***Methanococcus vulcanius* sp. nov., a novel hyperthermophilic methanogen isolated from East Pacific Rise, and identification of *Methanococcus* sp. DSM 4213^T as *Methanococcus fervens* sp. nov.**

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An autotrophic, hyperthermophilic methanogen (M7^T) was isolated from a deep-sea hydrothermal chimney sample collected on the East Pacific Rise at a depth of 2600 m. The coccoid-shaped cells are flagellated and exhibit a slight tumbling motility. The temperature range for growth at pH 6.5 was 49–89 °C, with optimum growth at 80 °C. The optimum pH for growth was 6.5, and the optimum NaCl concentration for growth was around 25 g l⁻¹. The new isolate used H₂ and CO₂ as the only substrates for growth and methane production. Tungsten, selenium and yeast extract stimulated growth significantly. In the presence of CO₂ and H₂, the organism reduced elemental sulphur to hydrogen sulphide. Growth was inhibited by chloramphenicol and rifampicin, but not by ampicillin, kanamycin, penicillin and streptomycin. The G+C content of the genomic DNA was 31 mol%. As determined by 16S rDNA gene sequence analysis, this organism was closely related to *Methanococcus jannaschii* strain JAL-1^T. However, despite the high percentage of similarity between their 16S rDNA sequences (97.1%), the DNA–DNA hybridization levels between these strains were less than 5%. On the basis of these observations and physiological traits, it is proposed that this organism should be placed in a new species, *Methanococcus vulcanius*. The type strain is M7^T (= DSM 12094^T). During the course of this study, the 16S rDNA sequence analysis placed *Methanococcus* sp. strain AG86^T (= DSM 4213^T) as a close relative of *M. jannaschii* strain JAL-1^T. However, the weak level of DNA–DNA hybridization with this strain (< 10%) allowed the proposal that strain AG86^T also constitutes a new species, *Methanococcus fervens*.

Keywords: deep-sea hydrothermal vents, thermophiles, *Archaea*, *Methanococcus*, *Methanococcus vulcanius*

INTRODUCTION

Within the order *Methanococcales*, the family *Methanococcaceae* contains one genus, *Methanococcus*, which is composed of eight species of mesophilic and thermophilic organisms (Burggraf *et al.*, 1990; Whit-

man *et al.*, 1992; Jeanthon *et al.*, 1998). The methanococci appear to be restricted to marine environments. *Methanococcus vannielii* was isolated from mud in San Francisco Bay (Stadtman & Barker, 1951). A number of strains of *Methanococcus voltae* and *Methanococcus maripaludis* were isolated from estuarine and salt marsh sediments (Balch *et al.*, 1979; Jones *et al.*, 1983a; Whitman *et al.*, 1986). The source of '*Methanococcus aeolicus*' has not been reported. Two strains of *Methanococcus thermolithotrophicus* were isolated from coastal geothermally heated sediments close to

The GenBank accession numbers for the 16S rDNA sequences of *Methanococcus vulcanius* M7^T and *Methanococcus fervens* AG86^T are AF051404 and AF056938, respectively.

Naples (Italy) (Huber *et al.*, 1982) and from reservoir water from a North Sea oilfield (Nilsen & Torsvik, 1996). The selenium-independent hyperthermophilic methanogen *Methanococcus igneus* was isolated from a shallow submarine hydrothermal vent (Kolbeinsey ridge, Iceland) (Burggraf *et al.*, 1990). Furthermore, *Methanococcus jannaschii* and *Methanococcus infernus* have been isolated from deep-sea hydrothermal vents from the East Pacific Rise at 21° N and Mid-Atlantic Ridge at 23° N (Jones *et al.*, 1983b; Jeanthon *et al.*, 1998). Close relatives to *M. jannaschii* that included strain AG86^T were also obtained from deep-sea sediments at Guaymas Basin (Zhao *et al.*, 1988; Jones *et al.*, 1989; Canganella & Jones, 1994). Physiological characteristics of strain AG86^T have been described and phenotypic and chromosomal differences with *M. jannaschii* have been observed (Zhao *et al.*, 1988). As part of this paper, we confirm that strain AG86^T constitutes a new species using DNA–DNA hybridization and 16S rDNA phylogenetic analysis. Additionally, we report the isolation and characterization of a novel hyperthermophilic *Methanococcus* sp. isolated from a deep-sea hydrothermal vent chimney collected at 13° N on the East Pacific Rise.

