Marinitoga okinawensis sp. nov., a novel thermophilic and anaerobic heterotroph isolated from a deep-sea hydrothermal field, Southern Okinawa Trough

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A novel thermophilic and sulfur-reducing heterotrophic bacterium, strain TFS10-5^T, was isolated from a deep-sea hydrothermal field in Yonaguni Knoll IV, Southern Okinawa Trough. Cells of strain TFS10-5^T were motile rods, 1.5–5 µm in length and 0.5–0.8 µm in width. Strain TFS10-5^T was an obligately anaerobic heterotroph and sulfur-reduction stimulated growth. Growth was observed between 30 and 70 °C (optimum at 55–60 °C), pH 5.0–7.4 (optimum at pH 5.5–5.8), 1.0–5.5 NaCl % (optimum at 3.0–3.5 %). The fatty acid content was C_{16:0} (71.0 %), C_{16:1} (6.0 %), C_{18:0} (21.4 %) and C_{18:1} (1.6 %). The G + C content of the genomic DNA was 28 mol%. 16S rRNA gene sequence analysis indicated that strain TFS10-5^T belongs to the genus *Marinitoga*. Based on the physiological and phylogenetic features of the new isolate, strain TFS10-5^T represents a novel species in the genus *Marinitoga* for which the name *Marinitoga okinawensis* sp. nov. is proposed. The type strain is TFS10-5^T (=JCM 13303^T=DSM 17373^T).

The order *Thermotogales* is composed of six genera: *Thermotoga*, *Thermosiphon*, *Fervidobacterium*, *Petrotoga*, *Geotoga* and *Marinitoga*. Members of these genera have been isolated from a variety of high temperature environments such as deep-sea hydrothermal vents, hot springs and oil reservoirs. All of these organisms are thermophilic or hyperthermophilic chemo-organotrophs, but do not utilize hydrogen or other inorganic compounds as electron donors (Davey *et al.*, 1993; Huber *et al.*, 1986, 1989; Patel *et al.*, 1985; Wery *et al.*, 2001). In the genus *Marinitoga*, three thermophilic chemo-organotrophs that have optimum temperatures for growth of between 55 and 65 °C have been isolated from deep-sea hydrothermal vents and characterized. *Marinitoga camini* (Wery *et al.*, 2001), the type species of this genus, was isolated from the Mid-Atlantic Ridge (MAR). The other two recognized species in the genus, *Marinitoga piezophila* (Alain *et al.*, 2002) and *Marinitoga hydrogenitolerans* (Postec *et al.*, 2005) were isolated from the East-Pacific Rise (EPR) and MAR,

respectively. A difference in hydrogen tolerance has been observed among these three species. *M. camini* and *M. piezophila* are strongly inhibited by hydrogen in the absence of sulfur but are able to grow under hydrogen in the presence of sulfur (Alain *et al.*, 2002; Wery *et al.*, 2001). *M. hydrogenitolerans* is able to grow in the presence of hydrogen even in the absence of sulfur (Postec *et al.*, 2005). In this study, we isolated and characterized a novel thermophilic bacterium which belongs to the genus *Marinitoga* and can grow under 100 % hydrogen in the absence of sulfur.

A large piece of sulfide flange from a black smoker chimney on the Tiger chimney mound (24° 50.938' N 122° 42.020' E) in the Yonaguni Knoll IV, Southern Okinawa Trough, was obtained with the manned submersible *Shinkai 6500* during the cruise YK03-05 (July 2003) of the R/V *Yokosuka*. The main vent emission of this black smoker contained 0.8 mM H₂ kg⁻¹, 1.8 mM CH₄ kg⁻¹ and 72 mM CO₂ kg⁻¹ (Konno *et al.*, 2006) and the maximum temperature was 330 °C but the geochemistry and temperature of the fluids under the flange structure were not determined. Subsamples were taken of the surface layer and orifice of the sulfide structure of the chimney structure as described previously (Takai *et al.*, 2001) and these were stored anaerobically (with or without 0.05 % neutralized Na₂S) in glass bottles under an atmo-

Abbreviations: EPR, East-Pacific Rise; MAR, Mid-Atlantic Ridge.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain TFS10-5^T is AB262395.

