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Thermococcus siculi sp. nov., a novel hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent at the Mid-Okinawa Trough

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Abstract A novel coccoid-shaped, hyperthermophilic, anaerobic archaeon, strain RG-20, was isolated from a deep-sea hydrothermal vent fluid sample taken at 1394-m depth at the Mid-Okinawa Trough (27°32.7′N, 126°58.5′E). Cells of this isolate occur singly or in pairs and are about 0.8 to 2 μm in diameter. Growth was observed at temperatures between 50° and 93°C, with an optimum at 85°C. The pH range for growth is 5.0–9.0, with an optimum around 7.0. Strain RG-20 requires 1%–4% of NaCl for growth, and cell lysis occurs at concentrations below 1%. The newly isolated strain grows preferentially in the presence of elemental sulfur on proteinaceous substrates such as yeast extract, peptone, or tryptone, and no growth was observed on carbohydrates, carboxylic acids, alcohols, or lipids. This microorganism is resistant to streptomycin, chloramphenicol, ampicillin, and kanamycin at concentrations up to 150 μg/ml, but is susceptible to rifampicin. Analysis of the hydrolyzed core lipids by thin-layer chromatography (TLC) revealed the presence of archaeol and caldarchaeol. The mol% G+C content of the DNA is 55.8. Partial sequencing of the 16S rDNA indicates that strain RG-20 belongs to the genus *Thermococcus*. Considering these data and on the basis of the results from DNA-DNA hybridization studies, we propose that this strain should be classified as a new species named *Thermococcus siculi* (si′cu.li. L. gen. n. *siculi*, of the deep-sea [*siculum*, deep-sea in literature of Ovid], referring to the location of the sample site, a deep-sea hydrothermal vent). The type strain is isolate RG-20 (DSM No. 12349).

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Introduction

Hyperthermophilic bacteria and archaea (temperature optimum >80°C) have attracted the attention of researchers because of their evolutionary significance, their ability to maintain their cellular integrity even at most inhospitable temperatures, and their biotechnological potential. These hyperthermophiles have been mainly isolated from terrestrial and shallow marine environments (Stetter 1996). As a result of the progress in deep-sea exploration systems, an increasing number of hyperthermophilic Archaea have been isolated recently from deep-sea hydrothermal vents (Tunnicliffe 1991). These deep-sea hydrothermal vent systems harbor a variety of archaeal species, including members of the genera *Methanococcus* (Jones et al. 1983), *Staphylothermus* (Fiala et al. 1986), *Desulfurococcus* (Jannasch et al. 1988), *Archaeoglobus* (Burggraf et al. 1990), *Pyrodictium* (Pley et al. 1991), and *Pyrococcus* (Fiala and Stetter 1986; Wirsen et al. 1992). Species belonging to the genus *Thermococcus* have been isolated from terrestrial freshwater (Ronimus et al. 1997), marine solfataric ecosystems, and deep-sea hydrothermal vents (Stetter 1996).

At present the genus *Thermococcus* comprises 14 species, namely *Thermococcus celer* (Zillig et al. 1983), *T. stetteri* (Miroshnichenko et al. 1989), *T. litoralis* (Neuner et al. 1990), *T. profundus* (Kobayashi et al. 1994), *T. peptonophilus* (González et al. 1995), *T. chitonophagus* (Huber et al. 1995), *T. alcaliphilus* (Keller et al. 1995), *T. fumicolans* (Godfroy et al. 1996), *T. hydrothermalis* (Godfroy et al. 1997), *T. zilligii* (Ronimus et al. 1997), *T. guaymasiensis*, *T. aggregans* (Canaganella et al. 1998), *T. gorgonarius*, and *T. pacificus* (Miroshnichenko et al. 1998). Like representatives of the genera *Pyrococcus* (Fiala and Stetter 1986), *Staphylothermus* (Fiala et al. 1986), and *Hyperthermus* (Zillig et al. 1991), most of the *Thermococcus* species are obligately anaerobic organotrophic thermophiles that prefer

to utilize proteinaceous substrates as carbon and energy source. Elemental sulfur is either stimulatory or necessary for the growth of these microorganisms. The sulfur is used as an electron acceptor to remove reducing equivalents that are produced during fermentation. In this paper we describe the morphology, physiology, and phylogenetic position of a novel hyperthermophilic, heterotrophic archaeal species, *Thermococcus siculi* sp. nov., that was isolated from a deep-sea hydrothermal vent at the region of Mid-Okinawa Trough in the southwest Pacific Ocean.