METHODS

Source of organisms. The new strain was isolated from a chimney sample collected from the 13° N hydrothermal field (12°48' N, 103°56' W) during the 'Hero' cruise (1991), on the East Pacific Rise at a depth of 2600 m. *Methanococcus infernus* strain ME^T (DSM 11812^T) was isolated by our laboratory (Jeanthon *et al.*, 1998). *Methanococcus jannaschii* strain JAL-1^T (DSM 2661^T), *Methanococcus* sp. strain AG86^T (DSM 4213^T), *Methanococcus igneus* strain Kol 5^T (DSM 5666^T) and *Methanococcus thermolithotrophicus* strain SN-1^T (DSM 2095^T) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany).

Enrichment, isolation and growth conditions. Enrichments were performed anaerobically in 50 ml or 100 ml vials containing 10 or 20 ml medium, respectively (Miller & Wolin, 1974). The enrichment and culture medium consisted of (per litre distilled water): 30 g sea salts (Sigma), 1 g NH₄Cl, 0.35 g KH₂PO₄, 3.46 g PIPES, 1 g NaHCO₃, 2 g Difco yeast extract, 0.5 g cysteine.HCl, 1 ml trace element mixture (Widdel & Bak, 1992), 30 mg tungstate, 0.5 mg selenate, 1 ml vitamin mixture (Widdel & Bak, 1992), 1 ml thiamin solution (Widdel & Bak, 1992), 0.05 mg vitamin B₁₂, 1 ml growth-stimulating factors (Pfennig *et al.*, 1981) and 1 mg resazurin. The pH was adjusted to 6.5 using 1 M HCl before autoclaving, and the medium was reduced by adding Na₂S.9H₂O (final concentration of 0.05%, w/v). H₂/CO₂ [80:20; 200 kPa (above atmospheric pressure)] was used as the gas phase. Unless indicated otherwise, cultures were incubated at 80 °C, and the pH of the medium was re-adjusted after 1 h incubation. Single colonies were obtained and purified by streaking on the same medium that was reduced with a titanium(III) citrate solution (Zehnder & Wuhrman, 1976) and solidified with 0.7% (w/v) Phytagel (a gellan gum from Sigma). Plates were incubated in anaerobic jars at 75 °C for 3 d under a H₂/CO₂ atmosphere (80:20; 300 kPa). Stock cultures of the isolate M7^T were stored in culture medium at 4 °C. For long-term storage, pure cultures

were stored at –80 °C in the same medium containing 20% (w/v) glycerol.

Determination of growth. Growth was monitored by measuring the increase in OD₆₀₀ with a Spectronic 20D spectrophotometer (Bioblock). Direct cell counts were determined using cells stained with acridine orange and epifluorescence microscopy using an ocular grid (Hobbie *et al.*, 1957). All growth experiments were performed in duplicate.

Determination of growth parameters. The pH range for growth was determined in the culture medium with various buffers at a concentration of 10 mM; acetate/acetic acid buffer for pH 4–5, MES for pH 5.5 and 6.0, PIPES buffer for pH 6.5 and 7.0, HEPES buffer for pH 7.5, Tris for pH 8 and 8.5. The pH of the medium was adjusted after 1 h incubation at the optimal temperature for growth. The effect of NaCl on growth was determined in the same medium containing 0, 1.25, 2.5, 3.75, 5.0, 6.25 and 7.5% (w/v) NaCl. The effects of pH and concentration of NaCl were determined at the optimal temperature for growth.

