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Methanothermococcus okinawensis sp. nov., a thermophilic, methane-producing archaeon isolated from a Western Pacific deep-sea hydrothermal vent system

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A novel thermophilic, methane-producing archaeon was isolated from a deep-sea hydrothermal vent chimney at the Iheya Ridge, in the Okinawa Trough, Japan. The cells were highly motile, irregular cocci, with a polar bundle of flagella. Growth was observed between 40 and 75 °C (optimum 60–65 °C; 30 min doubling time) and between pH 4·5 and 8·5 (optimum pH 6–7). The isolate was a strictly anaerobic autotroph capable of using hydrogen and carbon dioxide as sole sources of energy and carbon. Formate can serve as an alternative energy source. The G+C content of the genomic DNA was 33·5 mol%. Phylogenetic analysis based on 16S rDNA sequences and DNA–DNA hybridization analysis indicated that the isolate was closely related to members of the genera *Methanococcus* and *Methanothermococcus*. This isolate, however, could be differentiated from the previously described species of these genera on the basis of its physiological and molecular properties. The name *Methanothermococcus okinawensis* sp. nov is proposed, with the type strain IH1^T (=JCM 11175^T=DSM 14208^T).

Keywords: deep-sea hydrothermal vent, thermophile, methanogen, *Methanococcus*, archaea

INTRODUCTION

Hyperthermophilic or thermophilic methanogens have been isolated from a variety of marine hydrothermal systems (Burggraff *et al*., 1990; Huber *et al*., 1982, 1989; Jeanthon *et al*., 1998, 1999; Jones *et al*., 1983b, 1989; Kurr *et al*., 1991; Stetter, 1996; Zhao *et al*., 1988) and sub-sea-floor oil reservoirs (Nilsen & Torsvik, 1996; Orphan *et al*., 2000). Except for one isolate, *Methanopyrus kandleri* (Huber *et al*., 1989; Kurr *et al*., 1991), all of the methanogens isolated from marine hydrothermal vent systems and sub-sea-floor oil reservoirs are members of the order *Methanococcales*. *Methanococcus thermolithotrophicus* SN-1^T and *Methanococcus igneus* Kol 5^T were isolated from coastal hydrothermal systems of Italy and Iceland, respectively (Burggraff *et al*., 1990; Huber *et al*., 1982). *Methanococcus jannaschii* JAL-1^T, *Methanococcus vulcanius* M7^T, *Methanococcus fervens* AG86^T and *Methanococcus infernus* ME^T were isolated from deep-sea hydrothermal vent environments from the East Pacific Rise, Guaymas Basin and the Mid-Atlantic Ridge (Jeanthon *et al*., 1998, 1999; Jones *et al*., 1983b, 1989; Zhao *et al*., 1988). In addition, *Methanococcus thermolithotrophicus* ST22 and *Methanococcus* sp. strains vp183 and vp21, closely related to *Methanococcus thermolithotrophicus* SN-1^T, were isolated from deep sub-sea-floor oil reservoir waters in the North Sea (Nilsen & Torsvik, 1996) and off the shore of California (Orphan *et al*., 2000). Based on their widespread occurrence, hyperthermophilic or thermophilic members of the *Methanococcales* are likely to be cosmopolitan micro-organisms inhabiting global marine hydrothermal vent systems and subsurface oil reservoir environments like the hyperthermophilic members of the order *Thermococcales* (L'Haridon *et al*., 1995; Slobodkin *et al*., 1999; Stetter *et al*., 1993; Takai *et al*., 2000; Zillig *et al*., 1983). However, no thermophilic methanogen has yet been cultivated from the hydrothermal vent systems located in the Western Pacific, although a number of microbiological surveys have

The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of strain IH1^T is AB057722.

been performed in hydrothermal vent fields in the Western Pacific.

