Description of *Anaerobaculum hydrogeniformans* sp. nov., an anaerobe that produces hydrogen from glucose, and emended description of the genus *Anaerobaculum*

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A novel anaerobic, moderately thermophilic, NaCl-requiring fermentative bacterium, strain OS1^T, was isolated from oil production water collected from Alaska, USA. Cells were Gram-negative, non-motile, non-spore-forming rods (1.7–2.7 × 0.4–0.5 µm). The G + C content of the genomic DNA of strain OS1^T was 46.6 mol%. The optimum temperature, pH and NaCl concentration for growth of strain OS1^T were 55 °C, pH 7 and 10 g l⁻¹, respectively. The bacterium fermented D-fructose, D-glucose, maltose, D-mannose, α-ketoglutarate, L-glutamate, malonate, pyruvate, L-tartrate, L-asparagine, Casamino acids, L-cysteine, L-histidine, L-leucine, L-phenylalanine, L-serine, L-threonine, L-valine, inositol, inulin, tryptone and yeast extract. When grown on D-glucose, 3.86 mol hydrogen and 1.4 mol acetate were produced per mol substrate. Thiosulfate, sulfur and L-cystine were reduced to sulfide, and crotonate was reduced to butyrate with glucose as the electron donor. 16S rRNA gene sequence analysis indicated that strain OS1^T was related to *Anaerobaculum thermoterrenum* (99.7 % similarity to the type strain), a member of the phylum *Synergistetes*. DNA–DNA hybridization between strain OS1^T and *A. thermoterrenum* DSM 13490^T yielded 68 % relatedness. Unlike *A. thermoterrenum*, strain OS1^T fermented malonate, maltose, tryptone, L-leucine and L-phenylalanine, but not citrate, fumarate, lactate, L-malate, glycerol, pectin or starch. The major cellular fatty acid of strain OS1^T was iso-C_{15:0} (91 % of the total). Strain OS1^T also contained iso-C_{13:0} 3-OH (3 %), which was absent from *A. thermoterrenum*, and iso-C_{13:0} (2 %), which was absent from *Anaerobaculum mobile*. On the basis of these results, strain OS1^T represents a novel species of the genus *Anaerobaculum*, for which the name *Anaerobaculum hydrogeniformans* sp. nov. is proposed. The type strain is OS1^T (=DSM 22491^T =ATCC BAA-1850^T). An emended description of the genus *Anaerobaculum* is also given.

The bacterial phylum *Synergistetes* is an under-represented phylogenetic cluster of Gram-negative, anaerobic, rod-shaped bacteria that are related to *Synergistes jonesii* (Jumas-Bilak *et al.*, 2009). Members of this group have been isolated from a variety of environments including anaerobic digesters (LaPara *et al.*, 2000; Wu *et al.*, 2001), the termite hindgut (Ohkuma & Kudo, 1996), subgingival plaque (Munson *et al.*, 2004) and petroleum reservoirs (Orphan *et al.*, 2000; van der Kraan *et al.*, 2010; Voordouw *et al.*, 1996). The species in this group with validly published names display diverse physiological properties, but all currently cultivated strains catabolize amino acids (Jumas-Bilak *et al.*,

2009; Vartoukian *et al.*, 2007). It has been noted that these organisms, although underrepresented amongst cultivated taxa, are not uncultivable but are present at low cell numbers in microbial communities (Godon *et al.*, 2005). The genus *Anaerobaculum*, comprising *Anaerobaculum thermoterrenum* (Rees *et al.*, 1997) and *Anaerobaculum mobile* (Menes & Muxí, 2002), is a member of the phylum *Synergistetes*, but the ability of *Anaerobaculum* species to catabolize amino acids has not been studied. However, their capacity to produce hydrogen gas from sugars has been documented (Menes & Muxí, 2002).

Increasing global demand for fossil fuels and a need to decrease carbon dioxide emissions have driven research towards renewable, carbon-neutral energy sources. Hydrogen has the potential to be a sustainable alternative to some fossil fuels. It is a clean fuel that burns with no carbon dioxide emissions and the energy released can be easily converted to electricity by fuel cells (Davila-Vazquez

Abbreviation: FAME, fatty acid methyl ester.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain OS1^T is FJ862996.