Determination of growth requirements. The ability of the organism to utilize substrates other than H₂ was tested by using the culture medium without yeast extract amended with autoclaved or filter-sterilized substrates (see below). N₂/CO₂ (80:20; 200 kPa) was used as the gas phase. Acetate (2 g l⁻¹), formate (5 g l⁻¹), methanol (0.5%, v/v), monomethylamine (2 g l⁻¹) and yeast extract (2 g l⁻¹) were also tested as possible carbon sources in the medium without bicarbonate by using H₂ (100%; 200 kPa) as the gas phase. Selected nitrogenous compounds were tested for suitability as nitrogen sources using ammonium-free mineral medium (Widdel & Bak, 1992). Nitrogenous compounds were added at 10 mM final concentration. Sulphur (5 g l⁻¹), thiosulphate (20 mM) and sulphate (20 mM) were tested as potential electron acceptors in the same mineral medium where NH₄Cl (0.35 g l⁻¹) was added but sulphate was omitted. To determine their potential stimulatory effects on the growth yield, the vitamin mixture, trace element solution, yeast extract, tungstate and selenate were tested individually in the basal medium that consisted of (per litre distilled water): 30 g sea salts, 1 g NH₄Cl, 0.35 g KH₂PO₄, 3.46 g PIPES, 1 g NaHCO₃, 0.5 g cysteine.HCl and 1 mg resazurin. The pH was adjusted as before, and H₂/CO₂ (80:20; 200 kPa) was used as the gas phase.

Antibiotic susceptibility. Sensitivity of strains M7^T and AG86^T to chloramphenicol (75 µg ml⁻¹), penicillin G (200 µg ml⁻¹), streptomycin (200 µg ml⁻¹), kanamycin (200 µg ml⁻¹), ampicillin (200 µg ml⁻¹) and rifampicin (50 µg ml⁻¹) (all from Sigma) was tested at 80 °C. Simultaneous experiments were performed with the thermophilic methanogens *M. infernus* strain ME^T, *M. jannaschii* strain JAL-1^T and *M. igneus* strain Kol 5^T at 80 °C and *M. thermolithotrophicus* strain SN-1^T at 65 °C.

Light and electron microscopy. An Olympus BH-2 microscope equipped with an Olympus OM-2 camera was used routinely to observe and count the cells. For negative staining, 20 µl bacterial suspension fixed with 2% (w/v) glutaraldehyde was dropped on Formvar/carbon-coated grids (400 mesh) and stained with 4% (w/v) uranyl acetate. Preparation of cells for freeze-fracturing and ultrathin sectioning was performed as described previously (Sleytr *et al.*, 1988). Electron micrographs were taken using a model CM100 electron microscope (Philips) with an acceleration voltage of 80 kV.

H₂S production. H₂S production was evaluated by adding 500 µl of a solution of CuSO₄ (5 mM) and HCl (50 mM) to

250 µl culture grown at 85 °C. The dark-brown precipitate demonstrating the presence of sulphide was compared to that of the uninoculated medium incubated under the same conditions.

Methane determination. Methane was measured with a Girdel model 3000 gas chromatograph equipped with a thermal conductivity detector. Samples (0.5 ml) were injected onto a Poropak Q (80–100 mesh) column and eluted with helium at a flow rate of 12 ml min⁻¹. Temperatures were as follows: injector, ambient temperature; column, 60 °C; detector, 150 °C. These conditions allowed us to follow the methane production and hydrogen consumption simultaneously.

DNA extraction and base composition. Genomic DNA of strain M7^T was isolated by using the procedure described by Charbonnier & Forterre (1994). The DNA was purified on a caesium chloride gradient (Sambrook *et al.*, 1989), and purity was checked spectrophotometrically. The G+C content of the DNA was determined from the melting point according to Marmur & Doty (1962) using *Escherichia coli* DNA (52 mol% G+C; Sigma), *Clostridium perfringens* DNA (24 mol% G+C; Sigma) and *Micrococcus luteus* DNA (73 mol% G+C; Sigma) as standards.

