Methanobacterium petrolearium sp. nov. and *Methanobacterium ferruginis* sp. nov., mesophilic methanogens isolated from salty environments

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Two methane-producing archaea, designated Mic5c12^T and Mic6c05^T, were isolated from sludge deposited in a crude oil storage tank and a tubercle on the interior of a pipe transporting natural gas-containing brine, respectively. The isolates were Gram-staining-variable, non-motile rods and grew only on H₂/CO₂. Strain Mic6c05^T produced methane from some alcohols without showing any growth; strain Mic5c12^T did not utilize alcohols. The optimum growth conditions for strain Mic5c12^T were 35 °C, pH 6.5 and 0–0.68 M NaCl and for strain Mic6c05^T were 40 °C, pH 6.0–7.5 and 0.34 M NaCl. Strain Mic5c12^T was halotolerant and strain Mic6c05^T was halophilic. Comparative 16S rRNA gene sequence analysis revealed that strains Mic5c12^T and Mic6c05^T belonged to the genus *Methanobacterium* and their closest relative was *Methanobacterium subterraneum* A8p^T (97.3 and 97.9% 16S rRNA gene sequence similarity, respectively). The findings from the 16S rRNA gene sequence analyses were supported by analysis of *mcrA*, the alpha subunit of methyl-coenzyme M reductase. On the basis of phylogenetic analyses and phenotypic characteristics, two novel species are proposed, *Methanobacterium petrolearium* sp. nov. and *Methanobacterium ferruginis* sp. nov., with type strains Mic5c12^T (=NBRC 105198^T = DSM 22353^T) and Mic6c05^T (=NBRC 105197^T = DSM 21974^T), respectively.

The genus *Methanobacterium* contains some of the earliest-known hydrogenotrophic methanogens and 24 species have been described thus far (Boone, 2000; Cuzin *et al.*, 2001; Joulain *et al.*, 2000; Ma *et al.*, 2005; Shlimon *et al.*, 2004), however some have subsequently been transferred to other genera. Members of the genus *Methanobacterium* have been isolated from mesophilic, neutral and freshwater environments such as those found in anaerobic digesters; the exceptions are *Methanobacterium aarhusense* and *Methanobacterium alcaliphilum*, which were isolated from marine sediment (Shlimon *et al.*, 2004) and alkaline lake sediment (Worakit *et al.*, 1986), respectively. Recently, we isolated two hydrogenotrophic methanogens, strains Mic5c12^T and Mic6c05^T, both belonging to the genus *Methanobacterium*.

Two samples were used as isolation sources: a sludge sample from the bottom of a crude oil storage tank in

Japan, collected in June 2005, and a tubercle sample from the interior surface of a pipe transporting natural gas-containing brine in the southern Kanto gas field in Japan, collected in July 2005. In order to enrich and isolate hydrogen-consuming methanogens, we used a basal medium [containing l⁻¹: 0.26 g KH₂PO₄, 0.53 g K₂HPO₄, 3.05 g MgCl₂·6H₂O, 0.15 g CaCl₂·2H₂O, 0.54 g NH₄Cl, 30 g NaCl, 1.42 g sodium sulfate, 0.82 g sodium acetate, 0.02 g Bacto yeast extract (Difco), 1.5 g Na₂CO₃, 2 ml each of trace element solution and vitamin solution of NBRC medium 377 (NBRC, 2005), 1 mg resazurin, 0.5 g Na₂S·9H₂O, 0.5 g cysteine-HCl; about pH 7]. To enrich methanogens, a sample of approximately 1 g was used to inoculate 20 ml basal medium in a 50 ml serum bottle sealed with a butyl rubber stopper and an aluminium cap. The bottle was incubated at 25 °C for 4 weeks under a H₂/CO₂ atmosphere (4:1, v/v; 150 kPa). The cultures were serially diluted in the same medium and incubated again at 25 °C. The highest dilution in which microbial growth was observed was subsequently diluted and incubated in fresh medium at least three more times. Subsequently, colonies were isolated under H₂/CO₂ on basal medium solidified with 1.5% (w/v) agar. Thus, pure cultures of strains

The GenBank/EMBL/DDBJ accession numbers for strains Mic5c12^T and Mic6c05^T for the 16S rRNA gene sequences are AB542742 and AB542743 and for the *mcrA* sequences are AB542744 and AB542745, respectively.