A supplementary figure and a supplementary table are available with the online version of this paper.

et al., 2008). Hydrogen has an energy yield of 122 kJ g⁻¹, which is 2.75 times greater than that of hydrocarbon-derived fuels (Kapdan & Kargi, 2006). Presently, about 95 % of the world's hydrogen is produced via steam reformation of natural gas, which does not lower net carbon dioxide emissions, and is resource- and energy-intensive (Lee et al., 2008). The production of hydrogen from renewable resources such as biomass or waste materials is being explored as a sustainable global fuel source. 'Dark-fermentative' bacteria can produce hydrogen from carbohydrates and other waste products (Angenent et al., 2004; Han & Shin, 2004; Li & Fang, 2007) without the need for light input (Nandi & Sengupta, 1998), according to the equation

The Gibbs' free energy of formation given above was taken from Thauer et al. (1977), and is essentially the same as that given by Kengen et al. (2009).

Four hydrogen molecules can be produced per D-glucose molecule via this thermodynamically favourable reaction, with acetate and carbon dioxide as additional products. However, only a handful of organisms have been isolated that produce amounts of hydrogen close to this, such as *Acetomicrobium faecalis* (Winter et al., 1987), *Anaerobaculum mobile* (Menes & Muxí, 2002), *Caldicellulosiruptor saccharolyticus* (van Niel et al., 2002), *Thermotoga maritima* (Schröder et al., 1994) and *Thermotoga elfii* (van Niel et al., 2002). An objective of the United States Department of Energy is exploration of novel biological catalysts that can produce four hydrogen molecules per molecule of glucose (US Department of Energy, 2007).

The hydrogen-producing bacterium strain OS1^T isolated from oil production water is described in this study. On the basis of phenotypic and genotypic evidence, strain OS1^T is proposed to represent a new species of the genus *Anaerobaculum*. The description of the genus is also emended to include the amino acids catabolized by the three species reported to date. Strain OS1^T can produce almost four hydrogen molecules per molecule of D-glucose, which approaches the theoretical maximum via the aforementioned reaction. It also has the ability to produce hydrogen from a variety of amino acids and other organic acids.

Strain OS1^T was isolated from oil production water collected from Alaska, USA, as part of a study of the microbiology of a North Slope oil facility (Duncan et al., 2009). A most probable number (MPN) enumeration of general heterotrophs was conducted at 50 °C using anaerobic half-strength tryptic soy broth (TSB; Difco) with 1 % NaCl and 100 % nitrogen gas phase (Balch & Wolfe, 1976). Strain OS1^T was the dominant culturable heterotroph, present in the production water at 2.3 cells ml⁻¹ (MPN). The dominant culturable hydrogen oxidizer, *Methanothermobacter therm- autotrophicus*, was present at the same cell density (Duncan et al., 2009). The Hungate agar roll-tube method was used for isolation (Hungate, 1969). Colonies on anaerobic roll

tubes were small, circular, smooth and yellowish in colour. Exponential-phase cells grown on D-glucose were used for transmission electron microscopy. Cells were fixed with 1 % glutaraldehyde, spread onto carbon-coated Formvar grids and stained with 0.5 % phosphotungstate (pH 7.0). Cells were photographed using a JEOL JEM 2000 FX transmission electron microscope. Cells were Gram-negative, non-flagellated, non-motile, non-spore-forming rods, 1.7–2.7 × 0.4–0.5 µm, that occurred singly (see Fig. S1, available in IJSEM Online).

Strain OS1^T was cultivated routinely at 55 °C on anaerobic TSB plus 1 % NaCl. Cells grown under these conditions can be lyophilized for long-term preservation, provided adequate attention is given to the maintenance of anaerobic conditions. The temperature and NaCl range and optima were determined using TSB as the growth medium. Growth (optical density) was measured spectrophotometrically at 600 nm using a Spectronic 20D (Thermo Spectronic). Strain OS1^T grew optimally at 55 °C (range 40–65 °C) with 1 % NaCl (range 0.8–7 %). For determination of the pH range and optimum, TSB was amended with the following buffers (at 10 g l⁻¹): HOMOPIEPES, pH 4 and 5; MES, pH 6 and 6.5; TES, pH 7 and 7.5; TAPSO, pH 8; TAPS, pH 8.5; CAPSO, pH 9 and 9.5 (Tanner, 2007). The final pH was adjusted at 55 °C. Strain OS1^T grew optimally at pH 7.5 (range pH 6–9). A mineral medium was developed for routine growth, containing (l⁻¹) 10 ml mineral solution (Tanner, 2007), 10 ml vitamin solution (Tanner, 2007), 10 ml trace metal solution (Tanner, 2007), 10 g NaCl, 2 g yeast extract (Difco) and 3 g D-glucose. The medium was buffered with 10 g TES at pH 7.5. The medium was prepared using strict anaerobic technique under a final gas phase of 100 % nitrogen (Balch & Wolfe, 1976).