Graphs showing the effects of temperature, pH and NaCl concentration on the growth of strain TFS10-5^T are available as a supplementary figure in IJSEM Online.

sphere of 100 % N₂ (200 kPa) and sealed with butyl rubber stoppers for cultivation. The subsamples were used for serial dilution cultivation tests using various media. Using MMJYPS medium, consisting of MJ synthetic seawater (Sako *et al.*, 1996), 0.1 % yeast extract (Difco), 0.1 % tryptone peptone (Difco) and 0.3 % sulfur, at a pH adjusted to around 5.5 and with a head space gas mixture of 80 % H₂:20 % CO₂ (200 kPa) and an incubation temperature of 55 °C, growth of thin rods was observed from the most diluted series ($<2.0 \times 10^5$ cells g⁻¹) and thick rods from the second-most diluted series ($<2.0 \times 10^4$ cells g⁻¹) in jars inoculated with samples from the surface of the sulfide flange structure. Pure cultures of both thin and thick rods were obtained by performing a dilution to extinction procedure three times at 55 °C. When the purity of the isolates was tested by microscopic observations, it was apparent that the thin rods were members of the D subgroup of the *Epsilonproteobacteria* (*Lebetimonas* sp.) (data not shown). The thick rods were found to belong to the genus *Marinitoga* by partial sequencing of the 16S rRNA gene. The thick rod culture was further purified by the dilution to extinction technique (Takai *et al.*, 2000) and strain TFS10-5^T was obtained.

Since the growth of chemolithoautotrophs such as *Lebetimonas acidophila* (Takai *et al.*, 2005) would also be expected in MMJYPS medium as well as growth of chemo-organotrophs and chemolitho-organotrophs, the utilization of possible electron donors (H₂, yeast extract and tryptone peptone) and acceptors (sulfur, thiosulfate, sulfate, sulfite, L-cystine, nitrate, nitrite and O₂) was examined for strain TFS10-5^T. No differences in growth rate or final cell concentration (2.0×10^8 cells ml⁻¹) were found with samples grown under 80 % H₂:20 % CO₂ and 80 % N₂:20 % CO₂ in the presence of yeast extract, tryptone peptone or elemental sulfur. No growth occurred under any autotrophic conditions. The results indicate that the novel isolate does not use H₂ as an electron donor and is not sensitive to H₂ in the presence of sulfur. In the absence of electron acceptors, strain TFS10-5^T could grow by fermentation, but the final cell concentration was 10-fold lower than that recorded in the presence of elemental sulfur (2.0×10^7 cells ml⁻¹) under 80 % N₂:20 % CO₂. Only slight suppression of growth (1.0×10^7 cells ml⁻¹) was observed under 100 % H₂. Isolate TFS10-5^T utilized elemental sulfur and L-cystine as electron acceptors and reduced them to H₂S. The isolate was very sensitive to oxygen and could not grow in medium even slightly coloured by resazurin.

Cells were routinely observed with a light microscope (BX51; Olympus). Transmission electron microscopy (TEM) with negatively stained cells was performed as described by Zillig *et al.* (1990). Cells grown in MMJYPS medium at 55 °C in the late exponential growth phase were used for TEM observations. Cells of strain TFS10-5^T were straight rods, about 1.5–5 µm in length, 0.5–0.8 µm in width and motile with a polar flagellum. Negatively stained cells showed a thick outer membrane (Fig. 1).

Fig. 1. Electron micrograph of a negatively stained cell of strain TFS10-5^T. Bar, 1 µm.

Growth of the novel isolate was determined by direct cell counting under epifluorescence after staining with 4',6-diamidino-2-phenylindole (DAPI) (Porter & Feig, 1980) using an Olympus BX51 microscope. To determine the range of temperature, pH and NaCl concentration for growth, the cultures were grown in 15 ml test tubes containing 3 ml MMJYPS medium with shaking (100 r.p.m.) in a temperature-controlled drying oven. Strain TFS10-5^T grew over the temperature range of 30–70 °C and the optimum temperature for growth was 55–60 °C. The doubling time at the optimum temperature was 0.8 h and the maximum cell density was 2.0×10^8 cells ml⁻¹. No growth was observed at 25 or 75 °C. The effect of initial pH on growth was examined at 55 °C using MMJYPS medium adjusted to various pH values as described previously (Takai *et al.*, 2005). The pH range for growth was 5.0–7.4 and the optimum pH was 5.5–5.8. The optimum NaCl concentration for growth in MMJYPS medium was also tested and growth was observed at NaCl concentrations of between 1.0 and 5.5 % (w/v); the optimum concentration range was 3.0–3.5 % NaCl (see Supplementary Fig. S1 in IJSEM Online).

