Methanobrevibacter millerae sp. nov. and *Methanobrevibacter olleyae* sp. nov., methanogens from the ovine and bovine rumen that can utilize formate for growth

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Four formate-utilizing methanogens were isolated from ovine (strain KM1H5-1P^T) and bovine (strains AK-87, OCP and ZA-10^T) rumen contents. Based on 16S rRNA gene sequence analysis, the methanogen strains were found to belong to the order *Methanobacteriales* in the genus *Methanobrevibacter*. Strains ZA-10^T and KM1H5-1P^T gained energy for growth by the reduction of CO₂ to CH₄ using H₂ or formate exclusively as electron donors. Increasing formate concentrations to 220 mM in batch cultures increased the growth of strain KM1H5-1P^T but did not affect the growth of strain ZA-10^T. Substrate specificity and resistance to cell-wall lysis supported the affiliation of the strains to the genus *Methanobrevibacter*. Strains ZA-10^T and KM1H5-1P^T showed 16S rRNA gene sequence similarity of 98.0 and 98.6 % to their closest recognized relatives, *Methanobrevibacter thaueri* CW^T and *Methanobrevibacter ruminantium* M1^T, respectively. DNA–DNA hybridization experiments indicated that the strains were not affiliated at the species level to their closest recognized relatives, with DNA reassociation values of only 28 % between strains ZA-10^T and *Methanobrevibacter thaueri* CW^T and <25 % between strains KM1H5-1P^T and *Methanobrevibacter ruminantium* M1^T. Based on the data presented, the new strains are considered to represent two novel species of the genus *Methanobrevibacter*, for which the names *Methanobrevibacter millerae* sp. nov. (type strain ZA-10^T=DSM 16643^T=OCM 820^T) and *Methanobrevibacter olleyae* sp. nov. (type strain KM1H5-1P^T=DSM 16632^T=OCM 841^T) are proposed.

In an attempt to design anti-methanogen vaccines that would decrease methane emissions from ruminants (Wright *et al.*, 2004a), methanogens were enriched and isolated (Kicic, 1995; Munyard, 2000) from ovine and bovine rumen contents. Phylogenetic surveys of ovine rumen contents (Wright *et al.*, 2004b) were also conducted. The studies revealed that the majority of methanogens from the rumen

contents of the Western Australian sheep and cattle studied belonged to the genus *Methanobrevibacter*. The aim of this research was to determine whether the Western Australian isolates represented novel species of the genus *Methanobrevibacter* or were members of recognized species.

A polyphasic approach was taken to characterize four strains by using the minimal standards as detailed by Boone & Whitman (1988). Three strains were isolated from bovine rumen contents (strains AK-87, OCP and ZA-10^T) and one strain from ovine rumen contents (KM1H5-1P^T). The strains were purified by serial dilution on bottle plates with modified BRN medium with H₂/CO₂ (80:20) at 200 kPa (Balch *et al.*, 1979; Miller *et al.*, 1982). For purification of strain OCP, a mixture of four antibiotics was used (ml⁻¹:

The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequences of strains ZA-10^T and KM1H5-1P^T are AY196673 and AY615201.

Details of growth parameters and DNA–DNA hybridization and 16S rRNA gene sequence similarity values for strains ZA-10^T and M1H5-1P^T in comparison with *Methanobrevibacter* reference strains are available as supplementary material in IJSEM Online.

0.16 mg cephalothin, 0.04 mg clindamycin, 0.10 mg ampicillin and 0.10 mg vancomycin) in 5 ml medium.

All incubations were at 39 °C on an orbital shaker operating at 160 r.p.m. BRN medium was used for growth experiments, phenotypic tests and the production of cells for DNA extraction. A 2.5 % cysteine sulfide solution was used (0.02 ml^{-1}) to reduce the medium just prior to inoculation. Growth was determined by measuring the optical density at 660 nm (OD_{660}) with a Jenway 6300 spectrophotometer. Cultures were maintained by serial transfers in BRN medium every 3–4 weeks using a 10 % (v/v) inoculum. Long-term preservation followed the protocol of Miller (2001).

For 16S rRNA gene sequencing, DNA was extracted and purified following the methods of Wright *et al.* (2004b). PCR amplification and sequencing procedures were as detailed by Wright & Pimm (2003). The phylogenetic software package PHYLIP version 3.62c (Felsenstein, 2004) was used to calculate sequence similarity and evolutionary distances between pairs of nucleotide sequences of 13 methanogen strains by using the Kimura two-parameter correction model (Kimura, 1980). *Methanomicrobium mobile* BP^T was used as the outgroup. A distance-matrix tree was then constructed by using the Fitch–Margoliash method with the program FITCH. Bootstrap analysis (Felsenstein, 1985) (1000 replicates) was also performed.

