Pyrococcus kukulkanii sp. nov., a hyperthermophilic, piezophilic archaeon isolated from a deep-sea hydrothermal vent

Nolwenn Callac,[1,2,3,4,5]† Philippe Oger,[6] Françoise Lesongeur,[1,2,3] Jayne E. Rattray,[5] Pauline Vannier,[1,2,3] Grégoire Michoud,[1,2,3] Mickael Beauverger,[1,2,3] Nicolas Gayet,[7] Olivier Rouxel,[8] Mohamed Jebbar[1,2,3] and Anne Godfroy[1,2,3]

Correspondence
Anne Godfroy
anne.godfroy@ifremer.fr

[1]Université de Brest, UEB, IUEM, Laboratoire de Microbiologie des Environnements Extrêmes, UMR 6197, 4, rue Dumont d'Urville, 29280 Plouzané, France

[2]Ifremer, Laboratoire de Microbiologie des Environnements Extrêmes, UMR 6197, Technopôle Brest Iroise, CS10070, 29280 Plouzané, France

[3]CNRS, Laboratoire de Microbiologie des Environnements Extrêmes, UMR 6197, 4, rue Dumont d'Urville, 29280 Plouzané, France

[4]Université de Brest, Domaines Océaniques IUEM, UMR 6538, 4, rue Dumont d'Urville, 29280 Plouzané, France

[5]Department of Geological Sciences, Stockholm University, Stockholm, Sweden

[6]Univ Lyon, INSA de Lyon, CNRS UMR 5240, Lyon, France

[7]Ifremer, Laboratoire Environnements Profonds, Technopôle Brest Iroise, CS10070, 29280 Plouzané, France

[8]Ifremer, Laboratoire de Géochimie et de Métallogénie, Technopôle Brest Iroise, CS10070, 29280 Plouzané, France

A novel hyperthermophilic, piezophilic, anaerobic archaeon, designated NCB100^T, was isolated from a hydrothermal vent flange fragment collected in the Guaymas basin at the hydrothermal vent site named 'Rebecca's Roost' at a depth of 1997 m. Enrichment and isolation were performed at 100 °C under atmospheric pressure. Cells of strain NCB100^T were highly motile, irregular cocci with a diameter of ~1 μm. Growth was recorded at temperatures between 70 and 112 °C (optimum 105 °C) and hydrostatic pressures of 0.1–80 MPa (optimum 40–50 MPa). Growth was observed at pH 3.5–8.5 (optimum pH 7) and with 1.5–7 % NaCl (optimum at 2.5–3 %). Strain NCB100^T was a strictly anaerobic chemo-organoheterotroph and grew on complex proteinaceous substrates such as yeast extract, peptone and tryptone, as well as on glycogen and starch. Elemental sulfur was required for growth and was reduced to hydrogen sulfide. The fermentation products from complex proteinaceous substrates were CO_2 and H_2. The G+C content of the genomic DNA was 41.3 %. Phylogenetic analysis of the 16S rRNA gene sequence revealed that strain NCB100^T belongs to the genus *Pyrococcus*, showing 99 % similarity with the other described species of the genus *Pyrococcus*. On the basis of physiological characteristics, DNA G+C content, similarity level between ribosomal proteins and an average nucleotide identity value of 79 %, strain NCB100^T represents a novel species for which the name *Pyrococcus kukulkanii* sp. nov. is proposed. The type strain is NCB100^T (=DSM 101590^T=Souchothèque de Bretagne BG1337^T).

†**Present address:** Department of Geological Sciences, Stockholm University, Stockholm, Sweden.

The Genbank/EMBL/DDBJ accession number for the genome sequence of strain NCB100^T is CP010835; Bioproject PRJNA274214.

Organisms belonging to the order *Thermococcales* were shown to be key players in deep-sea hydrothermal vents. The order *Thermococcales* contains three genera: *Thermococcus* (Zillig *et al.*, 1983), *Pyrococcus* (Fiala & Stetter, 1986) and *Palaeococcus* (Takai *et al.*, 2000). Members of these genera are strict anaerobes, hyperthermophiles and chemo-organoheterotrophs growing on complex organic substrates by fermentation, often associated with sulfur reduction with a concomitant H_2S formation. The genus *Thermococcus* was shown to be one of the most abundant genera in the clone libraries and, so far, more than 30 species have been isolated and described (Flores *et al.*, 2011). The genera *Pyrococcus* and *Palaeococcus* are less abundant in molecular surveys and in culture collections. Only six species of the genus *Pyrococcus* have been described from shallow and deep-sea hydrothermal vents: *Pyrococcus furiosus* (Fiala & Stetter, 1986), *Pyrococcus woesei* (Zillig *et al.*, 1987), *Pyrococcus horikoshii* (González *et al.,* 1998), '*Pyrococcus abyssi*' (Erauso *et al.,* 1993), *Pyrococcus glycovorans* (Barbier *et al.*, 1999) and *Pyrococcus yayanosii* (Birrien *et al.*, 2011). Two other members of the genus *Pyrococcus* were isolated from terrestrial environments: *Pyrococcus* strain HT3 from the 'El Biban' hot spring in Algeria (Kecha *et al.*, 2007) and *Pyrococcus* strain Pikanate PK 5017 (Kanoksilapatham *et al.*, 2012) from sediment collected near the Pong Duet Hot Spring in Thailand. By contrast to the hydrothermal vent of Mariner (Mid-Atlantic Ridge) where species of the genus *Thermococcus* are dominant, members of the genus *Pyrococcus* appear to be key players in the Guaymas Basin hydrothermal vents (Jannasch *et al.*, 1992; Teske *et al.*, 2009). We describe here a novel species of the genus *Pyrococcus* isolated from a deep-sea hydrothermal vent of the Guaymas Basin.