Nitrate reduction and sulfide production were measured using CHEMetrics test kits (CHEMetrics, Inc). Nitrate, L-cystine, sulfate, thiosulfate and sulfite were added to the medium from sterile stock solutions. Elemental sulfur was added and sterilized as described previously (Rees et al., 1997). Crotonate reduction was measured by HPLC using a Shimadzu LC-20AT with an SPD-20A UV/Vis detector equipped with an Aminex HPX-87H ion exclusion column (Bio-Rad) using 0.001 M H₂SO₄ as the mobile phase at a flow rate of 0.9 ml min⁻¹. Strain OS1^T reduced elemental sulfur, thiosulfate and L-cystine to sulfide when grown in anaerobic TSB plus 1 % NaCl. Nitrate, sulfite and sulfate reduction were not observed. Strain OS1^T reduced crotonate to butyrate in the presence of glucose but did not ferment crotonate alone. Sulfur and thiosulfate reduction, as well as crotonate reduction in the presence of D-glucose, seems to be a universal phenotype of the genus *Anaerobaculum* (Menes & Muxí, 2002; Rees et al., 1997).

Genomic DNA was isolated from strain OS1^T using a modification of the Marmur procedure (Johnson, 1994). The G+C content of the genomic DNA was measured by HPLC, as described previously (Mesbah et al., 1989), with modifications described previously (Allen et al., 2008). 16S

rRNA gene sequencing was conducted using DNA as a template for PCR amplification using the universal primers 27f, 357f, 704f, 926f, 907r and 1492r corresponding to the *Escherichia coli* numbering system (Johnson, 1994). Sequencing was performed by the Oklahoma Medical Research Foundation (Oklahoma City, OK, USA). Primer sequences were aligned using the Sequencher software suite (Gene Codes Corporation), which resulted in a 1444-base contiguous DNA sequence. The phylogenetic position of strain OS1^T was assessed via maximum-likelihood analysis (Olsen *et al.*, 1994). A consensus tree was generated by bootstrapping at values greater than 90 % confidence (Felsenstein, 1985) (Fig. 1). The topology and major branching points of the phylogenetic tree were confirmed by neighbour-joining and maximum-parsimony analyses in the ARB software suite (Ludwig *et al.*, 2004) (not shown).

16S rRNA gene sequence analysis indicated that strain OS1^T was a member of the genus *Anaerobaculum* (Fig. 1). *A. thermoterrenum* and *A. mobile* are currently the two described members of this genus (Menes & Muxí, 2002; Rees *et al.*, 1997), which are members of the phylum *Synergistetes* (Hugenholtz *et al.*, 2009; Vartoukian *et al.*, 2007). The 16S rRNA gene of strain OS1^T was 99.7 % similar to that of the type strain of *A. thermoterrenum* and 97.8 % similar to that of the type strain of *A. mobile*. It may not be surprising to find a close relative to *A. thermoterrenum* in oil production water, because the type strain of this species was isolated from a similar environment (Rees *et al.*, 1997). Culture-independent surveys have shown the presence of *Anaerobaculum* strains in petroleum reservoirs (GenBank accession numbers GU357467 and EU573105) (Gieg *et al.*, 2010; Kaster *et al.*, 2009), mining wastewater (DQ256300) (Gihring *et al.*, 2006), a solid waste digester (EF559029) (Li *et al.*, 2009) and methanogenic reactors (AB234001, FN563242, FN563270 and AB274508) (Krakat *et al.*, 2011; Sasaki *et al.*, 2006, 2007). *A. mobile* was isolated from an anaerobic wastewater treatment lagoon (Menes & Muxí, 2002). This suggests that strains of *Anaerobaculum* may be present in many anaerobic environments, particularly thermophilic ones. The G+C content of the genomic DNA from strain OS1^T was 46.6 mol%, compared with 44 mol% for *A. thermoterrenum* (Rees *et al.*, 1997) and 51.5 mol% for *A. mobile* (Menes & Muxí, 2002).

