Methanoculleus horonobensis sp. nov., a methanogenic archaeon isolated from a deep diatomaceous shale formation

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A methanogenic organism from the domain Archaea, designated strain T10T, was isolated from groundwater sampled from a deep diatomaceous shale formation located in Horonobe, Hokkaido, Japan. The strain utilized H2/CO2 and formate as substrates for methanogenesis. Cells were strictly anaerobic, Gram-negative-staining, flagellated, irregular coccoids, 0.7–1.6 μm in diameter, and occurred singly. The strain grew at 25–45 °C (optimum 37–42 °C), at pH 5.8–8.2 (optimum pH 6.7–6.8) and in the presence of 0–1.3 M NaCl (optimum 0.1–0.2 M NaCl). The G+C content of the genomic DNA was 62.9 mol%. 16S rRNA gene sequencing revealed that, although the strain is a member of the genus Methanoculleus, it clearly differed from all described species of this genus (95.5–98.3 % sequence similarity). Values for DNA–DNA hybridization with type strains of closely related Methanoculleus species were less than 50 %. Phenotypic and phylogenetic features of strain T10T clearly indicate that it represents a novel species of the genus Methanoculleus, for which the name Methanoculleus horonobensis sp. nov. is proposed. The type strain is T10T (= DSM 21626T = JCM 15517T).

The groundwater sample was collected on 15 January 2005 from the Wakkani formation, a Miocene diatomaceous shale in northernmost Japan, by using methods described previously (Shimizu et al., 2006). The sample was extracted from 362.4–385.7 m below ground level through the survey borehole HDB-6, which was drilled by the Japan Atomic Energy Agency in the Horonobe area. Based on stable isotopic criteria (Strapoc et al., 2007), the δD value of CH4 (−197 ‰) and the δ13C difference between dissolved CO2 and CH4 (Δ13C–CH4 = 76 ‰) indicated that the dissolved methane in the groundwater of the Wakkani formation originated via microbial CO2 reduction.

Groundwater samples (100 μl) were inoculated into 20 ml JCM 262 medium (http://www.jcm.riken.go.jp/cgi-bin/jcm/jcm_grmd?GRMD=262) in 50 ml serum bottles (Maruemu) sealed with butyl rubber stoppers and aluminium caps in an ANX anaerobic chamber (Hirasawa). The samples were incubated at 37 °C for 4 weeks in a H2/CO2 atmosphere (80:20, v/v; 200 kPa). The enriched culture, which actively produced methane, was transferred periodically to fresh broth. Strain T10T was successfully isolated after repeated inoculations of a single colony that was serially diluted by using a JCM 262 slant culture containing 200 mg vancomycin l−1 and 1.5 % (w/v) agar in a H2/CO2 atmosphere.
The specific growth rate for strain T10T under optimal conditions based on the OD_{660} was 0.101–0.110 h^{-1}.

The strain used H_{2}/CO_{2} (80:20, v/v; 200 kPa) and formate (20 mM) as methanogenic substrates. The strain exhibited good growth with H_{2}/CO_{2} (80:20, v/v; 200 kPa), but slow growth with formate (the specific growth rate was 0.038 h^{-1}). Growth and methane formation were not observed on the following substrates: 2-propanol, 2-butanol, cyclopentanol, methanol, monomethylamine, dimethylamine, trimethylamine and acetate (20 mM each). Growth of the strain was stimulated by coenzyme M, acetate and yeast extract.

Genomic DNA was extracted (Marmur, 1961) and purified (Hamamoto & Nakase, 1995). The DNA G+C content was analysed using HPLC (Shimadzu LC-10A) (Katayama-Fujimura et al., 1984). The DNA G+C content of strain T10T was 62.9 ± 0.2 mol% (mean ± SD, n=3).

Phylogenetic analyses were performed as described previously (Shimizu et al., 2006, 2011). The phylogenetic tree, based on the almost-complete (1342 bp) 16S rRNA gene sequence, showed that strain T10T clustered with members of the genus Methanoculleus and was most closely related to Methanoculleus marisnigri JR1T, M. submarinus Nankai-1T and M. chikugoensis MG62T (Fig. 1). Bootstrap analysis indicated a clear branching of strain T10T from these species. BLAST searches using the almost-complete (1432 bp) 16S rRNA gene sequence of strain T10T revealed that the strain was most closely related to strains belonging to the genus Methanoculleus (95.5–98.3% similarity), and the highest similarity was observed between strain T10T and M. marisnigri JR1T (98.3%), M. submarinus Nankai-1T (98.3%) and M. chikugoensis MG62T (98.1%).

DNA–DNA hybridization was determined according to the method described by Ezaki et al. (1989) using an F0129005 SpectraFluor Plus (GENios). DNA–DNA relatedness between strain T10T and the type strains of the phylogenetically closest related species, M. marisnigri DSM 1498T, M. submarinus DSM 15122T and M. chikugoensis DSM 13459T, was respectively 23.0, 43.0 and 25.0%. These values were too low to classify strain T10T within one of these existing species of the genus Methanoculleus.
Table 1. Characteristics of strain T10T and type strains of species of the genus Methanoculleus

<table>
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*Data from Tian et al. (2010).

A comparison of the phenotypic characteristics of strain T10T and the type strains of Methanoculleus species is summarized in Table 1. The main differences were observed in the following parameters: (i) range of growth temperature and pH, which differed from those of all members of the genus Methanoculleus; (ii) range of NaCl concentration for growth, which differed from all other type strains except that of Methanoculleus receptaculi; (iii) optimum temperature and secondary alcohol utilization, which differed from those of the phylogenetically closest related species M. marisnigri, M. submarinus and M. chikugensis.

In conclusion, on the basis of phenotypic and phylogenetic data, strain T10T represents a novel species of the genus Methanoculleus, for which the name Methanoculleus horonobensis sp. nov. is proposed.

Description of Methanoculleus horonobensis sp. nov.

Methanoculleus horonobensis (ho.ro.no.ben’sis. N.L. masc. adj. horonobensis of or belonging to Horonobe, a town in Hokkaido, Japan, where the type strain was isolated).

Cells are irregular coccoids, 0.7–1.6 μm in diameter. Strictly anaerobic. Cells stain Gram-negative, are lysed by 0.01 % (w/v) SDS and are non-motile, despite the presence of flagella. Cells can utilize H2/CO2 or formate as substrates, but not 2-propanol, 2-butanol, cyclopentanol, methanol, dimethylamine, trimethylamine, dimethyl sulphide, acetate or monomethylamine. Grows at 25–45 °C (optimum 37–42 °C), at pH 5.8–8.2 (optimum pH 6.7–6.8) and in the presence of 0–1.3 M NaCl (optimum 0.1–0.2 M).

The type strain, T10T (=DSM 21626T =JCM 15517T), was isolated from deep subsurface groundwater from a diatomaceous shale formation in Horonobe, Japan. The genomic DNA G+C content of the type strain is 62.9 ± 0.2 mol%.

Acknowledgements

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References


