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 GluBS11T stained Gram-positive and were non-motile, straight rods, measuring 3.0–4.5 x 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, H₂ and CO₂. Neither sulfate nor nitrate served as an electron acceptor. Predominant cellular fatty acids (>10 %) were C₁₄ : ₀, C₁₆ : ₀, C₁₆ : ₁ω7c/iso-C₁₅ : ₀ 2-OH and C₁₈ : ₁ω7c. The DNA G+C content of strain GluBS11T was 44.1 mol%. Phylogenetic analysis based on 16S rRNA gene sequence data revealed that strain GluBS11T 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 GluBS11T 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 GluBS11T (=LMG 28619T =KCTC 15450T =DSM 29698T).

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. pasteuriunum, C. roseum, C. butyricum, ‘C. rubrum’ (C. beijerinckii ATCC 14949) and ‘C. butylicum’ (C. beijerinckii ATCC 14823) (Andreasen & 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/DDJB accession number for the 16S rRNA gene sequence of strain GluBS11T 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 metha-
nogenic sludge sample. To determine the taxonomic
position of strain GluBS11T, it was subjected to a
detailed physiological, chemotaxonomic and phylogenetic
characterization.

Strain GluBS11T 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 reac-
tor was diluted directly with anoxic medium and the
enrichment culture was started by inoculating a few milli-
litres 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; MgCl2·6H2O, 0.4;
KH2PO4, 0.2; KH2PO4, 0.2; NH4Cl, 0.25; KCl, 0.5; CaCl2·2H2O, 0.15;
NaHCO3, 2.5; Na2S·9H2O, 1 mM (Widdel & Bak, 1992).
The medium (excluding Na2S·9H2O and NaHCO3) was
autoclaved at 121 °C for 25 min and cooled under an
oxygen-free mixture of N2/CO2 (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 sele-
nate-tungstate (Tschech & Pfennig, 1984) and 1 ml seven-
vitamin solution (Pfennig, 1978) were added from concen-
trated stock solutions. The medium was adjusted to an initial
pH of 7.3 ± 0.1 with sterile 1 M NaOH or 1 M HCl. Cultiva-
tions and transfer of the strain were performed under a N2/
CO2 (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,
2002). Isolates were obtained from a biogas reactor in Odendorf,
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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. Photo-
graphs were taken using the agar slide technique (Pfennig
& Wagener, 1986). Gram-staining was performed using a
staining kit (Difco Laboratories) according to the manufac-
turer’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 MnSO4, 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. Opti-
mal 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 optimi-
zation. Sterile solutions of citric acid, Na2HPO4 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. Utiliza-
tion 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 alco-
hols. Reduction of sodium nitrate (5 mM) and sodium sul-
fate (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). Analy-
sis 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 auto-
matically integrated and fatty acid names and percentages
were 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 instruc-
tions. PCR amplification of the almost-complete 16S rRNA
genome 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 MgCl2 (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 frag-
ment of approximately 1.4 kb was purified using a DNA puri-
fication 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 ser-
vice (Kim et al., 2012), BLAST searches against the non-
redundant GenBank database (Altschul et al., 1990) and

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the Sequence_Match tool of the Ribosomal Database Project (RDP) (Cole et al., 2014). The taxonomic assignment of strain GluBS11T 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 GluBS11T 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 OD600 using a Jenway 6300 spectrophotometer. Utilization of each substrate and fermentation products were analysed and quantified with an HPLC system fitted with a RID detector (LC-prominence; Shimadzu) equipped with an Aminex HPX-87H ion-exchange column (Bio-Rad) and analysed at 60 °C, using 5 mM H2SO4 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 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 GluBS11T 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 ascuslin 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, casein, Casamino acids, gum arabic, pyruvate or lactate. Sulfate and nitrate did not serve as electron acceptors. Strain GluBS11T produced acetate (8.4 mM), formate (4.6 mM), ethanol (4.9 mM) and H2 (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 C12 : 0 (0.68 %), C13 : 0 3-OH/iso-C15 : 1 (4.88 %), C13 : 1 at 12–13 (1.11 %), C14 : 0 (27.74 %), C15 : 0 (0.36 %), C16 : 0 (21.74 %), C16 : 0 9c (4.85 %), C16 : 0 9c 5c (0.89 %), C16 : 1 ω7c alcohol (0.50 %), C16 : 1 ω7c iso-C15 : 0 2-OH (16.71 %), C17 : 1ω8c (1.12 %), iso-C17 : 1ω ante iso-C17 : 1 B (2.81 %), C18 : 0 (0.55 %), C18 : 1ω7c (11.24 %) and C18 : 1ω9c (2.86 %). The DNA G+C content of the strain was 44.1 mol%.

