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Anaerobium acetethylicum gen. nov., sp. nov., a strictly anaerobic, gluconate-fermenting bacterium isolated from a methanogenic bioreactor

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A novel strictly anaerobic, mesophilic bacterium was enriched and isolated with gluconate as sole substrate from a methanogenic sludge collected from a biogas reactor. Cells of strain GluBS11^T stained Gram-positive and were non-motile, straight rods, measuring 3.0–4.5 × 0.8–1.2 μm. The temperature range for growth was 15–37 °C, with optimal growth at 30 °C, the pH range was 6.5–8.5, with optimal growth at pH 7, and the generation time under optimal conditions was 60 min. API Rapid 32A reactions were positive for α-galactosidase, α-glucosidase and β-glucosidase and negative for catalase and oxidase. A broad variety of substrates was utilized, including gluconate, glucose, fructose, maltose, sucrose, lactose, galactose, melezitose, melibiose, mannitol, erythritol, glycerol and aesculin. Products of gluconate fermentation were ethanol, acetate, formate, H_2 and CO_2. Neither sulfate nor nitrate served as an electron acceptor. Predominant cellular fatty acids (>10 %) were $C_{14:0}$, $C_{16:0}$, $C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH and $C_{18:1}\omega7c$. The DNA G+C content of strain GluBS11^T was 44.1 mol%. Phylogenetic analysis based on 16S rRNA gene sequence data revealed that strain GluBS11^T is a member of subcluster XIVa within the order *Clostridiales*. The closest cultured relatives are *Clostridium herbivorans* (93.1 % similarity to the type strain), *Clostridium populeti* (93.3 %), *Eubacterium uniforme* (92.4 %) and *Clostridium polysaccharolyticum* (91.5 %). Based on this 16S rRNA gene sequence divergence (>6.5 %) as well as on chemotaxonomic and phenotypic differences from these taxa, strain GluBS11^T is considered to represent a novel genus and species, for which the name *Anaerobium acetethylicum* gen. nov., sp. nov. is proposed. The type strain of *Anaerobium acetethylicum* is GluBS11^T (=LMG 28619^T=KCTC 15450^T=DSM 29698^T).

Gluconic acid was discovered in 1870 by Hlasiwetz and Habermann (Röhr *et al.*, 1983). It is found naturally in fruit, honey, rice, meat and other foods (Ramachandran *et al.*, 2006) and is, by two electrons, more oxidized than glucose (Crueger & Crueger, 1990). It is used as a food and drink additive to act as an acidity regulator, in sterilization solutions or bleaching in food manufacturing, and as a salt in pharmaceutical products.

The genus *Clostridium* is one of the largest genera known among prokaryotes and comprises anaerobic, Gram-positive-staining, endospore-forming bacteria. In past decades, numerous species of the genus *Clostridium* capable of fermenting gluconate have been studied, e.g. *Clostridium aceticum*, *C. pasteurianum*, *C. roseum*, *C. butyricum*, '*C. rubrum*' (C. beijerinckii ATCC 14949) and '*C. butylicum*' (C. beijerinckii ATCC 14823) (Andreesen & Gottschalk, 1969; Bender *et al.*, 1971). Members of the genus *Clostridium* have been isolated from soil, sediment, decomposing biological material and the lower gut of mammals (Suresh *et al.*, 2007). Recently, Servinsky *et al.* (2014) studied a complete metabolic network in *Clostridium acetobutylicum* for utilization of glucose, gluconate and galacturonate using online databases, previous studies, genomic information and experimental data. Besides members of the genus *Clostridium*, *Escherichia coli* (Eisenberg & Dobrogosz, 1967), lactic acid bacteria such as *Lactobacillus reuteri* and *L. mucosae* and acid-utilizing bacteria such as *Megasphaera elsdenii* and *Mitsuokella multacida* (Tsukahara

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GluBS11^T is KP233894.

A supplementary figure is available with the online Supplementary Material.

et al., 2002) have been found to grow with gluconate. In the present study, we report the isolation of a gluconate-fermenting, strictly anaerobic bacterium from a methanogenic sludge sample. To determine the taxonomic position of strain GluBS11^T, it was subjected to a detailed physiological, chemotaxonomic and phylogenetic characterization.