Supplementary figures are available with the online version of this paper.

Mic5c12^T and Mic6c05^T were obtained from the sludge and tubercle samples, respectively.

Cells of strains Mic5c12^T and Mic6c05^T were non-motile rods ($0.3 \times 2.4\text{--}4.7$ and $0.3 \times 2.3\text{--}3.7$ μm , respectively; Supplementary Fig. S1, available in IJSEM Online) and showed autofluorescence under a AX70 epifluorescence microscope with U-MWU2 mirror unit (Olympus). The two isolates formed white colonies (approximately 0.5 mm in diameter after 3 weeks) on solid basal medium. Gram-staining and tests for susceptibility to SDS were performed as described by Boone & Whitman (1988). The isolates were Gram-staining-variable and were not lysed in 0.01 % (w/v) SDS or hypotonic (distilled) water.

Strains Mic5c12^T and Mic6c05^T were strictly anaerobic and grew with H₂/CO₂ (80:20, v/v). Formate (20 and 40 mM), acetate (20 and 40 mM), methanol (20 mM), ethanol (5 mM) and methylamine (10 mM) did not support growth or methane production under a N₂/CO₂ (80:20, v/v) atmosphere. Strain Mic6c05^T showed slight methane production from 2-propanol (5 mM), isobutanol (5 mM) and cyclopentanol (5 mM) under N₂/CO₂, but growth was not observed. Methane production from these alcohols was not observed in strain Mic5c12^T. Strain Mic5c12^T required yeast extract (0.02 g l⁻¹) and acetate (10 mM) for growth. In contrast, although strain Mic6c05^T required vitamins for growth, yeast extract and acetate were not required and did not stimulate growth.

The optimum temperature, pH and NaCl concentration for growth were determined by examining the time-course of methane production. The gas phase of the cultures was analysed by GC using a thermal conductivity detector and a Molecular Sieve 60/80 column (Shimadzu). Methane production with varying NaCl concentration is shown in Fig. 1 and with varying temperature and pH is shown in Supplementary Fig. S2. Strain Mic5c12^T was able to grow at 20–40 °C (optimum 35 °C), at pH 5.5–9.0 (optimum pH 6.5) and with 0–1.2 M NaCl (optimum 0–0.68 M NaCl) and doubling time under optimum conditions was 39.5 h. Strain Mic6c05^T was able to grow at 20–45 °C (optimum 40 °C), at pH 5.5–9.0 (optimum pH 6.0–7.5) and with 0–1.2 M NaCl (optimum 0.34 M NaCl) and doubling time under optimum conditions was 18.5 h. While both strains were able to grow with 1.2 M NaCl, strain Mic5c12^T showed optimal growth with 0–0.68 M NaCl, which indicated that it was halotolerant. To date, *M. aarhusense* and *M. subterraneum* have been reported as halotolerant species of the genus *Methanobacterium* (Kotelnikova *et al.*, 1998; Shlimon *et al.*, 2004) and the effects of NaCl on their growth are identical to those observed for strain Mic5c12^T. In contrast, strain Mic6c05^T showed optimal growth with 0.34 M NaCl, which indicated that it was halophilic. To the authors' knowledge, this is the first study to report a strain of the genus *Methanobacterium* that is acclimated to marine environments.

Genomic DNA was extracted and purified (Mori *et al.*, 2000) and G+C content was analysed using HPLC with a

Fig. 1. Effect of NaCl concentration on methane production of strain Mic5c12^T (open circles) and strain Mic6c05^T (solid circles) in basal medium at 35 °C and pH 7 under a H₂/CO₂ atmosphere.

reversed-phase column (Mesbah *et al.*, 1989). The G+C contents of strains Mic5c12^T and Mic6c05^T were 38.3 and 37.6 mol%, respectively.

