# *Methanotorris formicicus* sp. nov., a novel extremely thermophilic, methane-producing archaeon isolated from a black smoker chimney in the Central Indian Ridge

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A novel extremely thermophilic, methane-producing archaeon was isolated from a black smoker chimney at the Kairei field in the Central Indian Ridge. Cells of this isolate were irregular cocci with several flagella; motility was not observed. Growth was observed between 55 and 83 °C (optimum of 75 °C; 30 min doubling time) and between pH 6·0 and 8·5 (optimum of pH 6·7). The isolate was a strictly anaerobic, methanogenic autotroph capable of using hydrogen and carbon dioxide as sole energy and carbon sources. Formate was utilized as an alternative energy source. The G+C content of the genomic DNA was 33·3 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the isolate was most closely related to *Methanotorris igneus* strain Kol 5$^T$. The isolate, however, could be genetically differentiated from this species by DNA–DNA hybridization analysis and on the basis of its physiological properties. The name *Methanotorris formicicus* sp. nov. is proposed for this isolate; the type strain is Mc-S-70$^T$ (=JCM 11930$^T$=ATCC BAA-687$^T$).

A number of methanogens belonging to hyperthermophilic or thermophilic genera of the order *Methanococcales* have been obtained from a variety of marine hydrothermal systems (Burgraff *et al.*, 1990; Huber *et al.*, 1982; Jeanthon *et al.*, 1998, 1999a, b; Jones *et al.*, 1983b, 1989; L'Haridon *et al.*, 2003; Stetter, 1996; Takai *et al.*, 2002; Zhao *et al.*, 1988). A number of strains from deep-sea hydrothermal environments have recently been added to the list: *Methanocaldococcus jannaschii* strain JAL-1$^T$, *Methanocaldococcus vulcanius* strain M7$^T$, *Methanocaldococcus fervens* strain AG86$^T$ and *Methanocaldococcus infernus* strain ME$^T$ were isolated from the East Pacific Rise, Guaymas Basin and the Mid-Atlantic Ridge (MAR) (Jeanthon *et al.*, 1998, 1999a; Jones *et al.*, 1983b, 1989; Zhao *et al.*, 1988); *Methanocaldococcus indicus* strain SL43$^T$ from the Central Indian Ridge (CIR) (L'Haridon *et al.*, 2003); and *Methanothermococcus okinawensis* strain IH1$^T$ from the Western Pacific Okinawa Trough (Takai *et al.*, 2002). Although physiological and molecular properties have not been described, Jeanthon *et al.* (1999b) have also succeeded in isolating many hyperthermophilic methanogens potentially belonging to the genus *Methanotorris* from deep-sea hydrothermal environments in the Guaymas Basin and MAR.

Historically, the systematics of methanococci has been hindered by the absence of information on the reliability of phenotypic characters (Keswani *et al.*, 1996). Recently, Whitman *et al.* (2001) reclassified the order *Methanococcales* based on a polyphasic taxonomic characterization. The newly proposed classification fits well with phylogenetic relationships associated with thermophilic behaviour among the order *Methanococcales*, and the subsequent isolation of a novel species of the genus *Methanothermococcus* has strengthened the phylogenetic affiliation of members of the genus *Methanothermococcus* (Takai *et al.*, 2002). However, the genus *Methanotorris* is still represented by only one species, *Methanotorris igneus* strain Kol 5$^T$, which was isolated from a coastal hydrothermal environment in Iceland (Burggraf *et al.*, 1990). In addition, none of

Published online ahead of print on 12 December 2003 as DOI 10.1099/ijs.0.02887-0.

Abbreviations: CIR, Central Indian Ridge; MAR, Mid-Atlantic Ridge.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Mc-S-70$^T$ is AB100884.

Graphs showing the effects of temperature, pH and NaCl concentration on growth of *Methanotorris formicicus* are available as supplementary figures A, B and C, respectively, in IJSEM Online.

the extremely thermophilic members, which are defined as micro-organisms capable of growth above 80 °C and optimally above 70 °C, has been obtained from any habitat, although many hyperthermophilic or thermophilic entities are listed in the order *Methanococcales* (L'Haridon *et al.*, 2003; Takai *et al.*, 2002; Whitman *et al.*, 2001). In this study, an extremely thermophilic strain of the genus *Methanotorris* was isolated from a black smoker chimney at the Kairei field in the CIR.

