Thermococcus gammatolerans sp. nov., a hyperthermophilic archaeon from a deep-sea hydrothermal vent that resists ionizing radiation

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Enrichments for anaerobic organotrophic hyperthermophiles were performed with hydrothermal chimney samples collected at the Guaymas Basin (27° 01′ N, 111° 24′ W). Positive enrichments were submitted to γ-irradiation at a dose of 30 kGy. One of the resistant strains, designated strain EJ3^T, formed regular motile cocci. The new strain grew between 55 and 95 °C, with an optimum growth temperature of 88 °C. The optimal pH for growth was 6·0, and the optimum NaCl concentration for growth was around 20 g l^{-1}. Strain EJ3^T was an obligately anaerobic heterotroph that utilized yeast extract, tryptone and peptone. Elemental sulfur or cystine was required for growth and reduced to hydrogen sulfide. The G+C content of the genomic DNA was 51·3 mol%. As determined by 16S rRNA gene sequence analysis, the organism was most closely related to *Thermococcus celer*, *Thermococcus guaymasensis*, *Thermococcus hydrothermalis*, *Thermococcus profundus* and *Thermococcus gorgonarius*. However, no significant homology was observed between them by DNA–DNA hybridization. The novel organism also possessed phenotypic traits that differ from those of its closest phylogenetic relatives. Therefore, it is proposed that this isolate, which constitutes the most radioresistant hyperthermophilic archaeon known to date, should be described as the type strain of a novel species, *Thermococcus gammatolerans* sp. nov. The type strain is EJ3^T (=DSM 15229^T=JCM 11827^T).

All members of the archaeal order *Thermococcales* are strictly anaerobic hyperthermophiles. The *Thermococcaceae*, the single family of this order, is composed of three genera, *Thermococcus* (Zillig *et al.*, 1983), *Pyrococcus* (Fiala & Stetter, 1986) and *Palaeococcus* (Takai *et al.*, 2000). The main characteristics that distinguish these genera are their optimal growth temperature (between 75 and 88 °C for *Thermococcus* and *Palaeococcus* species and between 96 and 100 °C for *Pyrococcus* members) and the clustering of their 16S rRNA sequences as separate clades within the *Thermococcales* (Zillig & Reysenbach, 2001). The genus *Thermococcus* includes at present 20 species that share similar physiological characteristics and can be divided into two groups on the basis of their G+C content. Members of this genus grow heterotrophically by fermentation or sulfur respiration on a variety of organic compounds such as peptone, yeast extract, meat extract, casein, peptides, Casamino acids and starch. Some of them are able to grow in the absence of elemental sulfur, but this compound significantly stimulates their growth. Representative species of *Thermococcus* are widely distributed at deep-sea and shallow marine hydrothermal vents and have also been isolated from terrestrial thermal springs in New Zealand and deep oil reservoirs (Miroshnichenko *et al.*, 2001; Zillig & Reysenbach, 2001).

In the deep-sea hydrothermal environments of the East Pacific Rise, the polychaete *Alvinella* colonizes the walls of active chimneys and is exposed to natural radioactivity levels (^{210}Pb, ^{210}Po, ^{222}Rn) a hundred times higher than

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Data on the effect of temperature, pH and NaCl concentration on EJ3^T are available as supplementary material in IJSEM Online.

received on the surface of the Earth (Cherry *et al.*, 1992). Deep-sea hydrothermal vents could therefore represent an attractive milieu for studying the effects of ionizing radiation on thermophilic micro-organisms.

In this paper, a deep-sea hydrothermal vent chimney collected at the Guaymas Basin was used to isolate and characterize a novel *Thermococcus* species that resists high levels (30 kGy) of γ-irradiation.

The new organism was isolated from chimney samples collected by the submersible *Nautile* during the cruise 'Guaynaut' in 1991 in the Guaymas Basin [Gulf of California (27° 01′ N, 111° 24′ W)] at a depth of 2616 m. Samples were immediately transferred into flasks filled with sterile reduced artificial seawater. The vials were then closed tightly with butyl rubber stoppers and stored at 4 °C until used for further experiments.