For genotypic characterization of the strains, DNA was extracted and purified from 2–4 g wet cells by using a modified procedure of that of Marmur & Doty (1962). These modifications included incubation of cells overnight at 45 °C in proteinase K and at least 3 h incubation in 20 % SDS at room temperature, followed by mechanical disruption (4200 r.p.m. for 20 s repeated three times) on a mini bead-beater. The quality of DNA was checked by measuring the A_{260}/A_{280} ratio on a Varian Cary 1E spectrophotometer. The DNA G+C content was determined from thermal denaturation profiles (Sly *et al.*, 1986).

DNA reassociation was analysed between strains that showed ≥ 97 % 16S rRNA gene sequence similarity, between strains ZA-10^T and KM1H5-1P^T and their closest recognized relatives. The method of Bowman *et al.* (1998) was followed using *Methanobrevibacter ruminantium* M1^T as the reference strain. All samples were tested for control values, e.g. *Methanobrevibacter ruminantium* M1^T versus *Methanobrevibacter ruminantium* M1^T (hybridization value = 100 %) and mixed with other strains (hybridization value < 100 %) to determine the degree of DNA–DNA reassociation between strains. Hybridization values of < 25 % were not statistically significant as they were within values consistent with random hybridization. Hybridization was performed in $2 \times \text{SSC}$ at an optimal renaturation temperature of 73 °C (Huß *et al.*, 1983).

Cell walls were extracted from strains KM1H5-1P^T, AK-87 and ZA-10^T and reference strains *Methanobrevibacter*

arboriphilus DH-1^T, *Methanobrevibacter ruminantium* M1^T and *Methanobrevibacter smithii* PS^T, by using the method of König (1995). Protein concentrations of the cell walls and intracellular fractions were determined by using the Bio-Rad protein assay. The carbohydrate concentration of the cell walls was determined by using the Pierce glycoprotein carbohydrate estimation kit.

Phenotypic characterization of the strains included substrate utilization with an emphasis on optimizing growth on formate plus CO_2/N_2 (20:80), nutritional requirements, growth in bile salts and sodium chloride and the temperature and pH range for growth. Cell morphology, size, motility and Gram reaction (Gerhardt *et al.*, 1994) were determined by using a Leitz Aristoplan microscope with phase-contrast optics.

All strains were tested in duplicate for their ability to utilize 100 mM sodium formate, 50 mM sodium acetate, 50 mM methanol, 50 mM ethanol, 50 mM 2-propanol, 50 mM 2-butanol, 50 mM methylamine and 50 mM trimethylamine as growth substrates in the presence of CO_2/N_2 (20:80). To optimize the growth of two strains on formate plus CO_2 , growth rates and yields on H_2/CO_2 (80:20) as compared with formate plus CO_2 were determined. Strains ZA-10^T, KM1H5-1P^T and two reference strains (*Methanobrevibacter ruminantium* M1^T and *Methanobrevibacter smithii* PS^T) were grown in BRN medium in triplicate cultures with either H_2/CO_2 (80:20) pressurized to 200 kPa or sodium formate plus CO_2/N_2 (20:80) pressurized to 100 kPa. The concentration of sodium formate in the medium was increased in 70 mM increments from 150 to 440 mM to determine the optimum formate concentration for growth. Sodium tungstate (0.0016 mM) was also added to the trace minerals in formate-grown cultures. Gas production, pH and substrate consumption were measured. Doubling times and specific growth rates of exponentially growing cultures were determined from OD_{660} measurements over time by using the method of Prescott *et al.* (1999).

Gas pressures in the culture tubes were measured with a pressure transducer and the composition of the headspace in culture tubes was determined by injecting a gas sample into a Shimadzu 8A gas chromatograph, equipped with a thermal conductivity detector and data acquisition plotting and analysis software (DAPA chromatography software; Curtin University, Perth, Australia). The column used was 80/100 Carbosieve stainless steel [$15' \times 1/16''$ (outside diameter) (4.57 m $\times$ 1.6 mm); Supelco]. Operating conditions for the gas chromatograph were as follows: argon carrier gas, column head pressure, 400 kPa; column temperature, 190 °C; injector and detector temperature, 250 °C; and injection volume, 1.0 ml with a Velco valve injecting loop.

