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Anaerobaculum mobile sp. nov., a novel anaerobic, moderately thermophilic, peptide-fermenting bacterium that uses crotonate as an electron acceptor, and emended description of the genus *Anaerobaculum*

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A novel anaerobic, moderately thermophilic, peptide-fermenting bacterium, strain NGA^T, was isolated from an anaerobic wool-scouring wastewater treatment lagoon. The cells were Gram-negative, straight rods of 0·5–1·0 × 2·0–4·0 μm, motile by means of a single flagellum. The DNA G+C content was 51·5 mol%. The optimum pH and temperature range for growth were 6·6–7·3 and 55–60 °C, respectively. The optimum NaCl concentration was 0·08 g l^{-1}. The bacterium fermented organic acids (malate, tartrate, pyruvate, glycerol and fumarate), a few carbohydrates (starch, glucose, fructose and gluconate), Casamino acids, tryptone and yeast extract. Carbohydrates and organic acids were converted to acetate, hydrogen and CO_2. The bacterium oxidized leucine to isovalerate with crotonate as an electron acceptor, but not in co-culture with *Methanothermobacter thermoautotrophicus* DSM 3720^T. Thiosulfate, sulfur and cystine were reduced to sulfide and crotonate was reduced to butyrate with glucose and tryptone-yeast extract as electron donors. Phylogenetic analysis of the 16S rRNA gene indicated that strain NGA^T was related to *Anaerobaculum thermoterrenum* (98% similarity), the only described species of the genus. The DNA–DNA hybridization value for strain NGA^T and *A. thermoterrenum* ACM 5076^T was 40·8%. On the basis of these results, strain NGA^T is proposed as a novel species of the genus *Anaerobaculum*, namely *Anaerobaculum mobile* sp. nov. The type strain is NGA^T (= DSM 13181^T = ATCC BAA-54^T).

Keywords: anaerobic degradation, crotonate, electron acceptor, thermophile, *Anaerobaculum mobile* sp. nov.

INTRODUCTION

Anaerobic biological treatment is a frequently applied technology for decontamination of agroindustrial wastewaters by conversion of organic compounds to methane and carbon dioxide. The application of this treatment under thermophilic conditions has increased in recent years. However, when proteins are a major constituent of such wastes, their degradation is often incomplete (McInerney, 1988; Hansen *et al*., 1998) and the same situation arises when lipid-rich wastes are treated (Angelidaki *et al*., 1990). Isolation and physiological characterization of novel strains will help to explain their role in carbon flow in those anaerobic ecosystems.

In anoxic environments, the oxidation of certain amino acids such as alanine, valine, leucine and isoleucine, proceeds in association with hydrogen-scavenging organisms, due to the unfavourable $\Delta G°$ of the initial deamination step, which leads to the corresponding keto acid (Stams, 1994). However, acetate (Örlygsson *et al*., 1996), thiosulfate (Fardeau *et al*., 1997; Faudon *et al*., 1995; Mechichi *et al*., 2000) and crotonate (Girbal *et al*., 1997) have also been reported to act as

The EMBL accession number for the 16S rDNA sequence of strain NGA^T is AJ243189.

electron acceptors for amino acid utilization in anaerobiosis.

In this report, a novel thermophilic, peptide-fermenting bacterium within the genus *Anaerobaculum*, strain NGA^T, is described. Moreover, evidence of the use of crotonate as an electron acceptor by both *Anaerobaculum thermoterrenum* (Rees *et al*., 1997), the only currently described species within the genus, and strain NGA^T is presented.

METHODS

Strains. *Methanothermobacter thermoautotrophicus* DSM 3720^T was obtained from Deutsche Sammlung von Mikroorganismen and Zellkulturen, Braunschweig, Germany (DSMZ) and *A. thermoterrenum* ACM 5076^T was from the Australian Collection of Microorganisms, Brisbane, Australia.