Strain GluBS11^T was isolated from a sludge sample obtained from a biogas reactor in Odendorf, Germany. The reactor converts corn waste to methane gas at 40 °C. An anaerobic slurry/sludge sample obtained from the reactor was diluted directly with anoxic medium and the enrichment culture was started by inoculating a few millilitres of approximately 10-fold-diluted sludge sample into a freshwater medium containing gluconate (10 mM) as a car bon source. Isolation, cultivation and growth experiments were performed in anoxic, bicarbonate-buffered, sulfide-reduced freshwater mineral medium containing (unless indicated otherwise, in $g\,l^{-1}$): NaCl, 1.0; $MgCl_2.6H_2O$, 0.4; KH_2PO_4, 0.2; NH_4Cl, 0.25; KCl, 0.5; $CaCl_2.2H_2O$, 0.15; $NaHCO_3$, 2.5; $Na_2S.9H_2O$, 1 mM (Widdel & Bak, 1992). The medium (excluding $Na_2S.9H_2O$ and $NaHCO_3$) was autoclaved at 121 °C for 25 min and cooled under an oxygen-free mixture of N_2/CO_2 (80 : 20). Resazurin (0.4 mg l^{-1}) was added as a redox indicator. Further, 1 ml trace element solution SL-10 (Widdel *et al.*, 1983), 1 ml selenate-tungstate (Tschech & Pfennig, 1984) and 1 ml seven-vitamin solution (Pfennig, 1978) were added from concentrated stock solutions. The medium was adjusted to an initial pH of 7.3 ± 0.1 with sterile 1 M NaOH or 1 M HCl. Cultivations and transfer of the strain were performed under a N_2/CO_2 (80 : 20) atmosphere. The strain was cultivated in the dark at 30 °C.

Pure cultures were obtained by repeated agar (1 %) shake dilutions (Widdel & Bak, 1992). Agar shake tubes were incubated in an inverted position for 1–2 weeks until isolated, visible colonies appeared (Junghare & Schink, 2015). The strain was routinely examined for purity by light microscopy (Axiophot Zeiss) and also checked after growth with 10 mM gluconate plus 3 % (w/v) yeast extract. For physiological tests and chemotaxonomic analysis, it was cultivated in mineral freshwater medium containing 10 mM gluconate. Pure cultures were transferred every 4–5 weeks and stored in liquid medium at 4 °C. Photographs were taken using the agar slide technique (Pfennig & Wagener, 1986). Gram-staining was performed using a staining kit (Difco Laboratories) according to the manufacturer's instructions and also confirmed by the KOH test (Gregersen, 1978). Spore formation was checked by growing the strain with 30 mM glucose with 1 mM $MnSO_4$, a heat sporulation test (pasteurization at 80 °C for 30 min) and alternatively by growing the strain at acid pH (< pH 6).

Growth curves were recorded to determine gluconate utilization, product formation and the generation time. Optimal conditions for growth were investigated in mineral medium as described above. The strain was incubated in Hungate tubes at 15, 25, 30, 37, 40, 45 and 50 °C and at pH 4–9 (in increments of 0.5 pH units) for growth optimization. Sterile solutions of citric acid, NaH_2PO_4 and Tris/HCl (pH 7.2) were added to a final concentration of 10 mM as buffering agents to maintain the medium in the desired pH range for pH optimization studies. Utilization of different carbon sources was tested in freshwater medium with each substrate being added to 10 mM unless otherwise indicated. Carbon sources tested included monosaccharides, disaccharides, polysaccharides and alcohols. Reduction of sodium nitrate (5 mM) and sodium sulfate (10 mM) was checked via nitrite or sulfide formation.

Various biochemical properties were determined by using the API Rapid 32A strip by the Identification Service of the Deutsche Sammlung von Mikroorganismen and Zellkulturen (IS-DSMZ) (Braunschweig, Germany). Analysis of the DNA G+C content (Stackebrandt *et al.*, 2002) was performed by the HPLC method with DNA isolated from 1–2 *g* wet cell biomass (Cashion *et al.*, 1977; Mesbah *et al.*, 1989; Tamaoka & Komagata, 1984) by the IS-DSMZ. Fatty acid methyl esters were analysed from about 40 mg freeze-dried cells (harvested in late stationary phase) grown with 10 mM gluconate in the freshwater medium described above at 30 °C. Fatty acid methyl ester mixtures were separated using the Sherlock Microbial Identification System (MIS; MIDI, Microbial ID), which consisted of an Agilent model 6890N gas chromatograph fitted with a 5 % phenyl-methyl silicone capillary column (0.2 mm × 25 m), a flame-ionization detector, an Agilent model 7683A automatic sampler and an HP computer with MIDI database (Hewlett Packard). Peaks were automatically integrated and fatty acid names and percentages calculated by the MIS Standard Software (Microbial ID) (Kämpfer & Kroppenstedt, 1996; Kuykendall *et al.*, 1988; Miller, 1982) by the IS-DSMZ.