For phylogenetic analysis, partial sequences of the 16S rRNA gene and *mcrA* (alpha subunit of methyl-coenzyme M reductase) were used. The 16S rRNA genes for strains Mic5c12^T and Mic6c05^T were amplified using primers Ar0023mLF and Ar1530R (Mori *et al.*, 2008b) and amplification products were sequenced using the method described previously (Mori *et al.*, 2008a). Thus, almost-complete 16S rRNA gene sequences were determined for strains Mic5c12^T (1392 bp) and Mic6c05^T (1412 bp). The *mcrA* genes for strains Mic5c12^T and Mic6c05^T were amplified using primers MR1 (Simankova *et al.*, 2003) and ME2 (Hales *et al.*, 1996) and the amplification products were cloned using the TOPO TA Cloning kit (Invitrogen). Cloned *mcrA* sequences were amplified using the M13 primer set and sequences of amplification products were determined using M13 Forward, M13 Reverse, ME1, ME2 (Hales *et al.*, 1996), MCRf (Springer *et al.*, 1995) and MCRfr (5'-ARCCADATYTGRTCTA-3'). Thus, deduced amino acid sequences were obtained from *mcrA* sequences for strains Mic5c12^T (1148 bp) and Mic6c05^T (1148 bp). Because the *mcrA* sequences for strains of species with validly published names for the genus *Methanobacterium* that are available in public databases are short except for *M. aarhusense* H2-LR^T, the above method was used to determine *mcrA* sequences for *M. alcaliphilum* NBRC 105226^T (1145 bp), *M. congolense* NBRC 105227^T (1148 bp), *M. formicum* NBRC 100475^T (1145 bp), *M. oryzae* NBRC 105229^T (1145 bp) and *M. subterraneum* NBRC 105231^T (1148 bp). However, the combination of MR1 and ME2 generated sequences of *mrtA*, which encodes an isoenzyme of McrA, for *M. bryantii* NBRC 104951^T (1139 bp, AB542756), *M. espanolae* NBRC

Fig. 2. Neighbour-joining phylogenetic trees showing the relationships between strains Mic6c05^T and Mic5c12^T and type strains of species of the genus *Methanobacterium*, based on (a) 16S rRNA gene sequences (1240 bp) and (b) deduced McrA sequences (365 amino acids). Bootstrap values (>50%) are shown at branch nodes. Bars, 0.01 substitutions per position.

105228^T (1139 bp, AB542757), *M. ivanovii* NBRC 104952^T (1139 bp, AB542759), *M. palustre* NBRC 105230^T (1139 bp, AB542760) and *M. uliginosum* NBRC 105232^T (1139 bp, AB542761). Therefore, a new primer, MRbac1 (5'-AACACGCGCMATGCARCA-3'), was designed using the newly obtained *mcrA* sequences for the genus *Methanobacterium* and was used to substitute MR1 to determine *mcrA* sequences for *M. bryantii* NBRC 104951^T (1133 bp), *M. espanolae* NBRC 105228^T (1133 bp), *M. ivanovii* NBRC 104952^T (1133 bp), *M. palustre* NBRC 105230^T (1134 bp) and *M. uliginosum* NBRC 105232^T (1134 bp).

A phylogenetic tree based on almost-complete (1240 bp) 16S rRNA gene sequences was constructed with the neighbour-joining method using CLUSTAL_X (Saitou & Nei, 1987; Thompson *et al.*, 1997) after alignment using ARB (Ludwig *et al.*, 2004; Fig. 2a). Strains Mic5c12^T and Mic6c05^T were closely related and exhibited 97.9% 16S rRNA gene sequence similarity. The strains were closely affiliated with *M. subterraneum* A8p^T, *M. palustre* F^T and *M. formicicum* DSM 1535^T. 16S rRNA gene sequence similarities between strain Mic5c12^T and these type strains were 97.3, 97.0 and 96.5%, respectively, and those between strain Mic6c05^T and these type strains were 97.9, 96.6 and 97.4%, respectively. A phylogenetic tree based on 365 positions of the deduced McrA amino acid sequences was constructed with the neighbour-joining method after alignment using CLUSTAL_X (Fig. 2b). The McrA tree was topologically similar to the 16S rRNA gene tree: strains Mic5c12^T and Mic6c05^T were closely related to *M. subterraneum* NBRC 105231^T, *M. palustre* NBRC 105230^T and *M. formicicum* NBRC 100475^T. McrA sequence