Materials and methods

Reference strains

Thermococcus celer DSM 2476^T (T = type strain), *Thermococcus litoralis* DSM 5474^T, *Thermococcus stetteri* DSM 5262^T, *Pyrococcus furiosus* DSM 3638^T, and *Thermotoga maritima* DSM 3109^T were purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany. *Thermococcus profundus* DT 5432^T and *Thermococcus peptonophilus* JCM 9653 were provided by Japan Collection of Microorganisms (JCM, Wako-shi, Japan).

Collection of samples

Hydrothermal liquid samples were collected from a hydrothermal vent at 1394-m depth at the Mid-Okinawa Trough (27°32.7′N, 126°58.5′E) by employing the manned submersible *Shinkai 2000* during a scientific cruise aboard the R/V *Natsushima* in May 1996 (dive number, 2K#862). The collection site was a region of diffuse flow where hydrothermal fluids of approximately 110°–130°C were erupting from a flat bed of grayish hydrothermal sediment particles. The samples were brought to the mother vessel and stored at 4°C.

Culture conditions

Enrichment medium EM-1 contained (per liter of distilled water) NaCl, 22 g; Na_2SO_4, 4 g; KH_2PO_4, 0.5 g; $NaHCO_3$, 0.2 g; KBr, 0.1 g; H_3BO_3, 0.03 g; $MgCl_2{\cdot}6\ H_2O$, 10.8 g; $CaCl_2{\cdot}2H_2O$, 0.5 g; $SrCl_2{\cdot}6H_2O$, 0.03 g; trace element solution (Balch et al. 1979), 10 ml; vitamin solution (Wolin et al. 1964), 10 ml; yeast extract, 1 g; sulfur, 10 g; resazurin, 1 mg; $Na_2S{\cdot}9H_2O$ (25% solution wt/vol, pH 7.0), 3 ml. This medium was complemented with seperately sterilized solutions of tryptone, starch, and pyruvate (final concentration of each component, 1 g/l). Growth medium GM-1 contained (per liter of distilled water) NaCl, 20 g; Na_2SO_4, 4 g; KCl, 0.7 g; $NaHCO_3$, 0.2 g; KBr, 0.03 g; H_3BO_3, 0.03 g; $MgCl_2{\cdot}6H_2O$, 10.8 g; $CaCl_2{\cdot}\ 2H_2O$, 1.5 g; $SrCl_2{\cdot}6H_2O$, 0.0025 g; tryptone, 5 g; yeast extract, 1 g; sulfur, 10 g; resazurin, 1 mg; $Na_2S{\cdot}9H_2O$ (25% solution, wt/vol, pH 7.0), 3 ml. The media were prepared without Na_2S, boiled for 20 min, and cooled down on ice under N_2 atmosphere. The media were finally reduced by the addition of Na_2S. Unless otherwise mentioned, the media were adjusted to either pH 5.0 or 7.0 with 6 N HCl and dispensed into appropriate glass containers sealed with butyl-rubber stoppers under N_2 atmosphere. The media were sterilized by autoclaving them at 100°C for 1 h. Unless otherwise indicated, cultures were incubated under atmospheric pressure at 85°C. All chemicals were purchased from Merck (Darmstadt, Germany), except yeast extract and tryptone, which were obtained from Difco (Detroit, MI, USA).

Enrichment and isolation

After liquid samples were collected in sterilized plastic bags, they were immediately stored at 4°C without addition of reducing agents. The hydrothermal vent water samples were then concentrated 400 fold in a sterilized cross-flow device (Minisette Membrane Cassettes; Filtron, Northborough, MA, USA) equipped with a 50-kDa cutoff membrane. Enrichment cultures that were grown on EM-1 medium were incubated at temperatures between 70° and 100°C for 2–5 days. Isolates were obtained by successively growing endpoint dilutions at 85°C and pH 7.0 in medium EM-1. Purified isolates were stored in liquid culture at 4°C and could be used as inocula for at least 12 months. For long-term storage, pure cultures were stored under anaerobic conditions in the presence of 50% (wt/vol) glycerol at −20°C.