Anaerobic procedures were performed as described by Balch & Wolfe (1976). Enrichment cultures were performed anaerobically in Hungate tubes containing 10 ml YPS medium and incubated at 85 °C. The same conditions were used to cultivate routinely the reference strains and the new isolate (Table 1). The YPS medium contained per litre of distilled water: 35 g Sea Salts (Sigma), 3·46 g PIPES, 1 g yeast extract, 4 g peptone, 5 g elemental sulfur, 0·5 g NH_4Cl, 0·35 g KH_2PO_4, 0·2 g $CaCl_2$, 6·7 mg $FeCl_3$, 2·9 mg Na_2WO_4 and 0·1 mg resazurin. The pH was adjusted to 6·8 before autoclaving. Final anaerobiosis was achieved by adding sterile 5 % (w/v) $Na_2S.9H_2O$ to a final concentration of 0·025 %.

The positive enrichments obtained after 2 days incubation consisted of irregular motile and nonmotile coccoid cells. Aliquots of these cultures were irradiated at 30 kGy on ice with a γ-ray source (^{137}Cs) at a rate of 60 Gy min^{-1} (Institut Curie, Orsay, France). After irradiation, cultures were transferred in YPS medium and incubated at 85 °C for 3 days. Creamy colonies were obtained on YPS medium solidified with 1 % (w/v) gelrite and incubated in an anaerobic jar at 80 °C (gas phase N_2/CO_2, 80 : 20, 1 bar) (Erauso *et al.*, 1995). One colony was randomly picked and streaked on YPS-gelrite plates four times successively. The purity of the isolate (designated EJ3^T) was checked microscopically by a serial dilution step.

After purification, the survival rate to γ-irradiation of isolate EJ3^T was evaluated and compared to that of '*Pyrococcus abyssi*' GE5^T and *Thermococcus stetteri* DSM 5262^T. After irradiation at increasing doses, the surviving fraction was enumerated by the most probable number

Table 1. Characteristics that distinguish strain EJ3^T from its closest phylogenetic relatives

+, Positive; −, negative; ND, not determined; NR, not reported; R, required; S, stimulatory.

Property	*Thermococcus celer**	*Thermococcus profundus*†	*Thermococcus hydrothermalis*‡	*Thermococcus guaymasensis*§	*Thermococcus gorgonarius*‖	Strain EJ3^T
Mobility	+	+	+	−	+	+
Energy substrate						
Casein	+	+	+	+	ND	−
Amino acids	ND	ND	+	−	−	−
Starch	ND	+	−	+	−	−
Maltose	ND	+	−	+	−	−
Pyruvate	+	−	+	+	Weak	−
Sulfur requirement	S	R	S	R	R	R
Rifampicin resistance	+	−	+	+	ND	−
Growth temp. (°C)						
Range	⩽93	50–90	55–100	56–90	68–95	55–95
Optimum	88	80	85	88	80–88	88
NaCl concn (g l^{-1})						
Range	ND	10–60	20–80	ND	10–50	10–40
Optimum	40	20	30–40	18	20–35	20
pH						
Range	NR	4·4–8·5	3·5–9·5	5·6–8·1	5·8–8·5	4–8·5
Optimum	5·8	7·5	6·0	7·2	6·5–7·2	6·0
G+C content (mol%)	57	52·2	58	46	50·6	51·3

*Zillig *et al.* (1983).
†Kobayashi *et al.* (1994).
‡Godfroy *et al.* (1997).
§Canganella *et al.* (1998).
‖Miroshnichenko *et al.* (1998).

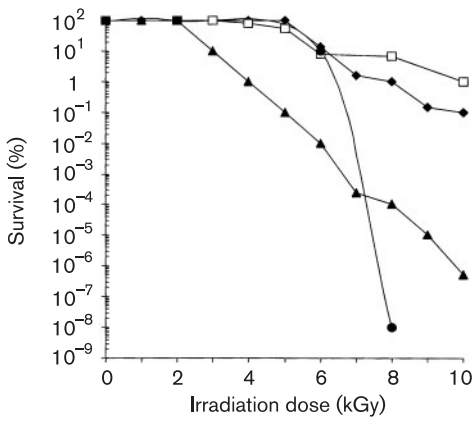

Fig. 1. Gamma-radiation survival curves. The new isolate, EJ3^T (□), was irradiated at the end of the exponential growth phase in YPS growth medium under anaerobic conditions. These values are a mean of two independent experiments. Survival curves of *Thermococcus stetteri* (●), '*Pyrococcus abyssi*' (▲) and *Deinococcus radiodurans* (◆) were taken from Kopylov *et al.* (1993), Battista (1997) and Gérard *et al.* (2001).

technique. The new isolate was found to resist 3 kGy without loss of cultivatability (Fig. 1). Contrary to '*P. abyssi*' and *Thermococcus stetteri*, its survival curve was close to that determined for *Deinococcus radiodurans* (Battista, 1997). Like *D. radiodurans*, a fraction of an end-exponential culture of the new isolate was able to grow after irradiation at 30 kGy. When tested for this ability, cells of '*P. abyssi*' and *Thermococcus stetteri* could not be cultivated after irradiation doses exceeding 11 and 18 kGy, respectively (data not shown).

