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

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17

18 **Subject:** NEW TAXA Archaea

19 **Running title:** *Thermococcus camini* sp. nov.

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.

27 The DDBJ[GenBank/ENA] accession number for the genome sequence of *Thermococcus camini*
28 Iri35c^T sp. nov. is LR881183.1. The complete 16S rRNA gene sequence of *T. camini* sp. nov. strain
29 Iri35c^T is available at GenBank/EMBL/DDBJ/PIR under accession no. MT921160.

30 Two supplementary figures and one supplementary table are available with the online version of this
31 paper.

32

33 **Abstract:** A coccoid-shaped, strictly anaerobic, hyperthermophilic and piezophilic
34 organoheterotrophic archaeon, strain Iri35c^T, was isolated from a hydrothermal chimney rock
35 sample collected at a depth of 2,300 m at the Mid-Atlantic Ridge (Rainbow vent field). Cells of
36 strain Iri35c^T grew at NaCl concentrations ranging from 1 to 5% (w/v) (optimum 2.0%), from
37 pH 5.0 to 9.0 (optimum 7.0-7.5), at temperatures between 50 and 90 °C (optimum 75-80 °C)
38 and at pressures from 0.1 to at least 50 MPa (optimum: 10-30 MPa). The novel isolate grew on
39 complex organic substrates, such as yeast extract, tryptone, peptone or beef extract, preferen-
40 tially in the presence of elemental sulfur or L-cystine; however, these molecules were not nec-
41 essary for growth. Its genomic DNA G+C content was 54.63 %. The genome has been annotat-
42 ed and the metabolic predictions are in accordance with the metabolic characteristics of the
43 strain and of *Thermococcales* in general. Phylogenetic analyses based on 16S rRNA gene se-
44 quences and concatenated ribosomal protein sequences showed that strain Iri35c^T belongs to
45 the genus *Thermococcus*, and is closer to the species *T. celericrescens* and *T. siculi*. Average
46 nucleotide identity scores and *in silico* DNA-DNA hybridization values between the genome of
47 strain Iri35c^T and the genomes of the type species of the genus *Thermococcus* were below the
48 species delineation threshold. Therefore, and considering the phenotypic data presented, strain

49 Iri35c^T is suggested to represent a novel species, for which the name *Thermococcus camini* sp.
50 nov. is proposed, with the type strain Iri35c^T (=UBOCC-M-2026^T =DSM 111003^T).

51

52 Contrary to many orders within the *Archaea*, the order *Thermococcales* has led to the isolation
53 of numerous cultured representatives that have been the subject of physiological and genomic
54 studies. *Thermococcales* populate a variety of high-temperature natural ecosystems (deep-sea
55 and shallow-marine hydrothermal vents, terrestrial hot springs, oil reservoirs, solfataric sys-
56 tems, etc.), the most important of which are marine hydrothermal vents, from which the largest
57 number of isolates originate (Godfroy *et al.*, in press). Among the 3 genera (*Thermococcus*,
58 *Pyrococcus* and *Palaeococcus*) of the order *Thermococcales* (Bertoldo & Antranikian, 2006), it
59 is the genus *Thermococcus* that has led to the isolation of the largest number of species, with
60 33 species (with names validly recognized by the ICSP, the International Committee on Sys-
61 tematics of Prokaryotes) recorded to date. Physiological studies of these species have shown
62 that this genus is composed of hyperthermophilic anaerobic taxa developing mainly through
63 chemoorganoheterotrophy by coupling the oxidation of peptides or sugars to the reduction of
64 elemental sulfur and protons (Bertoldo & Antranikian, 2006). This genus is also described to
65 contain carboxydrotrophic species (i.e. Sokolova *et al.*, 2004; Kozhevnikova *et al.*, 2016).
66 Thanks to the different genetic tools available to work on models of *Thermococcus* species,
67 such as *Thermococcus kodakarensis* or *Thermococcus barophilus* for example, many function-
68 al studies have been carried out and have allowed significant advances in our knowledge of