DNA–DNA reassociation analysis was necessary for taxonomic placement of strain OS1^T, and was conducted in the laboratory of Dr Peter Schumann at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) as described by De Ley *et al.* (1970), with the modifications described by Escara & Hutton (1980) and Huß *et al.* (1983). Analysis was performed using a model 2600 spectrophotometer equipped with a model 2257-R thermoprogrammer and plotter (Gilford Instrument Laboratories). Rates of renaturation were computed with the program TRANSFER.BAS (Jahnke, 1992). A DNA–DNA reassociation value of 68 ± 4 % was observed when strain OS1^T was hybridized to *A. thermoterrenum* DSM 13490^T, which is close to the threshold value of 70 % for the definition of species (Stackebrandt & Ebers, 2006), suggesting that strain OS1^T may belong to a species distinct from *A. thermoterrenum*.

A. thermoterrenum DSM 13490^T and *A. mobile* DSM 13181^T were obtained from the DSMZ and used in characterization assays. The basal medium used for routine growth of these strains was the mineral medium described above. Growth experiments were conducted in triplicate. All substrates were added from sterile anaerobic stock solutions to a final concentration of 3 g l⁻¹ before inoculation. D-Glucose-grown cells were used as the inoculum for substrate tests. Growth and end-product formation were compared with substrate-unamended controls. Chemicals used in this work were obtained from Sigma-Aldrich unless otherwise noted. Growth was measured spectrophotometrically at 600 nm (Spectronic 20D) (Balch & Wolfe, 1976). Phenotypic characteristics of strain OS1^T are listed in the species description and in Table 1. Several of these substrates were not tested in the original descriptions of *A. thermoterrenum* (Rees *et al.*, 1997) and *A. mobile* (Menes & Muxí, 2002), nor was the ability to utilize amino acids as substrates, a characteristic of members of the phylum *Synergistetes* (Vartoukian *et al.*, 2007). The list of substrates examined for the genus *Anaerobaculum*, as well as a comparison of the phenotypes of *A. thermoterrenum* DSM 13490^T, *A. mobile* DSM 13181^T and strain OS1^T, is given in Table 1.

As shown in Table 1, strain OS1^T can be readily differentiated from species of *Anaerobaculum* by several phenotypic characteristics, such as the ability to utilize malonate, maltose and inulin and the inability to catabolize

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequence analysis of strain OS1^T (*Anaerobaculum hydrogeniformans* sp. nov.) and other closely related micro-organisms within the phylum *Synergistetes*. Identical branches were present in phylogenetic consensus trees generated by the neighbour-joining, maximum-likelihood and maximum-parsimony algorithms. Bootstrap values are expressed as percentages of 1000 replications. Bar, 1 substitution per 10 nucleotide positions.

Table 1. Phenotypic comparison of *A. hydrogeniformans* sp. nov. OS1^T, *A. thermoterrenum* DSM 13490^T and *A. mobile* DSM 13181^T

Strains: 1, *A. hydrogeniformans* sp. nov. OS1^T; 2, *A. thermoterrenum* DSM 13490^T; 3, *A. mobile* DSM 13181^T. Data were obtained in this study unless indicated. All three strains utilized D-fructose, D-glucose, pyruvate, L-tartrate, Casamino acids, L-asparagine, L-cysteine, L-histidine, L-serine, L-threonine and L-valine. All three strains did not utilize D-arabinose, cellobiose, cellulose, D-galactose, lactose, melibiose, raffinose, D-rhamnose, sucrose, D-xylose, dextrin, xylan, adonitol, acetate, butyrate, L-aspartate, L-glutamine, glycine, L-lysine, L-methionine, L-proline, L-tryptophan or L-tyrosine.

Characteristic	1	2	3
Growth rate (h ⁻¹)	0.011	0.013	0.010
Temperature for growth (°C)			
Range	40–65	28–60	35–65
Optimum	55	55	55–60
pH for growth			
Range	6.0–9.0	5.5–8.6	5.4–8.7
Optimum	7.0	7.0–7.6	6.6–7.3
NaCl concentration for growth (g l ⁻¹)			
Range	0.8–70	0–20	0–15
Optimum	10	10	0.08
DNA G + C content (mol%)	46.6	44 ^{a*}	51.5 ^b
Motility	–	–	+
Growth on:			
α-Ketoglutarate	+	+	–
Citrate	–	+	–
Fumarate	–	+	–
D-Gluconate	–	–	+
L-Glutamate	+	+	–
Lactate	–	+	–
L-Malate	–	+	+
Malonate	+	–	–
Maltose	+	–	–
D-Mannose	+	+	–
Glycerol	–	+	+
Inositol	+	+	–
Tryptone	+	–	+
L-Arginine	–	–	+
L-Leucine	+	–	+
L-Phenylalanine	+	–	+
Inulin	+	–	–
Pectin	–	+	–
Starch	–	+	+

*Data taken from: a, Rees *et al.* (1997); b, Menes & Muxí (2002).