Samples from a black smoker vent were obtained from the Kairei field, CIR (25° 19·23′S; 70° 02·42′E), at a depth of 2421 m by the manned submersible *Shinkai 6500* in a dive (dive no. 659) performed in February 2002. The Kairei hydrothermal field in the CIR was discovered by Japanese scientists in 2000 (Hashimoto *et al.*, 2001). A bulk chimney sample with a vent emission temperature of $>250$ °C was brought to the sea surface in a sample box, which is part of the equipment of the *Shinkai 6500*, and immediately divided into two sections (surface layer of the chimney and vent orifice surface) as described by Takai *et al.* (2001). The chimney was mainly composed of crystalline or amorphous chalcopyrite. Each of the subsample sections (approx. 10 g) was suspended in 20 ml sterilized MJ synthetic sea water (Takai *et al.*, 1999) containing 0·05 % (w/v) sodium sulfide in a 100 ml glass bottle (Schott Glaswerke) and tightly sealed with a butyl rubber cap under a gas phase of 100 % $N_2$ (100 kPa). These suspended portions of the subsamples were inoculated (0·1 % volume of the medium) onto MMJ medium (Takai *et al.*, 2002) with 5 mM $CaCl_2$ under a gas phase of 80 % $H_2$ and 20 % $CO_2$ (300 kPa). The cultures were incubated at 70 and 85 °C in dry ovens onboard.

Growth of hyperthermophilic or thermophilic micro-organisms was observed in MMJ medium with 5 mM $CaCl_2$ after 2 days incubation at both 70 and 85 °C. Based on DAPI (4′,6-diamidino-2-phenylindole)-stained direct cell counting of the subsamples (Porter & Feig, 1980), the chimney surface layer contained $8{\cdot}9 \times 10^7$ cells (g wet weight)$^{-1}$, whereas the vent orifice surface had $1{\cdot}0 \times 10^5$ cells (g wet weight)$^{-1}$. These results were similar to previously demonstrated results on a black smoker chimney structure from the Manus Basin deep-sea hydrothermal field (Takai *et al.*, 2001). All enrichment cultures grown at 70 and 85 °C contained highly motile or non-motile irregular cocci. Since these irregular cocci had autofluorescence under UV- and blue-excitation by epifluorescence microscopy, the hyperthermophiles or thermophiles were probably methanogens, most likely members of the order *Methanococcales*. Characterization of the gas phase after growth using a GC (Micro GC CP2002; GL Sciences) revealed that methanogenesis occurred in all enrichment cultures grown at 70 and 85 °C. Pure cultures were obtained using the dilution-to-extinction technique at 70 or 85 °C with the same medium used for the enrichment (Takai & Horikoshi, 2000). At least five series of dilution-to-extinction purifications were performed. The partial sequence of the 16S rRNA gene from each of the isolates was determined and used for sequence similarity analysis as described previously (Takai *et al.*, 2001). One strain, designated Mc-S-70$^T$, was characterized further. The purity of the isolate was confirmed routinely by microscopic examination and by repeated partial sequencing of the 16S rRNA gene using several PCR primers (Lane, 1985). Potential contamination of heterotrophic thermophiles was also tested using media for strict heterotrophs such as MJYP and MJYPS (Takai *et al.*, 2000).

Cells were routinely observed under a phase-contrast Olympus BX51 microscope with the SPOT RT Slider CCD camera system (Diagnostic Instruments). Transmission electron microscopy of negatively stained cells and thin sections of cells was carried out as described by Zillig *et al.* (1990). Cells grown in MMJ medium supplemented with 5 mM $CaCl_2$ at 70 °C in the mid-exponential phase of growth were negatively stained with 2 % (w/v) uranyl acetate and observed under a JEOL JEM-1210 electron microscope at an accelerating voltage of 120 kV. Cells of strain Mc-S-70$^T$ were Gram-negative, irregular cocci, which were about 0·8–1·5 μm diameter in the exponential growth phase (Fig. 1a, b). Motility was not evident in laboratory cultures, although several thin, long flagella were observed by electron microscopy (Fig. 1a). These morphological features were quite similar to those of *Methanotorris igneus* strain Kol 5$^T$ (Burggraf *et al.*, 1990). In static culture with MMJ medium supplemented with 5 mM