Media and culture conditions. Enrichment and cultivation were performed in BC medium supplemented with yeast extract (0·2 g l^{-1}; Difco). The basal medium (BC) contained (l^{-1}): 0·3 g KH_2PO_4, 0·6 g NaCl, 0·1 g $MgCl_2 . 6H_2O$, 0·08 g $CaCl_2 . 2H_2O$, 2 mg resazurin, 1·0 g NH_4Cl, 3·6 g $KHCO_3$, 11·92 g HEPES and 10 ml trace element solution (Touzel & Albagnac, 1983). Media were prepared anaerobically under O_2-free N_2, the gas phase was replaced with N_2/CO_2 (70:30, v/v), adjusted to pH 7·0 and autoclaved for 15 min at 121 °C. Prior to inoculation, 1 ml vitamin solution (Touzel & Albagnac, 1983), 20 ml filter-sterilized sulfide-cysteine solution [1·25% (w/v) $Na_2S . 9H_2O$, 1·25% (w/v) cysteine.HCl] and the substrate were added.

Physiological characterization. Substrate studies were performed in duplicate in Hungate tubes with 5 ml medium (or in 125 ml vials with 20 ml medium). Substrates were added from anaerobic filter-sterilized stock solutions. Insoluble substrates were added to each tube or vial before media were dispensed. Soluble substrates were tested at concentrations of 5–10 mM and insoluble substrates at 2–5 g l^{-1}. The test was considered positive for substrate utilization when an increase in OD was detected and confirmed by substrate end product quantification. For insoluble substrates, the test was considered positive when an increase in end product formation was detected with respect to controls tubes with no added substrate. To study substrate degradation in co-culture with a methanogen, *M. thermoautotrophicus* DSM 3720^T was pre-grown in BC under 140 kPa H_2/CO_2 (80:20, v/v). After growth, the gas phase was replaced with N_2/CO_2 (70:30, v/v), substrate was added from stock solutions and vials were inoculated with strain NGA^T. Electron acceptor utilization, pH, temperature and NaCl growth range determinations were carried out in triplicate in Hungate tubes containing 10 ml modified PY broth – a basal medium containing peptone (5 g l^{-1}; Difco), tryptone (5 g l^{-1}; Difco) and yeast extract (10 g l^{-1}; Difco) (Smibert & Krieg, 1994), without sodium bicarbonate and prepared under an N_2 atmosphere. For electron acceptor utilization (at a final concentration of 10 mM, except for sulfite which was 2 mM), PY was reduced with 1·25% (w/v) cysteine.HCl solution (20 ml per l medium). For pH experiments, the following buffers were added from stock solutions to a final concentration of 20 mM: acetate buffer for pH 4·0 and 4·8; phosphate buffer for pH 5·8, 6·2, 6·8, 7·2 and 7·8; and Tris buffer for pH 8·0 and 8·8 (Smibert & Krieg, 1994). Solid media were prepared by the addition of agar (2%, w/v). All incubations were performed at 55 °C unless stated otherwise. All liquid media were inoculated (1%) with late exponential phase cultures in PY broth.

Enrichment and isolation. Sludge samples from an anaerobic wool-scouring wastewater treatment lagoon in Trinidad (Uruguay) were enriched for anaerobic, thermophilic, oleate-degrading bacteria. A crotonate-degrading enrichment was then obtained from the oleate-degrading consortium in BCYT (basal BC medium with tryptone and yeast extract, each at 0·5 g l^{-1}), supplemented with crotonate (15 mM; Sigma) as previously described (Menes *et al*., 2001).

After three subcultures, a stable enrichment culture was obtained which did not produce methane. This crotonate enrichment was used to inoculate tubes of BCYT agar supplemented with crotonate (15 mM) and incubated for 1 month. Culture purification was achieved by the agar-shake-dilution tube process. Purity was checked by phase-contrast microscopy (Axioplan; Zeiss).