A fragment of an active hydrothermal flange was collected, during the oceanographic cruise BIG (RV *L'Atalante* June–July 2010), on the 'Rebecca's Roost' vent site (27°00.634′ N 111°24.405′ W; 1997 m depth) in the Southern Trough area in the Guaymas Basin (Gulf of California, Mexico) using the manned submersible *Nautile* (dive 1747). The chimney fragment was brought to the surface in an aseptic insulated box previously filled on-board with sterile freshwater. Once on board, the sample (Pl 1747-BIG03E01) was immediately crushed and sub-sampled under sterile conditions in an anaerobic chamber (La Calhène) under a $N_2/H_2/CO_2$, 90 : 5 : 5, gas atmosphere and stored in sterile vials filled with sterile anaerobic NaCl solution (at 23 g l^{-1}) under anaerobic conditions ($N_2/H_2/CO_2$, 90 : 5 : 5) at 4 °C for further microbial cultivation. The chimney sample was used to inoculate anaerobic modified SME medium (10 %, v/v). The SME medium contained per litre of deionized water: 23 g NaCl; 1 g yeast extract; 1 g peptone; 3.46 g PIPES buffer; 10 ml magnesium solution (for 1 litre of deionized water: 180 g $MgSO_4 . 7H_2O$; 140 g $MgCl_26H_2O$), 1 mg resazurin; and 1 ml each of solutions A, B, C and D [composition for each solution, for 1 litre of deionized water: solution A: 9 g $MnSO_4 . 4H_2O$; 2.5 g $ZnSO_4 . 7H_2O$; 2.5 g $NiCl_2 . 6H_2O$; 0.3 g $AlK(SO_4)_2 . 12H_2O$; 0.3 g $CoCl_2 . 6H_2O$; 0.15 g $CuSO_4 . 5H_2O$; solution B: 5 g, 6 g $CaCl_2 . 2H_2O$; 25 g NaBr; 16 g KCl; 10 g KI; 4 g $SrCl_2 . 6H_2O$; solution C: 50 g K_2HPO_4; 7.5 g H_3BO_3; 3.3 g $Na_2WO_4 . 2H_2O$; 0.15 g $Na_2MoO_4 . 2H_2O$; 0.005 g Na_2SeO_3; and solution D: 10 g $FeCl_2 . 4H_2O$; all compounds from Sigma Aldrich, except yeast extract from Difco]. The pH was adjusted to pH 6.5 at room temperature. Once prepared, this medium was autoclaved and then cooled to room temperature. It was then transferred into an anaerobic chamber (La Calhène) containing a N_2/H_2 (90 : 10) gas atmosphere. Concentrated anaerobic, filter-sterilized solutions of poly-vitamins (Balch *et al.*, 1979) and elemental sulfur (sterilized by tyndallization at 100 °C for 30 min on two successive days) were added to the medium to final concentrations of 0.04 % (w/v) and 10 g l^{-1}, respectively. The medium was reduced with 10 ml of a sterile stock solution of $Na_2S . 9H_2O$ at 5 % (w/v) and then distributed into 50 ml penicillin vials closed with a butyl rubber and an aluminum cap. Initial cultures were incubated within a range of temperatures from 70 to 100 °C, at atmospheric pressure, in the dark and without shaking.

After 15 h of incubation, dense populations of coccoid, motile cells were observed under all culture conditions. A pure isolate, designated NCB100^T, was purified from the original culture at 100 °C using the dilution-to-extension technique and subculturing at 100 °C in SME medium. The purity of the isolate was confirmed by microscopic examination and by cloning and sequencing of the 16S rRNA gene. Cultures harvested in the exponential growth phase remained active for at a least 1 year at 4 °C. For long-term storage, cultures in SME medium were stored anaerobically in cryotubes at −80 °C after the addition of DMSO (5 %, v/v) as cryoprotectant.

Cells were observed using a light microscope equipped with a phase contrast oil-immersion objective (Olympus BX60) and appeared as slightly irregular cocci, appearing singly, occasionally in pairs, and divided by constriction. Cells were highly motile and archaella (archaeal flagella) were observed by scanning electron microscopy (Quanta 200; FEI) (Fig. 1). Cells diameter is 1.03±0.17 μm (n=130).

All further investigations were performed in SME medium. Growth rates were calculated using linear regression analysis of four points along the exponential portions of the resulting growth curves. Cells grown under specific conditions were collected at regular intervals and growth was estimated by direct cell counts using a Thoma chamber (depth 0.02 mm). All samples were tested in triplicate.