L-malate, glycerol and starch. Strain OS1^T had an absolute requirement for NaCl for growth, which the other two species do not share. In addition, strain OS1^T grew in medium containing up to 7 % NaCl, which is much higher than the maxima of 2.0 and 1.5 % observed for cultures of *A. thermoterrenum* (Rees *et al.*, 1997) and *A. mobile* (Menes & Muxí, 2002), respectively.

Cellular fatty acid methyl ester (FAME) and polar lipid analyses were conducted on strain OS1^T, *A. thermoterrenum* DSM 13490^T and *A. mobile* DSM 13181^T. Cells were grown at 55 °C in anaerobic TSB supplemented with 1 % NaCl containing a 100 % nitrogen gas phase. FAME and polar lipid analyses were carried out by the Identification Service of the DSMZ and Dr B. J. Tindall (DSMZ). The fatty acid profiles for all three strains are shown in Table 2. FAME analysis showed that all three strains contained iso-C_{15:0} and iso-C_{11:0}, with iso-C_{15:0} as the predominant fatty acid. Strain OS1^T contained both iso-C_{13:0} and iso-C_{13:0} 3-OH. However, *A. thermoterrenum* DSM 13490^T did not have iso-C_{13:0} 3-OH and *A. mobile* DSM 13181^T did not have iso-C_{13:0}. Polar lipid analysis results are summarized in Table S1. Polar lipid analysis showed that all three strains contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, three unknown phospholipids (PL1–PL3) and four unknown aminophospholipids (PN1–PN4). Strain OS1^T and *A. thermoterrenum* DSM 13490^T were shown to contain phospholipids PL4 and PL5, but these were not detected in *A. mobile* DSM 13181^T.

Both *A. thermoterrenum* and *A. mobile* produced hydrogen, acetate and carbon dioxide when grown in pure culture on D-glucose. *A. mobile* was shown to grow syntrophically with a methanogen using D-glucose as the substrate (Menes & Muxí, 2002). Syntrophic studies were conducted using strain OS1^T and the hydrogen-oxidizing methanogen *M. thermautotrophicus* DSM 1053^T. The syntrophic medium was the mineral medium described above, but 3 g NaHCO₃ was added after adjustment to pH 7.5. All syntrophic and pure-culture fermentation studies were carried out in 160 ml crimp-sealed serum bottles with 10 ml medium at 55 °C with D-glucose (3 g l⁻¹) as the substrate. Acetate was measured with a Shimadzu GC-8A equipped with a flame-ionization detector and a 2 m glass column packed with an 80/120 Carbopak B-DA/4 % Carbowax resin 20M (Supelco) with helium as the carrier gas at a flow rate of 35 ml min⁻¹. Methane, carbon dioxide and hydrogen were measured with a Shimadzu GC-8A GC equipped with a thermal conductivity detector and a Porapak Q (2 m stainless steel) column

Table 2. Cellular fatty acid compositions of *A. hydrogeniformans* OS1^T, *A. thermoterrenum* DSM 13490^T and *A. mobile* DSM 13181^T

Strains: 1, *A. hydrogeniformans* sp. nov. OS1^T; 2, *A. thermoterrenum* DSM 13490^T; 3, *A. mobile* DSM 13181^T. Data were obtained in this study. Values are percentages of total identified fatty acids. ND, Not detected.

Fatty acid	1	2	3
iso-C _{11:0}	4.4	13.0	20.7
iso-C _{13:0}	1.8	21.0	ND
iso-C _{13:0} 3-OH	3.3	ND	8.5
iso-C _{15:0}	90.5	66.1	70.7

(Alltech) using nitrogen as the carrier gas at a flow rate of 30 ml min⁻¹. Results from the pure and co-culture work are presented in Table 3. Strain OS1^T, *A. thermoterrenum* DSM 13490^T and *A. mobile* DSM 13181^T all produced close to four hydrogen molecules per molecule of D-glucose and one molecule of methane per molecule of D-glucose when grown syntrophically with a hydrogen-oxidizing methanogen. In pure culture, strain OS1^T produced up to 3.1–4.5 mmol hydrogen (l culture)⁻¹ using clarified raw sewage as the substrate (Maune & Tanner, 2008).

On the basis of the genotypic and phenotypic data presented, strain OS1^T is proposed to represent a novel species of the genus *Anaerobaculum*, for which the name *Anaerobaculum hydrogeniformans* sp. nov. is proposed.