Historically, the systematics of methanococci has been hindered by the absence of information on the reliability of phenotypic characters (Keswani *et al*., 1996). Based on the comparison of nearly complete 16S rRNA sequences from the members of *Methanococcus*, Boone *et al*. (1993) recommended that the genus *Methanococcus* should be further subdivided into four genera to reduce the genetic diversity within one genus. More recently, Whitman *et al*. (2001) have proposed the creation of two families and four genera within the order *Methanococcales*. According to the newly proposed taxonomy, the mesophilic methanococci, including the as-yet undescribed '*Methanococcus aeolicus*', belong to the genus *Methanococcus* and *Methanococcus jannaschii*, *Methanococcus infernus*, *Methanococcus vulcanius* and *Methanococcus fervens* belong to the genus *Methanocaldococcus* (Whitman *et al*., 2001). *Methanococcus igneus* is assigned to the genus *Methanotorris* and *Methanococcus thermolithotrophicus* to the genus *Methanothermococcus* (Whitman *et al*., 2001). This classification fits well the phylogenetic relationships associated with thermophily among the order *Methanococcales* recently provided by Keswani *et al*. (1996) and Jeanthon *et al*. (1999). However, both of the genera *Methanotorris* and *Methanothermococcus* are represented by single species and the phylogenetic affiliation of the deeply branched, mesophilic '*Methanococcus aeolicus*' is still uncertain. Hence, additional isolates will be helpful in elucidating the phylogenetic organization and taxonomy of the order *Methanococcales*.

In this study, we succeeded in isolating a thermophilic methanogen from a deep-sea hydrothermal vent chimney at the Iheya Ridge, in the Okinawa Trough, Japan. Phylogenetic analysis revealed that the novel isolate is a member of the order *Methanococcales*. The novel isolate had physiological properties very similar to those of *Methanothermococcus thermolithotrophicus*, but it is more closely related phylogenetically to the mesophile '*Methanococcus aeolicus*'. Based on its physiological properties and the results of DNA–DNA hybridization analysis, the isolate can be described as a novel species of *Methanothermococcus* that we have named *Methanothermococcus okinawensis* sp. nov.

METHODS

Sample collection. A sample from a deep-sea hydrothermal vent chimney was obtained from the hydrothermal field at Iheya Ridge in the Okinawa Trough, Japan (27° 47·220′ N, 126° 53·900′ E) at a depth of 972 m by means of the manned submersible *Shinkai 2000* in dive #1194, performed in June 2000. This deep-sea hydrothermal vent site is the same site from which *Thermosipho japonicus* was isolated (Takai & Horikoshi, 2000). The tip of the chimney was brought to the sea surface in a sample box.

Enrichment and purification. Immediately after the sample was retrieved from the submersible, portions of the fractured chimney were used to inoculate a series of media including MMJ (described below) medium supplemented with 10 mM sodium thiosulfate ($Na_2S_2O_3$. $5H_2O$) and 10 mM magnetite (Fe_3O_4) 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 on-board ship. The bottles of MMJ medium incubated at 70 °C became turbid after 2 days, but no growth was observed at 85 °C. To obtain a pure culture, the dilution-to-extinction technique was employed (Baross, 1995).

Sources of organisms. *Methanococcus* (*Methanocaldococcus*) *jannaschii* strain JAL-1^T (= JCM 10045^T), *Methanococcus* (*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 (JCM, Wako, Japan). All strains were cultivated under optimal conditions as described previously (Huber *et al*., 1982; Jones *et al*., 1983a, b).