PCR amplification and sequencing of 16S rDNA. The 16S rDNA was amplified by PCR as described previously (Reysenbach *et al.*, 1992). The double-strand PCR products were sequenced with an ABI 373 automated sequencer. The 16S rDNA sequences were aligned manually with a representative set of 16S rRNA sequences obtained from the Ribosomal Database Project (Maidak *et al.*, 1996) and from recent GenBank releases. The GenBank accession numbers for the 16S rRNA sequences reported in this paper are as follows: *M. infernus* ME^T, AF025822; *M. jannaschii* JAL-1^T, M59126; *M. thermolithotrophicus* SN-1^T, M59128; *M. igneus* Kol 5^T, M59125; *M. voltae* PS^T, M59290; and '*M. aeolicus*' A, U39016. The secondary structure was used as a guide to ensure that only homologous regions were compared. A total of 1424 (strain M7^T) and 1400 (strain AG86^T) nucleotides were sequenced and 1377 were used in the phylogenetic analysis. Distance trees were constructed by the least-squares algorithm of De Soete (1983). Maximum-likelihood trees and corresponding bootstrap proportions were constructed using the program fastDNAm1 (Olsen *et al.*, 1994). Bootstrap values were obtained for 100 replicate trees.

DNA hybridization. Genetic relatedness was investigated by slot-blot DNA–DNA hybridization by using a random-prime labelling and signal amplification system (Amersham Life Sciences) following the procedure described by Kristjánsson *et al.* (1994). Increasing amounts of target DNA (50–100 ng) denatured in 0.4 M NaOH were slotted onto a nylon hybridization membrane (Bio-Rad) and probed with 200 ng labelled tracer DNA. For each duplicate of DNA–DNA reassociation (15 h in buffer 4× SSC with formamide, 0.5% blocking agent, 5% dextran sulphate, 100 µg denatured sheared salmon sperm DNA ml⁻¹), the temperature of hybridization chosen was in the optimal range of the hybridization buffer (Johnson, 1984; Ivanova *et al.*, 1988). Final high stringent washes and signal amplification were performed according to the manufacturer's instructions. Hybridization signals were detected with a Storm fluorescent scanner (Molecular Dynamics) and analysed by using the IMAGE-QUANT program. Signal (maximum peak area) produced by self-hybridization of the probe with homologous target DNA was set as 100% and compared with the signal generated by heterologous DNA.

RESULTS

Enrichment and isolation

To enrich for methanogenic thermophiles, 10 ml enrichment medium was inoculated with approximately 1 ml of a chimney suspension. The enrichments were incubated at 80 °C in 50 ml vials with H₂/CO₂ as the gas phase (80:20; 200 kPa) without shaking. Within 2–3 d, turbidity due to cell growth was observed. This growth consisted of motile coccoid cells that fluoresced intense green at 420 nm. Pure cultures were obtained by streaking subcultures onto solidified culture medium and incubating in an anaerobic jar with the same gas phase at 75 °C. On solid medium, pale-yellow round colonies (1 mm in diameter) were visible after incubation for up to 3 d. One colony was randomly picked and was designated isolate M7^T.

Morphology

Cells of strain M7^T were irregular cocci, about 1–3 µm in diameter (Fig. 1a). Ultrathin sections of whole cells of strain M7^T possessed the typical archaeal cell envelope profile consisting of the cytoplasmic membrane and a single surface layer (S-layer) (Fig. 1b). They occurred singly or in pairs (Fig. 1a) and exhibited tumbling motility with three tufts of flagella, each tuft containing a large number of flagella (Fig. 1c). Since the strain was heavily flagellated, flagella were observed even in thin sections (Fig. 1b). The methods used to prepare the cells for freeze-fracturing did not allow us to recognize an S-layer lattice. From our experience, we know that the S-layer proteins are so delicate that the discrete lattice is disrupted during preparation. The cells were markedly fragile and readily lysed by detergents (in 1–2 min with 0.01% SDS or *N*-lauroylsarcosine) or by distilled water.

Determination of growth parameters

Strain M7^T grew between 49 and 89 °C with optimum growth around 80 °C, while no growth was detected at 48 and 90 °C (Fig. 2a). Growth was observed between pH 5.25 and 7.0, with optimum growth around pH 6.5 (data not shown). Growth occurred in NaCl concentrations ranging from 6.25 to 56.25 g l⁻¹ (Fig. 2b), with optimum growth at 25 g l⁻¹. No growth was observed at 3.1 and 62.5 g l⁻¹. Under optimal growth conditions (temperature, pH and NaCl) the doubling time of strain M7^T was approximately 45 min.