69 their metabolism, genomic maintenance mechanisms and biological adaptations, instrumental
70 in advancing our understanding of the biology of the *Thermococcales* and of the *Archaea* in
71 general (e.g. Thiel *et al.*, 2014; Atomi & Reeve, 2019). In addition to being important and
72 ubiquitous players in the hot areas of the hydrothermal ecosystems, *Thermococcus* species are
73 also of particular interest for learning more about the cellular processes at the limits of life, as
74 this genus contains extremophilic and polyextremophilic organisms, adapted to one or more
75 extreme physical or chemical conditions of their natural habitat. In this way, all taxa of the
76 genus *Thermococcus* are hyperthermophilic, capable for some of them to divide up to a maxi-
77 mal temperature of 100°C (e.g. *T. eurythermalis*, *T. kodakarensis*, *T. peptonophilus*) (González
78 *et al.*, 1995; Atomi *et al.*, 2004; Zhao *et al.*, 2015), tolerating in addition high doses of gamma
79 radiation for some of them (e.g. *T. gammatolerans* resist up to 30 kGy of γ -radiation) (Jolivet
80 *et al.*, 2003), and with better growth under high hydrostatic pressure (e.g. *T. barophilus*, *T.*
81 *piezophilus*; *T. piezophilus* holds the current record of pressure range for growth, growing from
82 atmospheric pressure to 130 MPa) (Marteinsson *et al.*, 1999; Dalmaso *et al.*, 2016) or under
83 alkaline pH conditions for others (e.g. *T. alcaliphilus*) (Keller *et al.*, 1995).

84 In this article, we describe a novel hyperthermophilic organoheterotrophic sulfur-reducer,
85 strain Iri35c^T, isolated from a hydrothermal rock sample from the Rainbow vent field, at the
86 Mid-Atlantic Ridge. Genotypic and phenotypic characteristics meet the standard nomenclatural
87 criteria to delineate a novel species. We propose to name this new species *Thermococcus*
88 *camini*.

89

90 A chimney rock sample was collected at a depth of 2,300 m from a hydrothermal vent at the
91 Rainbow vent field (36°13'N, 33°54'W), at the Mid-Atlantic Ridge, in June 2001, during the
92 IRIS oceanographic cruise. Onboard, the sample was preserved in a sealed sterile anoxic vial
93 and stored at 4 °C, since the objective was to cultivate (hyper)thermophiles from this sample.
94 Once in the lab, enrichment cultures followed by three series of dilutions-to-extinction were
95 performed at 85°C, in reduced TRM medium (pH 6.8), containing 5 g.L⁻¹ elemental sulfur, as
96 described elsewhere (Zeng *et al.*, 2009). A collection of pure strains was then deposited in the
97 UBOCC collection (<https://ent.univ-brest.fr/lm2e/home/#/>), at -80 °C with 5% (v/v) DMSO.
98 The strain Iri35c^T described here bears the accession number UBOCC-M-2026^T. The purity of
99 this isolate was confirmed routinely by microscopic examination, and by sequencing of its ge-
100 nome.

101

102 The genomic DNA of Iri35c^T was extracted using a phenol-chloroform procedure. The TruSeq
103 DNA PCR-free kit (Illumina, USA) was then used to prepare paired-end sequencing libraries
104 with an average insert size of 550 nt, before the genome was sequenced using the Illumina's
105 MiSeq technology (2 × 300 bp paired-reads, V3 chemistry), at the Marine Biological Laborato-
106 ry
107 (Woods Hole, MA, USA). Paired-end reads were filtered with the Python package illumina-
108 utils using the command “iu-filter-quality-minoche” and default parameters (Minoche *et al.*,

109 2011; Eren *et al.*, 2013). The *de novo* assembly of the genome was carried out using CLC Ge-
110 nomics Workbench v8.5.1 ([https://www.qiagenbioinformatics.com/products/clc-genomics-](https://www.qiagenbioinformatics.com/products/clc-genomics-workbench)
111 [workbench](https://www.qiagenbioinformatics.com/products/clc-genomics-workbench)). A total of 482 309 read pairs of 300bp were used for the genome assembly, repre-
112 senting a mean coverage of about 120×. The assembled genome was analyzed and annotated
113 with the MicroScope Microbial Genome Annotation and Analysis Platform (MaGe)
114 (<https://mage.genoscope.cns.fr/microscope/home/index.php>) using KEGG and BioCyc data-
115 bases (Vallenet *et al.*, 2020). It consists of one circular chromosome of 2,022,529 base pairs in
116 size, and has a G+C content of 54.63 %. CheckM estimated the genome to be 100% complete
117 based on the presence of default single-copy marker genes (four markers were missing) and
118 without any contamination. The genome consists of 2,204 encoding protein sequences, 46
119 tRNA genes, a single 16S-23S rRNA operon, 2 5S rRNA, and 14 miscellaneous RNA genes.
120 This genome is available in DDBJ/ENA/GenBank under the accession number LR881183.1
121 (BioProject: PRJEB40155).