Analytical techniques. Fermentation products were measured in centrifuged (6000 ***g***, 10 min) samples of the culture media. Values were corrected by subtracting the amount produced in control cultures without substrate. Volatile fatty acids and crotonate were measured by GC or HPLC. The GC (SRI) was equipped with a column packed with 10% SP-1000/1% H_3PO_4 on 100/120 Chromosorb W AW (Supelco) connected to an FID. N_2 was the carrier gas at a flow rate of 24 ml min^{-1} and the oven temperature was 150 °C. The HPLC (Waters) was equipped with an organic acid column (Chrompack) connected to UV and refractive index detectors (Waters, Millipore). The mobile phase was 0·005 M H_2SO_4 at a flow rate of 0·8 ml min^{-1} and the column temperature was 35 °C. Sulfide was quantified by the methylene blue method (Rand *et al*., 1975). Methane and hydrogen were quantified using a GC (SRI) equipped with a thermal conductivity detector and a molecular sieve 13X (80–100 mesh; Chrompack) packed column. Argon was used as the carrier gas at a flow rate of 20 ml min^{-1}. The detector temperature was 100 °C and the column temperature was 35 °C. Growth was measured at 660 nm by inserting Hungate tubes into the holder of a Genesys 5 (Spectronic; Milton Roy) spectrophotometer.

Electron microscopy. For electron microscopic studies, the culture was centrifuged at 3000 ***g*** for 6 min at 4 °C, the supernatant was discarded and the pellet was fixed in 2·5% glutaraldehyde and 2% paraformaldehyde in 0·1 M phosphate buffer (pH 7·4) for 2 h. Samples were then post-fixed in 1·0% osmium tetroxide, dehydrated in an ascending gradient of ethanol (50, 70, 80, 90 and 95%) and then impregnated in propylene oxide. Finally, they were embedded in Poly/Bed 812 resin (Polysciences 18976-2590). Ultrathin sections were cut with an ultratome Super Nova (Reichter-Jung), stained with uranyl acetate and lead citrate, and observed under a JEM-1200 EX II (JEOL). The negative staining photomicrographs were taken at the DSMZ.

DNA extraction and phylogenetic analysis. Samples (10 ml) from cells grown in PY were concentrated by centrifugation (6000 ***g***, 15 min), washed with NaCl solution (0·9 g l^{-1} in water) and resuspended in 500 µl lysozyme solution (10 mg ml^{-1} in 120 mM sodium phosphate buffer, pH 8·0). After incubation for 15 min at 37 °C, SDS was added (final concentration of 1%, w/v) and three cycles of freezing at −70 °C for 1 h and thawing at 37 °C for 45 min were applied to complete the lysis. Protein extraction and nucleic acid precipitation were performed according to Van Elsas & Smalla (1995). Amplification and 16S rDNA sequence

analysis were performed as reported by Fernández *et al.* (1999), except for the sequence data alignment, which was achieved using the CLUSTAL W package (Thompson *et al.*, 1994) and corrected by manual inspection. Sequencing of the purified PCR product was done at the University of Florida DNA Sequencing Core Laboratory, using ABI Prism Big Dye Terminator cycle sequencing protocols developed by Applied Biosystems (Perkin-Elmer). The fluorescently labelled extension products were analysed on an Applied Biosystems model 373 Stretch DNA sequencer or 377 DNA Sequencer (Perkin-Elmer). Oligo primers were designed using OLIGO 4.0 (National BioSciences) and synthesized by Gemini Biotech. Nucleotide sequences were aligned and assembled using programs in the SEQUENCER 3.0 software package (GeneCodes).

The new sequence was compared with other released sequences in the GenBank database using the BLAST program (Altschul *et al.*, 1997). The phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987) with the PHYLIP 3.5c package using the Kimura distance model (Felsenstein, 1993). Bootstrap resampling analysis for 100 replicates was performed to estimate the confidence of the tree topology. Only unambiguously aligned positions were used for phylogenetic analysis (1229 positions, positions 101–1413 by *Escherichia coli* numbering).

DNA base composition and DNA–DNA hybridization. The G+C content of the DNA was determined at the DSMZ by HPLC as described by Mesbah *et al.* (1989). Non-methylated λ DNA (Sigma) was used as the standard. Hybridization of strain NGA^T and *A. thermoterrenum* ACM 5076^T was carried out at the DSMZ.