Culture medium and conditions. The novel isolate was routinely cultivated in a standard medium, which was MMJ medium supplemented with 5 mM calcium chloride ($CaCl_2$), 10 mM sodium thiosulfate ($Na_2S_2O_3$. $5H_2O$) and 10 mM magnetite (Fe_3O_4). MMJ medium consisted of the following components (l^{-1} MJ synthetic seawater): 1 ml vitamin solution (Balch *et al*., 1979), 50 mg sodium selenite, 30 mg sodium tungstate, 1 mg resazurin, 20 g $NaHCO_3$, 0·5 g cysteine hydrochloride and 0·5 g Na_2S . $9H_2O$ (Sako *et al*., 1996; Takai *et al*., 1999). To prepare the standard medium, 50 mg sodium selenite, 30 mg sodium tungstate and 1 mg resazurin were dissolved in 1 l MJ synthetic seawater and the pH of the medium was adjusted to around 7·0 with NaOH before autoclaving. After autoclaving, a concentrated solution of vitamins (Balch *et al*., 1979), $NaHCO_3$, thiosulfate, magnetite, cysteine hydrochloride (pH adjusted to 7·0) and Na_2S (pH adjusted to 7·0) were added to the medium. These solutions were sterilized separately by autoclaving except for the vitamin solution, which was filter-sterilized. The pH of the medium was adjusted with H_2SO_4 or NaOH in an anaerobic chamber under 90 % N_2 and 10 % H_2 if needed. The medium was dispensed at 20 % of the total bottle or tube volume and the tubes and bottles were sealed tightly with butyl rubber stoppers under a gas phase of 80 % H_2 and 20 % CO_2 at 300 kPa. In order to test the oxygen sensitivity of the isolate, the gas phase was replaced by a gas mixture of 80 % H_2, 15 % CO_2 and 5 % O_2 or 80 % H_2, 19 % CO_2 and 1 % O_2.

All experiments described below were conducted in duplicate. In an attempt to examine whether other potential electron donors and acceptors supported or stimulated growth in place of H_2 and CO_2, organic compounds such as yeast extract, peptone, tryptone, Casamino acids, amino acids, formate, acetate, methanol, ethanol, dimethyl sulfide and trimethylamine were tested in the presence or absence of H_2 and CO_2. The potential requirement for thiosulfate, magnetite, elemental sulfur, selenate, selenite, tungstate and nitrogen sources (10 mM NH_4Cl, $NaNO_2$ or $NaNO_3$) for growth was also tested. To test the effect of pH on growth, the pH of MMJ medium was adjusted to various values with 10 mM acetate/acetic acid buffer (pH 4–5), MES (pH 5–6), PIPES (pH 6–7), HEPES (pH 7–7·5) or Tris (pH 8–9·5). After autoclaving, the pH of the medium was checked and readjusted with H_2SO_4 or NaOH to the desired pH at room temperature. The pH was found to be stable during the cultivation period. To test the effect of the sea salts concentration on growth, MMJ medium was prepared with varying dilutions of 4× MJ synthetic seawater (1× MJ synthetic seawater contains 30 g NaCl l^{-1}). H_2 and CO_2

consumption and CH_4 production during growth were measured by gas chromatography using a Micro GC CP2002 (GL Sciences).

Light and electron microscopy. Cells were routinely observed under visible, UV or blue light by phase-contrast microscopy using a Leica DMRB microscope with a Leica MPS 30 camera system. Transmission electron microscopy of negatively stained cells was carried out as described by Zillig *et al.* (1990). Cells grown in MMJ medium at 62 °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 80 kV.

Measurement of growth. Growth of the novel isolate was measured by direct cell counting after staining with 4′,6-diamidino-2-phenylindole (Porter & Feig, 1980) using a Nikon Optiphot microscope. Cultures were prepared in duplicate. The cultures were grown in 100 ml glass bottles (Schott Glaswerke), each containing 20 ml medium, with shaking (200 r.p.m.) in a temperature-controlled dry oven. The pH growth curve was determined at 62 °C and the growth conditions for all other cultivation tests were 62 °C and pH 7·0, unless otherwise noted.

Susceptibility to antibiotics and lysis. Sensitivity of strain IH1^T to 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 62 °C. A simultaneous experiment was performed with *Methanothermococcus thermolithotrophicus* SN-1^T at the same temperature. Susceptibility to lysis by SDS and by hypotonic solutions was tested as described previously (Boone & Whitman, 1988).