similarities between strain Mic5c12^T and these type strains were 96.2, 93.0 and 94.6%, respectively, and those between strain Mic6c05^T and these type strains were 95.7, 92.7 and 94.6%, respectively.

The differential characteristics of strains Mic5c12^T and Mic6c05^T and type strains of species of the genus *Methanobacterium* are summarized in Table 1 and as follows: (i) strains Mic5c12^T and Mic6c05^T differed from *M. beijingense*, *M. formicicum*, *M. oryzae* and *M. palustre* with regard to their inability to produce methane from formate; (ii) strains Mic5c12^T and Mic6c05^T differed from *M. alcaliphilum* and *M. subterraneum* with regard to the optimum pH required for growth and genomic DNA G+C content; and (iii) strains Mic5c12^T and Mic6c05^T differed from *M. aarhusense* and *M. ivanovii* with regard to their not requiring a moderately high temperature for growth. Additionally, strain Mic5c12^T differed from all type strains of species of the genus *Methanobacterium* because it required acetate for growth.

On the basis of the phylogenetic and physiological findings, we propose two novel species to accommodate strains Mic5c12^T and Mic6c05^T, with the names *Methanobacterium petrolearium* sp. nov. and *Methanobacterium ferruginis* sp. nov., respectively.

Description of *Methanobacterium petrolearium* sp. nov.

Methanobacterium petrolearium (pe.tro.le.a'ri.um. L. fem. n. *petra* rock; L. adj. *olearius* -a -um related to oil; N.L. neut. adj. *petrolearium* related to mineral oil).

Table 1. Characteristics of strains Mic5c12^T and Mic6c05^T and type strains of species of the genus *Methanobacterium*

Strains: 1, Mic5c12^T; 2, Mic6c05^T; 3, *M. aarhusense* H2-LR^T (Shlimon *et al.*, 2004); 4, *M. alcaliphilum* WeN4^T (Worakit *et al.*, 1986); 5, *M. beijingense* 8-2^T (Ma *et al.*, 2005); 6, *M. bryantii* M.o.H.^T (Boone, 2000); 7, *M. congolense* C^T (Cuzin *et al.*, 2001); 8, *M. espanolae* GP9^T (Patel *et al.*, 1990); 9, *M. formicicum* MF^T (Boone, 2000); 10, *M. ivanovii* Ivanov^T (Jain *et al.*, 1987); 11, *M. oryzae* FPi^T (Joulain *et al.*, 2000); 12, *M. palustre* F^T (Zellner *et al.*, 1989), 13, *M. subterraneum* A8p^T (Kotelnikova *et al.*, 1998); 14, *M. uliginosum* P2St^T (König, 1984). All strains utilized H₂/CO₂ as a substrate. +, Positive; –, negative; NG, alcohols are oxidized but no growth was observed; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Source	Crude oil storage tank sludge	Corroded pipe sediment	Marine sediment	Alkaline lake sediment	Anaerobic digester	Anaerobic digester	Anaerobic digester	Sludge	Sewage sludge digester	Rock core	Rice field	Peat bog	Deep granitic groundwater	Marshy soil
Growth with:														
Formate	–	–	–	–	+	–	–	–	+	–	+	+	–	–
Secondary alcohols	–	NG	–	ND	–	NG	NG	NG	–	–	–	+	–	–
Acetate requirement	+	–	–	–	–	–	ND	ND	–	–	ND	ND	–	–
Optimum growth at:														
Temperature (°C)	35	40	45	37	37	37–39	37–42	35	37–45	45	40	33–37	20–40	40
pH	6.5	6.0–8.0	7.5–8.0	8.1–9.1	7.2	6.9–7.2	7.2	5.6–6.2	ND	7.0–7.4	7.0	7.0	7.8–8.8	ND
NaCl range (M)	0–1.2	0–1.2	0.05–0.9	ND	0–0.5	ND	ND	ND	ND	ND	0–0.4	0–0.3	0.2–1.2	ND
DNA G+C content (mol%)*	38.3 (LC)	37.6 (LC)	34.9 (LC)	57 (BD)	38.9 (T _m)	33–38 (BD)	39.5 (LC)	34 (T _m)	41–42 (BD)	36.6 (T _m)	31 (LC)	34 (T _m)	54.5 (T _m)	33.8 (T _m)

