Herbinix hemicellulosilytica gen. nov., sp. nov., a thermophilic cellulose-degrading bacterium isolated from a thermophilic biogas reactor

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Phenotypic and phylogenetic studies were performed on new isolates of a novel Gram-stain-positive, anaerobic, non-sporulating, rod-shaped bacterium isolated from a thermophilic biogas plant. The novel organisms were able to degrade crystalline cellulose. 16S rRNA gene comparative sequence analysis demonstrated that the isolates formed a hitherto unknown subline within the family Lachnospiraceae. As a representative of the whole group of isolates, strain T3/55 T was further characterized. The closest relative of T3/55 T among the taxa with validly published names is Mobilbacteria sibirica, sharing 93.9 % 16S rRNA gene sequence similarity. Strain T3/55 T was catalase-negative, indole-negative, and produced acetate, ethanol and propionic acid as major end products from cellulose metabolism. The major cellular fatty acids (%): 16 : 0 dimethyl acetal, 16 : 0 fatty acid methyl ester and 16 : 0 aldehyde. The DNA G + C content was 36.6 mol%. A novel genus and species, Herbinix hemicellulosilytica gen. nov., sp. nov., is proposed based on phylogenetic analysis and physiological properties of the novel isolate. Strain T3/55 T (DSM 29228T = CECT 8801T), represents the type strain of Herbinix hemicellulosilytica gen. nov., sp. nov.

The order Clostridiales has traditionally been described by its morphological characteristics, such as spore formation and anaerobic growth. The classification and naming of novel species on the basis of 16S rRNA gene sequences has led to severe discrepancies concerning the relationship between taxa within the order Clostridiales (Collins et al., 1994). The recent taxonomic update of the phylum Firmicutes in Bergey’s Manual of Systematic Bacteriology divided the order Clostridiales into 10 named families. Nine additional families were identified as incertae sedis in an effort to regroup species positioned outside the named families (Collins et al., 1994; Ludwig et al., 2009). In the past decades, studies of 16S rRNA gene sequence diversity in metagenomic approaches have led to the identification of thousands of novel taxa, while knowledge about the roles of novel species in their environments is still limited (DeLong, 2009). For various groups with confusing taxonomic structure, such as the class Clostridia, linking the phylogeny of novel, uncultured taxa to possible ecological roles and physiological characteristics requires extensive isolation of new strains and their characterization – these are time-consuming experiments (Biddle et al., 2013).

Members of the family Lachnospiraceae, within the order Clostridiales, are often identified in mammalian gastrointestinal tract microbiomes (Meehan & Beiko, 2014), particularly in ruminants (Kittelmann et al., 2013) and humans (Gosalbes et al., 2011). The family Lachnospiraceae was first proposed by Rainey (2009) and is described on the basis of phylogenetic analysis of 16S rRNA gene sequences (Euzéby, 2010; Ludwig et al., 2009). It currently comprises 25 genera (Parle, 2014). The family is morphologically diverse and includes rods, vibrios and cocci. All species are strictly anaerobic and primarily non-spor-forming. As shown in the ‘Road map’ of Bergey’s Manual of Systematic Bacteriology, a number of the type strains of species with validly published names of the genera Acetivibrio, Anaerospirobacter, Bacteroides, Blautia, Clostridium, Desulfotomaculum,
**Eubacterium**, *Fusobacterium*, *Howardella*, *Lactobacillus*, *Lactonifactor* and *Ruminococcus* — currently classified in families other than *Lachnospiraceae* — are phylogenetically intermingled with members of the latter family. Yutin & Galperin (2013) proposed a new genus ‘*Lachnoclostridium*’ for the respective species of the genus *Clostridium*.

In this study, we succeeded in isolating a novel cellulose-degrading strain from a thermophilic biogas plant. The results of phylogenetic analyses based on 16S rRNA gene sequences revealed that strain T3/55T and other closely related isolates were members of a novel genus within the family *Lachnospiraceae*. In this communication, the taxonomic characterization of this new isolate is described and a novel genus and species are proposed.