Isolation and base composition of DNA. DNA was prepared as described by Marmur & Doty (1962). The G+C content of the DNA was determined by direct analysis of deoxyribonucleotides by HPLC (Tamaoka & Komagata, 1984). Non-methylated DNA from bacteriophage λ (49·8 mol% G+C; TaKaRa) (Sanger *et al.*, 1982) was used as a reference.

Amplification of the 16S rRNA gene and sequence determination. The 16S rRNA gene (rDNA) was amplified by PCR using primers Arch21F and 1492R (DeLong, 1992; Lane, 1985). The 1·5 kb PCR product was sequenced directly by the dideoxynucleotide chain-termination method using a DNA sequencer model 377 (Perkin Elmer/Applied Biosystems). The rDNA sequence was analysed using the gapped-BLAST search algorithm (Altschul *et al.*, 1997; Benson *et al.*, 1998) to estimate the degree of similarity to other archaeal 16S rDNA sequences.

Data analysis. The almost-complete sequence (1392 bp) of the 16S rDNA of strain IH1^T was aligned manually to 16S rDNA data from the RDP based on primary and secondary structure. Phylogenetic analyses were restricted to nucleotide positions that were unambiguously alignable in all sequences (Takai & Horikoshi, 1999a, b; Takai & Sako, 1999). Neighbour-joining analysis and maximum-likelihood analysis were accomplished using the PHYLIP package (version 3.5; obtained from J. Felsenstein, University of Washington, Seattle, WA, USA). Bootstrap analysis was used to provide confidence estimates for phylogenetic tree topologies.

DNA–DNA hybridization analysis. DNA–DNA hybridization was carried out at 42 °C for 3 h and was measured fluorometrically using photobiotin according to the method of Ezaki *et al.* (1989). *Methanocaldococcus jannaschii* JAL-1^T, *Methanothermococcus thermolithotrophicus* SN-1^T and *Methanococcus maripaludis* JJ^T were used as reference strains.

RESULTS AND DISCUSSION

Enrichment and purification

Growth of anaerobic, thermophilic methanogens from a black smoker chimney sample was observed in MMJ medium supplemented with 10 mM sodium thiosulfate ($Na_2S_2O_3 . 5H_2O$) and 10 mM magnetite (Fe_3O_4) after 2 days incubation at 70 °C. Cells were highly motile, irregular cocci and were fluorescent when viewed under UV and blue light excitation by epifluorescence microscopy. To obtain a pure culture, the dilution-to-extinction technique was employed. The culture in the tube showing growth at the highest dilution was designated strain IH1^T (= JCM 11175^T = DSM 14208^T) and investigated in detail.

Morphology

The cells were irregular cocci with a mean diameter of 1·0–1·5 μm (Fig. 1). As observed by light microscopy, the cells were highly motile, and a polar bundle of flagella was observed by electron microscopy (Fig. 1). Cells occurred singly and in pairs in all phases of growth. Although the novel isolate was observed to have slightly smaller cells, its morphological features were similar to those of *Methanothermococcus thermolithotrophicus* SN-1^T and '*Methanococcus aeolicus*' (Table 1).

Growth parameters

The novel isolate grew only under strictly anaerobic culture conditions and was strongly sensitive to oxygen. The isolate was found to be an autotrophic