DNA was extracted with a DNA extraction kit (catalogue no. 19060; Qiagen) according to the manufacturer's instructions. PCR amplification of the almost-complete 16S rRNA gene was performed using bacterial universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). The 50 µl reaction mixture consisted of 2 µl (approx. 20 ng) template DNA, 2 µl each primer (20 pmol), 3 µl $MgCl_2$ (25 mM), 5 µl 10× PCR buffer, 5 µl dNTPs (500 µM), 0.25 µl *Taq* polymerase (5 U μl^{-1}) and 30.75 µl molecular-grade PCR water. PCR was performed with an initial denaturation at 96 °C for 2 min followed by 32 cycles of 94 °C for 40 s, 54 °C for 40 s and 72 °C for 60 s, and final extension at 72 °C for 5 min. The amplified 16S rRNA gene fragment of approximately 1.4 kb was purified using a DNA purification kit (DNA clean and concentrator; Zymo Research) and sequenced at GATC Biotech AG (Konstanz, Germany).

The 16S rRNA gene sequences of closely related cultured micro-organisms were obtained using the EzTaxon-e service (Kim *et al.*, 2012), BLAST searches against the non-redundant GenBank database (Altschul *et al.*, 1990) and

the Sequence Match tool of the Ribosomal Database Project (RDP) (Cole *et al.*, 2014). The taxonomic assignment of strain GluBS11^T was performed within the ARB program package (Ludwig *et al.*, 2004). Phylogenetic trees were reconstructed based on 1242 unambiguously aligned sequence positions using the RAxML maximum-likelihood method (Stamatakis *et al.*, 2008) as implemented in ARB. Furthermore, the results of maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) methods also supported the phylogenetic placement of strain GluBS11^T within subcluster XIVa of the order *Clostridiales* (data not shown). Confidence in the resulting tree topology was evaluated by resampling 1000 bootstrap trees using the RAxML algorithm (Stamatakis *et al.*, 2008).

Growth was monitored by measuring the OD_{600} using a Jenway 6300 spectrophotometer. Utilization of each substrate and fermentation products were analysed and quantified with an HPLC system fitted with an RID detector (LC-prominence; Shimadzu) equipped with an Aminex HPX-87H ion-exchange column (Bio-Rad) and analysed at 60 °C, using 5 mM H_2SO_4 as the mobile phase at a flow rate of 0.6 ml min^{-1}. Analysis of traces of hydrogen gas was performed with a high-sensitivity Peak Performer 1 gas chromatograph (Peak Laboratories) equipped with a reducing compound photometer (RCP). Nitrogen was used as the carrier gas. Qualitative determination of nitrate and nitrite was performed by using colour-developing commercial strips (Qantofix; Macherey-Nagel). Sulfide was determined according to the method described by Cord-Ruwisch (1985). All tests were conducted independently in duplicate.

After 1 week of incubation in agar shake tubes at 30 °C, colonies were opaque, convex and circular with an entire margin and appeared yellow–white in the centre as the colony matured (Fig. S1, available in the online Supplementary Material). The strain did not grow aerobically on LB agar plates, and anoxic, reduced conditions were required for growth. Cells grown with 10 mM gluconate were 3.0–4.5 µm long (sometimes even 10 µm long) and 0.8–1.2 µm wide, non-motile, and occurred in chains, pairs or as single cells (Fig. 1). Spore formation was never observed, not even if cultures were grown with 1 mM manganese sulfate and 30 mM glucose for 2 weeks (further incubation led to cell lysis and death) or at < pH 6. Cells stained Gram-positive, as determined both by the Gram-staining reaction and by the KOH test. The strain reacted negative in tests for catalase and oxidase.