Determination of growth

Microbial growth was either monitored by direct cell counting using a hemocytometer and phase-contrast microscopy at a magnification of 400× or by measuring the optical density (OD) in a spectrophotometer at 600 nm. Growth rates (μ; h^{-1}) were estimated as $\mu = (\ln N_2 - \ln N_1)/(t_2\text{-}t_1)$, where N_2 and N_1 are the OD_{600} at time (in h) t_2 and t_1, respectively. Doubling times (t_d; h) were calculated as $t_d = (\ln 2)/\mu$.

Determination of growth parameters

To determine the optimum temperature, cells were grown in serum bottles containing 50 ml of GM-1 medium at temperatures between 70° and 95°C at pH 7.0. Determination of the optimum pH was performed at 85°C in GM-1 medium at pH values between 4.5 and 9.5. To determine the optimum NaCl concentration, cells were cultured in GM-1 medium prepared with NaCl concentrations from 10 to 50 g/l at 85°C and pH 7.0. To monitor growth, samples (in duplicate) were withdrawn every hour. Utilization of various substrates as energy and carbon source was tested in a modified GM-1 medium with 0.1 g/l yeast extract, 0.5 g/l ammonium chloride, 10 ml vitamin solution (Wolin et al. 1964), and 10 ml trace elements solution (Balch et al. 1979). Unless otherwise indicated, the carbon sources were tested at 5 g/l. Cultures containing no additional carbon source

served as control. The requirement for elemental sulfur was studied by comparing the growth of the isolate in GM-1 medium in shaken cultures (170 rpm) with and without sulfur. The ability of the isolate to grow in the presence of various electron acceptors was determined in sulfur-free GM-1 medium supplemented with either sodium thiosulfate (2 g/l), sodium sulfite (2 g/l), or cystine (10 g/l).

Antibiotic susceptibility

Susceptibility of the new isolate to the antibiotics streptomycin, chloramphenicol, rifampicin, ampicillin, and kanamycin at a final concentration of 150 μg/ml was tested under standard growth conditions at 80°C. The bacterium *Thermotoga maritima* was used as a control to ensure the effectiveness of antibiotics at elevated temperatures.

Electron microscopy

After growth for 15 h, cells were concentrated 50 fold by centrifugation. For transmission electron microscopy, cells were negatively stained with phosphotungstic acid, 0.3% (v/v), and examined with an electron microscope (model EM 301; Philips Eindhoven, The Netherlands) at 80 kV.

Lipid composition

Lipids were extracted from lyophilized cells as described previously (De Rosa and Gambacorta 1994) and were analyzed by thin-layer chromatography (TLC). Chloroform/methanol/H_2O (65:25:4, v/v) was used as solvent, and lipids obtained from other Archaea served as standards. The total lipid extract was hydrolyzed in 1 M methanolic HCl to cleave the polar headgroups. The core lipids were identified by TLC as previously described (De Rosa et al. 1983; Trincone et al. 1988) by using the following solvents: *n*-hexane/ethyl acetate (75:25, v/v) and *n*-hexane/ethyl acetate (78:22, v/v).

DNA base composition

Cells were cultured in 21 of GM-1 medium under optimal conditions and harvested at the end of the exponential growth phase. After centrifugation the cells were washed once with sterile medium (containing no sulfur) and subsequently resuspended in 2-propanol and stored at 4°C. Isolation of the DNA was performed according to the method decribed by Visuvanathan et al. (1989). The DNA base composition was determined by high-performance liquid chromatography (HPLC) (Mesbah et al. 1989; Tamaoka and Komagata 1984).

DNA analysis

DNA was extracted from overnight cultures following the method described by Charbonnier and Forterre (1995). The 16S ribosomal DNA gene was amplified by PCR using primers designed by DeLong (1992) and sequenced on an ABI 373S automated sequencer (Perkin Elmer/Applied Biosystems, Foster City, CA, USA). Nucleotide substitution rates (K_{nuc}) (Kimura 1980) were determined and a distance matrix tree was constructed by the neighbor-joining method (Saitu and Nei 1987) employing the program CLUSTAL W (Thompson et al. 1994). Alignment gaps and unidentified base positions were not taken into consideration for the calculations. The topology of the phylogenetic tree was evaluated by performing a bootstrap analysis with 1000 bootstrap trials. DNA-DNA hybridization was performed as described by Ezaki et al. (1989). The following reference species were used in the DNA-DNA hybridization studies: *Thermococcus peptonophilus* (JCM 9653), *Thermococcus celer* (DSM 2476), *Thermococcus litoralis* (DSM 5473), *Thermococcus profundus* (DSM 9503), *Thermococcus stetteri* (DSM 5262), and *Pyrococcus furiosus* (DSM 3638).