Formate concentrations were analysed via HPLC by using a Waters system equipped with two Aminex HPX-87H organic acid columns (300 $\times$ 7.8 mm) connected in series, with a microguard column and a column heater. Formic acid was eluted by using a mobile phase of 0.2 % phosphoric

acid at a flow rate of 0.7 ml min⁻¹, a column temperature of 65 °C and UV detection at 210 nm.

Nutritional supplements to determine the minimum requirements for growth were tested in the basal salts of BRN medium. Growth was confirmed after a third transfer culture had reached an OD₆₆₀ ≥ 0.13 in which a control tube containing inoculum but without substrate was used to 'zero' the spectrophotometer. The final concentrations of nutrients tested were 25 mM acetate, 0.04 mg yeast extract ml⁻¹ and 0.04 mg trypticase peptone ml⁻¹. A requirement for 0.01 mg 2-mercaptoethanesulfonic acid (co-enzyme M) ml⁻¹ and 0.02 ml of a fatty acids solution ml⁻¹ was also tested. The fatty acids solution contained valeric, isovaleric, 2-methylbutyric and isobutyric acid at 2.5 % (v/v) each.

Cells from exponentially growing cultures were used to test for susceptibility to lysis by using 10 % SDS and for bile sensitivity by using 2 % bovine bile (ox gall powder) and 0.1 % sodium deoxycholate. These were added prior to autoclaving the BRN medium as adding them after autoclaving caused precipitation. Growth was considered to be positive if transfer cultures reached an OD₆₆₀ ≥ 0.13. Growth in NaCl was tested at final NaCl concentrations of 0.1–0.5 M in 0.05 M increments.

Range and optimum temperature for growth were determined for strains KM1H5-1P^T, OCP, AK-87 and ZA-10^T and for reference strains *Methanobrevibacter smithii* PS^T, *Methanobrevibacter ruminantium* M1^T and *Methanobrevibacter gottschalkii* HO^T by using a temperature

gradient incubator (TGI). The TGI temperature range was 15–45 °C with 2–3 °C intervals at 150 r.p.m. The temperature of each of the 48 tube locations was checked by using a temperature logger. Finally, the range and optimum pH for growth of the strains at pH 5–10 in intervals of 0.5 pH units were determined.

The polyphasic approach taken to characterize the four new strains revealed that they were affiliated to the genus *Methanobrevibacter*. The DNA G + C contents were within the range previously given for members of the genus *Methanobrevibacter* (26–38 mol%) (Miller, 2001; Miller & Lin, 2002) (Table 1). Nutritional requirements, growth in bile salts, tolerance of NaCl and growth temperature range and pH range for the strains are also detailed in Table 1. Two of the strains (ZA-10^T and OCP) were able to grow at pH 5.5. Prior to this study, the only recognized *Methanobrevibacter* strains reported to be able to grow below neutral pH were *Methanobrevibacter ruminantium* M1^T and *Methanobrevibacter acididurans* ATM^T (Savant *et al.*, 2002). However, in the present study, it was determined that *Methanobrevibacter smithii* PS^T and *Methanobrevibacter gottschalkii* HO^T were also capable of growth below neutral pH (Table 1).

The ability of the strains to utilize only H₂/CO₂ (80:20) and in some cases formate plus CO₂ as a carbon and energy source is characteristic of the genus *Methanobrevibacter*. Two of the four strains in this study (ZA-10^T and KM1H5-1P^T) were able to utilize formate plus CO₂. *Methanobrevibacter ruminantium* M1^T is one of only three

Table 1. Differential characteristics between strains AK-87, OCP and ZA-10^T and KM1H5-1P^T and other related *Methanobrevibacter* species

Reference strains: 1, *Methanobrevibacter ruminantium* M1^T; 2, *Methanobrevibacter gottschalkii* HO^T; 3, *Methanobrevibacter thaueri* CW^T; 4, *Methanobrevibacter smithii* PS^T. ND, No data available.