Strain GluBS11^T grew at 15–37 °C, with an optimum at 30 °C, and at pH 6.5–8.5, with an optimum at pH 7.2±0.2. When grown on gluconate (10 mM), the doubling time was approximately 1 h at 30 °C (Fig. 2). Glucose, lactose, sucrose, fructose, maltose, xylose, galactose, melibiose, melezitose, gluconate, mannitol, erythritol, glycerol and aesculin were used as carbon sources. Starch, cellulose, cellobiose, pectin and glycogen were not utilized. No growth was observed with ribose, arabinose, rhamnose, raffinose, trehalose, inositol, gelatin,

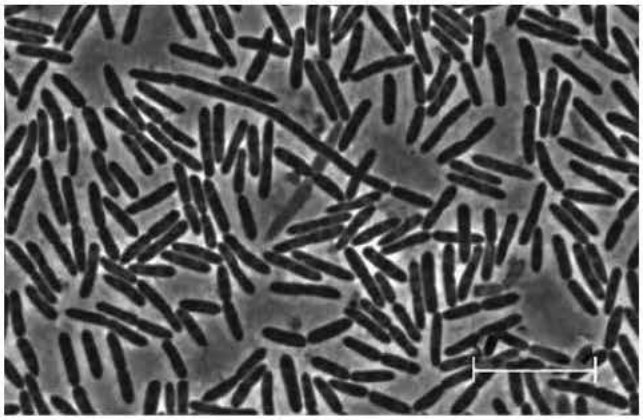

Fig. 1. Phase-contrast micrograph of cells of strain GluBS11^T grown with gluconate. Bar, 10 µm.

casein, Casamino acids, gum arabic, pyruvate or lactate. Sulfate and nitrate did not serve as electron acceptors. Strain GluBS11^T produced acetate (8.4 mM), formate (4.6 mM), ethanol (4.9 mM) and H_2 (2.1 mM) from the fermentation of gluconate (8.4 mM), as shown in Table 1 (lactate was occasionally detected in the range 0.5–1 mM). No butyrate, propionate, butanol, propanol or methane was formed. Cellular fatty acids included $C_{12:0}$ (0.68 %), $C_{13:0}$ 3-OH/iso-$C_{15:1}$ (4.88 %), $C_{13:1}$ at 12–13 (1.11 %), $C_{14:0}$ (27.74 %), $C_{15:0}$ (0.36 %), $C_{16:0}$ (21.74 %), $C_{16:1}\omega9c$ (4.85 %), $C_{16:1}\omega5c$ (0.89 %), $C_{16:1}\omega7c$ alcohol (0.50 %), $C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH (16.71 %), $C_{17:1}\omega8c$ (1.12 %), iso-$C_{17:1}$ I/anteiso-$C_{17:1}$ B (2.81 %), $C_{18:0}$ (0.55 %), $C_{18:1}\omega7c$ (11.24 %) and $C_{18:1}\omega9c$ (2.86 %). The DNA G+C content of the strain was 44.1 mol%.

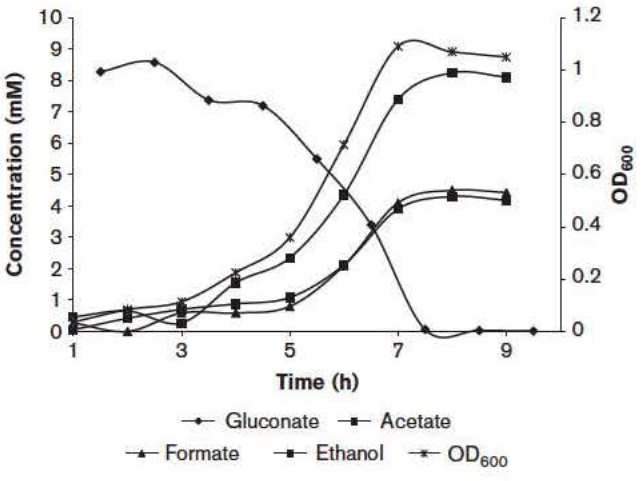

Fig. 2. Growth curve, gluconate consumption and fermentation product formation with time for strain GluBS11^T.