Nucleotide sequence accession number

The amplified 16S rDNA sequence reported in this paper has been deposited in the DDBJ, EMBL, and GenBank nucleotide sequence databases. The accession number of the sequence corresponding to the 16S rDNA of *Thermococcus siculi* strain RG-20 is AB010893. Accession numbers for the sequences used as references are as follows: *Thermococcus profundus*, Z75233; *Thermococcus hydrothermalis*, Z70244; *Thermococcus celer*, M21529; *Thermococcus stetteri*, Z75240; *Thermococcus peptonophilus*, D37982; *Thermococcus* sp. GE20, Z70251; *Thermococcus fumicolans*, Z70250; *Pyrococcus abyssi*, L19921; *Pyrococcus horikoshii*, D87344; *Pyrococcus furiosus*, U20163; *Methanothermus fervidus*, M11583; *Methanococcus vannielii*, M36507; *Halobacterium halobium*, M11583; *Pyrobaculum islandicum*, L07511; *Thermofilum pendens*, X14835; *Sulfolobus solfataricus*, D26490; *Pyrodictium occultum*, M21087; *Thermosphaera aggregans*, X99556; *Desulfurococcus mobilis*, M36474; and *Thermotoga maritima*, M21774.

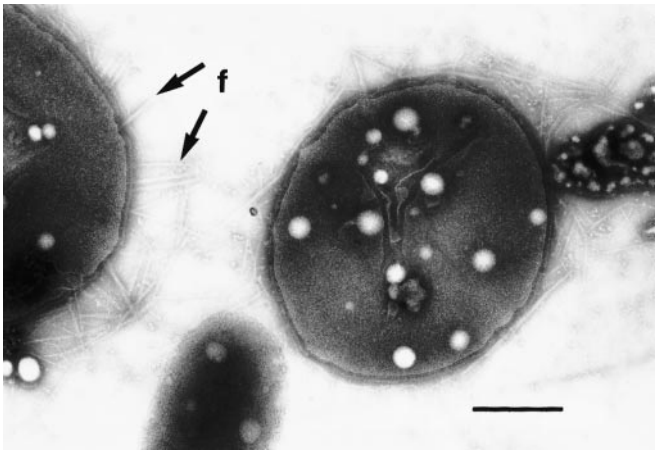
Results

Enrichment and isolation

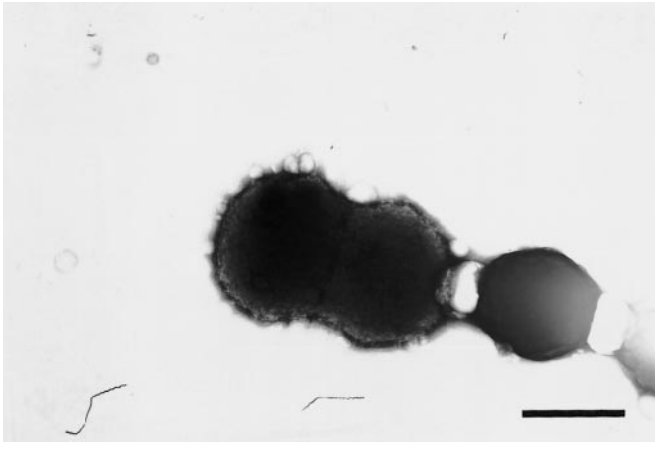
Growth of coccoid-shaped microorganisms was observed in enrichment cultures after 2 days of incubation of the concentrated hydrothermal vent fluids at 80°C. Pure cultures were obtained by repeated serial dilutions (six times, in 10^{-1} steps; final dilution, 10^{-12}). One of the isolates was designated RG-20 and was chosen for detailed studies.