122

123 Pairwise 16S rRNA gene sequence similarity was determined using the EzTaxon-e server
124 (<http://eztaxon-e.ezbiocloud.net/>; (Kim *et al.*, 2012)). 16S rRNA gene sequences were aligned
125 using MAFFT v7.427 (parameters -maxiterate 1000 -localpair) (Kato and Stanley, 2013), and
126 the alignment was trimmed with BMGE v1.12 (default parameters) (Criscuolo and Gribaldo,
127 2010). Then, PhyML v. 3.3.20190909 was used to build the tree thanks to the web-server
128 <http://www.atgc-montpellier.fr/phyml/> (Guindon *et al.*, 2010). The evolutionary model was

129 selected with the SMS algorithm (Lefort *et al.*, 2017) and the branch support was computed
130 with the aLRT SH-like method. The tree was visualized with iTOL (Letunic and Bork, 2019)
131 and rooted between the *Thermococcus* and *Pyrococcus* genera. Since the 16S rRNA gene se-
132 quence is highly conserved and therefore not very discriminating between *Thermococcales*, a
133 phylogenetic tree based on ribosomal proteins was also constructed. This phylogenomic tree
134 was based on the concatenation of 49 ribosomal proteins shared by all genomes. Each protein
135 was aligned and trimmed separately with MAFFT (parameters -globalpair -maxiterate 1000)
136 and BMGE (default parameters), respectively. Then, each alignment block was concatenated
137 into a single alignment that was submitted to PhyML. The evolutionary model was selected
138 with SMS and the branch support was computed with the aLRT SH-like method. The tree was
139 visualized with iTOL and rooted between the *Thermococcus* and *Pyrococcus* branches. Aver-
140 age Nucleotide Identity scores (ANI) was calculated using two methods: OrthoANIu from the
141 EzBioCloud web server (<https://www.ezbiocloud.net/tools/ani>) (Yoon *et al.*, 2017); and ANIb
142 values by JSpeciesWS Online Service (Richter *et al.*, 2016;
143 <http://jspecies.ribohost.com/jspeciesws/#analyse>). These ANI scores were calculated between
144 the genome of strain Iri35c^T and the genomes of the 4 closest type species whose genomes are
145 available: *T. celericrescens* TS2^T (NZ_LLYW000000000.1), *T. siculi* RG20^T
146 (NZ_CP015103.1), *T. cleftensis* CL1^T (NC_018015.1) and *T. pacificus* P-4^T
147 (NZ_CP015102.1). Digital DNA-DNA hybridization (dDDH) scores were also determined by

148 the genome-to-genome distance calculator (GGDC 2.1), using the formula 2 (Meier-Kolthoff *et*
149 *al.*, 2013).

150 Phylogenetic analyses of the 16S rRNA gene sequences and concatenated ribosomal proteins
151 both confirmed that the new isolate branched within the archaeal genus *Thermococcus* (Fig. 1
152 and Supplementary Fig. 1). The most closely related species of strain Iri35c^T were
153 *Thermococcus celericrescens* TS2^T (99.66% 16S rRNA gene sequence similarity), *T. siculi*
154 RG-20^T (99.26%), *T. barossii* SHCK-94^T (98.79%), *T. thioreducens* DSM 14981^T (98.79%), *T.*
155 *hydrothermalis* AL662^T (98.75%), *T. cleftensis* CL1^T (98.59%) and *T. pacificus* P-4^T (98.05%).