RESULTS AND DISCUSSION

Morphological and physiological characteristics

In a previous work, a stable thermophilic, crotonate-degrading enrichment was obtained for the isolation of long-chain fatty acid-degrading bacteria (Menes *et al.*, 2001). A 10^{-7} dilution of this crotonate enrichment was used to inoculate tubes of BCYT agar supplemented with crotonate and, after 1 week incubation, two types of colonies developed. Small colonies (0·5 mm) were light brown and consisted of non-motile rods that did not degrade crotonate. Large colonies (1·0–1·5 mm) were white, lens shaped, with entire margins and consisted of large (0·5 × 2·0–4·0 μm) motile rods. Five of these colonies were purified in BCYT-crotonate agar and a representative culture, designated NGA^T, was selected for further characterization. Cells of NGA^T were variable in size with longer cells (4–8 μm) being observed in older cultures. They stained Gram-negative and ultrathin sections

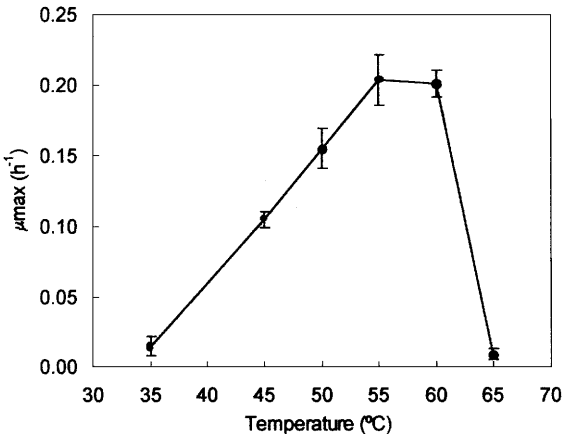

Fig. 2. Growth rate of strain NGA^T as a function of temperature. μ_{max}, Maximum growth rate. The experiment was performed in triplicate in PY broth. Bars, SD of three datasets for each temperature.

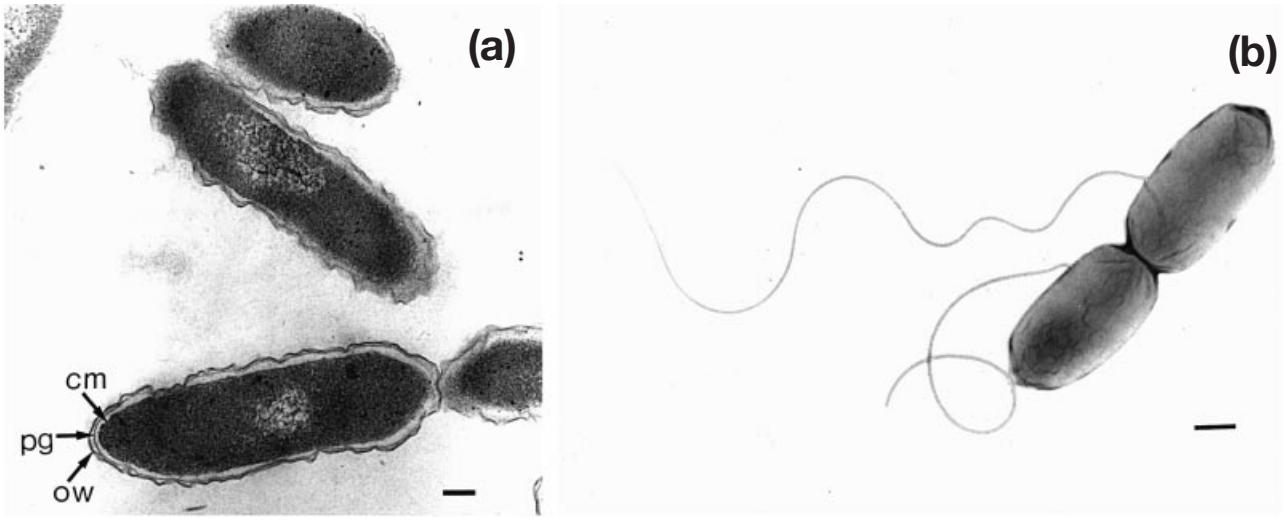

Fig. 1. Transmission electron micrographs of cells of strain NGA^T. (a) Ultrathin section showing typical Gram-negative cell wall structure (bar, 0·2 μm; ow, outer cell wall; pg, peptidoglycan layer; cm, cytoplasmic membrane). (b) Negatively stained preparation showing a dividing cell with single laterally inserted flagella (bar, 0·5 μm).