Strain NCB100^T grew only under strict anaerobic conditions within a temperature range from 70 to 112 °C, with optimum growth at 105 °C. It grew under high hydrostatic pressure within the range 0.1 to 80 MPa, with an optimum of approximately 50 MPa, when tested at optimal temperature (105 °C). Increased hydrostatic pressure did not allow growth at 115 °C or higher temperatures. The isolate grew

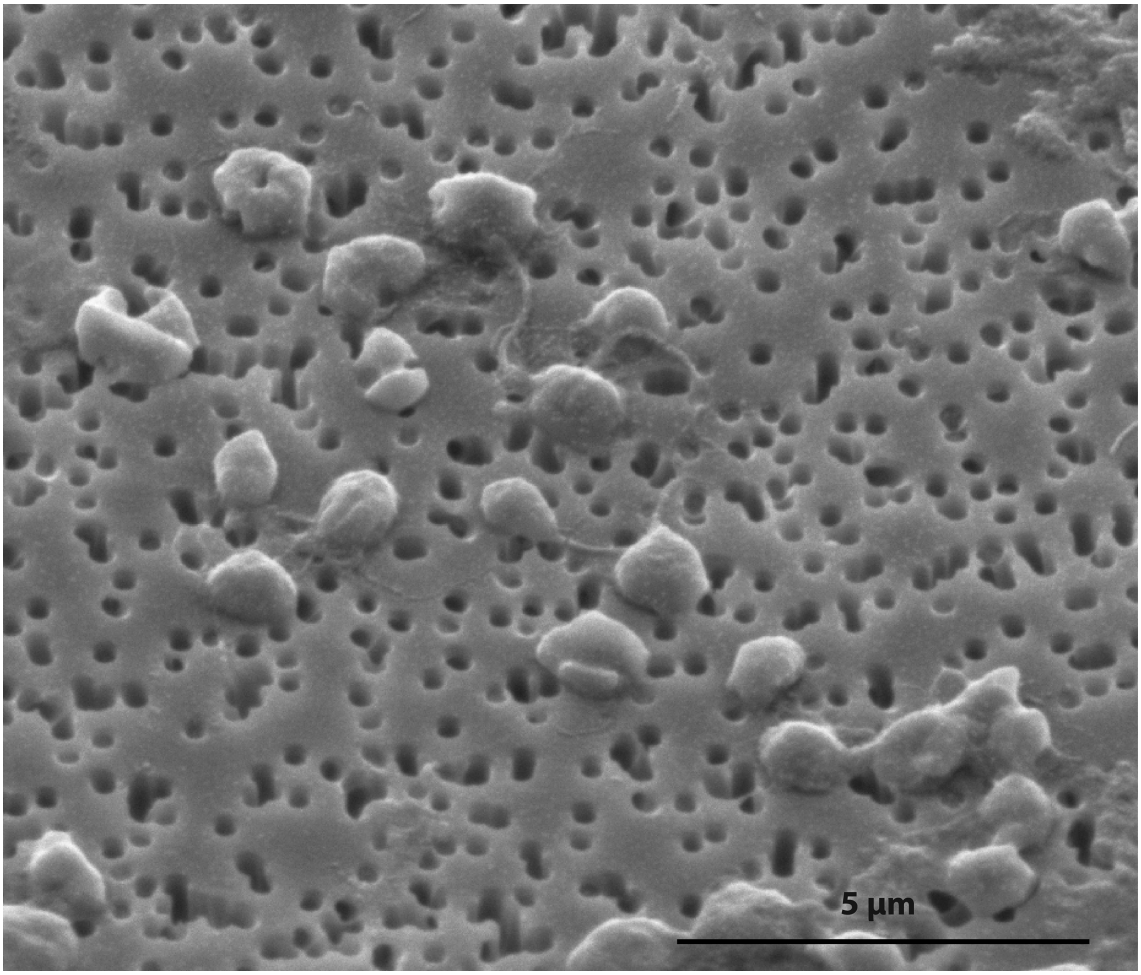

Fig. 1. Scanning electron micrograph of cells of strain NCB100^T in the mid-exponential phase of growth showing the archaella. Bar, 5 µm.

from pH 3.5 to 8.5, with an optimum of approximately pH 7.0. No growth was observed at pH 3.0 or at pH 9.0. The isolate required salt and grew in the presence of NaCl concentrations ranging from 1.5 to 7 % (w/v) with an optimum at ~2.5–3 %. No growth was observed with 1 % or 7.5 % NaCl.

Growth requirements and substrate utilization assays were performed in triplicate in 27 ml Hungate tubes containing 10 ml of SME medium at pH 6.5 and at 100 °C with the addition of elemental sulfur.