156 Due to the high degree of similarity between the sequences of 16S rRNA genes, overall ge-
157 nomes relatedness indices were calculated. The genomes of strain Iri35c^T and of its closest
158 relatives shared OrthoANIu values ranging from 79.45% to 88.20% (Table 1 and Supplemen-
159 tary Table 1), and ANIb values between 78.68% and 87.31% (Table 1). These values are far
160 below the ANI value of 95-96% generally accepted as a boundary for species delineation
161 (Richter & Rossello-Mora, 2009). Digital DNA-DNA hybridization scores were also well be-
162 low the DDH threshold level for species demarcation (70%), with values from 22.20% to
163 35.10% between the genome of strain Iri35c^T and the genomes of its closest neighbors, respec-
164 tively (Table 1 and Supplementary Table 1)(Wayne *et al.*, 1987). These results based on stand-
165 ard genomes relatedness indexes provide evidence that strain Iri35c^T represents a new genomic
166 species (Rosselló-Móra and Whitman, 2019).

167

168 Morphological characteristics of strain Iri35c^T were determined by light microscopy (Olympus
169 BX60 and CX40) and scanning electron microscopy (FEI Quanta 200) (Supplementary Fig. 2).
170 Cells were motile cocci that occurred generally singly and divided by constriction (Supplemen-
171 tary Fig. 2). Under optimal growth conditions and in the mid-exponential phase of growth,
172 cells occurred as irregular cocci of 0.8-1.7 µm in diameter (mean 1.1 ± 0.2 µm, n=31).

173

174 Unless stated otherwise, physiological assays were carried out anaerobically (N₂ headspace) in
175 modified Ravot medium, at 80°C, in duplicates, in the presence of elemental sulfur, as de-
176 scribed elsewhere (Dalmasso *et al.*, 2016). Growth tests were generally carried out as described
177 previously (Dalmasso *et al.*, 2016). Cells were routinely counted by direct cell counting using a
178 modified Thoma chamber (depth 10 µm), and checked by flow cytometry (CyFlowSpace,
179 Sysmex Partec, GmbH, Görlitz, Germany). Cells were fixed with 2.5% (v/v) glutaraldehyde
180 (Sigma) and stored at -80°C, before counting by the two methods described above. Determina-
181 tion of the temperature range for growth was carried out at 45, 50, 55, 60, 65, 70, 75, 80, 85,
182 90, 95 and 100 °C. The isolate was hyperthermophilic and grew between 50 and 90 °C with an
183 optimum at 75-80 °C. Salt tolerance was tested at 80 °C with various concentrations of NaCl
184 (0, 1, 2, 3, 4, 5, 6, 7 and 8 %, w/v). Strain Iri35c^T required NaCl for growth and grew at NaCl
185 concentrations between 1 and 5 % (optimum: 2%). The pH range for growth was tested from
186 pH 3.0 to pH 10.0 (initial pH at 20 °C) with increments to 1 unit near the limits of the pH
187 range, and with increments of 0.5 unit around the optimum. For this experiment, we used the

188 following buffers (each at 20 mM, Sigma-Aldrich): for pH 3.0, no buffer; for pH 4.0 and 5.0,
189 HOMOPIPES buffer; for pH 5.5-6.5, MES buffer; for pH 7.0, PIPES buffer; for pH 7.5-8.0,
190 HEPES buffer; for pH 8.5, TAPS buffer; for pH 9.0 and 10.0, CAPSO buffer. Growth of strain
191 Iri35c^T was observed from pH 5.0 to pH 9.0, with an optimum around 7.0-7.5. The pressure
192 range for growth of strain Iri35c^T was tested into high-pressure high-temperature reactors (Top
193 Industrie, Vaux-le-Pénil, France), at 0.1, 10, 20, 30, 40 and 50 MPa, as described previously
194 (Alain *et al.*, 2002). The novel isolate was piezophilic, growing from atmospheric pressure (0.1
195 MPa), to at least 50 MPa, and showed optimal growth at 10-30 MPa. Under optimal growth
196 conditions (80°C, pH 7.0, 2% NaCl, 5 g.L⁻¹ S^o, and 20 MPa), the doubling time of the novel
197 isolate was 80 minutes.