Emended description of the genus

Anaerobaculum Rees *et al.* 1997 emend. Menes and Muxí 2002

Chemo-organotrophic, Gram-negative anaerobes. Moderately thermophilic (28–65 °C) and halotolerant to halophilic (0–70 g NaCl l⁻¹). Straight to slightly curved rods. Motile by means of a single flagellum or non-motile. In complex media, some strains grow with a sheath-like material extending past the cell poles. Spores are not observed. Ferment organic acids and a limited number of carbohydrates to acetate, hydrogen and carbon dioxide. Peptone and yeast extract are also fermented. Some amino acids (e.g. L-asparagine, L-cysteine, L-histidine, L-serine, L-threonine or L-valine) can be used as substrates. Utilize a range of electron acceptors: thiosulfate, sulfur and L-cystine are reduced to sulfide, and crotonate is reduced to butyrate. Cellular fatty acid compositions of members of the genus include iso-C_{11:0} and iso-C_{15:0}, and may or may not contain iso-C_{13:0} and/or iso-C_{13:0} 3-OH. The polar lipid composition contains diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, three unknown phospholipids (PL1–PL3) and four unknown

aminophospholipids (PN1–PN3) and may or may not contain phospholipids PL4 and PL5. The DNA G+C content is 44–51.5 mol%. The type species is *Anaerobaculum thermoterrenum*.

Description of *Anaerobaculum hydrogeniformans* sp. nov.

Anaerobaculum hydrogeniformans (hy.dro.ge.ni.for'mans. N.L. n. *hydrogenum* hydrogen; L. part. adj. *formans* forming; N.L. part. adj. *hydrogeniformans* producing hydrogen).

Cells are 1.7–2.7 × 0.4–0.5 µm. Growth occurs at 40–65 °C (optimum 55 °C), pH 6–9 (optimum pH 7.0) and 0.8–7 % NaCl (optimum 1 %). The DNA G+C content of the type strain is 46.6 mol%. The cellular fatty acid composition includes iso-C_{15:0}, iso-C_{11:0}, iso-C_{13:0} 3-OH and iso-C_{13:0}. The polar lipids detected are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, unknown phospholipids PL1–PL5 and unknown aminophospholipids PN1–PN4. Sulfate, sulfite or nitrate are not reduced. Crotonate is reduced to butyrate when glucose is present. Growth occurs on D-fructose, D-glucose, maltose, D-mannose, α-ketoglutarate, L-glutamate, malonate, pyruvate, L-tartrate, L-asparagine, Casamino acids, L-cysteine, L-histidine, L-leucine, L-phenylalanine, L-serine, L-threonine, L-valine, inositol, inulin, tryptone and yeast extract. D-Arabinose, CM-cellulose, cellulose, cellobiose, D-galactose, lactose, melibiose, raffinose, D-rhamnose, sucrose, D-xylose, acetate, butyrate, citrate, fumarate, D-gluconate, lactate, L-malate, oleate, succinate, L-arginine, L-aspartate, L-glutamine, glycine, L-isoleucine, L-lysine, L-methionine, L-proline, L-tryptophan, L-tyrosine, adonitol, glycerol, dextrin, gelatin, pectin, starch and xylan do not support growth.

The type strain is OS1^T (=DSM 22491^T =ATCC BAA-1850^T), isolated from oil production water collected from Alaska, USA.

Table 3. Acetate, hydrogen and methane produced by *A. hydrogeniformans* OS1^T, *A. thermoterrenum* DSM 13490^T and *A. mobile* DSM 13181^T

Strains were grown with D-glucose in pure culture and in co-culture with *M. thermotrophicus* DSM 1053^T. Data for *A. mobile* DSM 13181^T were taken from Menes & Muxí (2002); other data were obtained in this study.

Strain	Glucose consumed (mmol l ⁻¹)	Fermentation products (mmol l ⁻¹)		
		H ₂	Acetate	Methane
Pure culture				
OS1 ^T	14.1	47.2	19.7	–
<i>A. thermoterrenum</i> DSM 13490 ^T	7.9	36.4	13.8	–
<i>A. mobile</i> DSM 13181 ^T	7.5	29.8	15.1	–
Co-culture				
OS1 ^T	8.4	–	17.6	8.1
<i>A. thermoterrenum</i> DSM 13490 ^T	8.3	–	16.3	8.7
<i>A. mobile</i> DSM 13181 ^T	8.5	–	16.9	8.5

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