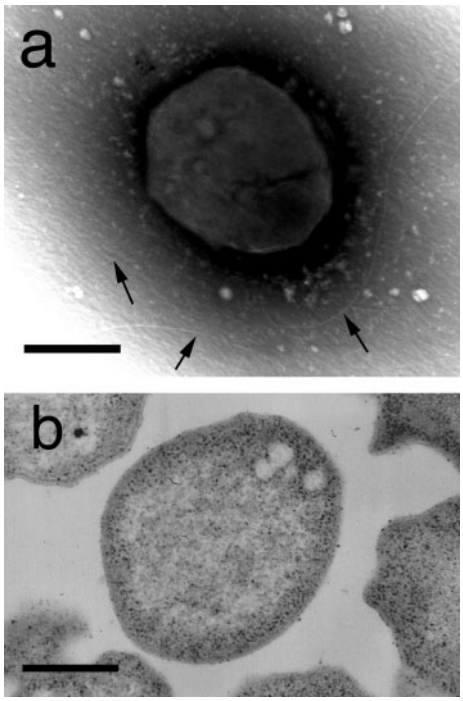


**Fig. 1.** Electron micrograph of negatively stained cells (a) and thin sections (b) of strain Mc-S-70$^T$ in the mid-exponential phase of growth. A few thin, long flagella (arrow heads) were observed (a). The cell envelope structure of the thin section shows that strain Mc-S-70$^T$ is Gram-negative. Bars, 500 nm.

$CaCl_2$, strain Mc-S-70$^T$ grew as an aggregate without causing turbidity in the liquid. Formation of an aggregate in static culture might be associated with non-motility of strain Mc-S-70$^T$. This was a distinctive growth characteristic, which was not common among other *Methanococcales* strains tested (*Methanocaldococcus jannaschii* strain JAL-1$^T$, *Methanotorris igneus* strain Kol 5$^T$, *Methanothermococcus okinawensis* strain IH1$^T$, *Methanothermococcus thermolithotrophicus* strain SN-1$^T$ and *Methanococcus maripaludis* strain JJ$^T$). *Methanocaldococcus jannaschii* strain JAL-1$^T$ (=JCM 10045$^T$), *Methanothermococcus thermolithotrophicus* strain SN-1$^T$ (=JCM 10549$^T$) and *Methanococcus maripaludis* strain JJ$^T$ (=JCM 10722$^T$) were obtained from the Japan Collection of Microorganisms (Wako, Japan) and *Methanotorris igneus* strain Kol 5$^T$ (=DSM 5666$^T$) was purchased from the Deutsche Sammlung von Mikrooganismen und Zellkulturen (Braunschweig, Germany). All strains were cultivated under optimal conditions, as described previously (Burggraf *et al.*, 1990; Huber *et al.*, 1982; Jones *et al.*, 1983a, b; Takai *et al.*, 2002).

Strain Mc-S-70$^T$ was routinely cultivated in MMJ medium supplemented with 5 mM $CaCl_2$. Growth was measured by direct cell counting after staining with DAPI (Porter & Feig, 1980) using an Olympus BX51 microscope with the SPOT RT Slider CCD camera system. All experiments described below were conducted in duplicate.