Trait	ZA-10 ^T	KM1H5-1P ^T	OCP	AK-87	1	2	3	4
Growth on formate	+	+	–	–	+	–	–	+
Nutritional requirements*								
Acetate only	–	+	–	–	–	–	–	–
Yeast extract plus trypticase peptone	+	–	+	+	+	+	+	–†
Co-enzyme M	–	–	–	+	+	–	–	–
Fatty acid solution	–	–	–	+	–	–	–	–
Growth with bile salts	–	–	–	–	–	+	–	+
Temperature range (°C)	33–43	28–42	28–42	36–43	33–42	27–41	ND	26–46
Optimal temperature (°C)	36–42	36–40	35–39	40–42	35–40	37–41	37‡	34–46
pH range	5.5–10.0	6.0–10.0	5.5–9.0	6.5–9.0	5.5–7.0	5.0–10.0	ND	5.0–8.5
pH optimum	7.0–8.0	7.5	7.5	7.5–8.5	6.0–7.0	6.5–7.0	7.0‡	5.5–7.0
Tolerance of 0.45 M NaCl (2.6 %)	+	+	+	–	–	+	–	+
Known habitat(s)§	BRC	ORC	BRC	BRC	BRC	EF, PF	BF	HF, S
DNA G + C content (mol%) (T _m)	31–32	27–29	29–30	28–29	31‡	29–31	35–37	30‡

*All strains require acetate for good growth.

†Miller & Lin (2002) report that *Methanobrevibacter smithii* PS^T requires B vitamins.

‡Data from Miller & Lin (2002).

§BF, Bovine faeces; BRC, bovine rumen contents; EF, equine faeces; HF, human faeces; ORC, ovine rumen contents; PF, porcine faeces; S, sewage.

strains of recognized *Methanobrevibacter* species isolated from ruminant sources (also *Methanobrevibacter thaueri* CW^T and *Methanobrevibacter wolinii* SH^T) that is able to use formate plus CO₂. This is surprising, given that formate may be an important substrate for methanogenesis in the rumen (Lovley *et al.*, 1984; Asanuma *et al.*, 1999).

Cell yields were 2–3 times less when strains ZA-10^T, KM1H5-1P^T, *Methanobrevibacter ruminantium* M1^T and *Methanobrevibacter smithii* PS^T were grown on formate instead of H₂/CO₂. Formate at concentrations greater than 150 mM increased growth of *Methanobrevibacter smithii* PS^T (OD₆₆₀ = 0.597 at 290 mM formate) and strain KM1H5-1P^T (OD₆₆₀ = 0.578 at 220 mM formate), but inhibited growth of *Methanobrevibacter ruminantium* M1^T (OD₆₆₀ = 0.09 at 220 mM formate) and did not increase growth of strain ZA-10^T (OD₆₆₀ = 0.25 at 220 mM formate). This may be due to the free energy available for methanogenesis being greater for hydrogen than for formate [–130 and –119 kJ (mol CH₄)^{–1}, respectively] (Muller *et al.*, 1993).

For *Methanobrevibacter smithii* PS^T, strain KM1H5-1P^T and strain ZA-10^T, the ratio of formate consumed to methane produced was in approximate agreement with that expected from the stoichiometry (4:1) of the methanogenic reaction (Muller *et al.*, 1993; Morii *et al.*, 1983). For *Methanobrevibacter ruminantium* M1^T, the ratio was not as expected (0.92:1). The substrate did not become limited and the pH of the formate-grown cultures was maintained for all strains within one unit of the optimal pH that Daniels *et al.* (1984) concluded was an acceptable range for good growth. It is possible that the growth was not coupled to methane production because the cells were generating methane for cell maintenance rather than growth (Sowers & Noll, 1995) due to increasing salt concentrations. Growth parameters for strains ZA-10^T, KM1H5-1P^T, *Methanobrevibacter smithii* PS^T and *Methanobrevibacter ruminantium* M1^T on H₂/CO₂ (80:20) and on formate plus CO₂ are given in Supplementary Table S1 (available in IJSEM Online).

The percentage cell-wall protein of the total cell protein for strains ZA-10^T, KM1H5-1P^T and AK-87 was 85.5, 86.0 and 85.8 %, respectively, and for the three reference strains *Methanobrevibacter ruminantium* M1^T, *Methanobrevibacter smithii* PS^T and *Methanobrevibacter arboriphilus* DH-1^T was 85.2, 85.9 and 86.6 %, respectively. The carbohydrate content of the cell walls varied between 21.7 and 44.0 % among these strains: ZA-10^T, 26.2 %; KM1H5-1P^T, no result; AK-87, 32.9 %; *Methanobrevibacter ruminantium* M1^T, 44.0 %; *Methanobrevibacter smithii* PS^T, 27.0 %; *Methanobrevibacter arboriphilus* DH-1^T, 21.7 %. These results compared favourably with those reported by Kandler & König (1985), who calculated the carbohydrate content of the cell walls of *Methanobrevibacter arboriphilus* DH-1^T, *Methanobrevibacter smithii* PS^T and *Methanobrevibacter ruminantium* M1^T to be 19, 27 and 41 %, respectively.

