***Methanoculleus chikugoensis* sp. nov., a novel methanogenic archaeon isolated from paddy field soil in Japan, and DNA–DNA hybridization among *Methanoculleus* species**

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A strictly anaerobic, irregularly coccoid, methanogenic archaeon, strain MG62^T (= JCM 10825^T = DSM 13459^T), was isolated from paddy field soil in Chikugo, Fukuoka, Japan. The cells stained Gram-negative, were 1.0–2.0 µm in diameter, were lysed by SDS and hypotonic solutions and were flagellated. Motility was not observed. The strain was able to use H₂/CO₂, 2-propanol/CO₂, formate, 2-butanol/CO₂ and cyclopentanol/CO₂ as substrates for methanogenesis, but did not utilize acetate, ethanol, methanol or methylamines. The optimum temperature and pH were 25–30 °C and 6.7–7.2. Analysis of lipid component parts (core lipids, phospholipid polar head groups and glycolipid sugar moieties) showed the characteristic pattern of members of the family *Methanomicrobiaceae* except for the absence of glucose as a glycolipid sugar moiety. The G+C content of the DNA was 62.2 mol%. Sequence analysis of the 16S rDNA revealed that the strain belonged to the genus *Methanoculleus*. The strain had DNA–DNA hybridization values of less than 50% with type strains of *Methanoculleus* species. On the basis of phenotypic, genotypic and phylogenetic characteristics, the name *Methanoculleus chikugoensis* sp. nov. is proposed for strain MG62^T (= JCM 10825^T = DSM 13459^T). The DNA hybridization study also revealed the close relationships of three species, *Methanoculleus olentangyi*, *Methanoculleus bourgensis* and *Methanoculleus oldenburgensis*, among *Methanoculleus* species.

Keywords: *Methanoculleus chikugoensis* sp. nov., methanogen, Archaea, paddy field soil

INTRODUCTION

Methane is one of the greenhouse gases, and paddy fields are reported to be an important source of atmospheric methane (Intergovernmental Panel on

Climate Change, 1995). Methane is produced by methanogenic archaea, and microbiological studies of methanogens are important for understanding methane emissions from paddy fields. In order to understand the ecology of the methanogenic archaea in paddy field soil, the characterization and identification of methanogenic isolates from paddy field soil are necessary. Almost all methanogens isolated so far from paddy field soil belong to the families *Methanobacteriaceae* and *Methanosarcinaceae* (Rajagopal *et al.*, 1988; Conrad *et al.*, 1989; Asakawa *et al.*, 1993, 1995; Chen *et al.*, 1993; Fetzer *et al.*, 1993; Min *et al.*, 1997; Großkopf *et al.*, 1998; Joulain *et al.*, 1998, 2000; Takeda, 1998; Adachi, 1999; Kubota & Takeda, 1999). Kudo *et al.* (1997) demonstrated by analysis of 16S rDNA clones amplified using PCR from

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The DDBJ accession number for the 16S rDNA sequence of strain MG62^T is AB038795.

DNA extracted from soil that *Methanogenium*-like methanogens existed in Japanese paddy soils, and a strain of *Methanoculleus marisnigri* has been isolated recently from a rice field in France by Joulain *et al.* (1998). These results suggest that members of the family *Methanomicrobiaceae* also exist in paddy field soil.

In this work, we isolated and characterized a methanogenic archaeon from a Japanese paddy field soil after enrichment with 2-propanol/CO₂. We selected 2-propanol/CO₂ as a substrate and used a slightly saline medium (6 g NaCl l⁻¹) in order to enrich methanogens belonging to the family *Methanomicrobiaceae*, since many species within this family are known to utilize secondary alcohols and they usually prefer slightly saline conditions (0.1 M NaCl or more) (Widdel *et al.*, 1988; Boone *et al.*, 1993). Phenotypic, genotypic and phylogenetic data show that the strain should be assigned as a novel species within the genus *Methanoculleus*, for which we propose the name *Methanoculleus chikugoensis* sp. nov. We also discuss genomic relationships among *Methanoculleus* species.

METHODS

Source of strain MG62^T and reference strains. The sampling site was a paddy field plot (Gray Lowland Soil) with long-term application of rice straw compost at the Kyushu National Agricultural Experiment Station, Chikugo, Fukuoka, Japan (Asakawa *et al.*, 1998). The sampling was performed on 29 August 1995 as described previously (Asakawa *et al.*, 1993).