Strain Mc-S-70$^T$ grew only under strictly anaerobic culture conditions and was strongly sensitive to oxygen. It was an autotrophic methanogen utilizing hydrogen and carbon dioxide as the sole energy and carbon sources. During growth, hydrogen and carbon dioxide levels in the headspace gas decreased and methane was produced (approx. 100 p.p.m. $H_2$, 3000 p.p.m. $CO_2$ and 95 % $CH_4$ in the gas phase after 2 days incubation). The maximum cell yield in the presence of $H_2$ and $CO_2$ in MMJ medium with 5 mM $CaCl_2$ was $5{\cdot}0\times10^8$ cells ml$^{-1}$. If the concentration of calcium ions in MMJ medium was decreased to 0·2 mM, the maximum cell yield was reduced to $7{\cdot}0\times10^7$ cells ml$^{-1}$. Mc-S-70$^T$ grew on formate (10 mM) in the absence of $H_2$ ($8{\cdot}0\times10^7$ cells ml$^{-1}$ after 2 days incubation) in MMJ medium with 5 mM $CaCl_2$, indicating that strain Mc-S-70$^T$ was able to utilize formate as an alternative energy source. Acetate (20 mM), methanol (0·05 %, v/v), ethanol (0·05 %, v/v), dimethyl sulfide (0·2 % v/v), trimethylamine (0·2 %, v/v), yeast extract (0·02 %, w/v), peptone (0·02 %, w/v), tryptone (0·02 %, w/v), Casamino acids (0·02 %, w/v) and an amino acid mixture (containing 0·001 %, w/v, each of 20 amino acids) did not replace the growth requirement for $H_2$ or stimulate growth in the presence of $H_2$ and $CO_2$. The following nitrogen sources were used to replace the 5 mM ammonium ion in MMJ medium (with 220 kPa $H_2$ and 80 kPa $CO_2$ in the gas phase): 5 mM nitrate; 0·5 mM nitrite; and 100 kPa $N_2$. Ammonium ion was the most effective nitrogen source: the maximum cell yield obtained was $5{\cdot}0\times10^8$ cells ml$^{-1}$. Both nitrate and $N_2$ also served as nitrogen sources and their maximum cell yields were $1{\cdot}6\times10^8$ and $2{\cdot}4\times10^8$ cells ml$^{-1}$, respectively. Nitrite did not serve as a nitrogen source. The presence of 10 mM magnetite ($Fe_3O_4$) and 10 mM thiosulfate ($S_2O_3^{2-}$) had no effect on growth and 3 % (w/v) elemental sulfur ($S^0$) slightly inhibited growth. Selenium, tungsten and vitamin mixture were neither stimulatory nor required for growth. Utilization of formate as a growth substrate and methanogenesis were distinctive features of strain Mc-S-70$^T$ compared to *Methanotorris igneus* strain Kol 5$^T$.

The effects of temperature, pH and NaCl concentration on growth were tested. With MMJ medium supplemented with 5 mM $CaCl_2$, strain Mc-S-70$^T$ grew at 55–83 °C, showing optimal growth at 75 °C; the generation time at 75 °C and pH 6·7 was about 30 min (see Fig. A, available as supplementary material in IJSEM Online). No growth was observed at 45 or 85 °C. To determine the effect of pH on growth, the pH of MMJ medium containing 5 mM $CaCl_2$ was adjusted to various levels with 10 mM acetate/acetic acid buffer (pH 4·0–5·0), MES (pH 5·0–6·0), PIPES (pH 6·0–7·0), HEPES (pH 7·0–7·5) and Tris (pH 8·0–9·5). Growth of strain Mc-S-70$^T$ at 70 °C occurred at pH 6·0–8·5, with optimum growth at about pH 6·7 (see Fig. B, available as supplementary material in IJSEM Online). The pH was stable during the cultivation period. The effect of NaCl concentration on growth was determined using MMJ medium with 5 mM $CaCl_2$ containing different amounts of NaCl. Strain Mc-S-70$^T$ grew in 4–60 g NaCl l$^{-1}$, with optimum growth at 24 g NaCl l$^{-1}$, 70 °C and pH 6·7 (see Fig. C, available as supplementary material in IJSEM Online). Compared with *Methanotorris igneus* strain Kol 5$^T$, strain Mc-S-70$^T$ had a lower optimal growth temperature, a lower shifted growth temperature range and a slightly higher shifted pH range.

The sensitivity of strain Mc-S-70$^T$ to antibiotics such as chloramphenicol (50, 100 and 200 μg ml$^{-1}$), streptomycin (100 and 200 μg ml$^{-1}$), kanamycin (100 and 200 μg ml$^{-1}$), ampicillin (100 and 200 μg ml$^{-1}$) and rifampicin (50 and 100 μg ml$^{-1}$) was tested at 70 °C. A simultaneous experiment was performed with *Methanotorris igneus* strain Kol 5$^T$ at 80 °C. *Methanotorris igneus* strain Kol 5$^T$ and strain Mc-S-70$^T$ showed the same antibiotic resistance pattern. Both strains were resistant to streptomycin (up to 200 μg ml$^{-1}$), kanamycin (up to 200 μg ml$^{-1}$) and rifampicin (up to 50 μg ml$^{-1}$), but sensitive to chloramphenicol (50 μg ml$^{-1}$) and rifampicin (100 μg ml$^{-1}$). Susceptibility to lysis by SDS and a hypotonic solution was tested as described previously (Boone & Whitman, 1988). Cells of strain Mc-S-70$^T$ lysed with 0·1 % (w/v) SDS solution and hypotonic solutions [$10^{-1}$ diluted MJ(−N) synthetic sea water (Takai *et al.*, 2000) and distilled water].