*DNA G+C content determined using HPLC (LC), buoyant density (BD) or melting point (T_m).

Cells are non-motile, Gram-staining-variable rods, 0.3 µm wide and 2.4–4.7 µm long, and are not lysed by 0.01 % SDS or hypotonic solution (distilled water). White colonies are formed on solid medium. Strictly anaerobic. H₂ is utilized for growth and methane production, but formate, acetate, methanol, methylamine, 2-propanol, isobutanol, cyclopentanol and ethanol are not. Acetate and yeast extract are essential for growth. Grows at 20–40 °C (optimum 35 °C), at pH 5.5–9.0 (optimum pH 6.5) and with 0–1.2 M NaCl (optimum 0–0.68 M NaCl).

The type strain, Mic5c12^T (=NBRC 105198^T=DSM 22353^T), was isolated from sludge deposited in a crude oil storage tank in Japan. The DNA G+C content of the type strain is 38.3 mol%.

Description of *Methanobacterium ferruginis* sp. nov.

Methanobacterium ferruginis (fer.ru.gi'nis. L. fem. n. ferrugo iron rust; L. gen. n. ferruginis of iron rust).

Cells are non-motile, Gram-staining-variable rods, 0.3 µm wide and 2.3–3.7 µm long, and are not lysed by 0.01 % SDS or hypotonic solution (distilled water). White colonies are formed on solid medium. Strictly anaerobic. H₂ is used for growth and methane production, but formate, acetate, methanol, methylamine and ethanol are not. Methane is produced from 2-propanol, isobutanol and cyclopentanol, but growth is not observed. Vitamins are essential for growth, but acetate is not. Grows at 20–45 °C (optimum 40 °C), at pH 5.5–9.0 (optimum pH 6.0–8.0) and with 0–1.2 M NaCl (optimum 0.34 M NaCl).

The type strain, Mic6c05^T (=NBRC 105197^T=DSM 21974^T), was isolated from an iron-corrosive tubercle on an interior pipe transporting natural-gas-containing brine in the southern Kanto gas field, Japan. The DNA G+C content of the type strain is 37.6 mol%.