Table 1. Stoichiometry of substrate fermentation and product formation by strain GluBS11^T at 30 °C after ~8 h of incubation

Maximum OD_{600} was recorded at ~8 h of growth. Cell dry mass was calculated from the OD_{600} as 1 OD_{600}=250 mg cell dry mass l^{-1}. Assimilation equation for gluconate: 17 $C_6H_{12}O_7 \rightarrow 22 <C_4H_7O_3> + 11\ H_2O + 14\ HCO_3^- + 14\ H^+$. Assimilation equation for glucose: 17 $C_6H_{12}O_6 \rightarrow 24 <C_4H_7O_3> + 6\ HCO_3^- + 12\ H_2O + 6\ H^+$.

Substrate utilized (mM)	Max. OD_{600}	Cell dry mass (mg)	Substrate assimilated (mM)	Substrate dissimilated (mM)	Fermentation products (mM)				Electron recovery (%)
					Acetate	Formate	Ethanol	H_2	
Gluconate									
8.42 (agitated)	1.091	272.7	2.04	6.37	8.40	4.64	4.90	2.10	99.4
7.03 (static)	0.958	239.5	1.79	5.24	6.99	3.33	3.14	2.37	97.0
Glucose									
3.71 (static)	0.958	239.5	1.79	1.92	2.87	2.80	2.87	1.84	97.8

Phylogenetic analysis of the almost-complete 16S rRNA gene sequence of strain GluBS11^T (1402 bp) using the maximum-likelihood method RAxML (Stamatakis *et al.*, 2008) revealed that it is a member of cluster XIVa within the order *Clostridiales* (Fig. 3). This was corroborated independently by using the neighbour-joining and maximum-parsimony algorithms as provided in ARB as well as by the EzTaxon server (Kim *et al.*, 2012) and RDP (Wang *et al.*, 2007) classifiers. On the basis of 16S rRNA gene sequence similarity, the type strains of *Clostridium populeti* (93.3 %), *Clostridium herbivorans* (93.1 %), *Eubacterium uniforme* (92.4 %) and *Clostridium polysaccharolyticum* (91.5 %) are most closely related to strain GluBS11^T. All of them belong to cluster XIVa within the order *Clostridiales* as defined by Collins *et al.* (1994), and our isolate represents a distinct phyletic line within this cluster. Strain GluBS11^T was not associated with the type species of the genus *Clostridium*, *Clostridium butyricum* (cluster I). The terminal branching between strain GluBS11^T and its closest relatives (as stated above) was not well resolved, as revealed by low bootstrap support (Fig. 3) and alternative phylogenetic tree reconstruction using the neighbour-joining and maximum-parsimony algorithms as implemented in ARB (Ludwig *et al.*, 2004) (data not shown). This indicates that the resolution of the 16S rRNA gene as a phylogenetic marker is not high enough to distinguish speciation events reliably within clostridial cluster XIVa (Warnick *et al.*, 2002). Clostridial cluster XIVa (Collins *et al.*, 1994) contains a diverse assortment of organisms, including the genera *Acetitomaculum*, *Anaerostipes*, *Bryantella*, *Butyrivibrio*, *Catonella*, *Coprococcus*, *Dorea*, *Hespellia*, *Johnsonella*, *Lachnospira*, *Lachnobacterium*, *Moryella*, *Oribacterium*, *Parasporobacterium*, *Pseudobutyrivibrio*, *Roseburia*, *Shuttleworthia*, *Sporobacterium* and *Syntrophococcus* in addition to a few misclassified clostridial species (Cotta *et al.*, 2009). Strain GluBS11^T forms a distinct lineage within this cluster, specifically within the family *Lachnospiraceae*, and does not display a particularly close affiliation to any of the aforementioned taxa (Fig. 3).

It is currently accepted that 5.5 % 16S rRNA gene sequence divergence is necessary for separation of novel genera from existing ones (Yarza *et al.*, 2014). Our pairwise comparison shows >6.5 % sequence divergence between strain GluBS11^T and the most closely related type strains. On the basis of this high 16S rRNA gene sequence divergence and the topologies of the phylogenetic trees, we propose strain GluBS11^T to represent a novel genus within the family *Lachnospiraceae*.