Cells of strain EJ3^T formed regular cocci occurring singly or in pairs. They were motile by means of polar flagella (Fig. 2).

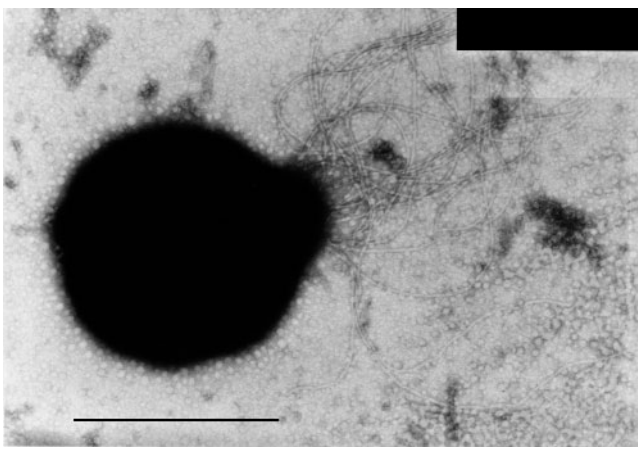

Fig. 2. Electron micrograph of a negatively stained cell of strain EJ3^T prepared as previously described (L'Haridon *et al.*, 1998). Bar, 1 μm.

The diameter of the cells ranged from 0·6 to 1·4 μm and remained relatively constant around 1 μm under optimal growth conditions. Cells appeared to divide by constriction.

Unless otherwise stated, YPS medium was used for growth experiments. The optimal pH for growth was determined at 85 °C as described by Marteinsson *et al.* (1999). To determine the optimal NaCl concentration for growth, increasing concentrations of NaCl were added to a medium that contained per litre of distilled water: 10·77 g $MgCl_2.6H_2O$, 3·97 g Na_2SO_4, 0·20 g $NaHCO_3$, 0·09 g KBr, 0·025 $SrCl_2.6H_2O$, 0·671 g KCl, 0·26 g H_3BO_3, 0·003 g NaF, 3·46 g PIPES, 1 g yeast extract, 4 g peptone, 5 g elemental sulfur, 0·5 g NH_4Cl, 0·35 g KH_2PO_4, 0·2 g $CaCl_2$, 6·7 mg $FeCl_3$, 2·9 mg Na_2WO_4 and 0·1 mg resazurin. The pH was adjusted to 6·8 before autoclaving. Final anaerobiosis was achieved by adding sterile 5 % (w/v) $Na_2S.9H_2O$ to a final concentration of 0·025 %. All the experiments were performed in duplicate.

Under these conditions, isolate EJ3^T grew between 55 and 95 °C and the optimum temperature for growth was 88 °C. No growth was detected at 50 and 96 °C. The optimum pH was between 5·5 and 6·5. No growth occurred at pH 3·0 and 8·5. The optimum NaCl concentration was 20 g l^{-1}. No growth was detected at NaCl concentrations of 0 and 40 g l^{-1}. Under optimal growth conditions (temperature, pH and NaCl), the doubling time of the isolate was around 95 min. See the supplementary data available in IJSEM Online at http://ijs.sgmjournals.org

The ability of the isolate to use single carbon sources for growth was tested at optimal growth temperature on YPS medium in which yeast extract and peptone were omitted. A filter-sterilized solution of vitamins (10 ml l^{-1}) (Widdel & Bak, 1992) was added and N_2 was used as headspace. Since no growth was observed in aerated conditions and in mineral medium supplemented with vitamins and a H_2/CO_2 (80 : 20) headspace, strain EJ3^T appeared to be an obligately anaerobic organotroph. Under anaerobic conditions, S° and cystine were necessary for growth and reduced to hydrogen sulfide. No growth was detected in the presence of thiosulfate (10 mM), sulfate (20 mM) or sulfite (10 mM). Significant growth was observed on yeast extract, peptone and tryptone (all at 0·2 %, w/v). Strain EJ3^T was not able to grow on a mixture of 20 amino acids. No growth was observed on Casamino acids, acetate, succinate, propionate, pyruvate (all at 0·2 %, w/v), vitamins, gelatin (0·5 %, w/v), sucrose, cellobiose, lactose, maltose, glycogen, xylose or starch (all at 0·5 %, w/v). No growth was observed in the basal medium using H_2/CO_2 (80 : 20; 200 kPa) as headspace.