The percentage of cell-wall protein compared with the total cell protein did not aid in delineating between the *Methanobrevibacter* species; however, the carbohydrate content of the cell walls varied considerably between the new and reference strains, with the exception of *Methanobrevibacter smithii* PS^T and strain ZA-10^T. A more thorough examination of the carbohydrate content of the cell walls of *Methanobrevibacter* species is warranted as this may provide another useful taxonomic tool.

Stackebrandt & Goebel (1994) determined that, for the domain *Bacteria*, >97 % 16S rRNA gene sequence similarity generally corresponded to >70 % DNA reassociation. Lin & Miller (1998) concluded that, for the genus *Methanobrevibacter*, >99 % 16S rRNA gene sequence similarity corresponded to >70 % DNA reassociation. This conclusion is generally supported by the data from the present study, as the four strains ZA-10^T, KM1H5-1P^T, OCP and AK-87 showed DNA reassociation values of >70 % when 16S rRNA gene sequence similarity was >99 %. An exception was between strains OCP and AK-87, which shared a 16S rRNA gene sequence similarity of 98.6 % and DNA reassociation value of 76 %.

The closest recognized relative to strain ZA-10^T was *Methanobrevibacter thaueri* CW^T (98.0 % 16S rRNA gene sequence similarity), followed by *Methanobrevibacter smithii* PS^T (97.9 %) and *Methanobrevibacter gottschalkii* HO^T (97.5 %). DNA–DNA hybridization experiments revealed only 28 % DNA reassociation between strains ZA-10^T and *Methanobrevibacter thaueri* CW^T, indicating that ZA-10^T was not a strain of *Methanobrevibacter thaueri*. ZA-10^T was also not related to *Methanobrevibacter smithii* or *Methanobrevibacter gottschalkii*, as it shared <25 % DNA reassociation with the type strains of these species. Based on these data, strain ZA-10^T appeared to be unique. Strain ZA-10^T grouped with the New Zealand sheep rumen isolate SM9 with a 16S rRNA gene sequence similarity of 99.3 % (Fig. 1).

The closest recognized relative to strain KM1H5-1P^T was *Methanobrevibacter ruminantium* M1^T, with a 16S rRNA gene sequence similarity of 98.6 %, followed by *Methanobrevibacter smithii* PS^T (94.5 %). However, there was <25 % DNA–DNA hybridization between strain KM1H5-1P^T and either *Methanobrevibacter ruminantium* M1^T or *Methanobrevibacter smithii* PS^T. The closest cultured organisms to strain KM1H5-1P^T were strains OCP and AK-87 with 16S rRNA gene sequence similarities of 99.5 and 99.1 %, respectively, and DNA reassociation values of 86 and 87 %, respectively. Based on these results, strain KM1H5-1P^T and strains OCP and AK-87 appeared to be related and to form a unique group. Levels of 16S rRNA gene sequence similarity and DNA–DNA hybridization values for the four new strains and the type strains of *Methanobrevibacter thaueri*, *Methanobrevibacter gottschalkii*, *Methanobrevibacter smithii* and *Methanobrevibacter ruminantium* are given in Supplementary Table S2 (available in IJSEM Online). The ability to utilize formate plus CO₂ appeared to be strain-specific, given that strain KM1H5-1P^T was able to utilize

Fig. 1. Fitch–Margoliash (FITCH) tree of strains ZA-10^T and KM1H5-1P^T plus related strains of *Methanobrevibacter* based on 16S rRNA gene sequences (1283 bp) using Kimura two-parameter genetic distances. *Methanomicrobium mobile* BP^T was used as the outgroup. Bootstrap support percentages (1000 replicates) >50% are shown on internal nodes and GenBank accession numbers are given in parentheses. Bar, 5 changes per 100 nt.

formate for methanogenesis, but strains OCP and AK-87 could not. Asakawa *et al.* (1993) also observed that strains of *Methanobrevibacter arboriphilus* varied in their ability to use formate.