Morphology

Isolate RG-20 was examined by employing phase-contrast microscopy (data not shown) and transmission electron microscopy (Fig. 1a,b). Cells were regular to slightly irregular



a



b

Fig. 1a,b. Transmission electron micrograph of strain RG-20, negatively stained with 0.3% phosphotungstic acid. *f*, fimbriae. *Bar* 0.5 μm (**a**) and 1 μm (**b**)

cocci and occurred singly or in pairs. The cells ranged from 0.8 to 2 μm in diameter and were apparently nonmotile. Transmission electron microscopy revealed the presence of fimbriae and indicates that the cells most likely divide by constriction (Fig. 1b).

Effect of temperature, pH, and salt concentration

Isolate RG-20 grew between 50° and 93°C, with an optimum temperature for growth of 85°C (Fig. 2a). The pH range for growth was 5.0 to 9.0, and the optimum pH was around 7.0 (Fig. 2b). Growth was observed in GM-1 medium containing 1%–4% of NaCl, with an optimum at 2% (Fig. 2c). In media containing less than 1% of NaCl, cell lysis was observed. Under optimal cultivation conditions the generation time for RG-20 was calculated to be 130 min. The final cell density in batch cultures reached a maximum of 3 to 5 $\times$ 10^8 cells/ml.

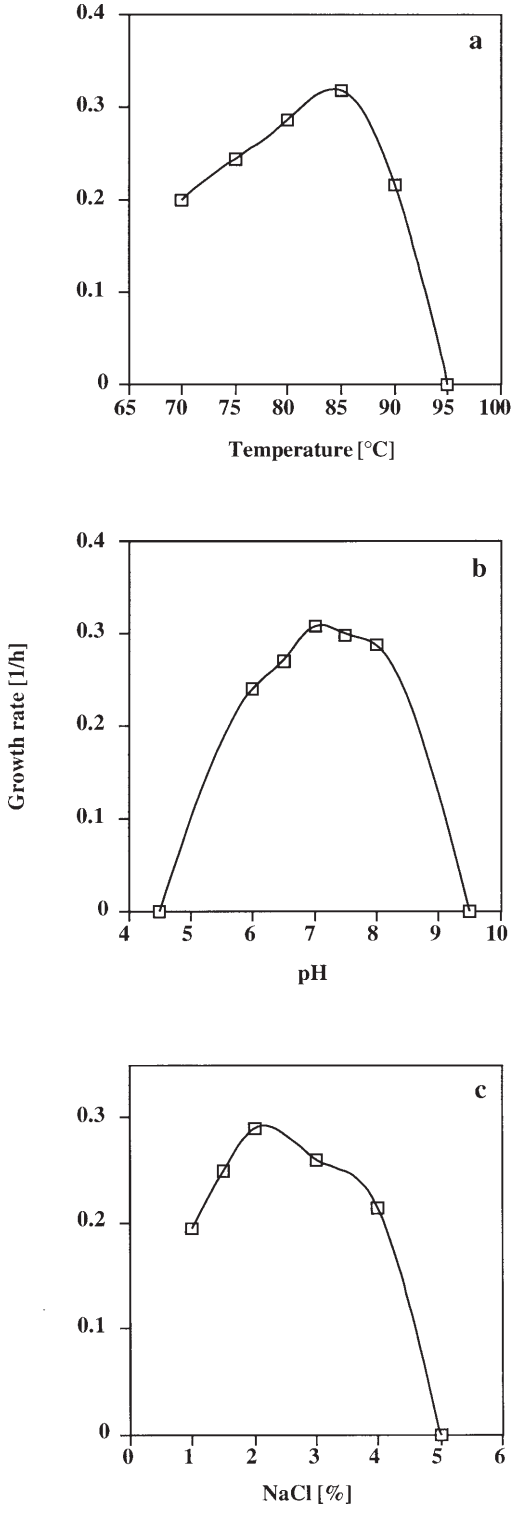


Fig. 2a–c. Temperature, pH, and NaCl optima for growth of strain RG-20 on GM-1 medium. Growth rates were plotted as a function of temperature (**a**), pH (**b**), and NaCl concentration (**c**). Growth rates were calculated as described in Materials and methods

Nutritional requirements

Growth of isolate RG-20 was observed with complex proteinaceous substrates such as yeast extract, tryptone, peptone, and hydrolyzed casein in the presence of elemental sulfur. As shown in Table 1, sugars, polysaccharides, carboxylic acids, and alcohols did not serve as substrates for growth with sulfur as an electron acceptor. The growth of

isolate RG-20 on yeast extract and tryptone in the absence of elemental sulfur was poor but could be enhanced when the cultures were shaken (170 rpm) during incubation. Unlike all other electron acceptors tested in combination with medium GM-1, cystine was found to support rapid growth of isolate RG-20 and was able to substitute for elemental sulfur (Table 1). The newly isolated organism grew strictly anaerobically under N_2 or N_2/CO_2 (80:20 v/v) atmosphere using Na_2S as a reducing agent.