Methanoculleus marisnigri JR1^T (= DSM 1498^T), *Methanoculleus thermophilus* CR-1^T (= DSM 2373^T), *Methanoculleus palmolei* INSULZ^T (= DSM 4273^T), *Methanoculleus oldenburgensis* CBI^T (= DSM 6216^T), *Methanoculleus bourgensis* MS2^T (= DSM 3045^T) and *Methanoculleus olentangyi* RC/ER^T (= DSM 2772^T) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany.

Media and growth conditions. The basal medium used for enrichment and isolation was a modification of LPBM (Zeikus, 1977) and contained (l⁻¹ distilled water): 0.75 g KH₂PO₄, 0.75 g K₂HPO₄, 1.0 g NH₄Cl, 0.36 g MgCl₂·6H₂O, 6.0 g NaCl, 1.0 g sodium acetate, 2.0 g yeast extract (Difco), 2.0 g Polypepton (Nihon Pharmaceutical), 10 ml 2-propanol, 9 ml trace mineral solution (Morii *et al.*, 1983), 10 ml vitamin solution (Balch *et al.*, 1979), 0.5 ml 0.2% (w/v) resazurin, 0.5 g L-cysteine hydrochloride hydrate, 0.5 g Na₂S·9H₂O and 4.8 g NaHCO₃. For roll tubes, 1.7% (w/v) agar was added to the basal medium. The gas phase was N₂/CO₂ (4:1, 203 kPa) and the pH was 7.0. The medium used for cultivation of the isolate and reference strains was DSM 141 medium (DSMZ, 1993) supplemented with Polypepton (Nihon Pharmaceutical) instead of Trypticase. The headspace was filled with H₂/CO₂ (4:1, 203 kPa) at pH 7.0 or N₂/CO₂ (4:1, 203 kPa) when the substrates 2-propanol, sodium formate, 2-butanol and cyclopentanol were used. These substrates, cysteine and Na₂S were added from anoxic, sterile stock solutions. The media were prepared anoxically, basically according to Hungate technique (Hungate, 1969; Balch *et al.*, 1979; Koga *et al.*, 1987). Cultures were grown in 120 ml serum bottles containing 10 ml medium or in 18 mm (outside diameter) by 180 mm test tubes with 5 ml medium.

Cells for lipid and DNA analyses were grown in 1 l serum bottles containing 100 ml medium at 27 °C for strain MG62^T, 37 °C for *Methanoculleus bourgensis*, *Methanoculleus marisnigri*, *Methanoculleus olentangyi*, *Methanoculleus palmolei* and *Methanoculleus oldenburgensis* and 55 °C for *Methanoculleus thermophilus*.

The specific growth rate was calculated by monitoring the OD₆₆₀. Media with different pH values were prepared by varying the ratio of the H₂/CO₂ mixture and the concentration of NaHCO₃ or Na₂CO₃. The H₂/CO₂ mixture was repeatedly repressurized to 203 kPa with the original ratio.

Methane production was determined by GC as described previously (Asakawa *et al.*, 1995).

Enrichment and isolation. Enrichment was performed in 1 l serum bottles sealed with butyl rubber stoppers and aluminium seals containing 100 ml medium with 2-propanol/CO₂ as growth substrate. A moist soil sample (about 20 g) was inoculated into the medium and the bottle was incubated with shaking (120 r.p.m.) at 30 °C. About 80% of the culture was replaced fortnightly by fresh medium. After seven successive transfers, roll tubes were prepared. Many colonies formed within a few weeks, but they did not produce methane after inoculating into liquid medium. Some of the colonies that formed after 1 month showed fluorescence when observed with an epifluorescence microscope. Therefore, these colonies were inoculated into liquid medium and methane production was examined. Isolation of colonies from roll tubes was repeated and well-isolated colonies were picked and inoculated into the liquid medium. To test for purity, a culture was inoculated into the basal medium without NaHCO₃ but containing 1% (w/v) glucose and VL medium as described previously (Asakawa *et al.*, 1993). The gas phase was N₂. The preparations were incubated statically for 43 d at 30 °C. No growth was obtained and no contaminants were detected microscopically. The pure culture was designated strain MG62^T.