Determination of growth requirements

Strain M7^T is a strictly anaerobic autotrophic organism. Its growth is prevented in the presence of low levels of oxygen, and H₂+CO₂ serve as the only substrates for growth. No growth was observed on acetate, formate, methanol, monomethylamine or yeast extract with an N₂/CO₂ (80:20; 200 kPa) or H₂ (100%; 200 kPa) headspace. As a control, *M. thermo-*

Fig. 1. Phase-contrast micrograph (a) of strain M7^T (bar, 5 µm) and electron micrographs (b, c) of strain M7^T. Ultrathin sections (b) of whole cells showed the typical archaeal cell envelope profile; bar, 100 nm. S, S-layer; CM, cytoplasmic membrane; F, flagellum. Freeze-etched and shadowed preparations (c) showed the flagella and the S-layer. Arrows indicate the presence of the S-layer lattice; bar, 100 nm.

lithotrophicus strain SN-1^T grew on formate at 65 °C in the presence of N₂/CO₂. In the presence of H₂ and CO₂, growth was accompanied by exponential methane production which paralleled growth (data not shown). Large amounts of methane (up to 32 µmol ml⁻¹) were produced when cells entered in the stationary phase (12 h incubation). Ammonium was preferably used as nitrogen source but significant growth also occurred in the presence of nitrate (data not shown). When supplemented individually in the basal medium, the vitamin mixture, tungstate solution, selenate solution and yeast extract stimulated the growth yield by two- to threefold. Final cell densities (two to three times lower than the control) revealed that the trace element solution was slightly inhibitory (data not shown). When sulphur was added to the medium in the presence of CO₂ and H₂, growth occurred and H₂S was produced. No dissimilatory reduction of sulphate and thiosulphate was observed.

Sensitivity to antibiotics

Strain M7^T, strain AG86^T, *M. jannaschii* strain JAL-1^T, *M. igneus* strain Kol 5^T, *M. infernus* strain ME^T and *M. thermolithotrophicus* strain SN-1^T were resistant to ampicillin and kanamycin (200 µg ml⁻¹) and were sensitive to chloramphenicol (75 µg ml⁻¹). Among these strains, only *M. thermolithotrophicus* strain SN-1^T was inhibited by penicillin and streptomycin (200 µg ml⁻¹). Finally, *M. jannaschii* strain JAL-1^T, *M. infernus* strain ME^T and strain M7^T were sensitive to rifampicin (50 µg ml⁻¹) whereas strain AG86^T, *M. igneus* strain Kol 5^T and *M. thermo-*

lithotrophicus strain SN-1^T were resistant to this compound at the same concentration.

DNA base composition

The G+C content of the DNA of strain M7^T determined by the thermal denaturation method was 31 mol%. As a control, the base composition of *Fervidobacterium nodosum* was determined to be 35 mol% [34 mol% by the *T_m* method reported by Patel *et al.* (1985) and by direct base analysis reported by Huber *et al.* (1990)].

16S rDNA sequence analysis

16S rDNA sequence analysis placed strain M7^T as a close relative of *M. jannaschii* strain JAL-1^T (97.6% similarity), strain AG86^T (96.9% similarity) and *M. infernus* (95.7% similarity) (Fig. 3). Strain AG86^T showed the highest similarity to the 16S rDNA sequence of *M. jannaschii* (97.7%). Phylogenetic trees generated using distance algorithms or maximum-likelihood analysis gave the same topology. The bootstrap values from 100 samplings confirmed the affiliation of strain M7^T to a clade (monophyletic taxon) that also included *M. jannaschii* strain JAL-1^T, strain AG86^T and *M. infernus* strain ME^T.

DNA-DNA hybridization

No significant hybridization was obtained between bulk cellular DNA of the isolate M7^T and that from *M. jannaschii* strain JAL-1^T (< 5%), strain AG86^T

Fig. 2. Growth rates (μ) of strain M7^T as a function of temperature (a) in the presence of NaCl (25 g l⁻¹) at pH 6.5 and as a function of concentration of NaCl (b) at 80 °C and at pH 6.5. Growth was followed (OD₆₀₀) in the culture medium under an atmosphere of H₂/CO₂ (80:20; 200 kPa).