Carbon source utilization was tested in SME medium prepared without yeast extract or peptone. Maltose, glucose, sucrose, galactose, cellobiose, starch, glycogen, acetate, formate, pyruvate, succinate, ethanol, glycerol, chitin, casein, casamino acids, yeast extract, peptone or tryptone were added to the medium (final concentration 5 g l^{-1} except ethanol and glycerol 5 ml l^{-1}). H_2S production was determined under all conditions using the Cord-Ruwisch test (Cord-Ruwisch, 1985). Nitrogen sources were tested in nitrogen-depleted SME medium, e.g. SME medium in which yeast extract and peptone were replaced by 5 g glycogen l^{-1} as carbon source and elemental sulfur (10 g l^{-1}) as electron acceptor. NH_4Cl (20 mM), nitrate (10 mM), urea (2 g l^{-1}), gelatin (2 g l^{-1}), yeast extract (0.5 %, w/v), peptone (0.5 %, w/v), tryptone (0.5 % w/v), a mixture of 20 amino acids each at 0.1 mM and N_2 gas phase were tested as potential nitrogen sources. To examine the ability of the isolate to grow in the absence of elemental sulfur, cells were cultured in SME medium with glycogen as a carbon source (5 g l^{-1}). Alternative electron acceptors were also tested: sulfate, thiosulfate, sulfite and nitrate at 10 mM; L-cystine at 10 g l^{-1}; ferric iron, and oxygen, in a sulfur-depleted medium under gas phase of N_2 or N_2/CO_2 (90 : 10, 150 kPa).

Growth was monitored over 2 days of incubation. Negative and positive controls were performed for each substrate. For all tests, positive cultures were transferred into the test medium for confirmation of growth twice, after which they were considered positive for growth.

Strain NCB100^T used a variety of proteinaceous substrates and carbohydrates (yeast extract, peptone, tryptone, starch and glycogen) as carbon and energy sources. Sulfate, nitrate and thiosulfate were not used as electron acceptors. Growth was observed with all nitrogen sources tested except with NH_4Cl. Growth was possible only in the presence of elemental sulfur with the concomitant production of hydrogen sulfide. The fermentation products of complex proteinaceous substrates were analysed using HPLC/DAD (210 nm) and according to Phenomenex application note 23365. Organic acids were eluted on an F5 column isocratically with 20 mM potassium phosphate (pH 2.9), and the sample was compared with organic acid standard solutions. Headspace gasses, except hydrogen, were analysed using an SRI GC fitted with an FID detector. Culture headspace gas was injected into helium-pre-flushed Exetainer vials containing 1 ml 20 % ZnAc (bubbling through to precipitate H_2S), and subsequently 3 ml of the headspace was extracted and injected onto the GC. The hydrogen, collected directly from the headspace gasses, was analysed using a Peak Performer 1 gas analyser. The main fermentation products were CO_2 and H_2, no organic acids were produced during the growth of the strain on proteinaceous compounds.

For the core lipid analysis, a small pellet of the biomass was hydrolysed using acid methanolysis. Briefly, the sample was extracted using a 10 % mixture of HCl (37 %) in methanol and placed in the oven at 110 °C for 2 h. After cooling and pH adjustment, the mixture was extracted using a 1 : 1 mixture of hexane and dimethyl chloromethane (DCM). The solvent supernatant was combined and dried under N_2. Lipid analysis was performed using HPLC/MS for the analysis of glycerol dialkyl tetraethers. Archaeol (diphytanylglycerol diether) was the dominant core lipid identified in the hydrolysed lipid extracts and no caldarchaeol was observed. In addition a range of glycerol dialkyl glycerol tetraethers were identified with the masses; m/z 1302, m/z 1300, m/z 1298, m/z 1296 and m/z 1292.

The genomic DNA G+C content was determined from the melting point according to the method of Marmur & Doty (1962), by the Identification Service of the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany). The DNA G+C content of strain NCB100^T was 44.6 mol%.

For genomic analysis, DNA was extracted from cells grown to late exponential phase as described previously (Barbier *et al.*, 1999).

The 16S rRNA gene was amplified using the archaeal 16S rRNA gene universal primers A8f (5′-CGGTGGATCC TGCCGGA-3′) and ARC915R (5′-CTGCTCCCCCGCCAA TTCCT-3′). Prior to cloning, positively amplified PCR products were gel-purified using a NucleoSpin Gel and PCR Clean-up kit (Macherey Nagel) according the manufacturer's instructions. The 16S rRNA gene clone libraries were