Hydrocarbon chains in core ether lipids were analysed by a combined method described by Koga *et al.* (1993) and DeLong *et al.* (1998). Total lipid was extracted from lyophilized cells of strain Mc-S-70$^T$ (50 mg) harvested in late-exponential growth phase by the method of Nishihara & Koga (1987) and Koga *et al.* (1993). Then, hydrocarbon

chains were prepared by HI degradation followed by $LiAlH_4$ reduction as described by DeLong *et al.* (1998). The resulting hydrocarbons were analysed by GLC (model GC-380; GL-Science) equipped with a mass spectrometer (GCMS-QP5050; Shimadzu) at a temperature increasing from 100 to 320 °C at a rate of 4 °C $min^{-1}$. A simultaneous experiment was performed with *Methanotorris igneus* strain Kol $5^T$, *Methanocaldococcus jannaschii* strain JAL-$1^T$ and *Methanothermococcus okinawensis* strain IH$1^T$. The hydrocarbon chains of strain Mc-S-$70^T$ were $C_{20}$ (76·9 %), derived from archaeol and hydroxyarchaeol, and $C_{40}$ (23·1 %), derived from caldarchaeol and cyclic archaeol. No $C_{40}$ isoprenoid containing cyclopentane and cyclohexane rings was detected in strain Mc-S-$70^T$. Compared to hydrocarbons in *Methanotorris igneus* strain Kol $5^T$ ($C_{20}$, 55·4 %; $C_{40}$, 44·6 %), *Methanocaldococcus jannaschii* strain JAL-$1^T$ ($C_{20}$, 19·6 %; $C_{40}$, 80·4 %) and *Methanothermococcus okinawensis* strain IH$1^T$ ($C_{20}$, 37·8 %; $C_{40}$, 63·2 %), a lower proportion of $C_{40}$ isoprenoid was found in strain Mc-S-$70^T$.

Genomic DNA of strain Mc-S-$70^T$ was prepared as described by Marmur & Doty (1962). The DNA G+C content was determined by direct analysis of deoxyribonucleotides by HPLC (Tamaoka & Komagata, 1984). The G+C content of the genomic DNA of strain Mc-S-$70^T$ was 33·3 mol%, which is slightly higher than that of *Methanotorris igneus* strain Kol $5^T$ (Table 1).

The 16S rRNA gene was amplified by PCR using Arch 21F and 1492R primers (DeLong, 1992; Lane, 1985) as described previously (Takai *et al.*, 2002). The nearly complete sequence (1374 bp) of the 16S rRNA gene from strain Mc-S-$70^T$ was directly sequenced from both strands using the dideoxynucleotide chain-termination method with a DNA sequencer model 3100 (Perkin Elmer/Applied Biosystems). The 16S rRNA gene sequence was analysed using the gapped-BLAST search algorithm (Altschul *et al.*, 1997; Benson *et al.*, 1998) and was most closely related (97·8 %) to that of *Methanotorris igneus* strain Kol $5^T$ (Burggraf *et al.*, 1990). This suggested that strain Mc-S-$70^T$ belongs to the genus *Methanotorris*.

The nearly complete sequence was manually realigned to 16S rRNA gene data from the Ribosomal Data Project II (Maidak *et al.*, 2000), based on alignments determined using the SEQUENCE ALIGNER program of RDP-II. Phylogenetic analyses were restricted to nucleotide positions that could be unambiguously aligned. Evolutionary distance matrix analysis (using the Kimura two-parameter method, the least-squares distance method and transition/transversion rate of 2·0) and neighbour-joining analysis were performed using the PHYLIP package (version 3.5; obtained from J. Felsenstein, University of Washington, Seattle, WA, USA) (Fig. 2). Bootstrap analysis was performed to provide confidence estimates for phylogenetic tree topologies. The phylogenetic tree indicated that strain Mc-S-$70^T$ is closely related to *Methanotorris igneus* strain Kol $5^T$, as determined by sequence similarity analysis (Fig. 2).