Fig. 3. Phylogenetic relationship of strains M7^T and AG86^T and other *Methanococcales* produced by maximum-likelihood analysis. The sequence of the M7^T small subunit was aligned with other rRNAs from the Ribosomal Database Project (Maidak *et al.*, 1996). The scale bar represents the expected number of changes per sequence position.

and *M. infernus* strain ME^T (< 2%). Moreover, the distinct species status of strain AG86^T was demonstrated by weak levels of DNA-DNA hybridization with *M. jannaschii* strain JAL-1^T (< 10%), *M. infernus* strain ME^T (< 2%) and M7^T (< 2%).

DISCUSSION

The novel marine hyperthermophilic strain M7^T belongs to the domain *Archaea* on the basis of the cell envelope composition, its resistance to antibiotics and the 16S rDNA sequence (Hilpert *et al.*, 1981; Woese *et al.*, 1990; Sleytr *et al.*, 1996). The morphology, metabolism, G+C content and the 16S rDNA sequence indicate that strain M7^T belongs to the genus *Methanococcus*.

Strain M7^T differs from *M. thermolithotrophicus* strain SN-1^T in its inability to use formate and in its temperature range and optimum for growth (Table 1) (Huber *et al.*, 1982; Jones *et al.*, 1989). It differs from *M. igneus* strain Kol 5^T in its motility, its temperature optimum for growth, its ability to grow in the presence of sulphur and its positive response to the effect of yeast extract and selenium (Burggraf *et al.*, 1990). The newly described strain M7^T is most similar to *M. jannaschii* strain JAL-1^T, strain AG86^T and *M. infernus* strain ME^T with respect to motility, temperature range for growth (Table 1) and susceptibility to antibiotics. However, strain M7^T is distinct from the above strains by its optimum temperature for growth and by other slight differences. Contrary to *M. infernus* strain ME^T, strain M7^T can grow below 55 °C, but not at 90 °C. The flagellar system of strain JAL-1^T consists of two bundles of flagella inserted close to the same pole (Jones *et al.*, 1983b) whereas that of strain M7^T appears to be arranged in three tufts of flagella, two of them inserted in polar positions. As reported for *M. infernus* strain ME^T, the growth of strain M7^T and of AG86^T in basal medium was enhanced by the presence of yeast extract. Yeast extract has no stimulatory effect on growth of strain JAL-1^T (Jones *et al.*, 1983b; Jeanthon *et al.*, 1998).

The 16S rDNA analysis indicated that M7^T is closely related to other deep-sea vent methanococci isolates, namely *M. jannaschii*, strain AG86^T and *M. infernus* strain ME^T. However, a low level of genetic relationship between strains M7^T, ME^T, AG86^T and JAL-1^T was determined by DNA-DNA hybridization studies, indicating that these organisms cannot be assigned to the same species (Johnson, 1984). Similarly, weak levels of DNA-DNA hybridization were measured between *M. jannaschii* strain JAL-1^T and strain AG86^T. Phenotypically, strain AG86^T was distinct from *M. jannaschii* strain JAL-1^T by its pH range for growth (Table 1) and in its response to the stimulatory effects on growth of Casamino acids and yeast extract (Zhao *et al.*, 1988). Here, we determined that AG86^T was resistant to rifampicin (50 µg ml⁻¹) whereas *M. jannaschii* strain JAL-1^T was sensitive.

Table 1. Characteristics of *Methanococcus* species and strains M7^T and AG86^T

Organism	Temp. (°C)		pH range	Stimulatory compounds			Resistance to rifampicin*	Reference
	Range	Optimum		Se	WO ₄	Yeast extract		
Strain M7 ^T	49–89	80	5.25–7.0	+	+	+	—	This work.
Strain AG86 ^T	48–92	85	5.50–7.6	+	+	+	+	Zhao <i>et al.</i> (1988)
<i>M. infernus</i> strain ME ^T	55–91	85	5.25–7.0	+	+	+	—	Jeanthon <i>et al.</i> (1998)
<i>M. jannaschii</i> strain JAL-1 ^T	50–86†	85	5.20–7.0	+	ND‡	—	—	Jones <i>et al.</i> (1983b)
<i>M. igneus</i> strain Kol 5 ^T	45–91	88	5.00–7.5	—	ND	+	+	Burggraf <i>et al.</i> (1990)
<i>M. thermolithotrophicus</i> strain SN-1 ^T	30–70	65	6.00–8.0	ND	—	ND	+	Huber <i>et al.</i> (1982)

ND, Not determined.