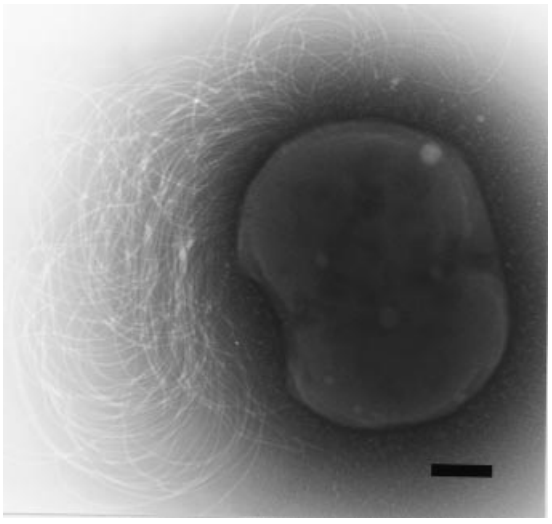

Fig. 1. Electron micrograph of negatively stained cells in the mid-exponential phase of growth. The polar bundle of flagella is observed. Bar, 400 nm.

Table 1. Comparison of properties between *Methanothermococcus okinawensis* sp. nov. and closely related strains

All three taxa use both H_2/CO_2 and formate as substrates for methane synthesis and use ammonium ions as a source of nitrogen. Data were taken from this study and from Huber *et al.* (1992) (*Methanothermococcus thermolithotrophicus*), Keswani *et al.* (1996) ('*Methanococcus aeolicus*') and Whitman *et al.* (2001) (both taxa).

Character	*Methanothermococcus okinawensis* IH1^T	*Methanothermococcus thermolithotrophicus* SN-1^T	'*Methanococcus aeolicus*' A
Cell diameter (μm)	1·0–1·5	1·5	1·7
Stimulatory for growth:			
Selenium	+	ND	+
Magnetite	+	+*	ND
Nitrogen sources:			
N_2	ND	+	+
NO_2^-	−	ND	ND
NO_3^-	−	+	ND
Temperature range (°C)	40–75	17–70	< 20–45
Temperature optimum (°C)	60–65	60–65	ND
pH range	4·5–8·5	4·9–9·8	6·5–8·0
pH optimum	6·0–7·0	5·1–7·5	ND
NaCl range (%, w/v)	0·5–6·0	0·6–9·4	1·0–>5·0
NaCl optimum (%, w/v)	1·5–3·0	2·0–4·0	1·0–2·0
G+C content of genomic DNA (mol%)	33·5	32·5	32

* Checked in this study.

methanogen, utilizing H_2 and CO_2 as sole energy and carbon sources. During growth, H_2 and CO_2 in the headspace gas decreased and methane (CH_4) was produced (approx. 800 p.p.m. H_2, 8000 p.p.m. CO_2 and 90 % CH_4 in the gas phase after 2 days incubation). The maximum cell yield with H_2 and CO_2 in the standard medium was obtained after 2 days incubation and was $7{\cdot}0 \times 10^8$ cells ml^{-1}. Strain IH1^T grew on formate (20 mM) in the absence of H_2 ($4{\cdot}0 \times 10^8$ cells ml^{-1} after 2 days incubation), indicating that formate was 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 a mixture of 20 amino acids (containing 0·001 %, w/v, of each) did not support growth in the absence of H_2 and did not stimulate growth in the presence of H_2 and CO_2. The novel isolate utilized ammonium ions as a nitrogen source but could not use nitrite or nitrate. The presence of magnetite (Fe_3O_4) improved growth while thiosulfate (S_2O_3) had no effect on growth and elemental sulfur (S^0) inhibited growth. Selenium was stimulatory to growth, while the novel isolate did not require tungsten for growth. The vitamin mixture was not required for growth.

The isolate grew over the temperature range 40–75 °C, showing optimal growth at 60–65 °C, and the generation time at 62 °C was about 30 min at pH 7·0 (Fig. 2). No growth was observed at 30 or 80 °C. Growth of the novel isolate at 62 °C occurred between pH 4·5 and 8·5, with optimum growth at about pH 6–7 (Fig. 2). No growth was detected below pH 4·5 or above pH 8·5. The novel isolate required sea salts for growth. It grew over the concentration range 12–96 g sea salts l^{-1}, with optimum growth at 25–50 g sea salts l^{-1} at 62 °C and pH 7·0 (Fig. 2).