Table 1. Substrates degraded by strain NGA^T in pure culture and in co-culture with *M. thermoautotrophicus* DSM 3720^T

Carbon balances were calculated assuming the production of 2 mol CO_2 per mol glucose, tartrate and malate, and 1 mol CO_2 per mol pyruvate and glycerol degraded. Experiments were performed in BC medium supplemented with yeast extract (0·2 g l^{-1}) and incubated for 30 d at 55 °C, except for fumarate (90 d). The concentration of fermentation products was corrected by subtracting the values obtained in control vials without substrate. No degradation was observed in uninoculated controls. ND, Not detected.

Substrate	Substrate consumed (mM)	Fermentation products (mmol l^{-1}):			Electron balance (%)	Carbon balance (%)
		Acetate	Hydrogen	Methane		
In pure culture:						
Glucose (8·5 mM), R = 1·8*	1·6	3·5	6·7		108	109
Glucose (8·5 mM), R = 27*	7·5	15·1	29·8		100	100
Pyruvate (10 mM)	4·3	3·5	4·1		84	81
Glycerol (11·4 mM)	3·0	3·1	6·4		90	103
Tartrate (4·2 mM)	4·2	3·9	3·8		92	93
Malate (9·0 mM)	2·1	1·8	4·6		94	86
In co-culture:						
Glucose (8·5 mM)	8·5	16·9	ND	8·5	100	100
Fumarate (6·0 mM)	4·3	5·0	ND	2·4	112	113
Malate (9·0 mM)	9·0	8·6	ND	4·6	98	96

* R, Ratio of headspace volume to culture medium volume.

revealed a typical Gram-negative cell wall structure (Fig. 1a). They were motile and a single flagellum, inserted in the lateral region of the cell body, was observed in negatively stained electron micrographs (Fig. 1b). During the exponential growth phase, most of the cells grew in pairs. Spores or sheath formation were never observed under our culture conditions. Strain NGA^T was a strict anaerobe, as shown by its inability to grow in PY under aerobic conditions. Growth occurred at NaCl concentrations of up to 15 g l^{-1}, with an optimum in PY broth with no added NaCl (0·08 g l^{-1}). The optimum temperature for growth was 55–60 °C; no growth was observed above 65 °C (Fig. 2). The optimum pH for growth was 6·6–7·3 and growth occurred between pH 5·8 and 8·8. Strain NGA^T fermented glucose, organic acids (pyruvate, tartrate and malate) and glycerol to acetate, hydrogen and, presumably, CO_2 (Table 1). Glucose and malate degradation were enhanced in co-culture with *M. thermoautotrophicus*. Furthermore, the absence of reduction products other than hydrogen and the enhancement of glucose utilization by an increase in the headspace to medium volume ratio (Table 1), suggest hydrogen inhibition of glucose utilization. Fumarate fermentation was not observed in pure culture or in co-culture with *M. thermoautotrophicus* in 30 d. However, after 90 d incubation, a very slow fermentation was detected by acetate and methane production (Table 1). The bacterium did not ferment crotonate, but could reduce it to butyrate in the presence of yeast extract and tryptone, glucose, Casamino acids and leucine (Table 2). The observed stoichiometric oxidation of leucine to isovalerate coupled to crotonate reduction to butyrate (Table 2) indicates that crotonate was used as electron acceptor, as has been reported for *Clostridium acetireducens*. However, the latter was also capable of fermenting it in the absence of electron donors (Girbal *et al.*, 1997). To our knowledge, this is the first reported bacterium that uses crotonate exclusively as an electron acceptor. Strain NGA^T, like *C. acetireducens* (Girbal *et al.*, 1997), could not perform leucine oxidation by interspecies hydrogen transfer. Crotonate, sulfur, cystine and thiosulfate addition also enhanced growth from tryptone and yeast extract (PY medium), Casamino acids and glucose, as indicated by an increase in OD and by production of reduction compounds (butyrate and sulfide, respectively) (Table 2). The ability to reduce thiosulfate, sulfur and cystine may represent a means of eliminating inhibition by hydrogen, as has been suggested for thiosulfate reduction by *Thermoanaerobacter* spp. (Faudon *et al.*, 1995), members of the *Thermotogales* (Jeanthon *et al.*, 1995), *Coprothermobacter platensis* (Etchebehere & Muxí, 2000) and *A. thermoterrenum* (Rees *et al.*, 1997).