Microscopy. Phase-contrast and epifluorescence microscopy were carried out by using a Nikon Optiphot microscope and an Olympus VANOX microscope, respectively. Gram staining was carried out by the Hucker method. Cells for electron microscopy were collected from a late logarithmic culture at 10 000 g for 2 min. Resuspended cells were stained negatively with 2% phosphotungstic acid and observed with a Hitachi H-7000 electron microscope.

Lipid analysis. Cells were harvested at late exponential phase and stored at -20 °C until use. Extraction of total lipids and analysis of lipid component parts were conducted as described previously (Koga *et al.*, 1993, 1998). The nomenclature for archaeal ether lipids proposed by Nishihara *et al.* (1987) is used in this paper.

G + C content of DNA and DNA-DNA hybridization. Cells of methanogenic strains harvested at late exponential phase were used for DNA isolation. DNA was isolated and purified according to the method of Sowers (1995). The G + C content of the DNA was determined by HPLC (Tamaoka & Komagata, 1984) with the equipment described previously (Asakawa *et al.*, 1995).

DNA-DNA hybridization was performed according to the microtitration plate method (Ezaki *et al.*, 1989; Sawada *et al.*, 1995). Aliquots of 100 µl denatured DNA solution (3 µg ml⁻¹) in PBS-Mg buffer (137 mM NaCl, 2.7 mM KCl, 8 mM Na₂HPO₄, 1.5 mM KH₂PO₄, 0.1 M MgCl₂) were added to each well and the plate was kept at 30 °C overnight for immobilization of DNA. The solution was then dis-

carded and the plate was dried at 55 °C for 24 h. DNAs from methanogenic strains were labelled with digoxigenin (DIG; Roche Diagnostics) according to the protocol of the DIG DNA labelling and detection kit (Roche Diagnostics). Hybridization of DIG-labelled DNA to immobilized DNA was performed at 48 °C (optimal conditions for DNAs of 60% G + C content) with the hybridization mixture in $2 \times$ SSC ($1 \times$ SSC is 0.15 M NaCl, 0.015 M sodium citrate; pH not adjusted) with 50% formamide for 38 h according to the method described by Sawada *et al.* (1995) with the modifications that the amount of DIG-labelled DNA added into each well was 50 ng and salmon sperm DNA was added to the hybridization mixture to a final concentration of $100 \mu\text{g ml}^{-1}$. Hybridized DNA was detected colorimetrically using *p*-nitrophenyl phosphate as a substrate following the method of Sawada *et al.* (1995) and the protocol of the kit (Roche Diagnostics). A_{405} was measured with a model 550 Microplate Reader (Bio-Rad).

16S rDNA sequence analysis. 16S rDNA was amplified by PCR using primers 0025eF (5'-CTGGTTGATCCTGCC-AG-3'; *Escherichia coli* positions 9–25) and 1525R (5'-AAGGAGGTGATCCAGCC-3'; *E. coli* positions 1541–1525) (Achenbach & Woese, 1995) according to the method described by Rainey *et al.* (1996) by using a GeneAmp PCR reagent kit with AmpliTaq DNA polymerase and a GeneAmp PCR 9700 System (Perkin Elmer). The PCR products were purified with Micro Spin S-400HR columns (Amersham Pharmacia Biotech) and sequenced with an ABI 373S automated sequencer (Applied Biosystems). Sequencing reactions were carried out using a PRISM Dye Terminator cycle sequencing ready reaction kit with AmpliTaq DNA polymerase FS (Applied Biosystems). Sequencing primers used were 0112aR (5'-CCACGTGT-TACTSAGC-3'), 0348aF (5'-TCCAGGCCCTACGGG-3'), 0690aR (5'-TTACAGGATTTCACT-3'), 0802aF (5'-ATTAGATACCCGGGTA-3'), 1068F (5'-GCATGGCY-GYCGTCAG-3'), 1100aR (5'-TGGGTCTCGCTCGTTG-3'), 1392R (5'-ACGGGCGGTGTGTRC-3') and 1406F (5'-TGYACACACCGCCCGT-3') (*E. coli* positions 128–112, 333–348, 704–690, 787–802, 1053–1068, 1115–1100, 1406–1392 and 1391–1406; Achenbach & Woese, 1995) together with 0025eF and 1525R described above. Sequences were compared with the BLAST program (NCBI).