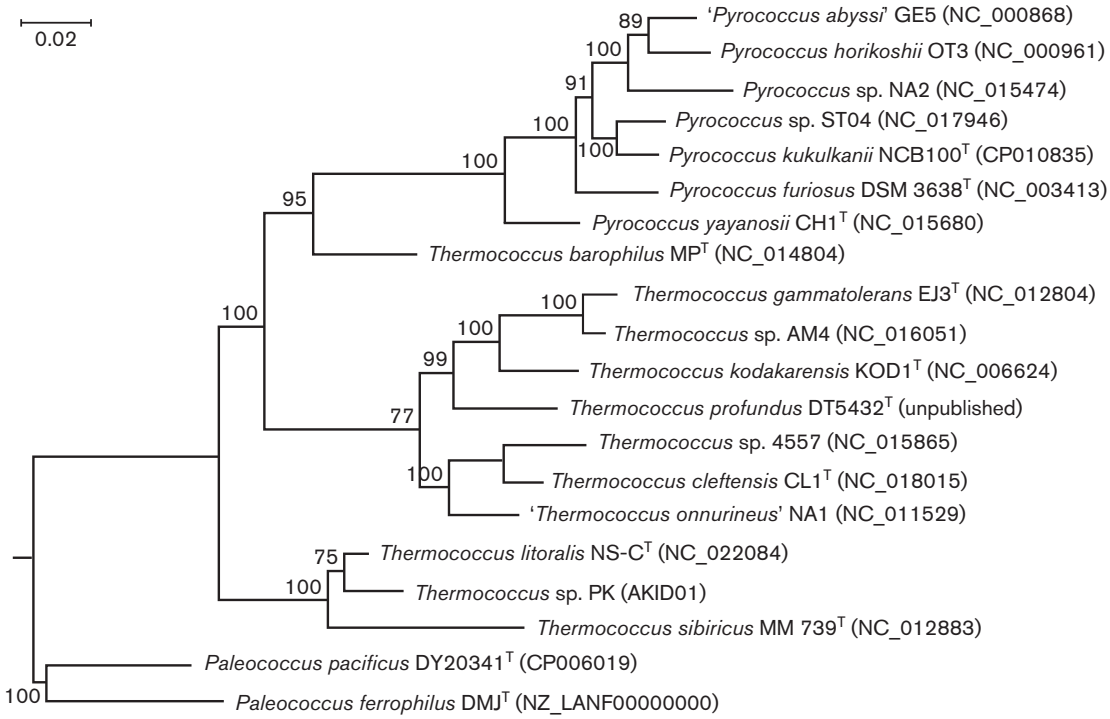

Fig. 2. Phylogenetic tree based on concatenated ribosomal proteins. Accession numbers for all genomes are shown in parentheses. The *Palaeococcus ferrophilus* DMJ^T genome is available from the Joint Genome Institute (JGI). Numbers at nodes represent percentage bootstrap values based on 1000 replicates; only those above 50% are displayed. Bar, 2 nt substitution per 100 nt branch length.

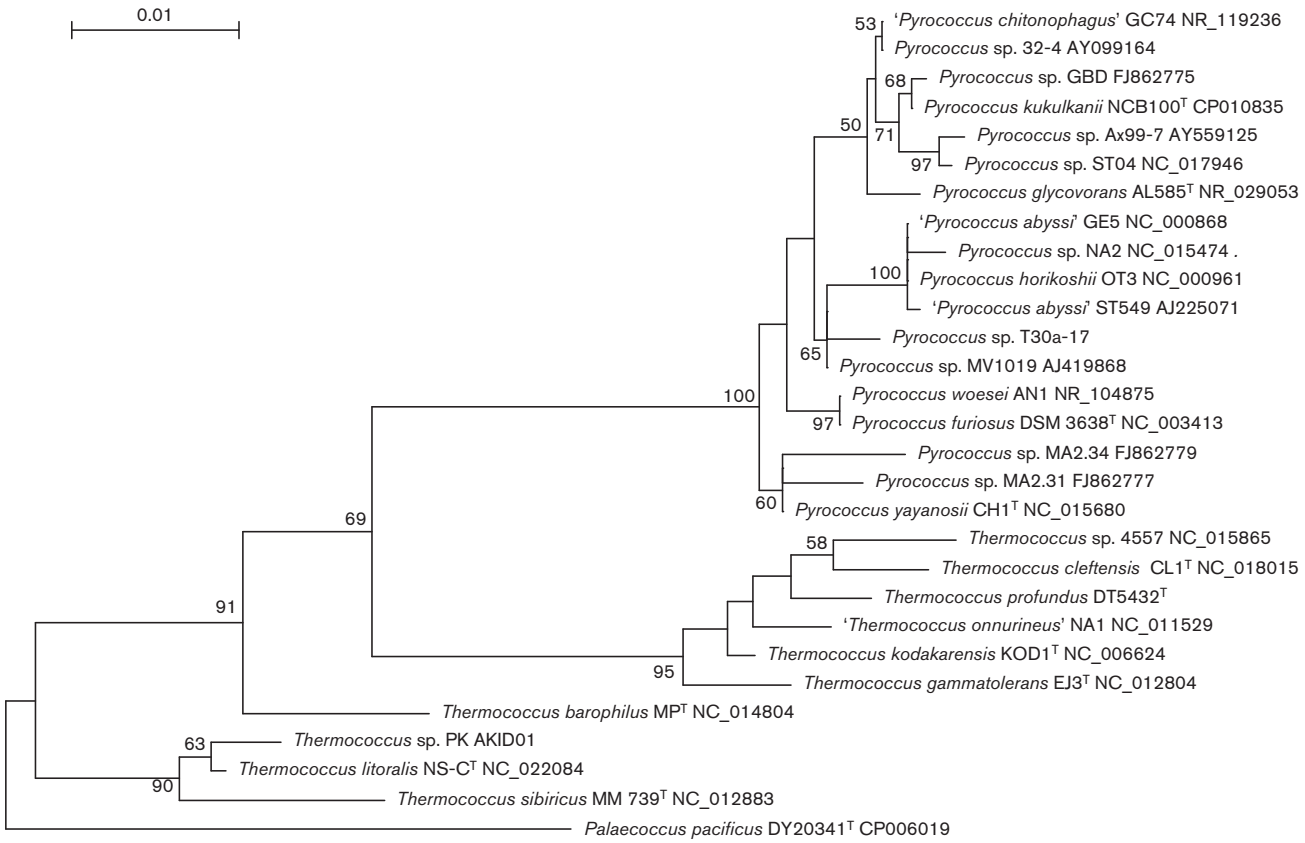

Fig. 3. Phylogenetic tree based on partial 16S ribosomal gene sequences. Accession numbers are indicated. Numbers at nodes represent percentage bootstrap values based on 1000 replicates; only those above 50% are displayed. Bar, 1 nt substitution per 100 nt branch length.

prepared with the TOPO XL cloning kit (Invitrogen) following the manufacturer's recommendations. Positive clones were processed for sequencing at GATC Biotech190 (Konstanz, Germany) using M13F primers. The analysis of the 16S rRNA gene sequence of strain NCB100^T (730 bp) showed that the strain belonged to the genus *Pyrococcus* and was most closely related to all the other described species of the genus *Pyrococcus*,sharing 99 % sequence similarity.