Differential characteristics of strain GluBS11^T from the most closely related type strains in clostridial subcluster XIVa (Collins *et al.*, 1994) are summarized in Table 2. Strain GluBS11^T may be distinguished from three of its closest cellulolytic relatives, *C. herbivorans* (Varel *et al.*, 1995), *C. polysaccharolyticum* (van Gylswyk, 1980; van Gylswyk *et al.*, 1980) and *C. populeti* (Sleat & Mah, 1985), by its inability to utilize cellulose and cellobiose and its fermentation end product pattern (no butyrate produced). *C. herbivorans*, *C. polysaccharolyticum* and *C. populeti* produce butyrate as a fermentation major product, whereas strain GluBS11^T produces formate, acetate and ethanol. Strain GluBS11^T can be differentiated phenotypically from the type strains of these three species by its lack of motility (absence of flagella) and its inability to form spores. Strain GluBS11^T is also distantly related to *E. uniforme* (van Gylswyk & van der Toorn, 1985), but can be readily distinguished from it by the fact that *E. uniforme* has a much lower DNA G+C content (35 mol%), hydrolyses starch and cellobiose and produces lactate as a major fermentation product in addition to formate, acetate and ethanol (Table 2). According to our 16S rRNA gene phylogenetic analyses, strain GluBS11^T is more closely related to *C. herbivorans* and *C. populeti* (as many members of the *Lachnospiraceae* are polysaccharolytic and typically produce butyrate as one of the fermentation end products) but, in terms of fermentation pattern, strain GluBS11^T appears to be more similar to *E. uniforme* (van Gylswyk & van der Toorn, 1985), as neither taxon produces butyrate at all. The fatty acid patterns of *C. herbivorans* and strain GluBS11^T show remarkable differences, in particular the presence of a higher percentage of $C_{16:0}$ (21.7 %) fatty acid in strain GluBS11^T, which was present at only 5.3 % in cells of *C. herbivorans* (Varel *et al.*, 1995). Later, $C_{14:0}$