RESULTS

Morphology

Cells of strain MG62^T were irregularly coccoid and 1–2 μm in diameter (Fig. 1). The cells stained Gram-negative. Motility was not observed, but the cells were flagellated (Fig. 1b). SDS and hypotonic conditions lysed cells when the method described by Boone & Whitman (1988) was used. This indicates that strain MG62^T has a proteinaceous cell wall.

Surface colonies on agar medium in roll tubes were 0.3–1.0 mm in diameter, round or ellipsoidal, entire, convex, smooth and yellow to ochre-yellow.

Physiology

Strain MG62^T grew on 2-propanol (134 mM)/CO₂ and was also able to utilize H₂/CO₂, formate (40 mM), 2-butanol (109 mM)/CO₂ and cyclopentanol

Fig. 1. (a) Phase-contrast micrograph of strain MG62^T. Bar, 5 μm . (b) Electron micrograph of negatively stained cells of strain MG62^T. Bar, 1 μm .

(110 mM)/CO₂ but not acetate (80 mM), methanol (250 mM), ethanol (110 mM) or trimethylamine (40 mM) as substrates for growth and methanogenesis. Growth on cyclopentanol/CO₂ was very slow. Yeast extract or Polypepton (Nihon Pharmaceutical), which is equivalent to Trypticase Peptone (BBL) and Casitone (Difco), and acetate were required for growth and methane production when growing on H₂/CO₂. The generation time was 46 h (specific growth rate 0.015 h^{-1}) in 100 ml DSM 141 medium in a 1 l bottle with H₂/CO₂ (4:1, 203 kPa) at pH 7 and 27 °C.

Fig. 2 shows the effect of temperature on growth and methane production. Strain MG62^T grew at 15–40 °C. Growth was fastest at 25–30 °C. Growth occurred between pH 6.7 and 8.0 and was fastest at pH 6.7–7.2 (data not shown). Strain MG62^T grew well in the presence of 0–0.3 M NaCl and optimum growth was observed at 0.1 M (data not shown).

Fig. 2. Effect of temperature on growth and methane production of strain MG62^T cultivated in test tubes with 5 ml DSM 141 medium on H₂/CO₂. Specific growth rate (○) and methane production after 13 d of incubation (●) are shown.

Lipids

Strain MG62^T contained archaeol and caldarchaeol as core lipids, galactose as glycolipid sugar and aminopentanetetrol and glycerol as phospholipid polar head groups. Glucose was not detected. Aminopentane-tetrol was methylated.

G + C content and DNA–DNA hybridization

The G + C content of DNA from strain MG62^T was 62.2 ± 1.1 % (mean ± standard deviation, *n* = 3).

Strain MG62^T exhibited DNA–DNA hybridization values of less than 50 % with type strains of *Methanoculleus* species (Table 1). *Methanoculleus olentangyi* RC/ER^T, *Methanoculleus bourgensis* MS2^T and *Methanoculleus oldenburgensis* CB1^T had values of 67–104 % relatedness to each other.

16S rDNA sequence analysis

The 16S rDNA sequence of strain MG62^T (1430 bp) was compared with those available in DNA databases. The highest 16S rDNA similarity, of 98.3 %, was

obtained between strain MG62^T and *Methanoculleus marisnigri* strains JR1^T and CoCam (Joulain *et al.*, 1998), followed by 97.4 % to *Methanoculleus palmolei* INSLUZ^T. Strain MG62^T showed similarities of 94.5–96.3 % to the other species of the genus *Methanoculleus* and at most 91.9 % to genera within the families *Methanomicrobiaceae*, *Methanoplanaceae* and *Methanocorpusculaceae*.