The utilization of carbon sources was tested at 55 °C using MMJYPS medium without yeast extract and tryptone peptone but supplemented with a vitamin mixture (Balch *et al.*, 1979) and the following carbon sources: yeast extract, tryptone peptone, peptone, Casamino acids (Difco), gelatin, chitin, starch, glucose, fructose, maltose, galactose, lactose, cellobiose, xylose, sucrose, rhamnose, mannose, ethanol, methanol, glycerol, acetate, propionate, pyruvate, formate, fumarate, citrate, malate, succinate, tartrate, glutamate, glycine, alanine and xylan. Each substrate was tested at concentrations of 0.01 % and 0.1 %. Strain TFS10-5^T was only able to utilize proteinaceous complex substrates such as yeast extract, tryptone peptone and peptone. In order to test the utilization of carbon sources other than proteinaceous complex substrates, the substrates mentioned above were supplemented with 0.02 % yeast extract. Growth with each substrate was compared with growth in a medium that contained 0.02 % yeast extract with minerals. Starch, glucose and glycerol improved growth of strain TFS10-5^T when

Table 1. Comparison of characteristics of members of the genus *Marinitoga*

Strains/species: 1, TFS10-5^T (data from this study); 2, *M. camini* MV1075^T (Wery *et al.*, 2001); 3, *M. piezophila* KA3^T (Alain *et al.*, 2002); 4, *M. hydrogenitolerans* AT1271^T (Postec *et al.*, 2005). Substrates were tested in the presence of yeast extract (0.1 g l⁻¹ for *M. camini* and *M. hydrogenitolerans*; 0.2 g l⁻¹ for *M. piezophila* and strain TFS10-5^T. MAR, Mid-Atlantic Ridge; EPR, East-Pacific Rise; SOT, Southern Okinawa Trough. +, Positive; -, negative; (+), weakly positive; ND, not determined.

Characteristic	1	2	3	4
Origin	SOT, Yonaguni Knoll IV	MAR, Menez-Gwen site	EPR, Grandbonum site (13° N)	MAR, Rainbow site
Location	24° 51' N 122° 42' E; 1365 m	37° 51' N 31° 31' W; 980 m	12° 48' N 103° 56' W; 2630 m	36° 13' N 33° 54' W; 2275 m
Motility	+	+	+	(+)
pH for growth:				
Range	5.0–7.4	5–9	5–8	4.5–8.5
Optimum	5.5–5.8	7	6	6
Temperature for growth (°C):				
Range	30–70	25–65	45–70	35–65
Optimum	55–60	55	65	60
NaCl concentration for growth (% w/v):				
Range	1.0–5.5	1.0–4.5	1.0–5.0	1.0–6.5
Optimum	3.0–3.5	2.0	3.0	3.0–4.0
Optimal doubling time (h)	0.8	1.7	0.35	1.9
Utilization of:				
Acetate	–	ND	(+)	–
Casamino acids	–	–	+	–
Casein	–	–	+	+
Cellobiose	–	(+)	(+)	–
Fructose	–	(+)	(+)	–
Galactose	–	–	(+)	–
Glucose	+	+	(+)	–
Pyruvate	–	+	–	+
Starch	+	+	(+)	–
Tryptone	+	+	+	–
Growth without electron acceptors under 100 % H ₂	+	–	–	+
Tolerance of O ₂	<0 %	ND	ND	<4 %
Electron acceptors	Sulfur, L-cystine	Sulfur, L-cystine	Sulfur, thiosulfate, L-cystine	Sulfur, thiosulfate, L-cystine
DNA G + C content (mol %)	28	29 ± 1	29 ± 1	28

*Slight inhibition was observed.

combined with 0.02 % yeast extract, but other substrates did not support growth even when supplemented with yeast extract.

The cellular fatty acid content of cells of strain TFS10-5^T was analysed with cells grown in MMJYPS medium at 55 °C in the late exponential phase. The fatty acid extraction and analysis methods used were as described previously (Takai *et al.*, 2003). The fatty acid content was C_{16:0} (71.0 %), C_{16:1} (6.0 %), C_{18:0} (21.4 %) and C_{18:1} (1.6 %).