Isolate EJ3^T was resistant to chloramphenicol, ampicillin, penicillin, kanamycin, vancomycin and streptomycin at a concentration of 150 μg ml^{-1}, but this isolate was sensitive to rifampicin at the same concentration. *Thermotoga maritima*, used as control, exhibited the expected pattern of antibiotic susceptibility at 80 °C (Huber *et al.*, 1986).

The G+C content of the DNA of isolate EJ3^T determined by the thermal denaturation method as described by Jeanthon *et al.* (1998) was 51·3 mol%.

16S rDNA was amplified by PCR with *Taq* polymerase (Promega), using the genomic DNA from strain EJ3^T as template and two primers: one specific for archaea (4F primer: 5′-TCC GGT TGA TCC TGC CGG-3′) and one universal (1492R primer: 5′-GGT TAC CTT GTT ACG ACT T-3′). PCR reactions were typically carried out in a volume of 50 µl containing 50–100 ng template, 100 ng of each of the two specific primers, 250 µM dNTP, 1±5 mM $MgCl_2$, 1× buffer (Promega) and 2·5 U polymerase. The different steps of PCR were as follows: 5 min at 95 °C; then 25 cycles of 1·5 min at 95 °C, 1·5 min at 53 °C and 2·5 min at 72 °C; then finally a polymerization step of 8 min at 72 °C. PCR products were cloned in vector PCRII2.1 and several clones were sequenced to ensure the sequence quality, using Texas-red-labelled primers, a Thermosequenase kit (RPN 2444; Amersham) and a Vistra 725 automated sequencer. Phylogenetic analysis of the 16S rDNA gene sequence was realized as described by Corre *et al.* (2001). The sequence of strain EJ3^T has been deposited in the GenBank database under accession number AF479014.

The 16S rDNA sequence analysis placed strain EJ3^T within the genus *Thermococcus* (Fig. 3). The highest levels of similarity between the 16S rDNA sequence of EJ3^T and those of other *Thermococcus* species were as follows: *Thermococcus gorgonarius*, 98·9 %; *Thermococcus celer* and *Thermococcus guaymasensis*, 98·4 %; *Thermococcus profundus*, 98·3 %; and *Thermococcus hydrothermalis*, 97·6 %, *Pyrococcus furiosus*, 96·4 %, and *Palaeococcus ferrophilus*, 94·0 %.

Considering the high levels of similarity (more than 98 %) existing between strain EJ3^T and *Thermococcus gorgonarius*, *Thermococcus celer*, *Thermococcus guaymasensis*, *Thermococcus profundus* and *Thermococcus hydrothermalis*, quantitative DNA–DNA hybridizations between the isolate and its closest relatives were performed as described by Jeanthon *et al.* (1998). When strain EJ3^T was used as the labelled probe, the levels of DNA reassociation were as follows: *Thermococcus gorgonarius*, 18·3 %; *Thermococcus celer*, 19·2 %; *Thermococcus guaymasensis*, 23·2 %; *Thermococcus profundus*, 19·5 %; and *Thermococcus hydrothermalis*, 22·1 %.

When a number of different taxonomic parameters were compared, strain EJ3^T differed from its closest phylogenetic relatives (Table 1). It differs from most of them by its temperature range for growth and its inability to grow on casein and pyruvate. Moreover, strain EJ3^T differs strongly from *Thermococcus celer*, *Thermococcus hydrothermalis* and *Thermococcus guaymasensis* in its G+C content and its rifampicin sensitivity, and from *Thermococcus gorgonarius* in its salinity range and its optimum pH for growth. Finally, it can be distinguished from *Thermococcus profundus* by its salinity range and its inability to use starch and maltose.

On the basis of its phenotypical and genetic characteristics, strain EJ3^T represents a novel species within the genus *Thermococcus*. We propose to name it *Thermococcus gammatolerans* according to its high degree of tolerance to γ-irradiation.

Description of *Thermococcus gammatolerans* sp. nov.

Thermococcus gammatolerans (ga.mma.to′le.rans. *gamma* referring to gamma rays used as selection pressure for isolation; L. pres. part. *tolerans* tolerating; N.L. adj. *gammatolerans* referring to its ability to tolerate high levels of γ-rays).