Fumarate, acetate, sulfate, sulfite and nitrate were not used as electron acceptors in PY broth. Other substrates tested but not utilized by strain NGA^T were: sorbitol, melibiose, adonitol, cellobiose, arabinose, dextrin, polygalacturonate, xylan and inulin. Crotonate, butyrate, oleate and benzoate were not utilized in co-culture with *M. thermoautotrophicus* DSM 3720^T.

Table 2. Electron acceptors used by strain NGA^T and crotonate reduction by *A. thermoterrenum* strain ACM 5076^T

Experiments were performed in 10 ml PY broth or BC medium supplemented with yeast extract (0·2 g l^{-1}) in 15 ml tubes and incubated for 30 d at 55 °C. The concentration of fermentation products in BC was corrected by subtracting the values obtained in control tubes supplemented with electron acceptor but without substrate. ND, Not detected.

Medium	Crotonate consumed (mM)	OD_{660}	Products (mM):		
			Acetate	Butyrate	Sulfide
Strain NGA^T					
PY medium					
No acceptor		0·149	17·0	ND	1·0
Crotonate (26 mM)	26·0	0·577	22·0	26·9	1·1
Thiosulfate (20 mM)		0·853	–	–	16·0
Sulfur (4 g l^{-1})		0·401	–	–	9·8
Cystine (2 g l^{-1})		0·515	–	–	13·0
BC + glucose (8·5 mM)					
No acceptor		0·051	1·8	ND	0·7
Crotonate (23 mM)	6·8	0·149	4·6	6·2	–
Thiosulfate (20 mM)		0·096	3·6	ND	2·5
BC + Casamino acids (1 g l^{-1})					
No acceptor		0·040	0·8	ND	–
Crotonate (13 mM)	5·9	0·113	2·2	5·4	–
BC + leucine* (6 mM)					
No acceptor		0·020	0·3	ND	–
Crotonate (26 mM)	13·5	0·128	0·3	12·8	–
Strain ACM 5076^T					
PY medium					
No acceptor		0·060	14·5	ND	0·5
Crotonate (25 mM)	5·7	0·070	17·6	3·9	–
Thiosulfate (20 mM)		0·610	ND	ND	14·0
Sulfur (4 g l^{-1})		0·167	ND	ND	10·0

* Only isovalerate (5·6 mM) was detected in the culture with crotonate.

Phylogenetic analysis

The 16S rDNA sequence (1367 bp) of strain NGA^T corresponded to positions 32–1413 of the 16S rDNA of *E. coli*. This sequence was compared with all sequences currently available in the GenBank database. Phylogenetic analysis (Fig. 3) revealed that the closest relatives of strain NGA^T were *A. thermoterrenum* (Rees *et al*., 1997), with a sequence similarity of 98·0 %, and *Aminobacterium colombiense* (Baena *et al*., 1998), with a sequence similarity of 84·4 %. Clearly, strain NGA^T clustered with *A. thermoterrenum*, a result supported by the high bootstrap value (100 % of 100 replicates). Due to the high level of similarity (98·0 %) with the only described species of the genus *Anaerobaculum*, DNA–DNA hybridization was performed and a value of 40·8 % was obtained. At present, the phylogenetic definition of a species generally includes strains with 70 % or greater DNA–DNA relatedness (Wayne *et al*., 1987). From the value of 40·8 % obtained, it is therefore concluded that strain NGA^T belongs to a new species within the genus *Anaerobaculum*. Furthermore, the difference in DNA G + C content between strain NGA^T (51·5 mol %) and *A. thermoterrenum* (44 mol %; Rees *et al*., 1997) is larger than 3 %, the maximum range generally accepted for members of a well-defined species (Stackebrandt & Liesack, 1993).