**Fig. 1.** Phase-contrast micrograph of cells of strain GluBS11T grown with gluconate. Bar, 10 μm.

**Fig. 2.** Growth curve, gluconate consumption and fermentation product formation with time for strain GluBS11T.
Table 1. Stoichiometry of substrate fermentation and product formation by strain GluBS11<sup>T</sup> at 30 °C after ~8 h of incubation

Maximum OD<sub>600</sub> was recorded at ~8 h of growth. Cell dry mass was calculated from the OD<sub>600</sub> as 1 OD<sub>600</sub> = 250 mg cell dry mass l<sup>-1</sup>. Assimilation equation for gluconate: 17 C<sub>6</sub>H<sub>12</sub>O<sub>7</sub> → 22 C<sub>4</sub>H<sub>10</sub>O<sub>5</sub> + 11 H<sub>2</sub>O + 14 HCO<sub>3</sub> <sup>-</sup> + 14 H<sup>+</sup>. Assimilation equation for glucose: 17 C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> → 24 C<sub>2</sub>H<sub>4</sub>O<sub>4</sub> + 6 HCO<sub>3</sub> <sup>-</sup> + 12 H<sub>2</sub>O + 6 H<sup>+</sup>.

<table>
<thead>
<tr>
<th>Substrate utilized (mM)</th>
<th>Max. OD&lt;sub&gt;600&lt;/sub&gt;</th>
<th>Cell dry mass (mg)</th>
<th>Substrate assimilated (mM)</th>
<th>Substrate dissimilated (mM)</th>
<th>Fermentation products (mM)</th>
<th>Electron recovery (%)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Acetate</td>
<td>Formate</td>
</tr>
<tr>
<td>Gluconate 8.42 (agitated)</td>
<td>1.091</td>
<td>272.7</td>
<td>2.04</td>
<td>6.37</td>
<td>8.40</td>
<td>4.64</td>
</tr>
<tr>
<td>Gluconate 7.03 (static)</td>
<td>0.958</td>
<td>239.5</td>
<td>1.79</td>
<td>5.24</td>
<td>6.99</td>
<td>3.33</td>
</tr>
<tr>
<td>Glucose 3.71 (static)</td>
<td>0.958</td>
<td>239.5</td>
<td>1.79</td>
<td>1.92</td>
<td>2.87</td>
<td>2.80</td>
</tr>
</tbody>
</table>

Phylogenetic analysis of the almost-complete 16S rRNA gene sequence of strain GluBS11<sup>T</sup> (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 rDNA 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<sup>T</sup>. All of them belong to cluster XIVa within the order Clostridiales as defined by Collins et al. (1994), and our isolate represents a distinct phytic line within this cluster. Strain GluBS11<sup>T</sup> was not associated with the type species of the genus Clostridium, Clostridium butyricum (cluster I). The terminal branching between strain GluBS11<sup>T</sup> 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, Butyrivibrio, Catenola, Coprococcus, Dorea, Hespella, Johnsonella, Lachnospira, Lachnobotrium, Moryella, Oribacterium, Parasporobacterium, Pseudobutyrovibrio, Roseburia, Shuttleworthia, Sporobacterium and Syntrophococcus in addition to a few misclassified clostridial species (Cotta et al., 2009). Strain GluBS11<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup> to represent a novel genus within the family Lachnospiraceae.

Differential characteristics of strain GluBS11<sup>T</sup> from the most closely related type strains in clostridial subcluster XIVa (Collins et al., 1994) are summarized in Table 2. Strain GluBS11<sup>T</sup> 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<sup>T</sup> produces formate, acetate and ethanol. Strain GluBS11<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup> show remarkable differences, in particular the presence of a higher percentage of C<sub>16</sub>: 0 (21.7 %) fatty acid in strain GluBS11<sup>T</sup>, which was present at only 5.3 % in cells of C. herbivorans (Varel et al., 1995). Later, C<sub>14</sub>: 0
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\textsuperscript{T}. Besides this, C. pasteurianum and C. butyricum ferment glycerol to butanol and 1,3-propanediol as main fermentation products, whereas strain GluBS11\textsuperscript{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\textsuperscript{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\textsuperscript{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‘i.um. Gr. pref. an indicating a negation; Gr. n. aer air; Gr. n. bios life; N.L. neut. n. Anaer-obium an anaerobic life form).

Cells are strictly anaerobic, long rods, found singly or in pairs. Non-spore-forming. Growth is observed with glucose 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\textsubscript{+}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
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 (Fig. S1) when grown in agar-shake tubes. Cells stain Gram-positive. They measure 3.0–4.5 | 0.8–1.2 mm (sometimes cells even 10 mm 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, arabitol, 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$_{16:1}$ω7c/ω3c, C$_{15:0}$ 2-OH and C$_{18:1}$ω7c.

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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