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-10^7) 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 H_2/CO_2 (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}:
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 (OD₆₆₀) 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 BP¹ 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 A₂₆₀/A₂₈₀ ratio on a Varian Cary 1E spectrophotometer. The DNA G + C content was determined from thermal denaturation profiles (Sly et al., 1986).

DNA reassocation was analysed between strains that showed ≥ 97 % 16S rRNA gene sequence similarity, between strains ZA-10ᵀ and KM1H5-1Pᵀ and their closest recognized relatives. The method of Bowman et al. (1998) was followed using Methanobrevibacter ruminantium M1ᵀ as the reference strain. All samples were tested for control values, e.g. Methanobrevibacter ruminantium M1ᵀ versus Methanobrevibacter ruminantium M1ᵀ (hybridization value = 100 %) and mixed with other strains (hybridization value < 100 %) to determine the degree of DNA–DNA reassocation between strains. Hybridization values of < 25 % were not statistically significant as they were within values consistent with random hybridization. Hybridization was performed in 2 × SSC at an optimal renaturation temperature of 73 °C (Huß et al., 1983).

Cell walls were extracted from strains KM1H5-1Pᵀ, AK-87 and ZA-10ᵀ and reference strains Methanobrevibacter arboriphilus DH-1ᵀ, Methanobrevibacter ruminantium M1ᵀ and Methanobrevibacter smithii PSᵀ, 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 CO₂/N₂ (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 CO₂/N₂ (20 : 80). To optimize the growth of two strains on formate plus CO₂, growth rates and yields on H₂/CO₂ (80 : 20) as compared with formate plus CO₂ were determined. Strains ZA-10ᵀ, KM1H5-1Pᵀ and two reference strains (Methanobrevibacter ruminantium M1ᵀ and Methanobrevibacter smithii PSᵀ) were grown in BRN medium in triplicate cultures with either H₂/CO₂ (80 : 20) pressurized to 200 kPa or sodium formate plus CO₂/N₂ (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 OD₆₆₀ 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’ × 1/16’’ (outside diameter) (4.57 m x 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 x 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 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 OD\(_{660} \geq 0.13\) 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 trypsinpeptone 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 OD\(_{660} \geq 0.13\). 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 PST\(^T\), 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 PST\(^T\) and Methanobrevibacter gottschalkii HO\(^T\) were also capable of growth below neutral pH (Table 1).

The ability of the strains to utilize only H\(_2/\)CO\(_2\) (80:20) and in some cases formate plus CO\(_2\) 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 CO\(_2\). Methanobrevibacter ruminantium M1\(^T\) is one of only three

![Table 1](image)

**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 PST\(^T\). ND, No data available.

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

*All strains require acetate for good growth.
†Miller & Lin (2002) report that Methanobrevibacter smithii PST\(^T\) 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 CWT 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-10T, KM1H5-1P T, Methanobrevibacter ruminantium M1T 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 M1T (OD660 = 0.09 at 220 mM formate) and did not increase growth of strain ZA-10T (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-10T, 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 M1T, 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-10T, KM1H5-1P T, Methanobrevibacter smithii PS T and Methanobrevibacter ruminantium M1T 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-10T, KM1H5-1P T and AK-87 was 85.5, 86.0 and 85.8 %, respectively, and for the three reference strains Methanobrevibacter ruminantium M1T, 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-10T, 26.2 %; KM1H5-1P T, no result; AK-87, 32.9 %; Methanobrevibacter ruminantium M1T, 44.0 %; Methanobrevibacter smithii PS T, 27.0 %; Methanobrevibacter arboriphilus DH-1 T, 21.7 %. These results compared favourably with those reported by Kandler & Konig (1985), who calculated the carbohydrate content of the cell walls of Methanobrevibacter arboriphilus DH-1 T, Methanobrevibacter smithii PS T and Methanobrevibacter ruminantium M1T 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-10T. 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 reassocitation. Lin & Miller (1998) concluded that, for the genus Methanobrevibacter, > 99 % 16S rRNA gene sequence similarity corresponded to > 70 % DNA reassocitation. This conclusion is generally supported by the data from the present study, as the four strains ZA-10T, KM1H5-1P T, OCP and AK-87 showed DNA reassocitation 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 reassocitation value of 76 %.

The closest recognized relative to strain ZA-10T was Methanobrevibacter thaueri CWT (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 reassocitation between strains ZA-10T and Methanobrevibacter thaueri CWT, indicating that ZA-10T was not a strain of Methanobrevibacter thaueri. ZA-10T was also not related to Methanobrevibacter smithii or Methanobrevibacter gottschalkii, as it shared < 25 % DNA reassocitation with the type strains of these species. Based on these data, strain ZA-10T appeared to be unique. Strain ZA-10T 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 M1T, with a 16S rRNA gene sequence similarity of 98.6 %, followed by Methanobrevibacter smithii PST (94.5 %). However, there was < 25 % DNA–DNA hybridization between strain KM1H5-1P T and either Methanobrevibacter ruminantium M1T or Methanobrevibacter smithii PST. 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 reassocitation 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
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 I provide support for the creation of two novel species to accommodate the new strains described here. Strain ZA-10T differed from its closest relative Methanobrevibacter thaueri CWT 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 CWT was also quite high (35–37 mol%) compared with strain ZA-10T (31–32 mol%). Strain KM1H5-1P and Methanobrevibacter ruminantium M1T were isolated from ovine and bovine rumen contents, respectively, and they differed in their nutritional requirements. Strain KM1H5-1P was able to grow in a salts medium plus H₂/CO₂ (80 : 20) with only the addition of acetate, whereas Methanobrevibacter ruminantium M1T required the addition of yeast extract and trypticase peptone for growth. Strain KM1H5-1P was also able to fulfils its own requirements for co-enzyme M, whereas Methanobrevibacter ruminantium M1T lacked this ability.

The results presented herein indicate that strains ZA-10T and KM1H5-1P 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’er.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, methyamine 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% (Tₘ).

The type strain, ZA-10T (= DSM 16643 = OCM 820), 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 and from formate plus CO₂. Reference strains OCP and AK-87 cannot utilize formate plus CO₂. Acetate, methanol, ethanol, 2-propanol, 2-butanol, methyamine 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 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 %.
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% (Tm).

The type strain, KM1H5-1P7 (=DSM 16632T=OCM 841T), was isolated from enrichments of sheep rumen contents. Reference strains OCP and AK-87 were isolated from enrichments of bovine rumen contents.

Acknowledgements

We thank Dr Terry Miller (Wadsworth Center, Albany, NY, USA) for providing us with strain ZA-10T and Ms Clare Auckland and Mr Louis Klein for their technical assistance. We also thank Dr Wendy Robertson and Dr Stuart Denham for critical comments on the manuscript.

References