Several phenotypic properties support this conclusion. Both strains are able to utilize tryptone, Casamino acids, yeast extract, starch, malate, tartrate, pyruvate, glycerol, glucose and fructose, and unable to grow on lactose, xylose, cellulose, CM-cellulose, gelatin, maltose, sucrose, galactose, rhamnose, raffinose, Gum Arabic, malonate, lactate or succinate. They share the ability to reduce thiosulfate, sulfur and cystine with tryptone and yeast extract as electron donors and, as shown in Table 2, *A. thermoterrenum* ACM 5076^T is also able to reduce crotonate to butyrate in PY medium supplemented with crotonate. However, the extent and rate of crotonate reduction and the increase in OD are lower than those observed for strain NGA^T. Experiments performed with different amounts of crotonate always showed that NGA^T was a more efficient crotonate reducer than ACM 5076^T, even using media

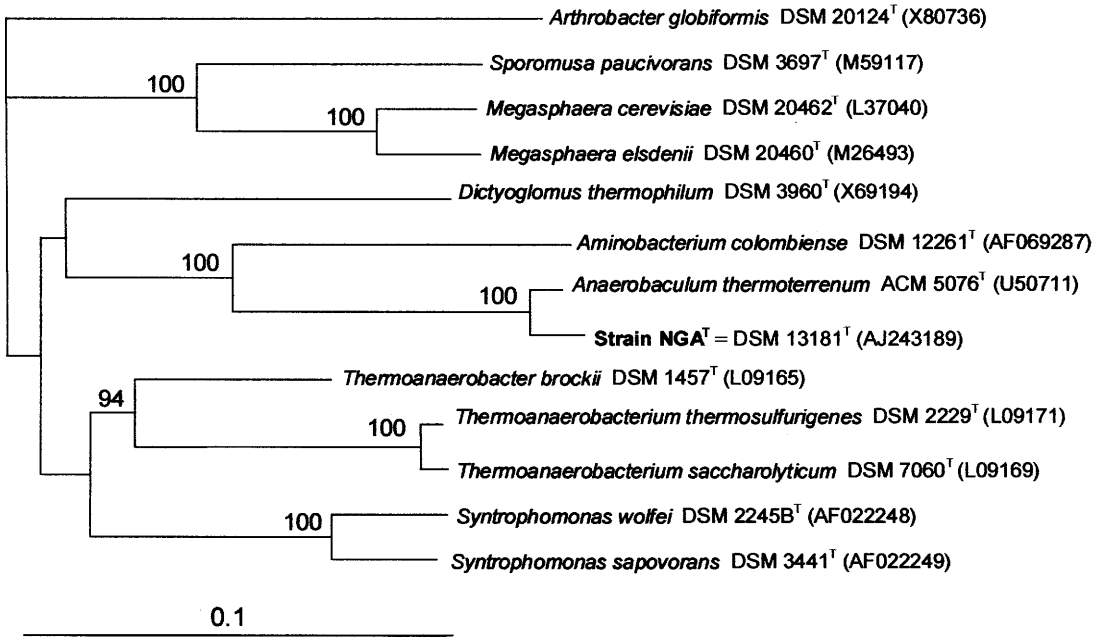

Fig. 3. Phylogenetic tree derived from the analysis of the 16S rDNA sequences of strain NGA^T and other related species. Bar, 10 nt substitutions per 100 nt. Bootstrap values of 100 resamplings are shown for the closest relatives.