DISCUSSION

The morphological and physiological features of strain MG62^T, such as cell form, flagellation, proteinaceous cell wall, substrate utilization and growth requirements, are similar to those found in members of the genera *Methanogenium*, *Methanoculleus*, *Methanofollis* and *Methanocorpusculum* (Romesser *et al.*, 1979; Zellner *et al.*, 1987, 1989, 1999; Maestrojuán *et al.*, 1990). The lipid component composition of strain MG62^T exhibited the characteristic features, i.e. caldarchaeol as a core lipid, galactose as a glycolipid sugar, aminopentane-tetrol and glycerol as phospholipid polar head groups, of the order *Methanomicrobiales* (Koga *et al.*, 1998), which has been proposed to be separated from ‘*Methanosarcinales*’ by Boone *et al.* (1993). This suggests strongly that strain MG62^T belongs to the order *Methanomicrobiales*. The G + C content of DNA of the genera *Methanogenium* and *Methanocorpusculum* is 47–52 mol %, while that of the genera *Methanoculleus* and *Methanofollis* ranges from 49 to 62 mol % (Romesser *et al.*, 1979; Rivard & Smith, 1982; Corder *et al.*, 1983; Ferguson & Mah, 1983; Harris *et al.*, 1984; Zabel *et al.*, 1984, 1985; Ollivier *et al.*, 1985, 1986; Zellner *et al.*, 1987, 1989, 1990, 1998, 1999; Widdel *et al.*, 1988; Xun *et al.*, 1989; Zhao *et al.*, 1989; Maestrojuán *et al.*, 1990; Blotevogel *et al.*, 1991). The DNA G + C content of strain MG62^T was 62 mol %, which indicates that the strain belongs to the genus *Methanoculleus* or *Methanofollis*. 16S rDNA sequence analysis revealed that strain MG62^T should be assigned to the genus *Methanoculleus* and that it was most closely related to *Methanoculleus marisnigri*, although the similarity was no more than 98.3 %. The results of DNA–DNA hybridization

Table 1. DNA–DNA hybridization between *Methanoculleus chikugoensis* MG62^T and type strains of *Methanoculleus* species

Values are percentage hybridization with DIG-labelled DNA from the species shown, and are means of four replicates.

Strain	1	2	3	4	5	6	7
1. <i>Methanoculleus chikugoensis</i> MG62 ^T (= JCM 10825 ^T = DSM 13459 ^T)	100	39	16	40	30	21	42
2. <i>Methanoculleus marisnigri</i> JR1 ^T (= DSM 1498 ^T)	47	100	12	26	34	23	40
3. <i>Methanoculleus thermophilus</i> CR-1 ^T (= DSM 2373 ^T)	16	22	100	21	35	20	31
4. <i>Methanoculleus palmolei</i> INSLUZ ^T (= DSM 4273 ^T)	18	15	7	100	18	19	30
5. <i>Methanoculleus oldenburgensis</i> CB1 ^T (= DSM 6216 ^T)	25	16	6	25	100	77	104
6. <i>Methanoculleus bourgensis</i> MS2 ^T (= DSM 3045 ^T)	14	20	7	15	67	100	84
7. <i>Methanoculleus olentangyi</i> RC/ER ^T (= DSM 2772 ^T)	19	23	15	19	76	80	100

Table 2. Phenotypic characters of type strains of *Methanoculleus* species

Strains are listed as: 1, *Methanoculleus chikugoensis* MG62^T (= DSM 13459^T); 2, *Methanoculleus marisnigri* JR1^T (= DSM 1498^T); 3, *Methanoculleus thermophilus* CR-1^T (= DSM 2373^T); 4, *Methanoculleus palmolei* INSLUZ^T (= DSM 4273^T); 5, *Methanoculleus oldenburgensis* CB1^T (= DSM 6216^T); 6, *Methanoculleus bourgensis* MS2^T (= DSM 3045^T); 7, *Methanoculleus olentangyi* RC/ER^T (= DSM 2772^T). Data were taken from this study (*M. chikugoensis*); Romesser *et al.* (1979) and Maestrojuán *et al.* (1990) (*M. marisnigri*); Rivard & Smith (1982) (*M. thermophilus*); Zellner *et al.* (1998) (*M. palmolei*); Blotevogel *et al.* (1991) (*M. oldenburgensis*); Ollivier *et al.* (1986) and Maestrojuán *et al.* (1990) (*M. bourgensis*); and Corder *et al.* (1983) and Maestrojuán *et al.* (1990) (*M. olentangyi*). ND, Not determined.