198 Utilization of various individual substrates for growth was tested in a basal medium supple-
199 mented with 0.05 % (w/v) yeast extract (YE) as growth factor, and without this growth factor,
200 as described previously (Dalmasso *et al.*, 2016). The following substrates were tested, at the
201 final concentrations shown in brackets: tryptone (0.5% w/v), peptone (0.5% w/v), yeast extract
202 (0.5% w/v), beef extract (0.5% w/v), casamino acids (0.4% w/v), casein (0.5% w/v), formate
203 (20 mM), acetate (20 mM), pyruvate (20 mM), fumarate (20 mM), propionate (20 mM), suc-
204 cinate (20 mM), maltose (20 mM), fructose (20 mM), lactose (20 mM), ribose (20 mM),
205 galactose (20 mM) and glucose (20 mM). Positive controls were performed for all tests, and
206 unsupplemented media were used as negative controls. To examine the ability of the strain to
207 grow in the absence of elemental sulfur, cells were cultivated in modified Ravot medium with-

208 out sulfur. Alternative electron acceptors were also tested in a sulfur-depleted medium, under a
209 gas phase of N₂ (100%, 150 kPa): L-cystine (5 g.L⁻¹), polysulfides (0.5 mM), thiosulfate (20
210 mM), sulfate (20 mM), sulfite (5 mM), nitrate (20 mM), nitrite (5 mM) and dioxygen (0.5%,
211 5%, 20% v/v). Growth was monitored over 3 days of incubation. The results were considered
212 positive when growth was still observed after 2 successive subcultures (1/100th subculturing),
213 on the test medium. All tests were performed in duplicates and growth was confirmed after
214 microscopic observation. Hydrogen sulfide production was monitored with a colorimetric test
215 as described previously (Cord-Ruwisch, 1985). Gas concentrations in the headspace phase
216 were determined using a modified INFICON/MicroGC FUSION Gas Analyser (INFICON,
217 Basel, Switzerland) fitted with a pressure gauge (CTE8005AY0, Sensortech GmbH) and
218 two conductivity detectors. Separation was performed using two columns: molecular sieve 10
219 m column and argon as a carrier gas; and a RT-Q12 m using helium as a carrier gas. Cations
220 and anions produced from peptone and yeast extract fermentation were identified by ionic
221 chromatography on a Dionex ICS-900 Ion Chromatography System (Dionex, Camberley UK)
222 coupled with a CERS 500 4 mm suppressor and a DS5 conductivity detector (40°C) and fitted
223 with a RFC-10 Reagent-Free Controller™, an ASDV autosampler, and an IonPac CS16 col-
224 umn maintained at 60°C in a UltiMate™ 3000 Thermostated Column Compartment (Thermo
225 Scientific, Waltham, MA, USA).

226 In the presence of elemental sulfur and under strict anaerobic conditions, complex carbon
227 sources such as yeast extract, peptone, tryptone and beef extract supported fast and significant

228 growth. Cations and anions produced by the fermentation of peptone and yeast extract included
229 formate, acetate, propionate, isobutyrate, succinate or malate, isovalerate, thiosulfate, ammo-
230 nia, carbon dioxide and hydrogen sulfide. The presence of all or part of these organic acids has
231 already been reported as products of amino acid catabolism in other *Thermococcus* species (*T.*
232 *gorgonarius*, *T. alcaliphilus*, *T. piezophilus*...) (Keller *et al.*, 1995; Miroshnichenko *et al.*,
233 1998; Dalmaso *et al.*, 2016). The production of ammonium, already reported in *T. alcaliphilus*
234 for example, probably results from the transamination or oxidation of amino acids (Keller *et*
235 *al.*, 1995). In the absence of elemental sulfur, the strain produced hydrogen by fermentation of
236 peptone and yeast extract. Under our experimental conditions, no obvious growth was ob-
237 served with the other carbon sources tested, with the exception of maltose which slightly en-
238 hanced growth. Poor growth was observed on peptone and yeast extract under pure fermenta-
239 tion conditions, in the absence of sulfur species. Although not necessary for growth, L-cystine
240 and elemental sulfur clearly stimulated the growth of the strain, and were both reduced to hy-
241 drogen sulfide. None of the other sulfur species tested (sulfate, thiosulfate, sulfite,
242 polysulfides) had an effect on growth. Nitrate, nitrite and oxygen (aerobic to microaerophilic
243 conditions) were not used by the cells as terminal electron acceptors. Growth by
244 carboxydutrophy was not tested as the gene encoding the carbon monoxide dehydrogenase
245 CooF, one central protein in the *Thermococcales*'s carbon monoxide metabolism, was absent
246 from the genome. Similarly, growth on chitin and starch has not been tested because the degra-
247 dation pathways of these compounds are incomplete based on the MetaCyc database.