Since most species of the genus *Pyrococcus* have very similar 16S rRNA gene sequences, the complete genome sequence of strain NCB100^{T}was determined to examine its proximity to other known species of the genus *Pyrococcus*. The genome of strain NCB100^T is composed of a single circular chromosome, 1977 kb in length, with no extrachromosomal elements, encoding 2242 putative coding sequences. The proximity with other known species was estimated by the comparison of concatenated ribosomal protein sequences as described previously (Matte-Tailliez *et al.*, 2002; Brochier-Armanet *et al.*, 2008; Birrien *et al.*, 2011). Common ribosomal proteins (CRP) from the known members of the order *Thermococcales* were concatenated to form a single protein sequence of approximately 8100 residues. CRP were aligned in Seaview with Muscle (Gouy *et al.*, 2010). A phylogenetic tree based on CRP sequences was reconstructed using the PHYML algorithm with the two species of the genus *Palaeococcus* as an outgroup (Fig. 2). This phylogenetic tree clearly separated the different species of the order *Thermococcales*, placing strain NCB100^T on a branch with another undescribed *Pyrococcus* clone named ST04. This branch is most closely related to *Pyrococcus furiosus*, with a highly supported tree topology with bootstrap values close to 100 % for all branches. The tree topology was affected only marginally when an alternate sequence aligner or different phylogenetic reconstruction algorithms were used. The local topology of the CRP phylogenetic tree around the NCB100^T branch was also congruent with the one obtained by using 16S rRNA gene sequences (Fig. 3). In addition, *in silico* hybridization yielded an ANI value (average nucleotide identity of protein-coding genes shared at $\geq$60 % nucleotide identity and $\geq$70 % coverage) of less than 79 % between strain NCB100^T and any of the species of the genus *Pyrococcus*. This ANI value is lower than the 95–96 % cut-off value shown to correspond to the 70 % DNA–DNA hybridization level accepted to delimit microbial species.

Table 1. Comparison of characteristics of strain NCB100^T and related members of the genus *Pyrococcus*

Data for *Pyrococcus yayanosii* were taken from Birrien *et al.* (2011); *Pyrococcus glycovorans* from Barbier *et al.* (1999); '*Pyrococcus abyssi*' from Erauso *et al.* (1993); *Pyrococcus horikoshii* from González *et al.* (1998); *Pyrococcus furiosus* from Fiala & Stetter (1986); *Pyrococcus* sp. strain GB-D from Jannasch *et al.* (1992); *Pyrococcus* sp. strain HT3 from Kecha *et al.* (2007) and *Pyrococcus* sp. strain Pikanate PK 5017 from Kanoksilapatham *et al.* (2012). All taxa show strictly anaerobic metabolism. +, Positive; −, negative; W, weakly positive growth; ND, not determined.

Characteristic	NCB 100^T	*Pyrococcus yayanosii*	*Pyrococcus* glycovorans	'*Pyrococcus* abyssi'	*Pyrococcus* horikoshii	*Pyrococcus* furiosus	Strain GB-D	Strain HT3	Strain Pikanate PK 5017
Geographical origin	Rebecca's Roost, Guaymas Basin	Ashadze, Mid-Atlantic Ridge	13° N, East Pacific Rise	North Fidji Basin, Pacific Ocean	Okinawa Trough, Pacific Ocean	Vulcano, Italy	Guaymas Basin	El Biban hot spring, Algeria	Pong Duet hot spring, Thailand
Depth (m)	1997	4100	2650	2000	1395	0	2020	0	1000 above sea level
Motility/morphology	Motile and irregular cocci	Motile cocci	Motile cocci	Motile cocci	Irregular cocci	Motile and irregular cocci	Irregular cocci	Motile cocci	Irregular cocci
Size (μm)	1.03±0.17	0.6–1.4	0.5–1.5	0.8–2	0.8–2	0.8–2.5	0.8–1	ND	0.7–1.2
Growth conditions									
Temperature range (°C)	70–112	70–108	75–104	67–102	80–102	70–103	65–103	70–95	75–105
Optimum temperature (°C)	105	98	95	96	98	100	95	85	95–100
pH range	3.5–8.5	6–9.5	2.5–9.5	4–8.5	5–8	5–9	ND	6–9	5–7.8
Optimum pH	7	7.5–8	7.5	6.8	7	7	ND	7.5	7.2
NaCl range (%, w/v)	1.5–7	2.5–5.5	1.7–5.2	0.7–5	1–5	0.5–5	ND	1–2.5	0.25–4
Optimum NaCl range (%, w/v)	2.5–3	3.5	2.6	3	2.4	2	ND	2	2.4
DNA G+C content (mol%)	44.6	51.6	47	44.7	41.9	40.8	39.47	43	42.5
Carbon sources									
Yeast extract	+	+	+	+	+	+	+	+	+
Peptone	+	+	+	+	+	+	+	+	+
Tryptone	+	+	ND	+	+	+	+	+	ND
Casein	−	+	−	+	+	+	ND	+	+
20 Amino acids	−	ND	+	+	ND	ND	ND	+	ND
Chitin	−	+	+	−	ND	−	ND		ND
Maltose	−	−	+	+	−	+	−	+	+
Glucose	−	W	+	−	−	−	−	+	ND
Sucrose	−	+	+	+	−	−	ND	+	ND
Galactose	−	−	ND	−	ND	ND	−	−	ND
Starch	+	+	+	+	−	+	−	+	W
Glycogen	+	−	ND	+		ND	ND	+	ND
Cellobiose	−	+	+	−	+	−	ND	+	ND
Acetate	−	+	ND	ND	−	−	ND	−	ND
Pyruvate	−	+	−	+	−	−	ND	+	ND
Formate	−	−	ND	−	ND	ND	ND	ND	ND
Ethanol	−	−	−	−	ND	ND	ND	ND	ND
Glycerol	−	+	ND	ND	−	−	ND	ND	ND
Growth with elemental sulfur	Necessary	Enhanced	Enhanced	Enhanced	Enhanced	Enhanced	Necessary	Enhanced	Enhanced