Table 1. Growth of strain RG-20 on various carbon sources and electron acceptors

	Growth[a]
Substrates	
Yeast extract + tryptone (medium GM-1)	+++
Yeast extract	++
Tryptone	++
Peptone	++
Casein (hydrolyzed)	+
Gelatin	−
Collagen	−
Glucose	−
Cellobiose	−
Lactose	−
Fructose	−
Maltose	−
Xylose	−
Starch	−
Pullulan	−
Xylan	−
Carboxy-methyl cellulose	−
Olive oil	−
Ethanol	−
Acetate	−
Pyruvate	−
Electron acceptors[b]	
Sulfur (10 g/l)	+++
None (shaken cultures)	+
Cystine (10 g/l)	+++
Sodium thiosulfate (2 g/l)	−
Sodium sulfite (2 g/l)	−

[a] +++, $>1 \times 10^8$ cells per ml (final concentration); ++, 5×10^7 to 1×10^8; +, 1×10^7 to 5×10^7; −, $<1 \times 10^7$
[b] Tested on medium GM-1 containing 1 g/l yeast extract and 5 g/l tryptone

Antibiotic susceptibitlity

Isolate RG-20 was resistant to streptomycin, chloramphenicol, ampicillin, and kanamycin, each at 150 μg/ml. Growth, on the other hand, was inhibited by the addition of rifampicin (150 μg/ml).

DNA base composition and 16S rDNA sequence analysis

The mol% G+C content of the DNA of isolate RG-20 was 55.8 ± 0.1 as determined by HPLC. The partial sequence (897 bases; *E. coli* positions 24–950) of the 16S rRNA-encoding gene of strain RG-20 was determined and aligned with other available 16S rDNA sequences of various species belonging to the archaeal and bacterial domains. A phylogenetic tree generated by employing the program CLUSTAL W (Thompson et al. 1994) is shown in Fig. 3. Comparison of the 16S rDNA sequences unequivocally revealed that strain RG-20 belongs to the genus *Thermococcus*. The highest level of similarity was found between the newly isolated strain and *Thermococcus stetteri* (98.5%).

DNA-DNA homology

The results of DNA-DNA reassociation experiments between strain RG-20 and closely related *Thermococcus* species are presented in Table 2. The new isolate did not exhibit significant DNA homology with the type strains of *Thermococcus celer*, *T. litoralis*, *T. peptonophilus*, *T. profundus*, *T. stetteri*, and *Pyrococcus furiosus*. The highest