In many of its physiological properties, the novel isolate resembles *Methanothermococcus thermolithotrophicus* SN-1^T (Huber *et al.*, 1982). However, compared with *Methanothermococcus thermolithotrophicus* SN-1^T, the novel isolate has a slightly higher temperature range for growth and does not utilize nitrate as a sole nitrogen source (Table 1). In addition, thermophily was a significant physiological feature that differentiated the novel isolate from mesophilic *Methanococcus* species (Table 1).

Sensitivity to antibiotics and lysis

Methanothermococcus thermolithotrophicus SN-1^T and the novel isolate showed the same antibiotic resistance pattern except for streptomycin. The novel isolate was resistant to streptomycin (100 μg ml^{-1}). Cells of the novel isolate were lysed by 0·1 % (w/v) SDS and hypotonic solutions [10^{-1}-diluted MJ(−N) synthetic seawater and distilled water].

DNA base composition

The G+C content of the genomic DNA of strain IH1^T was found to be 33·5 mol %, which was slightly higher than those of *Methanothermococcus thermolithotrophicus* SN-1^T and '*Methanococcus aeolicus*' (Table 1).

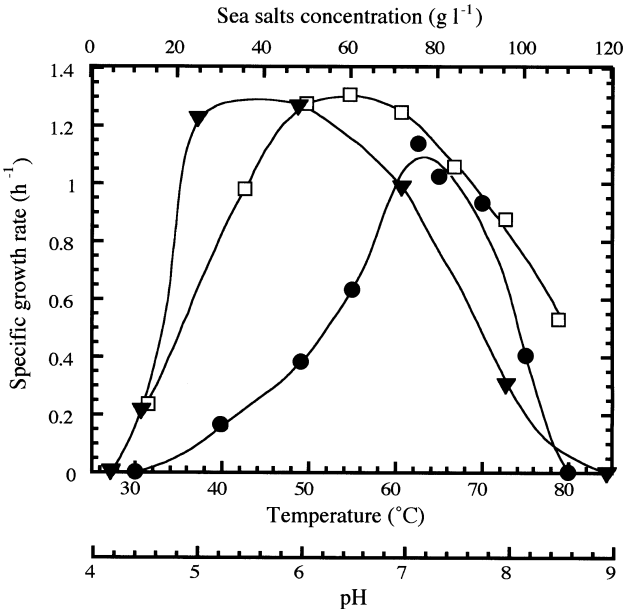

Fig. 2. Effects of temperature (●), pH (□) and sea salts concentration (▼) on growth of *Methanothermococcus okinawensis* sp. nov. Growth curves at different temperatures were determined in the standard medium at pH 7·0. The effect of pH on growth was determined in MMJ medium containing various buffers at 62 °C. The pH was adjusted at room temperature. The effect of sea salts concentration on growth was determined in varying dilutions of 4× MJ synthetic sea water.

Phylogenetic analyses and DNA–DNA hybridization

The almost-complete sequence (1392 bp) of the 16S rRNA gene from strain IH1^T was determined. The rDNA sequence of strain IH1^T was most closely related to those of members of the family *Methanococcaceae* (Whitman *et al.*, 2001) such as '*Methanococcus aeolicus*' (94·5%) (Schmid *et al.*, 1984; Keswani *et al.*, 1996), *Methanothermococcus thermolithotrophicus* SN-1^T (Huber *et al.*, 1982) (94·8%) and *Methanococcus vannielii* SB^T (93·2%) (Stadtman & Barker, 1951; Keswani *et al.*, 1996). This result indicated that the novel isolate is a member of the family *Methanococcaceae*, represented by the genera *Methanothermococcus* and *Methanococcus*.