248 The annotation of the Iri35c^T genome confirmed that the strain has the genetic potential to
249 grow organoheterotrophically from peptides, amino acids and carbohydrates. Concerning the
250 catabolism of peptides and amino acids, the genome encodes several proteases and two central
251 enzymes involved in the oxidation of amino acids into their respective organic acids: the ala-
252 nine aminotransferase (AlaAT; TIRI35C_0605) and the glutamate dehydrogenase (GDH;
253 TIRI35C_0530). It has been proposed that these two enzymes, AlaAT and GDH, may act in a
254 coordinated manner to maintain the redox balance in *Thermococcales* metabolism and to form
255 an electron sink, by modulating the transformation of pyruvate towards acetate or alanine; ala-
256 nine could be accumulated as an end-product under high H₂ partial pressure and in the absence
257 of sulfur (Kengen & Stams, 1994; Ward *et al.*, 2000). Based on MicroCyc, the degradation
258 pathways for 6 amino acids are complete in the genome of strain Iri 35c^T: alanine, arginine,
259 asparagine, aspartate, glutamine and glycine. These results are congruent with the growths ob-
260 served on complex proteinaceous substrates. With regard to the catabolism of sugars, the ge-
261 nome contains notably an ABC-type maltose/maltodextrin transport system MalEFGK
262 (TIRI35C_0120- TIRI35C_0122-0124), a cyclomaltodextrinase (TIRI35C_0125), three alpha-
263 amylases (TIRI35C_1801; TIRI35C_1925; TIRI35C_2170), one pullullanase (TIRI35C_0121)
264 and a complete Embden-Meyerhof-Parnas pathway V (including three enzymes unique to this
265 modified version of the glycolysis pathway, typically found within *Thermococcales* (Sakuraba
266 *et al.*, 2004), namely: an ADP-dependent glucokinase (locus tag TIRI35C_0588), an ADP-
267 dependent phosphofructokinase (TIRI35C_1487) and a glyceraldehyde-3-phosphate:ferredoxin

268 oxidoreductase (TIRI35C_2006)). It encodes also full degradation pathways for glycerol, D-
269 mannose and melibiose. In *Thermococcales* the catabolism of carbohydrates produces reducing
270 equivalents as reduced ferredoxins, while the catabolism of amino acids produces both
271 NADPH and reduced ferredoxins. Reduced ferredoxins are used by two main types of mem-
272 brane-bound hydrogenases, Mbh and Mbs (previously termed Mbx), which conserve energy by
273 creating an ion gradient across the membrane, and this gradient can then be used by an ATP
274 synthase (TIRI35C_0071-0079) to produce energy (Lipscomb *et al.*, 2017). These two main
275 types of membrane-bound hydrogenases, which are respectively hydrogenogenic (Mbh) and
276 sulfidogenic (Mbs) are mobilized according to whether or not there is sulfur in the culture me-
277 dium (Lipscomb *et al.*, 2017). Both are present in the genome of strain Iri35c^T (Mbh:
278 TIRI35C_2078 – 2097; Mbs: TIRI35C_0918-0921, TIRI35C_0406-0409). In addition to these
279 membrane-bound hydrogenases, the genome encodes also cytosolic hydrogenases. The gene
280 cluster encoding the formate hydrogenlyase complex which is present in several
281 *Thermococcales*, combines H₂ oxidation with CO₂ reduction to form formate (which could
282 mitigate H₂ saturation under hydrogenogenic growth conditions) (Topçuoğlu *et al.*, 2018), is
283 incomplete in this draft genome, which suggests that formate is produced by another pathway
284 in this strain. However, the genome codes for the pyruvate formate lyase activating-enzyme
285 (TIRI35C_0782) which allows the conversion of pyruvate (the end-product of glycolysis) into
286 formate and acetyl Co-A, and might explain the formate production as a catabolic end-product.