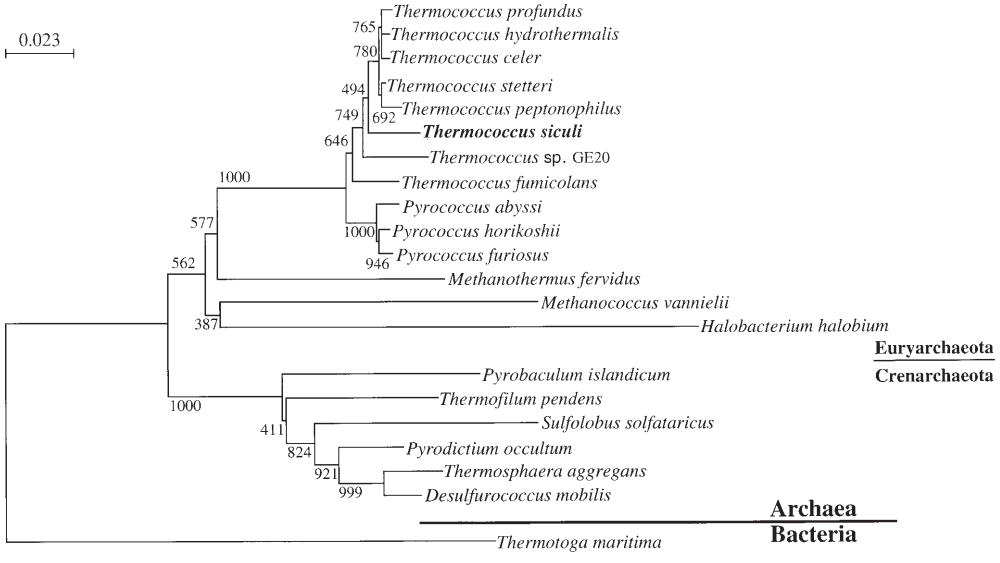


Fig. 3. Phylogenetic tree showing the position of *Thermococcus siculi* within the order *Thermococcales* and the archaeal domain. The tree was constructed by maximum-likelihood analysis using the program CLUSTAL W (Thompson et al. 1994). The *scale bar* represents the expected number of changes per sequence position (K_{nuc}). Accession numbers are as mentioned in Materials and methods

Table 2. DNA homology between strain RG-20 and other Archaea belonging to the order *Thermococcales*

Strain	Hybridization (%) to the DNA of		
	RG-20	*Thermococcus profundus*	*Thermococcus stetteri*
RG-20	100	4	21
Thermococcus celer	23	6	32
T. litoralis	3	1	6
T. peptonophilus	5	6	27
T. profundus	24	100	36
T. stetteri	18	ND	100
Pyrococcus furiosus	1	1	4

ND, not determined

Table 3. Characteristics of strain RG-20 in comparison to other archaeal species belonging to the genus *Thermococcus*

Species	G+C content (mol%)	Growth temperature (°C)		NaCl concentration (g/l)		pH		Carbon source	S^0 effect	Rifampicin resistance	Reference
		Range	Optimum	Range	Optimum	Range	Optimum				
T. celer	57	≤93	88	ND	40	ND	5.8	Peptides, sucrose	E	+	Zillig et al. 1983
T. litoralis	38	65–95	88	18–65	25	6.2–8.5	7.2	Peptides, pyruvate	E	−	Neuner et al. 1990
T. stetteri	50	60–85	75	10–40	25	5.7–7.2	6.5	Peptides, starch, pectin	R	−	Miroshnichenko et al. 1989
T. profundus	52.2	50–90	80	10–60	20	4.4–8.5	7.5	Peptides, starch, maltose, pyruvate	R	−	Kobayashi et al. 1994
T. peptonophilus	52	60–100	85	10–50	30	4.0–8.0	6.0	Peptides	E	−	González et al. 1995
T. alcaliphilus	42.4	54–91	85	10–60	20–30	6.5–10.5	9.0	Peptides	E	ND	Keller et al. 1995
T. chitonophagus	46.5	60–93	85	8–80	20	3.5–9.0	6.7	Chitin, peptides	E	ND	Huber et al. 1995
T. fumicolans	54–55	73–103	85	6–40	13–26	4.5–9.5	8.0	Peptides, pyruvate	E	−	Godfroy et al. 1996
T. hydrothermalis	58	55–100	85	20–80	30–40	3.5–9.5	6.0	Peptides, maltose, pyruvate, cellobiose	E	+	Godfroy et al. 1997
T. guaymasiensis	46	56–90	88	ND	18	5.6–8.1	7.2	Peptides, dextrose, maltose, starch	E	+	Canganella et al. 1998
T. aggregans	42.0	60–94	88	ND	18	5.6–7.9	7.0	Peptides, dextrose, maltose, starch	E	+	Canganella et al. 1998
T. zilligii	46.2	55–85	75–80	≤11.6	2.9	5.4–9.2	7.4	Peptides	R	+	Ronimus et al. 1997
T. gorgonarius	50.6	68–95	80–88	10–50	20–35	5.8–8.5	6.5–7.2	Peptides	R	ND	Miroshnichenko et al. 1998
T. pacificus	53.3	70–95	80–88	10–60	20–35	6.0–8.0	6.5	Peptides, starch	R	ND	Miroshnichenko et al. 1998
Strain RG–20	55.8	50–93	85	10–40	20	5.0–9.0	7.0	Peptides	E	−	This publication

E, enhanced; R, required; +, positive; −, negative; ND, not determined

level of DNA-DNA reassociation was found between strain RG-20 and *Thermococcus profundus* (24%). However, these hybridization values were significantly lower than that accepted as the phylogenetic definition of a species (<70% [Wayne et al. 1987]).