The sensitivity of the novel strain to various antibiotics was tested at 55 °C. Growth was inhibited by ampicillin,

chloramphenicol, erythromycin, penicillin G, novobiocin, vancomycin and rifampicin at concentrations of 25 µg ml⁻¹ and by tetracycline at 100 µg ml⁻¹. Strain TFS10-5^T was resistant to kanamycin, streptomycin and spectinomycin at 100 µg ml⁻¹.

Genomic DNA was prepared as described by Lauerer *et al.* (1986). The G + C content of genomic DNA was determined by direct analysis of deoxyribonucleotides by HPLC (Tamaoka & Komagata, 1984). The G + C content of strain TFS10-5^T was 28 mol% and this value is similar to that found previously for recognized species of the genus *Marinitoga* (Table 1).

The 16S rRNA gene of strain TFS10-5^T was amplified by PCR using primers Bac27F and 1492R (DeLong, 1992; Lane, 1991). The sequence of about 1.5 kb of amplified fragment was determined directly by the deoxynucleotide chain-termination method with a DNA sequencer (model 3100; Perkin Elmer/Applied Biosystems). The almost complete 16S rRNA gene sequence (1476 bp) was analysed by the FASTA algorithm (<http://www.ddbj.nig.ac.jp/search/fasta-j.html>). The most similar gene sequences to strain TFS10-5^T were those of *M. hydrogenitolerans* and *M. camini* (95.8 % similarity), species that were isolated from the Menez-Gwen and Rainbow sites, respectively, on the MAR (Postec *et al.*, 2005; Wery *et al.*, 2001). The next most closely related sequence was that of *M. piezophila* (95.3 % similarity), a species isolated from the Grandbonum site on EPR (13° N) (Alain *et al.*, 2002). A phylogenetic analysis based on 16S rRNA gene sequences clearly showed that strain TFS10-5^T belonged to genus *Marinitoga* and the most closely related organism was *M. piezophila* (Fig. 2).

The characteristics of strain TFS10-5^T, such as the temperature, pH and NaCl concentration for growth, the utilization of proteinaceous complex substrates and reduction of elemental sulfur and L-cystine, are consistent with those of the previously characterized species of the genus *Marinitoga*. However, several differences were observed between them (Table 1). In terms of oxygen sensitivity, strain TFS10-5^T requires strict anaerobic conditions for growth and so is different from *M. hydrogenitolerans*, which can survive with up to 4 % oxygen in the head space gas (Postec *et al.*, 2005). On the other hand, with regard to the ability to grow under a head space gas of 100 % H₂ in the

absence of electron acceptors (Postec *et al.*, 2005), the new isolate differs from *M. camini* and *M. piezophila* (Alain *et al.*, 2002; Wery *et al.*, 2001), but is similar to *M. hydrogenitolerans*. Fermentative growth of *M. camini* and *M. piezophila* does not occur under head space gas concentrations of 40 and 60 % H₂, respectively, whereas strain TFS10-5^T can grow under 100 % H₂ with only slight inhibition and the fermentation of *M. hydrogenitolerans* is not sensitive to hydrogen (Postec *et al.*, 2005) (Table 1). The sensitivity to hydrogen among *Marinitoga* species is consistent with the hydrogen concentrations found in their respective habitats. The hydrogen contents of the vent emissions at the Rainbow site on MAR and the Yonaguni Knoll IV, from where *M. hydrogenitolerans* and strain TFS10-5^T were collected, are extraordinary high at 13 and 0.8–5.5 mmol kg⁻¹, respectively (Donval *et al.*, 1997; Konno *et al.*, 2006). The hydrogen concentrations of the Menez Gwen site on MAR and 13° N on EPR, from where *M. camini* and *M. piezophila* were isolated, are 44 and 143 µmol kg⁻¹, respectively (Charlou *et al.*, 1996, 2000). In fact, *Marinitoga* species may acquire tolerance to hydrogen after inhabiting a hydrogen-enriched hydrothermal field. Given the low levels of 16S rRNA gene sequence similarity between strain TFS10-5^T and the other recognized *Marinitoga* species and the differences in physiological features (Table 1), the new strain represents a novel species of the genus *Marinitoga* for which the name *Marinitoga okinawensis* is proposed.

