textsubscript{2} partial pressure and in the absence of sulfur (Kengen & Stams, 1994; Ward \textit{et al.}, 2000). Based on MicroCyc, the degradation pathways for 6 amino acids are complete in the genome of strain Iri 35c\textsuperscript{T}: alanine, arginine, asparagine, aspartate, glutamine and glycine. These results are congruent with the growths observed on complex proteinaceous substrates. With regard to the catabolism of sugars, the genome contains notably an ABC-type maltose/maltodextrin transport system MalEFGK (TIRI35C\_0120- TIRI35C\_0122-0124), a cyclomaltodextrinase (TIRI35C\_0125), three alpha-amylases (TIRI35C\_1801; TIRI35C\_1925; TIRI35C\_2170), one pullulanase (TIRI35C\_0121) and a complete Embden-Meyerhof-Parnas pathway V (including three enzymes unique to this modified version of the glycolysis pathway, typically found within Thermococcales (Sakuraba \textit{et al.}, 2004), namely: an ADP-dependent glucokinase (locus tag TIRI35C\_0588), an ADP-dependent phosphofructokinase (TIRI35C\_1487) and a glyceraldehyde-3-phosphate:ferredoxin
oxidoreductase (TIRI35C_2006)). It encodes also full degradation pathways for glycerol, D-mannose and melibiose. In *Thermococcales* the catabolism of carbohydrates produces reducing equivalents as reduced ferredoxins, while the catabolism of amino acids produces both NADPH and reduced ferredoxins. Reduced ferredoxins are used by two main types of membrane-bound hydrogenases, Mbh and Mbs (previously termed Mbx), which conserve energy by creating an ion gradient across the membrane, and this gradient can then be used by an ATP synthase (TIRI35C_0071-0079) to produce energy (Lipscomb *et al*., 2017). These two main types of membrane-bound hydrogenases, which are respectively hydrogenogenic (Mbh) and sulfidogenic (Mbs) are mobilized according to whether or not there is sulfur in the culture medium (Lipscomb *et al*., 2017). Both are present in the genome of strain Iri35cT (Mbh: TIRI35C_2078 – 2097; Mbs: TIRI35C_0918-0921, TIRI35C_0406-0409). In addition to these membrane-bound hydrogenases, the genome encodes also cytosolic hydrogenases. The gene cluster encoding the formate hydrogenlyase complex which is present in several *Thermococcales*, combines H2 oxidation with CO2 reduction to form formate (which could mitigate H2 saturation under hydrogenogenic growth conditions) (Topçuoğlu *et al*., 2018), is incomplete in this draft genome, which suggests that formate is produced by another pathway in this strain. However, the genome codes for the pyruvate formate lyase activating-enzyme (TIRI35C_0782) which allows the conversion of pyruvate (the end-product of glycolysis) into formate and acetyl Co-A, and might explain the formate production as a catabolic end-product.
In summary, like many *Thermococcales*, strain Iri35c<sup>T</sup> is a strict anaerobic archaeon growing chemoorganoheterotrophically on complex proteinaceous substrates, whose growth is very largely stimulated by the presence of elemental sulfur or L-cystine to detoxify the dihydrogen produced by its metabolism (deleterious to its growth) and thus produce a high biomass in culture.

As is very often observed in the genus *Thermococcus*, which forms a fairly homogeneous group in terms of physiology, there are very few phenotypic differences between strain Iri35c<sup>T</sup> and its phylogenetically closely related species. These differences are summarized in Table 1. Strain Iri35c<sup>T</sup> is distinguishable from *T. pacificus* by the fact that it does not require the presence of sulfur to grow. In addition, the novel isolate has a slightly lower optimal growth temperature than the majority of its closest relatives.

Therefore, from the clear genotypic distance, the many physiological similarities and some phenotypical differences, we comply with the phylo-phenetic concept that currently prevails for the description of a new species. Thus, we proposed to assign strain Iri35c<sup>T</sup> to a novel species, for which the name *Thermococcus camini* sp. nov. is proposed.

**Description of Thermococcus camini sp. nov.**

*Thermococcus camini* (ca.mi’ní. L. gen. n. camini of a furnace, referring to the isolation of the type strain from a hydrothermal chimney).
Cells are irregular motile cocci (diameter: 1.1 ± 0.2 µm). Obligately anaerobic. Growth is observed at temperatures between 50 and 90 °C (optimum 75-80 °C), at NaCl concentration from 1 to 5% (optimum 2 %) and at pH from 5.0 to 9.0 (optimum 7.0-7.5). Piezophilic growing optimally under 10-30 MPa. S° or L-cystine is not required for growth but definitely stimulates growth. Does not use sulfate, thiosulfate, sulfite, polysulfide, 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) and growth is slightly enhanced by maltose addition. Fermentation products (on peptone, yeast extract and S°) include isovalerate, isobutyrate, acetate, propionate, formate, succinate/or malate, ammonia, thiosulfate, carbon dioxide and hydrogen sulfide.

The type strain Iri35cT (=UBOCC-M-2026T =DSM 111003T) was isolated from a deep-sea chimney rock sample collected at a depth of 2,300 m from a hydrothermal chimney at the Rainbow vent field, Mid-Atlantic Ridge. The genomic DNA G+C content of the type strain is 54.63 %.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and draft genome sequences are MT921160 and LR881183.1, respectively.

Funding information
This work was supported by the program MERLIN Abyss to K.A, the "Laboratoire d'Excellence" LabexMER Axis 3 programs ENDIV and CULTIVENT (ANR-10-LABX-1) to K.A., the Sino-French IRP 1211 MicrobSea to K.A. The study was supported by a grant from the French Ministry of Higher Education and Research, to D.C.
The authors thank the co-chiefs, crew and shipboard scientific party of the oceanographic expedition IRIS. We are grateful to Erwan Roussel for his expert advice on ionic chromatography analyses. 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. The LABGeM (CEA/Genoscope & 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.

**Author contributions**

Author contributions: Formal analysis, D.C. and K.A.; Funding acquisition, L.M. and K.A.; Investigation, D.C., E.V., N.G., X.P. and K.A.; Supervision, L.M., Z.S. and K.A.; Validation, D.C., E.V., N.G. and K.A.; Writing—original draft, D.C. and K.A.; Writing—review & editing, D.C., L.M., Z.S. and K.A. All authors have read and agreed to the published version of the manuscript.

**Conflict of interest**

We declare no conflict of interest and no source of indirect financial support.

**References**


**Figure and Table legends**

**Fig. 1.** Phylogenetic tree of the strain Iri35cT and representatives of *Thermococcales*, based on 49 ribosomal proteins (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). The tree was built by maximum likelihood (PhyML). Branch support, shown on the tree, was computed with the aLTR SH-like method. Bar, 0.1 amino-acid substitution rate.

**Table 1.** Characteristics differentiating strain Iri35cT from closest species of the genus *Thermococcus*.

| Strains: | 1. Iri35cT (data from this study); 2. *T. celericrescens* TS2T (Kuwabara et al., 2007); 3. *T. siculi* RG20T (Grote et al., 1999); 4. *T. cleftensis* CL1T (Holden et al., 2001; Hensley et al., 2014); 5. *T. pacificus* P-4T (Miroshnichenko et al., 1998). |
| Characteristics are scored as: +, positive; –, negative; S, Stimulatory; R, required; ND, not determined; §, data from the literature; *, data obtained for the five strains under same experimental laboratory conditions. |