DNA–DNA hybridization was carried out between the genomic DNA of strain Mc-S-$70^T$ and *Methanotorris igneus* strain Kol $5^T$ at 42 °C for 3 h and was measured fluorometrically using photobiotin according to the method of Ezaki *et al.* (1989). The mean hybridization value was 5·1 %, indicating that strain Mc-S-$70^T$ could be genotypically differentiated from the previously described species of the genus *Methanotorris*.

Strain Mc-S-$70^T$ was isolated from a black smoker chimney of a deep-sea hydrothermal vent at a depth of 2421 m at the Kairei field, CIR. Phylogenetic analysis indicated that strain Mc-S-$70^T$ is most closely related to *Methanotorris igneus* strain Kol $5^T$, which was isolated from a coastal hydrothermal environment in Iceland (Burggraf *et al.*, 1990), and was probably a member of the genus *Methanotorris*. However, many of the physiological characteristics of strain Mc-S-$70^T$ differed from those of *Methanotorris igneus* strain Kol $5^T$ (Table 1). Strain Mc-S-$70^T$ is the first extreme thermophile found within the order *Methanococcales* that grows optimally at 75 °C, which is approximately 10 °C lower than the optimum of *Methanotorris igneus* strain Kol $5^T$ (Table 1). In addition, *Methanotorris igneus* strain Kol $5^T$ is known as the most acidiphilic thermophilic methanogen (Wiegel, 2002), having an optimal pH for growth of pH 5·7,

**Table 1.** Properties that differentiate strains of *Methanotorris formicicus* and *Methanotorris igneus*

| Character | *Methanotorris formicicus* strain Mc-S-$70^T$ | *Methanotorris igneus* strain Kol $5^T$ |
|---|---|---|
| Isolation environment | Deep-sea black smoker chimney, CIR | Coastal hydrothermal system, Iceland |
| Cell diameter (μm) | 0·8–1·5 | 1·0–2·0 |
| Formate as a substrate for methane synthesis | + | − |
| Yeast extract stimulation of growth | − | + |
| Temperature range (optimum) (°C) | 55–83 (75) | 45–91 (88) |
| pH range (optimum) | 6·0–8·5 (6·7) | 5·0–7·5 (5·7) |
| NaCl range (optimum) (%, w/v) | 0·4–6·0 (2·4) | 0·9–5·4 (1·8) |
| Genomic DNA G+C content (mol%) | 33·3 | 31 |
| References | This study | Burggraf *et al.* (1990); Whitman *et al.* (2001) |

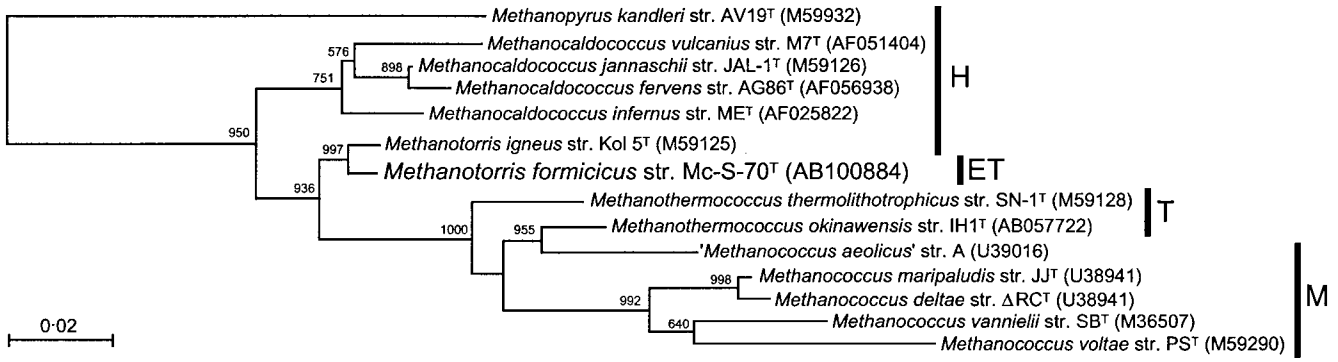


**Fig. 2.** Phylogenetic tree of representative members of the order *Methanococcales* inferred from 16S rRNA gene sequences by the neighbour-joining method using 1208 homologous sequence positions for each organism. The number at each node represents the bootstrap value (1000 replicates). Bar, 2 substitutions per 100 nt. H, Hyperthermophilic; ET, extremely thermophilic; T, thermophilic; M, mesophilic.