Cells are cocci (diameter 0·6–1·4 µm) that are motile by the presence of polar flagella. Cell division occurs by constriction. Obligately anaerobic. Growth occurs at 55–95 °C, and the optimum temperature is 88 °C. Grows optimally in the presence of 20 g NaCl l^{-1} and at pH around 6·0. Obligately organotrophic. Grows preferentially on proteolysis products such as yeast extract, tryptone and peptone. Does not grow on Casamino acids, acetate, succinate, propionate,

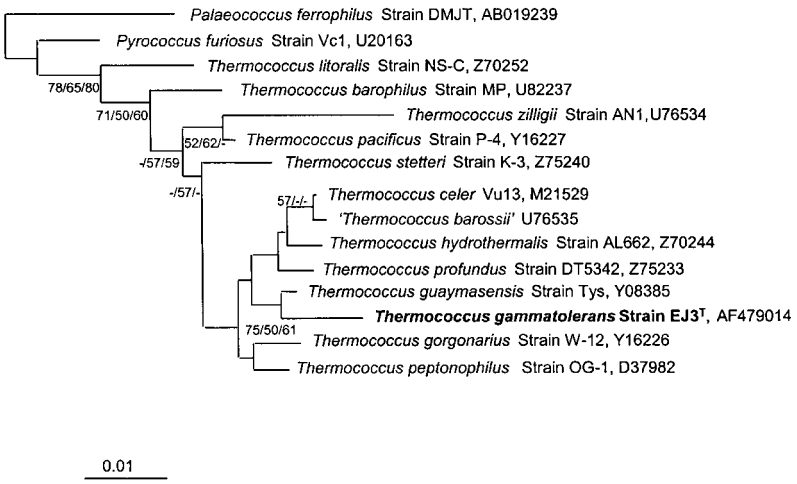

Fig. 3. Phylogenetic position of strain EJ3^T (in boldface) amongst some representatives of the family *Thermococcaceae*; 1320 nucleotides were used in the phylogenetic analysis. Numbers after the strain names are GenBank accession numbers of 16S rDNA sequences. The topology shown is the tree obtained using the neighbour-joining method (Jukes and Cantor distance correction). Numbers at the nodes refer to the bootstrap values (100 replicates) in distance, maximum-likelihood and maximum-parsimony analyses, respectively. Bootstrap values below 50 % were not represented or represented by dashes. The scale bar represents the expected number of changes per sequence position.

pyruvate, gelatin, glucose, maltose or starch. Sulfur or cystine are necessary for growth and reduced to hydrogen sulfide. Thiosulfate, sulfate and sulfite are not used as electron acceptors. Resistant to chloramphenicol, ampicillin, penicillin, kanamycin, vancomycin and streptomycin at 150 μg ml^{-1}, but sensitive to 150 μg rifampicin ml^{-1}. The results of 16S rDNA sequence comparisons place *Thermococcus gammatolerans* in the *Thermococcales*.

The type strain, EJ3^T (=DSM 15229^T=JCM 11827^T), was isolated from an active chimney recovered from a hydrothermal site in Guaymas Basin (27° 01′ N and 111° 24′ W) at a depth of 2616 m.

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References

Balch, W. E. & Wolfe, R. S. (1976). New approach to the cultivation of methanogenic bacteria: 2-mercaptoethane-sulfonic acid (HS-CoM)-dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere. *Appl Environ Microbiol* **32**, 781–791.

Battista, J. R. (1997). Against all odds: the survival strategies of *Deinococcus radiodurans*. *Annu Rev Microbiol* **18**, 203–224.

Canganella, F., Jones, W. J., Gambacorta, A. & Antranikian, G. (1998). *Thermococcus guaymasensis* sp. nov. and *Thermococcus aggregans* sp. nov., two novel thermophilic archaea isolated from the Guaymas Basin hydrothermal vent site. *Int J Syst Bacteriol* **48**, 1181–1185.

Cherry, R. D., Desbruyères, M., Heyraud, M. & Nolan, C. (1992). High levels of natural radioactivity in hydrothermal vent polychaetes. *C R Acad Sci Paris* **III**, 21–26.

Corre, E., Reysenbach, A. L. & Prieur, D. (2001). Epsilon-proteobacterial diversity from a deep-sea hydrothermal vent on the Mid-Atlantic Ridge. *FEMS Microbiol Lett* **205**, 329–335.