Characteristic	1	2	3	4	5	6	7
Cell size (µm)	1–2	1–3	1.0–1.3	1.25–2.0	1.0	1–2	1.0–1.5
Flagellation	+	+	+	–	–	–	–
Substrates*	H, F, P, B	H, F, P, B	H, F	H, F, P, B	H, F	H, F, P, B	H, F
Optimum growth conditions:							
Temperature (°C)	25–30	40	55	40	45	37	37
pH	6.7–7.2	8.0	7.0	6.9–7.5	8.0	6.7	ND
NaCl (M)	0.1	0.19	0.25	ND	0.04–0.17	0.17	0.17
G + C content (mol %) [†]	62.2 (Lc)	61.2 (Bd)	59 (Bd)	59.5 (Lc), 59 (<i>T_m</i>)	48.6 (<i>T_m</i>)	59 (Bd)	54.4 (Bd)

* H, H₂/CO₂; F, formate; P, 2-propanol/CO₂; B, 2-butanol/CO₂.

[†] Determined by: Lc, HPLC; Bd, buoyant density; *T_m*, thermal denaturation.

experiments not only supported this assignment but also revealed that the genomic relatedness between strain MG62^T and the type strains of each of the *Methanoculleus* species was too low to identify strain MG62^T as belonging to one of the existing species of the genus *Methanoculleus* (Table 1). In addition, strain MG62^T exhibited distinctive features, i.e. optimum temperature and lipid component parts. The optimum temperature (25–30 °C) for growth of strain MG62^T is the lowest among the *Methanoculleus* species (Table 2). *Methanoculleus marisnigri* JR1^T was originally reported with a temperature optimum of 20–25 °C (Romesser *et al.*, 1979); however, this has been emended to 40 °C by Maestrojuán *et al.* (1990). Strain MG62^T did not contain glucose as a glycolipid sugar, which has been detected in all strains of the order *Methanomicrobiales* studied so far (Koga *et al.*, 1998). On the basis of the phenotypic, genotypic and phylogenetic characteristics described above, we propose the name *Methanoculleus chikugoensis* sp. nov. for strain MG62^T.

DNA–DNA hybridization also revealed that *Methanoculleus olentangyi* RC/ER^T, *Methanoculleus bourgensis* MS2^T and *Methanoculleus oldenburgensis* CB1^T were genomically closely related (Table 1). Xun *et al.* (1989) also reported a DNA hybridization value of 66% between *Methanoculleus olentangyi* RC/ER^T and *Methanoculleus bourgensis* MS2^T. The 16S rRNA sequence similarity between *Methanoculleus olentangyi* RC/ER^T and *Methanoculleus bourgensis* MS2^T was 97.7% (the 16S rRNA sequence of *Methanoculleus oldenburgensis* CB1^T is not available), which also supports their close relationship. There seem to be few distinguishing phenotypic features of these three species (Table 2), with the single exception that the

G + C content of *Methanoculleus oldenburgensis* CB1^T is 48.6 mol%. These results indicate that not only *Methanoculleus olentangyi* and *Methanoculleus bourgensis*, as reported already by Xun *et al.* (1989) and Boone *et al.* (1993), but also *Methanoculleus oldenburgensis* CB1^T are subjective synonyms. Since *Methanoculleus olentangyi* is the senior synonym, *Methanoculleus olentangyi* must be the name of the united species. However, as *Methanoculleus bourgensis* is the type species of the genus *Methanoculleus*, the genus would be lost. Therefore, we postpone the proposal of this synonymy until the genus *Methanoculleus* is conserved by an Opinion of the Judicial Commission. We will submit a Request for an Opinion on this matter later.

Description of *Methanoculleus chikugoensis* sp. nov.

Methanoculleus chikugoensis (chi.ku.go.en'sis. N. L. adj. *chikugoensis* of Chikugo, the city in Fukuoka, Japan, from where the strain was isolated).

Cells are irregularly coccoid, 1.0–2.0 µm in diameter, with a proteinaceous cell wall, and flagellated. Strictly anaerobic. Cells grow on H₂/CO₂, formate, 2-propanol/CO₂, 2-butanol/CO₂ and cyclopentanol/CO₂ as substrates for methanogenesis. Acetate, ethanol, methanol and methylamines are not utilized. The optimum temperature and pH for growth are 25–30 °C and 6.7–7.2. Cells contain archaeol and caldarchaeol as core lipids, aminopentane-tetrol and glycerol as phospholipid polar head groups and galactose as glycolipid sugar, but glucose is absent. The type strain is MG62^T (= JCM 10825^T = DSM 13459^T), which was isolated from a paddy field soil in Chikugo, Fukuoka, Japan.

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