whereas strain Mc-S-70$^T$ grows optimally at neutral pH (Table 1). Another distinctive feature is the utilization of formate as a substrate for growth and methanogenesis in strain Mc-S-70$^T$ (Table 1). All the hyperthermophilic members of the order *Methanococcales* known so far, other than a hyperthermophilic isolate (strain CS-1) from the Guaymas Basin (Jones *et al.*, 1989), are unable to use formate as a sole energy source (Whitman *et al.*, 2001). In sharp contrast, all thermophilic and mesophilic entities of the order *Methanococcales* can utilize formate as a sole energy source, providing a similar growth yield to $H_2$ (Whitman *et al.*, 2001). In strain Mc-S-70$^T$, formate gives considerably lower growth yield than $H_2$, but can serve as an alternative energy source (Table 1). The extremely thermophilic behaviour and formate utilization of strain Mc-S-70$^T$ are important physiological features that support genetic differentiation between strain Mc-S-70$^T$ and *Methanotorris igneus* strain Kol 5$^T$ by DNA–DNA relatedness. These may also represent intermediate traits during evolution of the order *Methanococcales* from hyperthermophile to mesophile. On the basis of these physiological and genetic properties, it is proposed that the isolate is classified as a novel species of the genus *Methanotorris*, *Methanotorris formicicus* sp. nov., with Mc-S-70$^T$ as the type strain.

### Description of *Methanotorris formicicus* sp. nov.

*Methanotorris formicicus* (for.mi′ci.cus. N.L. neut. n. *acidum formicum* formic acid; N.L. neut. adj. *formicicus* pertaining to formic acid).

Irregular coccus, mean diameter of 0·8–1·5 μm. Cells occur singly or in pairs. Non-motile, but with a few thin, long flagella, which are easily removed from the cell. Strictly anaerobic and obligately methanogenic. The temperature range for growth is 55–83 °C (optimum of 75 °C). The pH range for growth is pH 6·0–8·5 (optimum of pH 6·7). NaCl is required for growth, which occurs in 4–60 g NaCl $l^{-1}$ (optimum growth at 24 g $l^{-1}$). Grows using molecular hydrogen or formate as an electron donor and carbon dioxide as an electron acceptor and a carbon source. Ammonium, molecular nitrogen and nitrate serve as nitrogen sources. Vitamins, selenium, tungsten, magnetite ($Fe_3O_4$) and thiosulfate do not stimulate growth. Resistant to ampicillin (200 μg $ml^{-1}$), kanamycin (200 μg $ml^{-1}$), rifampicin (50 μg $ml^{-1}$) and streptomycin (200 μg $ml^{-1}$), but sensitive to chloramphenicol (50 μg $ml^{-1}$) and rifampicin (100 μg $ml^{-1}$). Susceptible to lysis by 0·1 % (w/v) SDS solution and hypotonic solutions. Major hydrocarbon chains of core lipids are $C_{20}$ (76·9 %) and $C_{40}$ (23·1 %). 16S rRNA gene sequence exhibits 97·8 % similarity to that of *Methanotorris igneus* strain Kol 5$^T$. DNA–DNA relatedness to *Methanotorris igneus* strain Kol 5$^T$ is low.

The type strain is Mc-S-70$^T$ (=JCM 11930$^T$=ATCC BAA-687$^T$), isolated from a black smoker chimney in the Kairei field, Central Indian Ridge, Indian Ocean. Its genomic DNA G+C content is 33·3 % (HPLC method).

## Acknowledgements

We would like to thank Dr K. Uematsu for assistance in preparing electron micrographs. We are very grateful to the *R/V Yokosuka* crews and the *Shinkai 6500* operation team for helping us to obtain deep-sea hydrothermal vent samples.

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