The neighbour-joining and maximum-likelihood methods yielded almost identical topologies, indicating that the novel isolate was phylogenetically related to '*Methanococcus aeolicus*', while the sequence similarity of 16S rDNA was almost the same with '*Methanococcus aeolicus*' and *Methanothermococcus thermolithotrophicus* SN-1^T (Fig. 3). Base composition disparities in the 16S rDNA sequences among *Methanothermococcus* and *Methanococcus* strains were relatively small (from a G+C content of 54·3% in the *Methanococcus voltae* PS^T DNA sequence to 57·2% in the *Methanothermococcus thermolithotrophicus* SN-1^T rDNA sequence) (Fig. 3) and had little influence on the tree topology, supported by transversion distance-matrix analysis (Woese *et al.*, 1991). In addition, the bootstrap analysis revealed considerable confidence in the placement of IH1^T (Fig. 3).

Based on the phylogenetic analysis, the novel isolate was most closely related to '*Methanococcus aeolicus*' (Schmid *et al.*, 1984). This archaeon has not been validly described; however, DNA hybridization data and 16S rRNA sequence analysis suggested that it represents a novel species of the genus *Methanococcus*

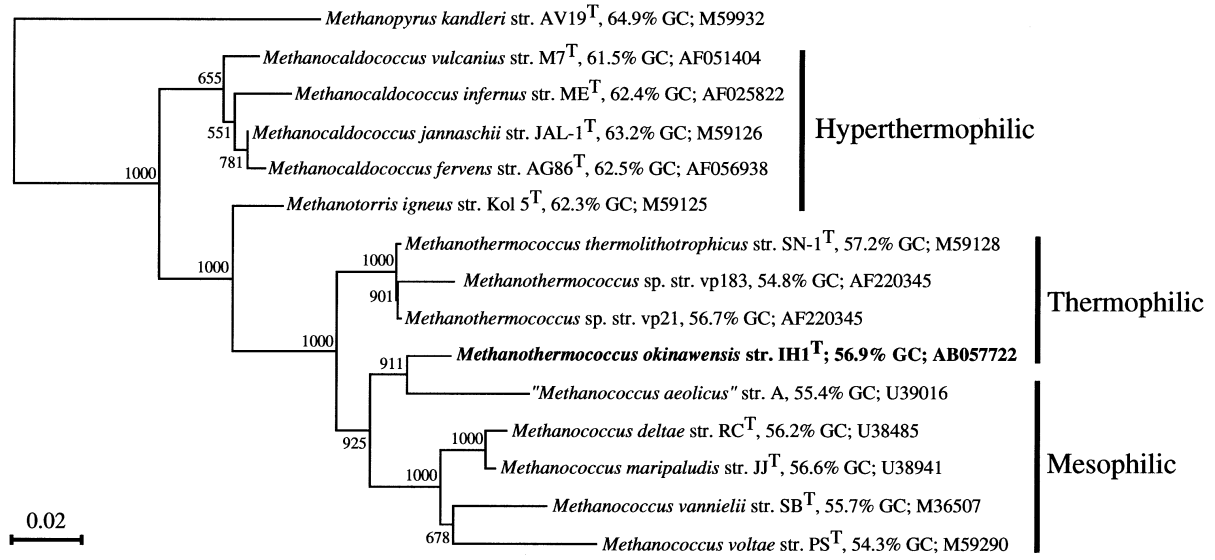

Fig. 3. Phylogenetic tree of representative members of the order *Methanococcales* inferred from 16S rDNA sequences by the neighbour-joining method using 689 homologous sequence positions for each organism. The numbers at each node represent bootstrap values (1000 replicates). Bar, 2 substitutions per 100 nucleotides. The G+C content of the sequences analysed is given.