287 In summary, like many *Thermococcales*, strain Iri35c^T is a strict anaerobic archaeon growing
288 chemoorganoheterotrophically on complex proteinaceous substrates, whose growth is very
289 largely stimulated by the presence of elemental sulfur or L-cystine to detoxify the dihydrogen
290 produced by its metabolism (deleterious to its growth) and thus produce a high biomass in cul-
291 ture.

292

293 As is very often observed in the genus *Thermococcus*, which forms a fairly homogeneous
294 group in terms of physiology, there are very few phenotypic differences between strain Iri35c^T
295 and its phylogenetically closely related species. These differences are summarized in Table 1.
296 Strain Iri35c^T is distinguishable from *T. pacificus* by the fact that it does not require the pres-
297 ence of sulfur to grow. In addition, the novel isolate has a slightly lower optimal growth tem-
298 perature than the majority of its closest relatives.

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

303

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

305 *Thermococcus camini* (ca.mi'ni. L. gen. n. *camini* of a furnace, referring to the isolation of the
306 type strain from a hydrothermal chimney).

307 Cells are irregular motile cocci (diameter: $1.1 \pm 0.2 \mu\text{m}$). Obligately anaerobic. Growth is ob-
308 served at temperatures between 50 and 90 °C (optimum 75-80 °C), at NaCl concentration from
309 1 to 5% (optimum 2 %) and at pH from 5.0 to 9.0 (optimum 7.0-7.5). Piezophilic growing op-
310 timally under 10-30 MPa. S^o or L-cystine is not required for growth but definitely stimulates
311 growth. Does not use sulfate, thiosulfate, sulfite, polysulfide, nitrate, nitrite or oxygen (0.5%,
312 5%, 20% v/v) as electron acceptors. Chemoorganoheterotrophic growth occurs on complex
313 proteinaceous substrates (yeast extract, peptone, tryptone, beef extract) and growth is slightly
314 enhanced by maltose addition. Fermentation products (on peptone, yeast extract and S^o) in-
315 clude isovalerate, isobutyrate, acetate, propionate, formate, succinate/or malate, ammonia, thi-
316 osulfate, carbon dioxide and hydrogen sulfide.

317 The type strain Iri35c^T (=UBOCC-M-2026^T =DSM 111003^T) was isolated from a deep-sea
318 chimney rock sample collected at a depth of 2,300 m from a hydrothermal chimney at the
319 Rainbow vent field, Mid-Atlantic Ridge. The genomic DNA G+C content of the type strain is
320 54.63 %.

321 The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and draft genome se-
322 quences are MT921160 and LR881183.1, respectively.

323

324

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342 **Author contributions**

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348

349 **Conflict of interest**

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

351

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474

475 **Figure and Table legends**

476 **Fig. 1.** Phylogenetic tree of the strain Iri35c^T and representatives of *Thermococcales*, based on
477 49 ribosomal proteins (Proteins associated with the large ribosomal subunit: L1, L2, L3, L4,
478 L6, L7AE, L11, P1 (=L12P), L13, L15, L15E, L18, L18A, L18E, L19E, L21E, L22, L24, L29,
479 L30, L30E, L31E, L32E, L37AE, L37E, L39E, L40E, L44E; Proteins associated with the small
480 ribosomal subunit: S2, S3, S3AE, S4, S4E, S5, S6E, S7, S8, S8E, S9, S10, S12, S13, S15, S17,
481 S17E, S19, S19E, S27E, S28E). The tree was built by maximum likelihood (PhyML). Branch
482 support, shown on the tree, was computed with the aLTR SH-like method. Bar, 0.1 amino-acid
483 substitution rate.

484

485 **Table 1.** Characteristics differentiating strain Iri35c^T from closest species of the genus
486 *Thermococcus*.

487 Strains: 1, Iri35c^T (data from this study); 2, *T. celericrescens* TS2^T (Kuwabara *et al.*, 2007); 3,
488 *T. siculi* RG20^T (Grote *et al.*, 1999) ; 4, *T. cleftensis* CL1^T (Holden *et al.*, 2001 ;Hensley *et al.*,
489 2014); 5, *T. pacificus* P-4^T (Miroshnichenko *et al.*, 1998).

490 Characteristics are scored as: +, positive ; –, negative; S, Stimulatory; R, required; ND, not
491 determined; §, data from the literature; *, data obtained for the five strains under same experi-
492 mental laboratory conditions.

493