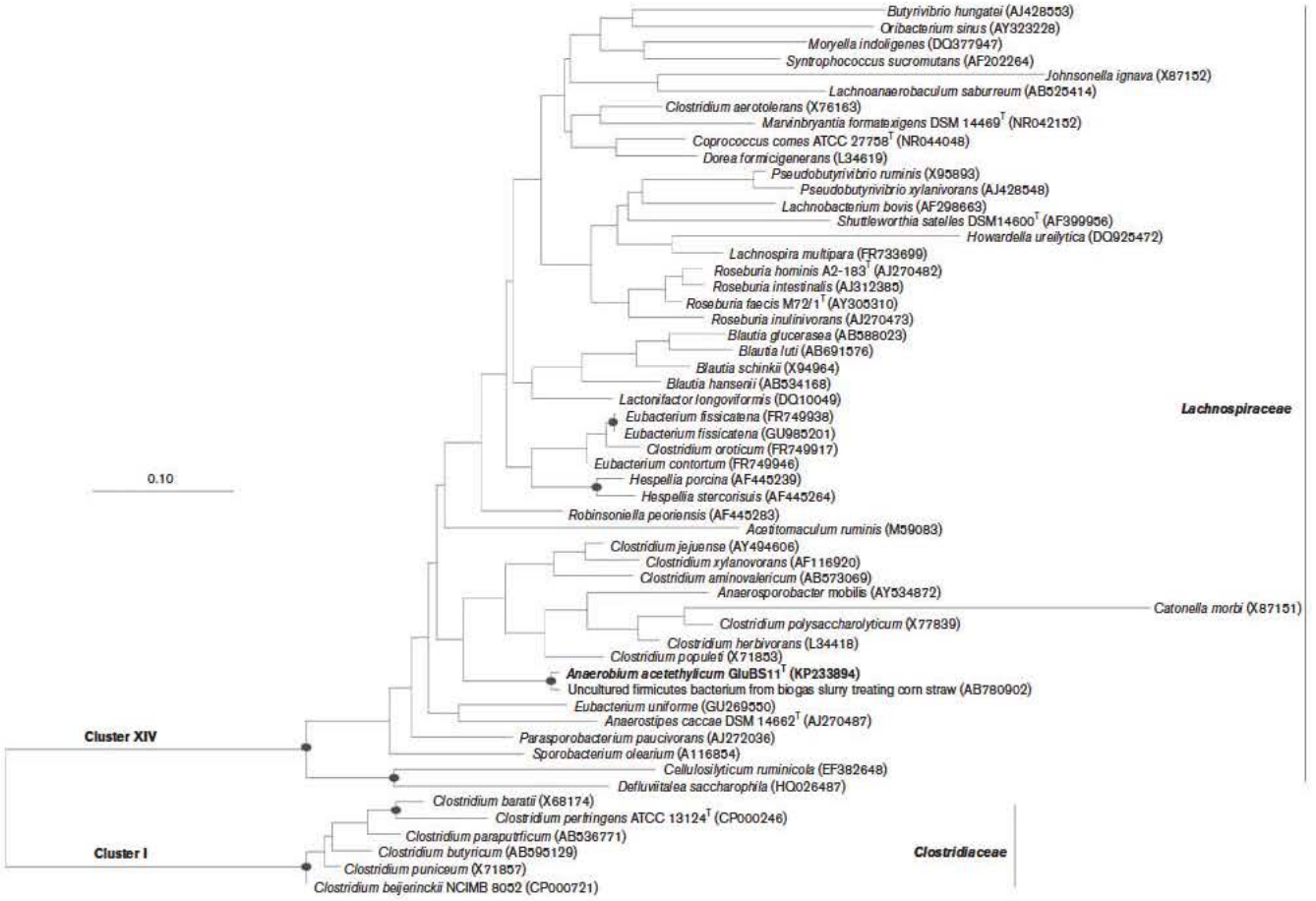

Fig. 3. Maximum-likelihood tree showing the phylogenetic placement of the 16S rRNA gene sequences of strain GluBS11^T and members of the family *Lachnospiraceae* generated using the RAxML algorithm (Stamatakis *et al.*, 2008). Filled circles indicate that the corresponding nodes have more than 90% bootstrap support. Where strain names are not given, sequences were obtained from the type strain. Accession numbers are given in parentheses. Bar, 10% estimated sequence divergence.

aldehyde (10.2 %) and $C_{14:0}$ dimethyl acetal (22.1 %) (Varel *et al.*, 1995) fatty acids were present in cells of *C. herbivorans* in considerable amounts, but these were completely absent from strain GluBS11^T. Besides this, *C. pasteurianum* and *C. butyricum* ferment glycerol to butanol and 1,3-propanediol as main fermentation products, whereas strain GluBS11^T ferments glycerol to acetate, formate, ethanol and hydrogen (no butanol formation was detected). Rarely, a very small amount of 1,3-propanediol was detected if the fermentation time was extended for several days.

In conclusion, the novel strain GluBS11^T described in our study shows significant differences in phenotype, phylogeny and chemotaxonomic characteristics from previously described closely related type strains within clostridial subcluster XIVa and in particular within the family *Lachnospiraceae* (Collins *et al.*, 1994). Therefore, we propose that strain GluBS11^T represents a novel genus within the family *Lachnospiraceae* in the order *Clostridiales*, for which the name *Anaerobium acetethylicum* gen. nov., sp. nov. is proposed.

Description of *Anaerobium* gen. nov.

Anaerobium (An.a.e.ro'bi.um. Gr. pref. *an* indicating a negation; Gr. n. *aer* air; Gr. n. *bios* life; N.L. neut. n. *Anaerobium* an anaerobic life form).

Cells are strictly anaerobic, long rods, found singly or in pairs. Non-spore-forming. Growth is observed with gluconate and glucose in addition to a wide range of carbohydrates. The major end products of fermentation include formate, acetate and ethanol. The whole-cell fatty acids consist mainly of a mixture of straight-chain saturated and monounsaturated acids. The DNA G+C content of the type strain of the type species is 44.1mol%. Comparative analysis of the 16S rRNA gene sequence shows that the genus *Anaerobium* represents a separate lineage

Table 2. Physiological and chemotaxonomic characteristics that differentiate strain GluBS11^T from its nearest phylogenetic relatives

Strains/species: 1, GluBS11^T (this study); 2, *C. herbivorans* (data from Varel *et al.*, 1995); 3, *C. populeti* (Sleat & Mah, 1985); 4, *C. polysaccharolyticum* (van Gylswyk, 1980; van Gylswyk et al., 1980); 5, *E. uniforme* (van Gylswyk & van der Toorn, 1985). ND, No data available/not determined.