(Keswani *et al*., 1996). In this study, we did not obtain this methanogen from public culture collections. Therefore, the DNA–DNA hybridization analysis was conducted among the novel isolate, *Methanocaldococcus jannaschii* JAL-1^T, *Methanothermococcus thermolithotrophicus* SN-1^T and *Methanococcus maripaludis* JJ^T. The mean hybridization values comparing the novel isolate with *Methanocaldococcus jannaschii* JAL-1^T, *Methanothermococcus thermolithotrophicus* SN-1^T and *Methanococcus maripaludis* JJ^T were respectively 10·7, 17·6 and 13·4%. These results indicated that the novel isolate could be differentiated genotypically from the previously described species of the hyperthermophilic, thermophilic and mesophilic methanococci.

Comparison with related species

The phylogenetic analysis indicated that strain IH1^T was most closely related to '*Methanococcus aeolicus*' (Schmid *et al*., 1984), which has not been validly described. According to Whitman *et al*. (2001), this methanogen is mesophilic, growing at temperatures between < 20 and 45 °C (Table 1). In addition, the cell size (1·0–1·5 μm diameter), pH range for growth (4·5–8·5), optimal salinity for growth (2–5%, w/v) and G+C content of the genomic DNA (33·5 mol%) differentiate the novel isolate from '*Methanococcus aeolicus*' (Whitman *et al*., 2001) (Table 1). In many physiological properties, the novel isolate resembles *Methanothermococcus thermolithotrophicus* SN-1^T (Huber *et al*., 1982) (Table 1). The slightly higher temperature range for growth and the inability to use nitrate as a sole nitrogen source may be physiological characteristics that separate them (Table 1). However, phylogenetic analysis based on 16S rDNA and the DNA hybridization data clearly reveal that the novel isolate can be genetically differentiated from *Methanothermococcus thermolithotrophicus* SN-1^T and other *Methanothermococcus* strains isolated from sub-seafloor oil reservoirs (Orphan *et al*., 2000), at least at the species level. On the basis of the physiological and genetic properties of the novel isolate, we propose that the isolate be classified as *Methanothermococcus okinawensis* sp. nov., and the type strain is IH1^T (= JCM 11175^T = DSM 14208^T).

Description of *Methanothermococcus okinawensis* sp. nov.

Methanothermococcus okinawensis (o.ki.na.wen′sis. N.L. adj. *okinawensis* of Okinawa, a region of Japan).

Irregular cocci, with a mean diameter of 1·0–1·5 μm. Cells occur singly or in pairs. Exhibits vigorous motility with a polar bundle of flagella. Strictly anaerobic and obligately methanogenic. The temperature range for growth is 40–75 °C, with the optimum being 60–65 °C. The pH range for growth is 4·5–8·5, with optimum growth occurring at pH 6–7. Sea salts are required for growth and the range is 12–96 g l^{-1}, with optimum growth occurring at 20–50 g l^{-1}. Growth occurs with H_2 or formate as an electron donor and CO_2 as an electron acceptor and a carbon source. Ammonium serves as a nitrogen source. Selenium and magnetite (Fe_3O_4) are stimulatory for growth. Resistant to ampicillin (200 μg ml^{-1}), kanamycin (200 μg ml^{-1}), rifampicin (100 μg ml^{-1}) and streptomycin (200 μg ml^{-1}), but sensitive to chloramphenicol (50 μg ml^{-1}). Cells are susceptible to lysis by 0·1% (w/v) SDS and hypotonic solutions. The G+C content of genomic DNA is 33·5% (HPLC). The 16S rDNA sequence exhibits 94·8 and 94·5% similarity to those of *Methanothermococcus thermolithotrophicus* SN-1^T and '*Methanococcus aeolicus*'. The DNA–DNA relatedness to *Methanocaldococcus jannaschii* JAL-1^T, *Methanothermococcus thermolithotrophicus* SN-1^T and *Methanococcus maripaludis* JJ^T is low.

The type strain, IH1^T (= JCM 11175^T = DSM 14208^T), was isolated from a deep-sea hydrothermal vent chimney at the Iheya Ridge, in the Okinawa Trough, Japan.

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