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References

- Boone, D. R. (2000). Genus I. *Methanobacterium*. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, pp. 215–218. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. New York: Springer.
- Boone, D. R. & Whitman, W. B. (1988). Proposal of minimal standards for describing new taxa of methanogenic bacteria. *Int J Syst Bacteriol* **38**, 212–219.
- Cuzin, N., Ouattara, A. S., Labat, M. & Garcia, J. L. (2001). *Methanobacterium congolense* sp. nov., from a methanogenic fermentation of cassava peel. *Int J Syst Evol Microbiol* **51**, 489–493.
- Hales, B. A., Edwards, C., Ritchie, D. A., Hall, G., Pickup, R. W. & Saunders, J. R. (1996). Isolation and identification of methanogen-specific DNA from blanket bog peat by PCR amplification and sequence analysis. *Appl Environ Microbiol* **62**, 668–675.
- Jain, M. K., Thompson, T. E., Conway de Macario, E. & Zeikus, J. G. (1987). Speciation of *Methanobacterium* strain Ivanov as *Methanobacterium ivanovii*, sp. nov. *Syst Appl Microbiol* **9**, 77–82.
- Joulian, C., Patel, B. K., Ollivier, B., Garcia, J. L. & Roger, P. A. (2000). *Methanobacterium oryzae* sp. nov., a novel methanogenic rod isolated from a Philippines ricefield. *Int J Syst Evol Microbiol* **50**, 525–528.
- König, H. (1984). Isolation and characterization of *Methanobacterium uliginosum* sp. nov. from a marshy soil. *Can J Microbiol* **30**, 1477–1481.
- Kotelnikova, S., Macario, A. J. L. & Pedersen, K. (1998). *Methanobacterium subterraneum* sp. nov., a new alkaliphilic, eurythermic and halotolerant methanogen isolated from deep granitic groundwater. *Int J Syst Bacteriol* **48**, 357–367.
- Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadukumar, Buchner, A., Lai, T., Steppi, S. & other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.
- Ma, K., Liu, X. & Dong, X. (2005). *Methanobacterium beijingense* sp. nov., a novel methanogen isolated from anaerobic digesters. *Int J Syst Evol Microbiol* **55**, 325–329.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Mori, K., Yamamoto, H., Kamagata, Y., Hatsu, M. & Takamizawa, K. (2000). *Methanocalculus pumilus* sp. nov., a heavy-metal-tolerant methanogen isolated from a waste-disposal site. *Int J Syst Evol Microbiol* **50**, 1723–1729.
- Mori, K., Maruyama, A., Urabe, T., Suzuki, K. & Hanada, S. (2008a). *Archaeoglobus infectus* sp. nov., a novel thermophilic, chemolithoheterotrophic archaeon isolated from a deep-sea rock collected at Suiyo Seamount, Izu-Bonin Arc, western Pacific Ocean. *Int J Syst Evol Microbiol* **58**, 810–816.
- Mori, K., Sunamura, M., Yanagawa, K., Ishibashi, J., Miyoshi, Y., Iino, T., Suzuki, K. & Urabe, T. (2008b). First cultivation and ecological investigation of a bacterium affiliated with the candidate phylum OP5 from hot springs. *Appl Environ Microbiol* **74**, 6223–6229.
- NBRC (2005). *NBRC Catalogue of Biological Resources: microorganisms, genomic DNA clones, and cDNAs*, 1st edn. Chiba, Japan: National Institute of Technology and Evaluation (NITE).
- Patel, G. B., Sprott, G. D. & Fein, J. E. (1990). Isolation and characterization of *Methanobacterium espanolae* sp. nov., a mesophilic, moderately acidiphilic methanogen. *Int J Syst Bacteriol* **40**, 12–18.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Shlimon, A. G., Friedrich, M. W., Niemann, H., Ramsing, N. B. & Finster, K. (2004). *Methanobacterium aarhusense* sp. nov., a novel methanogen isolated from a marine sediment (Aarhus Bay, Denmark). *Int J Syst Evol Microbiol* **54**, 759–763.
- Simankova, M. V., Kotsyurbenko, O. R., Lueders, T., Nozhevnikova, A. N., Wagner, B., Conrad, R. & Friedrich, M. W. (2003). Isolation and characterization of new strains of methanogens from cold terrestrial habitats. *Syst Appl Microbiol* **26**, 312–318.
- Springer, E., Sachs, M. S., Woese, C. R. & Boone, D. R. (1995). Partial gene sequences for the A subunit of methyl-coenzyme M reductase (mcrI) as a phylogenetic tool for the family *Methanosarcinaceae*. *Int J Syst Bacteriol* **45**, 554–559.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Worakit, S., Boone, D. R., Mah, R. A., Abdel-Samie, M.-E. & El-Halwagi, M. M. (1986). *Methanobacterium alcaliphilum* sp. nov., an H₂-utilizing methanogen that grows at high pH values. *Int J Syst Bacteriol* **36**, 380–382.

Zellner, G., Bleicher, K., Braun, E., Kneifel, H., Tindall, B. J., Conway de Macario, E. & Winter, J. (1989). Characterization of a new mesophilic, secondary alcohol-utilizing methanogen, *Methanobacterium palustre* spec. nov. from a peat bog. *Arch Microbiol* **151**, 1–9.