Table 3. Phenotypic differential characteristics of strain NGA^T and *A. thermoterrenum* ACM 5076^T

Characteristic	Strain NGA^T	*A. thermoterrenum**
Size (μm)	0·5–1·0 × 2·0–4·0	0·75 × 2·00
Morphology	Straight rods	Straight or slightly curved rods
Motility	+	−
Flagellum	+	−
Optimum NaCl concn for growth (g l^{-1})	0·08	10
DNA G+C content (mol%)	51·5	44
Sheath formation	−	+
Utilization of:		
Fumarate	−	+
Citrate	−	+
2-Oxoglutarate	−	+
Glutamate	−	+
Mannose	−	+
Pectin	−	+
Gluconate	+	NR

NR, Not reported.

* Data from Rees *et al.* (1997).

supplemented with NaCl (10 g l^{-1}) for the latter (data not shown).

Strain NGA^T differs from *A. thermoterrenum* in its motility (presence of a single flagellum), its inability to ferment citrate, 2-oxoglutarate, glutamate, mannose and pectin, and in optimum NaCl concentration (Table 3). Citrate fermentation was reported as a distinctive trait for *A. thermoterrenum* (Rees *et al.*, 1997), but this ability could not be shown for strain NGA^T even using different culture media: BC medium, Brackish bicarbonate-buffered, sulfide-reduced medium (Rees *et al.*, 1997) or in co-culture with *M. thermoautotrophicus* after 90 d incubation. The habitats from which both organisms were isolated were also different: *A. thermoterrenum* was isolated

from the production water of a petroleum reservoir, with high salinity, which may explain its high optimum NaCl concentration (Rees *et al.*, 1997), whereas NGA^T was enriched and isolated from an anaerobic lagoon treating wool-scouring effluent.

The phenotypic characteristics and the results of 16S rDNA sequence analysis, DNA G+C content and DNA–DNA hybridization confirm that strain NGA^T represents a novel species within the genus *Anaerobaculum*, for which the name *Anaerobaculum mobile* sp. nov. is proposed.

Emended description of the genus *Anaerobaculum* (Rees *et al.* 1997)

Chemo-organotrophic anaerobe. Moderately thermophilic. 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. No spores observed. Gram-negative. Ferments organic acids and a limited number of carbohydrates to acetate, hydrogen and CO_2. Peptone and yeast extract also fermented. Utilizes a range of electron acceptors: thiosulfate, sulfur and cystine are reduced to sulfide and crotonate is reduced to butyrate. The DNA G+C content is 44·0–51·5 mol%. The type species is *Anaerobaculum thermoterrenum*.

Description of *Anaerobaculum mobile* sp. nov.

Anaerobaculum mobile (mo′bi.le. L. masc. adj. *mobile* motile).

Straight rods, 0·5–1·0 × 2·0–4·0 μm, motile by means of a single flagellum inserted in the lateral region of the cell body, occur singly or in pairs. Gram-negative. Strictly anaerobic and chemo-organotrophic. No spores observed. Moderately thermophilic. Growth occurs at 35–65 °C, with an optimum between 55 and 60 °C, and at pH 5·4–8·7, with an optimum between 6·6 and 7·3. The optimum NaCl concentration is 0·08 g l^{-1} (in media with no added NaCl), but growth occurs up to 15 g NaCl l^{-1}. Ferments malate, tartrate, pyruvate, glycerol, starch, glucose, fructose, gluconate, Casamino acids, tryptone and yeast extract. Carbohydrates and organic acids are converted to acetate, hydrogen and CO_2. Oxidizes leucine to isovalerate with crotonate as electron acceptor, but not in co-culture with a methanogenic partner. Thiosulfate, sulfur and cystine are reduced to sulfide. Crotonate is reduced to butyrate. Sulfate, sulfite, fumarate, acetate and nitrate are not reduced. No growth occurs on citrate, 2-oxoglutarate, glutamate, mannose, pectin, lactose, xylose, galactose, maltose, sucrose, rhamnose, raffinose, malonate, lactate, succinate, xylan, dextrin, inulin, melibiose, adonitol, cellobiose, arabinose, polygalacturonate, cellulose, gelatin, butyrate or oleate. The DNA G+C content is 51·5 mol%. The type strain is NGA^T (= DSM 13181^T = ATCC BAA-54^T). Isolated from the sludge of an anaerobic lagoon treating wool-scouring wastewater from Uruguay.

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