Description of *Marinitoga okinawensis* sp. nov.

Marinitoga okinawensis (o.ki.na.wen'sis. N.L. fem. adj. *okinawensis* of Okinawa, a region of Japan).

Cells are rod-shaped and motile with polar flagella. Growth occurs at 30–70 °C (optimum temperature is 55–60 °C), at pH 5.0–7.4 (optimum of pH 5.5–5.8) and at NaCl concentrations of 1.0–5.5 % (optimum of 3.0–3.5 %). The doubling time under optimum conditions is 0.8 h and cell concentrations reach 2.0 × 10⁸ cells ml⁻¹. Obligately anaerobic. Not sensitive to hydrogen in the presence of sulfur; fermentation is suppressed, but not completely inhibited by hydrogen. Obligately chemo-organotrophic. Grows with proteinaceous complex compounds such as yeast extract, tryptone peptone and peptone. Several carbohydrates, starch, glucose and glycerol, also support growth with yeast extract. Growth is greatly enhanced by the addition of sulfur and L-cystine. The fatty acid content is C_{16:0} (71.0 %), C_{16:1} (6.0 %), C_{18:0} (21.4 %) and C_{18:1} (1.6 %). The G + C content of genomic DNA is 28 mol% (HPLC). 16S rRNA gene sequence analysis indicates that strain TFS10-5^T is a member of the genus *Marinitoga* of the order *Thermotogales*. 16S rRNA gene sequence similarity is 95.8 % with *M. camini*.

The type strain, TFS10-5^T (=JCM 13303^T=DSM 17373^T), was isolated from a sulfide chimney structure at the Yonaguni Knoll IV hydrothermal field, Southern Okinawa Trough (24° 50.938' N 122° 42.020' E).

Fig. 2. Phylogenetic tree of species of the order *Thermotogales* based on 16S rRNA gene sequences constructed by the neighbour-joining method using 1050 homologous sequence positions for each organism. Numbers at nodes indicate bootstrap values from 100 replications. GenBank accession numbers are given in parentheses. Bar, 2 substitutions per 100 nucleotides.