Characteristic	1	2	3	4	5
Isolation source*	Biogas slurry	Intestinal tract of pig	Woody biomass digester	Sheep rumen	Sheep rumen
Cell morphology	Straight rods	Straight rods	Slightly curved rods	Rod shaped	Short rods
Motility	Non motile	Motile	Motile	Motile	Non motile
Gram stain type	Positive	Positive	Negative	Positive	Positive
Optimum growth temperature (°C)	30	39 42	36	30 38	38 45
Fermentation products†	F, A, E	F, B, e	B, a, l	F, B, e	F, A, L, E
Sporulation	No	Yes	Yes	Yes	No
DNA G+C content (mol%)	44.1	38*	28*	41.6*	35.2±2
16S rRNA gene sequence similarity to strain GluBS11^T (%)*	(100)	93.1	93.3	91.5	92.4
Substrate utilization					
Glucose	+		+		ND
Fructose	+		+	ND	+
Maltose	+	+	+	ND	ND
Sucrose	+		+		ND
Lactose	+				ND
Xylose	+		+	ND	+
Galactose	+		+		+
Ribose			ND	ND	ND
Starch		+		+	+
Gluconate	+	ND	ND	ND	ND
Arabinose			+	ND	+
Rhamnose					ND
Raffinose			ND		ND
Trehalose					ND
Cellulose		+	+	+	ND
Cellobiose		+	+	+	+
Mannitol	+		ND		ND
Erythritol	+	ND		ND	ND
Glycerol	+				ND
Inositol			ND		ND
Pectin			+	ND	ND
Pyruvate				ND	ND
Lactate					ND
Xylan	ND		+	+	+

*Data for reference taxa are from the type strains.

†A/a, Acetate; B/b, butyrate; E/e, ethanol; F/f, formate; L/l, lactate. Upper case letters indicate major end products and lower case letters indicate minor end products.

within the family *Lachnospiraceae*. The type species of the genus is *Anaerobium acetethylicum*.

Description of *Anaerobium acetethylicum* sp. nov.

Anaerobium acetethylicum (a.ce.te.thy′li.cum. L. n. *acetum* vinegar; N.L. root *ethyl-* referring to the ethyl moiety, e.g. ethyl alcohol, ethanol; N.L. neut. adj. *acetethylicum* referring to its main fermentation products, acetate and ethanol).

Displays the following properties in addition to those given for the genus. Cells form opaque, circular, convex colonies with entire margins (Fig. S1) when grown in agar-shake tubes. Cells stain Gram-positive. They measure 3.0–4.5 × 0.8–1.2 μm (sometimes cells even 10 μm long are observed; Fig. 1). Cells are non-motile. With 10 mM gluconate at 30 °C, the doubling time is about 1 h (Fig. 2). Growth occurs at 15–37 °C, with an optimum at 30 °C; no growth at 40 or 45 °C. The pH range for growth (at 30 °C) is pH 6.5–8.5, with an optimum at pH 7.0. Positive

API Rapid 32A reactions are obtained for α-galactosidase, α-glucosidase and β-glucosidase, and weakly positive reactions are observed for β-galactosidase, α-arabinosidase and β-glucuronidase. Negative API Rapid 32A reactions for urease, arginine dihydrolase, β-galactosidase-6-phosphate, *N*-acetyl-β-glucosaminidase, mannose fermentation, raffinose fermentation, glutamic acid decarboxylase, α-fucosidase, nitrate reduction, indole production, alkaline phosphatase, arginine arylamidase, proline arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, leucine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase, glutamyl glutamic acid arylamidase and serine arylamidase. Grows with glucose, lactose, sucrose, fructose, maltose, xylose, galactose, melibiose, melezitose, gluconate, mannitol, erythritol, glycerol and aesculin. No growth with starch, cellulose, cellobiose, pectin, ribose, arabinose, rhamnose, raffinose, trehalose, inositol, gelatin, casein, Casamino acids, gum arabic, glycogen, pyruvate or lactate. Sulfate and nitrate are not reduced. Products of gluconate fermentation are acetate, formate, ethanol and H_2. Major cellular fatty acids (>10 %) are $C_{14:0}$, $C_{16:0}$, $C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH and $C_{18:1}\omega7c$.

The type strain is GluBS11^T (=LMG 28619^T=KCTC 15450^T=DSM 29698^T), which was isolated from a methanogenic biogas reactor fed with corn silage.

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