Strain NCB100^T is currently the most thermophilic member of the genus *Pyrococcus*, growing at an optimal temperature of 105 °C versus 100 °C for *Pyrococcus furiosus*, the former record-holder among the members of the genus *Pyrococcus*, and has the widest temperature range for growth. Strain NCB100^T differs from other members of the genus *Pyrococcus* on the basis of numerous physiological and metabolic features (Table 1). In conclusion, owing to all the distinctive properties, it is proposed that strain NCB100^T is a representative of a novel species belonging to the genus *Pyrococcus*, for which the name *Pyrococcus kukulkanii* sp. nov. is proposed.

Description of *Pyrococcus kukulkanii* sp. nov.

Pyrococcus kukulkanii (ku.kul.kan′i.i. N.L. gen. n. *kukulkanii* for Kukulkan, the Pre-Columbian divinity born in the ocean, the divinity of the four elements, the creation, resurrection and reincarnation in the Mayan mythology).

Cells are motile, irregular rods, approximately 1 µm in diameter, with archaella (flagella). At atmospheric pressure, optimal growth occurs at 105 °C, with a growth temperature range of 70–112 °C. The pH range for growth is pH 3.5–8.5 (optimum pH 7.0) and the NaCl range for growth is 1.5–7 % (w/v) (optimum, 2.5–3 %, w/v, NaCl). Piezophilic and able to grow under hydrostatic pressure of 20, 30, 40 or 50 MPa at 100 °C and 105 °C, and at 20 and 30 MPa (not tested at higher pressure) at 110 °C. Growth occurs under strictly anaerobic conditions by fermentation of complex proteinaceous substrates such as yeast extract, peptone and tryptone as well as glycogen and starch along with sulfur reduction. Elemental sulfur is required for growth as the electron acceptor and is reduced to hydrogen sulfide. The fermentation products from complex proteinaceous substrates are CO_2 and H_2. Maltose, glucose, sucrose, galactose, cellobiose, acetate, formate, pyruvate, succinate, casein, casamino acids, chitin, ethanol, glycerol and a mixture of 20 amino acids are not used. Sulfate, thiosulfate, sulfite, nitrate and L-cystine at 10 g l^{-1} each and ferric iron [poorly crystalline Fe(III) oxides] are not used as electron acceptors. Nitrate, urea, gelatin, yeast extract, peptone, tryptone and a mixture of 20 amino acids are used as nitrogen sources. The type strain, NCB100^T (=DSM 101590^T), was isolated from the flange of an active deep-sea hydrothermal chimney at the depth of 1997 m at the Rebecca's Roost site on the Guaymas Basin (27°00.634′ N 111°24.405′ W), Mexico. The type strain is also available on request from the 'Souchothèque de Bretagne' (catalogue Ifremer) culture collection (no BG1337^T); http://wwz.ifremer.fr/umr6197/Souchotheque. The genomic DNA G+C content of strain NCB100^T is 41.3 mol%.

Acknowledgements

The authors acknowledge the BIG shipboard cruise party for their work and support during the BIG cruise: officers, crew and technicians of the R/V *L'Atalante*, the DSV *Nautile* team and scientific team, in particular Céline Rommevaux-Jestin for her precious help during the hydrothermal flange sampling and processing. This cruise was funded by IFREMER (France) and has benefited from a work permit in Mexican waters (DAPA/2/281009/3803, 28 October 2009). We also thank Anna Neubeck for her help with the hydrogen quantification and Ernest Chi Fru for his helpful comments. This work was supported by Ifremer, the GIS Europôle Mer, UEB, CNRS, INSU and has benefited from state aid managed by the Agence Nationale de la Recherche under the program 'Investments for the Future' with the reference ANR-10-LabX-19-01.