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References

- Alain, K., Marteinsson, V. T., Miroshnichenko, M. L., Bonch-Osmolovskaya, E. A., Prieur, D. & Birrien, J.-L. (2002). *Marinitoga piezophila* sp. nov., a rod-shaped thermo-piezophilic bacterium isolated under high hydrostatic pressure from a deep-sea hydrothermal vent. *Int J Syst Evol Microbiol* **52**, 1331–1339.
- 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.
- Charlou, J. L., Fouquet, Y., Donval, J. P., Auzende, J. M., Jean-Baptiste, P., Stievenard, M. & Michel, S. (1996). Mineral and gas chemistry of hydrothermal fluids on an ultrafast spreading ridge: East Pacific Rise, 17° to 19° S (Naudur cruise, 1993) phase separation processes controlled by volcanic and tectonic activity. *J Geophys Res* **101**, 15899–15919.
- Charlou, J. L., Donval, J. P., Douville, E., Jean-Baptiste, P., Radford-Knoery, J., Fouquet, Y., Dapoigny, A. & Stievenard, M. (2000). Compared geochemical signatures and the evolution of Menez Gwen (37° 50' N) and Lucky Strike (37° 17' N) hydrothermal fluids, south of the Azores Triple Junction on the Mid-Atlantic Ridge. *Chem Geol* **171**, 49–75.
- Davey, M. E., Wood, W. A., Key, R., Nakamura, K. & Stahl, D. (1993). Isolation of three species of *Geotoga* and *Petrogoga*: two new genera, representing a new lineage in the bacterial line of descent distantly related to the 'Thermotogales'. *Syst Appl Microbiol* **16**, 191–200.
- DeLong, E. F. (1992). Archaea in coastal marine environments. *Proc Natl Acad Sci U S A* **89**, 5685–5689.
- Donval, J. P., Charlou, J. L., Douville, E., Knoery, J., Fouquet, Y., Poncevera, E., Jean-Baptiste, P., Stievenard, M., German, C. R. & other authors (1997). High H₂ and CH₄ content in hydrothermal fluids from Rainbow site newly sampled at 36° 14' N on the AMAR segment, Mid-Atlantic Ridge (diving FLORES cruise, July 1997): comparison with other MAR sites. *Trans Am Geophys Union* **78**, Supplement, F832 (abstract).
- Huber, R., Langworthy, T. A., König, H., Thomm, M., Woese, C. R., Sleytr, U. B. & Stetter, K. O. (1986). *Thermotoga maritima* sp. nov. represents a new genus of uniquely extremely thermophilic eubacteria growing up to 90 °C. *Arch Microbiol* **144**, 324–333.
- Huber, R., Woese, C. R., Langworthy, T. A., Fricke, H. & Stetter, K. O. (1989). *Thermosipho africanus* gen. nov., represents a new genus of thermophilic eubacteria within the "Thermotogales". *Syst Appl Microbiol* **12**, 32–37.
- Konno, U., Tsunogai, U., Nakagawa, F., Nakashima, M., Ishibashi, J., Nunoura, T. & Nakamura, K. (2006). Liquid CO₂ venting on seafloor: Yonaguni Knoll IV hydrothermal system, Okinawa Trough. *Geophys Res Lett* **33**, L16607. doi:10.1029/2006GL026115
- Lane, D. J. (1991). 16S-23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.
- Lauerer, G., Kristjansson, J. K., Langworthy, T. A., König, H. & Stetter, K. O. (1986). *Methanothermus sociabilis* sp. nov., a second species within the *Methanothermaceae* growing at 97 °C. *Syst Appl Microbiol* **8**, 100–105.
- Patel, B. K. C., Morgan, H. W. & Daniel, R. M. (1985). *Fervido-bacterium nodosum* gen. nov. and spec. nov., a new chemoorganotrophic, caldoactive, anaerobic bacterium. *Arch Microbiol* **141**, 63–69.
- Porter, K. G. & Feig, Y. S. (1980). The use of DAPI for identifying and counting aquatic microflora. *Limnol Oceanogr* **25**, 943–948.
- Postec, A., Le Breton, C., Fardeau, M. L., Lesongeur, F., Pignet, P., Querellou, J., Ollivier, B. & Godfroy, A. (2005). *Marinitoga hydrogenitolerans* sp. nov., a novel member of the order Thermotogales isolated from a black smoker chimney on the Mid-Atlantic Ridge. *Int J Syst Evol Microbiol* **55**, 1217–1221.
- Sako, Y., Takai, K., Ishida, Y., Uchida, A. & Katayama, Y. (1996). *Rhodothermus obamensis* sp. nov., a modern lineage of extremely thermophilic marine bacteria. *Int J Syst Bacteriol* **46**, 1099–1104.
- 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.
- Takai, K., Komatsu, T., Inagaki, F. & Horikoshi, K. (2001). Distribution of archaea in a black smoker chimney structure. *Appl Environ Microbiol* **67**, 3618–3629.
- Takai, K., Nakagawa, S., Sako, Y. & Horikoshi, K. (2003). *Balnearium lithotrophicum* gen. nov., sp. nov., a novel thermophilic, strictly anaerobic, hydrogen-oxidizing chemolithoautotroph isolated from a black smoker chimney in the Suiyo Seamount hydrothermal system. *Int J Syst Evol Microbiol* **53**, 1947–1954.
- Takai, K., Hirayama, H., Nakagawa, T., Suzuki, Y., Nealson, K. H. & Horikoshi, K. (2005). *Lebetimonas acidiphila* gen. nov., sp. nov., a novel thermophilic, acidophilic, hydrogen-oxidizing chemolithoautotroph within the 'Epsilonproteobacteria', isolated from a deep-sea hydrothermal fumarole in the Mariana Arc. *Int J Syst Evol Microbiol* **55**, 183–189.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Wery, N., Lesongeur, F., Pignet, P., Derennes, V., Cambon-Bonavita, M. A., Godfroy, A. & Barbier, G. (2001). *Marinitoga camini* gen. nov., sp. nov., a rod-shaped bacterium belonging to the order Thermotogales, isolated from a deep-sea hydrothermal vent. *Int J Syst Evol Microbiol* **51**, 495–504.
- Zillig, W., Holz, I., Janekovic, D., Klenk, H. P., Imsel, E., Trent, J., Wunderl, S., Forjaz, V. H., Coutinho, R. & other authors (1990). *Hyperthermus butylicus*, a hyperthermophilic sulfur-reducing archaeobacterium that ferments peptides. *J Bacteriol* **172**, 3959–3965.