Lipid composition

As shown by TLC, strain RG-20 possesses three phospholipids, one major phospholipid exhibiting a R_f of 0.35 and two minor lipids with R_f of 0.27 and 0.1. At the level of TLC

analysis, glycolipids and aminolipids were absent in the newly isolated strain. TLC examinations of the hydrolyzed core lipids revealed the presence of archaeol and caldarchaeol. The R_f in TLC eluted with *n*-hexane/ethyl acetate (75:25, v/v) were 0.80 and 0.41 for archaeol and caldarchaeol, respectively. In *n*-hexanol/ethyl acetate (78:22, v/v), the same compounds had a R_f of 0.77 (archaeol) and 0.27 (caldarchaeol).

Discussion

Based on the high temperature optimum for growth, the characteristic archaeal lipid composition, and resistance to the antibiotics chloramphenicol, kanamycin, and ampicillin, the novel hyperthermophilic marine isolate RG-20 is a member of the archaeal domain. The 16S rDNA partial sequence and G+C content indicate that strain RG-20 belongs to the genus *Thermococcus*. This classification is also supported by the morphological and physiological characteristics of strain RG-20, which resemble those of the genus *Thermococcocus*. This genus comprises to date 14 representatives, but as shown in Table 3 strain RG-20 is not identical to these previously described species. *Thermoccocus litoralis*, *T. stetteri*, *T. alcaliphilus*, *T. chitonophagus*, *T. guaymasiensis*, *T. aggregans*, *T. gorgonarius*, and *T. zilligii* have considerably lower G+C contents, ranging between 38 and 50 mol%. *Thermococcus peptonophilus*, *T. fumicolans*, and *T. hydrothermalis* differ from isolate RG-20 by their higher temperature tolerance. These species can grow at temperatures up to 103°C, whereas the newly isolated microorganism exhibits a maximum temperature for growth at 93°C. On the other hand, the isolate described in this study is able to grow at 50°C and shows a broad temperature range for growth (Δ 43°C). Similar results have been reported only for *T. profundus*, but this species is different from strain RG-20 because of its requirement for elemental sulfur and its ability to metabolize a broader range of carbon sources. The substrate utilization of strain RG-20 is limited to proteinaceous compounds. This is in contrast to the nutritional versality of *Thermococcus celer*, *T. litoralis*, *T. stetteri*, *T. chitonophagus*, *T. fumicolans*, *T. hydrothermalis*, *T. guaymasiensis*, *T. aggregans*, and *T. pacificus*, which can utilize a more extensive variety of substrates including different carbohydrates.

Based on its morphological and physiological properties, along with its phylogenetic relationships determined by partial 16S rDNA sequence analysis and DNA-DNA hybridization studies, the newly isolated strain RG-20 can be classified as a new species of the genus *Thermococcus*, which we propose to designate *Thermococcus siculi*.

Description of *Thermococcus siculi* sp. nov.

Thermococcus siculi Grote and Antranikian (si′cu.li. L. gen. n. *siculi*, of the deep-sea [*siculum*, deep-sea in literature of Ovid], referring to the location of the sample site, a deep-sea hydrothermal vent). Cells are regular to slightly irregular, nonmotile cocci (0.8–2 μm in diameter) that occur singly or in pairs and divide by constriction. Obligately anaerobic. Susceptible to rifampicin. Growth occurs between 50° and 93°C, with an optimum temperature of 85°C. Grows optimally at pH 7.0 (range, 5.0–9.0) and 20 g/l of NaCl (range, 10–40 g/l). Utilizes yeast extract, tryptone, peptone, and casein. Obligately heterotrophic. Sulfur is not required for growth but significantly stimulates growth. Cystine supports rapid growth and is able to substitute elemental sulfur. Core lipids contain archaeol and caldarchaeol. The mol% G+C content of the DNA is 55.8. Type strain RG-20 (DSM No. 12349) was isolated from hydrothermal vent fluids collected at Mid-Okinawa Trough in the southwest Pacific Ocean.

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