References

Balch, W. E., Fox, G. E., Magrum, L. J., Woese, C. R. & Wolfe, R. S. (1979). Methanogens: reevaluation of a unique biological group. *Microbiol Rev* **43**, 260–296.

Barbier, G., Godfroy, A., Meunier, J. R., Quérellou, J., Cambon, M. A., Lesongeur, F., Grimont, P. A. & Raguénès, G. (1999). *Pyrococcus glycovorans* sp. nov., a hyperthermophilic archaeon isolated from the East Pacific Rise. *Int J Syst Bacteriol* **49**, 1829–1837.

Birrien, J. L., Zeng, X., Jebbar, M., Cambon-Bonavita, M. A., Quérellou, J., Oger, P., Bienvenu, N., Xiao, X. & Prieur, D. (2011). *Pyrococcus yayanosii* sp. nov., an obligate piezophilic hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent. *Int J Syst Evol Microbiol* **61**, 2827–2881.

Brochier-Armanet, C., Boussau, B., Gribaldo, S. & Forterre, P. (2008). Mesophilic Crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota. *Nat Rev Microbiol* **6**, 245–252.

Cord-Ruwisch, R. (1985). A quick method for the determination of dissolved and precipitated sulfides in cultures of sulfate-reducing bacteria. *J Microbiol Methods* **4**, 33–36.

Erauso, G., Reysenbach, A.-L., Godfroy, A., Meunier, J.-R., Crump, B., Partensky, F., Baross, J., Marteinsson, V., Barbier, G, Erauso, G., Partensky, F. & other authors (1993). *Pyrococcus abyssi* sp. nov., a new hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent. *Arch Microbiol* **160**, 338–349.

Fiala, G. & Stetter, K. O. (1986). *Pyrococcus furiosus* sp. nov. represents a novel genus of marine heterotrophic archaebacteria growing optimally at 100 °C. *Arch Microbiol* **145**, 56–61.

Flores, G. E., Campbell, J. H., Kirshtein, J. D., Meneghin, J., Podar, M., Steinberg, J. I., Seewald, J. S., Tivey, M. K., Voytek, M. A. & other authors (2011). Microbial community structure of hydrothermal deposits from geochemically different vent fields along the Mid-Atlantic Ridge. *Environ Microbiol* **13**, 2158–2171.

González, J. M., Masuchi, Y., Robb, F. T., Ammerman, J. W., Maeder, D. L., Yanagibayashi, M., Tamaoka, J. & Kato, C. (1998). *Pyrococcus horikoshii* sp. nov., a hyperthermophilic archaeon isolated from a hydrothermal vent at the Okinawa Trough. *Extremophiles* **2**, 123–130.

Gouy, M., Guindon, S. & Gascuel, O. (2010). SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol Biol Evol* **27**, 221–224.

Jannasch, H. W., Wirsen, C. O., Molyneaux, S. J. & Langworthy, T. A. (1992). Comparative physiological studies on hyperthermophilic archaea isolated from deep-sea hot vents with emphasis on *Pyrococcus* strain GB-D. *Appl Environ Miocrobiol* **58**, 3472–3481.

Kanoksilapatham, W., Pasomsup, P., Portillo, M. C., Keawram, P. & Gonzalez, J. M. (2012). Identification and Characterization of a Freshwater *Pyrococcus* sp. Strain PK 5017 and Identification of Pfu-Like IS Elements in *Thermococcus sibiricus* MM 739. *Int J Biol* **4**, 11–22.

Kecha, M., Benallaoua, S., Touzel, J. P., Bonaly, R. & Duchiron, F. (2007). Biochemical and phylogenetic characterization of a novel terrestrial

hyperthermophilic archaeon pertaining to the genus *Pyrococcus* from an Algerian hydrothermal hot spring. *Extremophiles* **11**, 65–73.

Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.

Matte-Tailliez, O., Brochier, C., Forterre, P. & Philippe, H. (2002). Archaeal phylogeny based on ribosomal proteins. *Mol Biol Evol* **19**, 631–639.

Takai, K., Sugai, A., Itoh, T. & Horikoshi, K. (2000). *Palaeococcus ferrophilus* gen. nov., sp. nov., a barophilic, hyperthermophilic archaeon from a deep-sea hydrothermal vent chimney. *Int J Syst Evol Microbiol* **50,** 489–500.

Teske, A., Edgcomb, V., Rivers, A. R., Thompson, J. R., de Vera Gomez, A., Molyneaux, S. J. & Wirsen, C. O. (2009). A molecular and physiological survey of a diverse collection of hydrothermal vent Thermococcus and Pyrococcus isolates. *Extremophiles* **13**, 905–915.

Zillig, W., Holz, I., Janekovic, D., Schäfer, W. & Reiter, W. D. (1983). The archaebacterium *Thermococcus celer* represents, a novel genus within the thermophilic branch of the archaebacteria. *Syst Appl Microbiol* **4**, 88–94.

Zillig, W., Holz, I., Klenk, H. P., Trent, J., Wunderl, S., Janekovic, D., Imsel, E. & Haas, B. (1987). *Pyrococcus woesei*, sp. nov., an ultra-thermophilic marine archaebacterium, representing a novel order, Thermococcales. *Syst Appl Microbiol* **9**, 62–70.