* This test was performed in this study. The concentration tested was 50 µg ml⁻¹. +, Resistant; -, sensitive.

† In our laboratory, the maximum growth temperature measured was 91 °C. No growth was obtained at 93 °C (Jeanthon *et al.*, 1998).

‡ In our laboratory, tungstate (30 mg l^{-1}) was found to stimulate growth.

From the above results, we concluded that strain M7^T and strain AG86^T represent new *Methanococcus* species. We propose to name strain M7^T as *Methanococcus vulcanius* and strain AG86^T as *Methanococcus fervens*, reflecting their high temperature of growth and the nature of their extreme habitat, the deep-sea hydrothermal vents.

Description of *Methanococcus vulcanius* sp. nov.

Methanococcus vulcanius (vul.ca'ni.us. L. masc. adj. *vulcanius* referring to Vulcanus, the Roman fire god, and to the place of isolation, the deep-sea hydrothermal vents).

Cells exhibit a tumbling motility by means of three tufts of flagella. They are cocci (diameter 1–3 μm) and occur singly and in pairs. Pale-yellow round colonies about 1 mm in diameter formed on 'Phytigel' plates. Growth occurs between 49 and 89 $^{\circ}\text{C}$, with an optimum around 80 $^{\circ}\text{C}$ (doubling time 45 min). Growth occurs between pH 5.25 and 7 with an optimum of approximately pH 6.5 and with NaCl concentrations between 6.25 and 56.25 g l^{-1} with an optimum of 25 g l^{-1} . Obligately anaerobic. Chemolithotrophic. Uses H_2 and CO_2 as energy and carbon sources to produce methane. Growth is stimulated by selenate, tungstate and yeast extract. Sulphur is reduced to hydrogen sulphide in the presence of CO_2 and H_2 . Growth is inhibited by chloramphenicol (75 $\mu\text{g ml}^{-1}$) and rifampicin (50 $\mu\text{g ml}^{-1}$) but not by streptomycin, penicillin G, kanamycin or ampicillin (all at 200 $\mu\text{g ml}^{-1}$). DNA base composition of the type strain is 31 mol % G+C (as determined by the thermal denaturation method). The type strain is *Methanococcus vulcanius* M7^T, which was obtained from a deep-sea hydrothermal vent chimney on the East Pacific Rise (13° N). Strain M7^T has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen as DSM 12094^T.

Description of *Methanococcus fervens* sp. nov.

Methanococcus fervens (fer'vens. L. part. adj. *fervens* boiling hot, referring to its high growth temperature).

Cells exhibit a tumbling motility. They are regular to irregular cocci (diameter 1–2 μm) and occur singly and in pairs. Whitish, translucent and round colonies about 0.5 mm in diameter formed on Gelrite plates. Growth occurs between 48 and 92 $^{\circ}\text{C}$, with an optimum around 85 $^{\circ}\text{C}$ (doubling time 20–30 min). Growth occurs between pH 5.5 and 7.6 with an optimum about pH 6.5 and with NaCl concentrations between 0.5 and 5% (w/v) with an optimum of approximately 3%. Obligately anaerobic. Chemolithotrophic. Uses H_2 and CO_2 as energy and carbon sources to produce methane. Growth is stimulated by yeast extract, Casamino acids, trypticase, selenite and tungstate. Growth is inhibited by chloramphenicol (75 $\mu\text{g ml}^{-1}$) but not by streptomycin, penicillin G, kanamycin or ampicillin (all at 200 $\mu\text{g ml}^{-1}$) or rifampicin (50 $\mu\text{g ml}^{-1}$). DNA base composition of the type strain is 33 mol% G + C (as determined by the thermal denaturation method). The type strain is *Methanococcus fervens* AG86^T, which was obtained from a deep-sea hydrothermal vent core sample from Guaymas Basin, Gulf of California, at a depth of 2003 m. Strain AG86^T is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen as DSM 4213^T.

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