Erauso, G. & Prieur, D. (1995). Plate cultivation technique for strictly anaerobic, thermophilic, sulfur-metabolizing *archaea*. In *Archaea: a Laboratory Manual*, vol. 3, pp. 25–29. Edited by E. M. Fleischmann, A. R. Place, F. T. Robb & H. J. Schreider. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Fiala, G. & Stetter, K. (1986). *Pyrococcus furiosus* sp. nov. represents a new genus of marine heterotrophic archaebacteria growing optimally at 100 °C. *Arch Microbiol* **145**, 338–349.

Gérard, E., Jolivet, E., Prieur, D. & Forterre, P. (2001). DNA protection is not involved in the radioresistance of the hyperthermophilic archaea *Pyrococcus abyssi* and *Pyrococcus furiosus*. *Mol Genet Genom* **266**, 72–78.

Godfroy, A., Lesongeur, F., Raguénès, G., Quérellou, J., Antoine, E., Meunier, J.-R., Guezennec, J. & Barbier, G. (1997). *Thermococcus hydrothermalis* sp. nov., a new hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent. *Int J Syst Bacteriol* **47**, 622–626.

Huber, R. J., Langworthy, T. A., Konig, H., Thomm, M., Woese, C. R., Sleytr, U. B. & Stetter, K. O. (1986). *Thermotoga maritima* sp. nov. represents a new genus of unique extremely thermophilic eubacteria growing up to 90 °C. *Arch Microbiol* **144**, 324–333.

Jeanthon, C., L'Haridon, S., Reysenbach, A. L., Vernet, M., Messner, P., Sleytr, U. B. & Prieur, D. (1998). *Methanococcus infernus* sp. nov., a novel hyperthermophilic lithotrophic methanogen isolated from a deep-sea hydrothermal vent. *Int J Syst Bacteriol* **48**, 913–919.

Kobayashi, T., Kwak, Y. S., Akiba, T., Kudo, T. & Horikoshi, K. (1994). *Thermococcus profundus* sp. nov., a new hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent. *Syst Appl Microbiol* **17**, 232–236.

Kopylov, V. M., Bonch-Osmolovskaya, E. A., Svetlichnyi, V. A., Miroshnicheko, M. L. & Skobkin, V. S. (1993). Gamma-irradiation resistance and UV-sensitivity of extremely thermophilic archaebacteria and eubacteria. *Mikrobiologiya* **62**, 90–95.

L'Haridon, S., Cilia, V., Messner, P., Raguénès, G., Gambacorta, A., Sleytr, U. B., Prieur, D. & Jeanthon, C. (1998). *Desulfurobacterium thermolithotrophum* gen. nov., sp. nov., a novel autotrophic, sulphur-reducing bacterium isolated from a deep-sea hydrothermal vent. *Int J Syst Bacteriol* **48**, 701–711.

Marteinsson, V. T., Birrien, J.-L., Reysenbach, A.-L., Vernet, M., Marie, D., Gambacorta, A., Messner, P., Sleytr, U. & Prieur, D. (1999). *Thermococcus barophilus* sp. nov., a new barophilic and hyperthermophilic archaeon isolated under high hydrostatic pressure from a deep-sea hydrothermal vent. *Int J Syst Bacteriol* **49**, 351–359.

Miroshnichenko, M. L., Gongadze, G. M., Rainey, F. A., Kostyukova, A. S., Lysenko, A. M., Chernyh, N. A. & Bonch-Osmolovskaya, E. A. (1998). *Thermococcus gorgonarius* sp. nov. and *Thermococcus pacificus* sp. nov.: heterotrophic extremely thermophilic archaea from New Zealand submarine hot vents. *Int J Syst Bacteriol* **48**, 23–29.

Miroshnichenko, M. L., Hippe, H., Stackebrandt, E., Kostrinika, N. A., Chernyh, N. A., Jeanthon, C., Nazina, T. N., Belyaev, S. S. & Bonch-Osmolovskaya, E. A. (2001). Isolation and characterization of *Thermococcus sibiricus* sp. nov., from western siberia high-temperature oil reservoir. *Extremophiles* **5**, 85–91.

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.

Widdel, F. & Bak, F. (1992). Gram-negative mesophilic sulfate-reducing bacteria. In *The Prokaryotes*, 2nd edn, pp. 3352–3378. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Shleifer. New York: Springer.

Zillig, W. & Reysenbach, A.-L. (2001). Class IV. Thermococci *class. nov.* In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, pp. 342–346. Edited by D. R. Boone & R. W. Castenholz. New York: Springer.

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.