In addition to the 16S rRNA gene sequence similarity data and DNA reassociation values, the data in Table 1 provide support for the creation of two novel species to accommodate the new strains described here. Strain ZA-10^T differed from its closest relative *Methanobrevibacter thaueri* CW^T in its ability to tolerate 2.6% NaCl and by its ability to use formate plus CO₂ as carbon and energy source. The G+C content of the DNA for *Methanobrevibacter thaueri* CW^T was also quite high (35–37 mol%) compared with strain ZA-10^T (31–32 mol%). Strain KM1H5-1P^T and *Methanobrevibacter ruminantium* M1^T were isolated from ovine and bovine rumen contents, respectively, and they differed in their nutritional requirements. Strain KM1H5-1P^T was able to grow in a salts medium plus H₂/CO₂ (80:20) with only the addition of acetate, whereas *Methanobrevibacter ruminantium* M1^T required the addition of yeast extract and trypticase peptone for growth. Strain KM1H5-1P^T was also able to fulfil its own requirements for co-enzyme M, whereas *Methanobrevibacter ruminantium* M1^T lacked this ability.

The results presented herein indicate that strains ZA-10^T and KM1H5-1P^T should be classified as representing separate novel species of the genus *Methanobrevibacter*. Neither strain had >70% DNA reassociation with recognized species of the genus *Methanobrevibacter* or

with each other. Levels of 16S rRNA gene sequence similarity were <99% in comparisons with recognized species of the genus *Methanobrevibacter* and in comparisons with each other, which was shown by Lin & Miller (1998) and in this study generally to correspond to <70% DNA reassociation. We therefore propose the creation of two novel *Methanobrevibacter* species, *Methanobrevibacter millerae* sp. nov. and *Methanobrevibacter olleyae* sp. nov. With the description of these two novel species, the number of *Methanobrevibacter* species isolated from ruminant sources that can grow on formate plus CO₂ increases from one to three (also *Methanobrevibacter ruminantium*).

Description of *Methanobrevibacter millerae* sp. nov.

Methanobrevibacter millerae (mil'ler.ae. N.L. gen. n. *millerae* of Miller, named for Dr Terry Miller for her contributions to the taxonomy of methanogens, in particular the genus *Methanobrevibacter*).

Cells occur singly or in pairs or chains and are coccobacilli (0.5–1.2 µm) with rounded ends. Cells stain Gram-positive, are non-motile and are resistant to lysis by 10% SDS. Grows and produces methane from H₂/CO₂ and from formate plus CO₂. Acetate, methanol, ethanol, 2-propanol, 2-butanol, methylamine and trimethylamine are not utilized as substrates. Requires acetate and one or more components of yeast extract and trypticase peptone for growth. Does not require co-enzyme M or fatty acids for growth but fatty acids can stimulate growth. Bile-sensitive. Mesophilic. No growth in BRN medium below 33 °C or above 43 °C; optimum temperature range for growth is 36–42 °C. pH range for growth is 5.5–10.0; optimum pH is 7.0–8.0. The maximum salt tolerance for growth is 2.6%. The DNA G+C content is 31–32 mol% (*T_m*).

The type strain, ZA-10^T (=DSM 16643^T=OCM 820^T), was isolated from enrichments of bovine rumen contents.

Description of *Methanobrevibacter olleyae* sp. nov.

Methanobrevibacter olleyae (ol'ley.ae. N.L. gen. n. *olleyae* of Olley, named for Dr June Olley for her contributions to food microbiology).

Cells occur singly or in pairs and are coccobacilli (0.3–1.0 µm) with rounded ends. Cells stain Gram-positive, although some variability is evident. Non-motile and resistant to lysis by 10% SDS. Grows and produces methane from H₂/CO₂ (80:20) and from formate plus CO₂ (reference strains OCP and AK-87 cannot utilize formate plus CO₂). Acetate, methanol, ethanol, 2-propanol, 2-butanol, methylamine and trimethylamine are not utilized as substrates. Requires acetate for growth and does not require co-enzyme M or fatty acids. Strain OCP requires yeast extract and peptone in addition to acetate, and strain AK-87 requires co-enzyme M and fatty acids in addition to these. Bile-sensitive. Mesophilic. No growth in BRN

medium below 28 °C or above 42 °C, although the temperature range for strain AK-87 is restricted to 36–43 °C. The optimum temperature range for growth is 28–42 °C. pH range for growth is 6.0–10.0; optimum pH is 7.5. The maximum salt tolerance for growth is 2.6 %, although growth is inhibited in strain AK-87 at this concentration. The DNA G+C content is 27–29 mol% (T_m).

The type strain, KM1H5-1P^T (=DSM 16632^T=OCM 841^T), was isolated from enrichments of sheep rumen contents. Reference strains OCP and AK-87 were isolated from enrichments of bovine rumen contents.

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