The strain T3/55T has been deposited in the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) and the Belgium Coordinated Collections of Microorganisms (BCCM/LMG, Gent, Belgium). The strains *Clostridium thermocellum* DSM 1237T (= ATCC 27405T) and *Clostridium stercorarium* DSM 8532T were used for reference. They were obtained from the DSMZ. GS2 medium (Johnson et al., 1981) was used in all experimental cultures with the following composition per litre: 6 g yeast extract, 2.9 g K$_2$HPO$_4$, 2.1 g urea, 1.5 g KH$_2$PO$_4$, 2.9 g trisodium citrate dihydrate, 1.0 g cysteine hydrochloride monohydrate, 10 g MOPS, 0.1 g MgCl$_2$, 6H$_2$O, 0.015 g CaCl$_2$, 2H$_2$O, 0.125 mg FeSO$_4$.7H$_2$O and 0.01 % resazurin. Media were prepared in butyl-rubber-stoppered serum bottles under anaerobic conditions and autoclaved.

New strains were isolated from a thermophilic biogas reactor in Viersen, Germany (GPS coordinates: 51° 13' 36.5" N 6° 19' 35.8" E). A total of 1 ml of the sludge was suspended in 50 ml NaCl solution (0.2 M) and serially diluted to 10$^{-8}$. Each dilution was freshly inoculated into autoclaved, anaerobic GS2 medium with 0.5 % (w/v) filter paper (Whatman No. 1) as the main carbon source and 0.5 % (v/v) rumen extract to enrich cellulolytic microbes. The culture was incubated at 55 °C until the filter paper showed obvious degradation. The highest dilution with filter paper degradation was diluted to 10$^{-8}$ again and transferred into the same medium. After four repetitions of dilution and growth in fresh medium, the resultant enriched culture was serially diluted up to 10$^{-4}$. An aliquot (0.1 ml) of the dilution was plated on a GS2 agar plate (2 % agar) containing 0.05 % (w/v) cellulose as the carbon source and overlaid with GS2 agar (2 %, w/v) containing 0.5 % (w/v) PASC (phosphoric acid swollen cellulose) (Wood, 1988) in an anaerobic chamber (95 % N$_2$ + 5 % H$_2$; Coy Laboratory Products). The agar plates were incubated anaerobically at 55 °C. Colonies producing clear haloes due to cellulolytic activity were streaked to single colonies three times. Single colonies were picked and inoculated into GS2 medium with filter paper to confirm cellulose degradation and purity.

Genomic DNA was extracted from the culture broth using a bacterial genomic DNA kit (Molzym). PCR amplification and sequencing of the 16S rRNA genes were carried out as described previously (Koeck et al., 2014). The resulting nucleotide sequences were inserted into a 16S rRNA ARB (Ludwig et al., 2004) seed database containing an alignment of a non-redundant dataset comprising about 40 000 complete high-quality primary structures extracted from the SILVA 119 SSU Ref NR release (Quast et al., 2013). All available 16S rRNA gene sequences representing type strains of species with validly published names were selected according to the Living Tree Project (LTP) (Munoz et al., 2011) and List of Prokaryotic Names with Standing in Nomenclature (LPSN; Parte, 2014). Quality-checking and filtering were performed by applying the quality, chimera and operational taxonomic unit (OTU) tools of the ARB software package (Westram et al., 2011); these were also performed manually. A subset of about 3000 sequences comprising all sequences representing members of the family *Lachnospiraceae* in the seed database as well as primary structures from selected representatives of other major groups of the phylum *Firmicutes* and the other bacterial and archaeal phyla was used for tree reconstruction. A selection of different conservation profile-based (alignment) column filters, alternative models (of evolution) in combination with distance matrix-, maximum-parsimony-, and maximum-likelihood-based treeing tools (Ludwig et al., 2004; Stamatakis, 2006) available within the ARB package were used for tree generation. Evaluation of significance and support of tree topologies, as well as tree presentation, was performed by applying the consensus tree tool of the ARB package. Positioning of branches representing lower quality type strain sequences of relevance for the family *Lachnospiraceae* in evaluated and optimized trees was performed by applying the ARB parsimony tool with the option of maintaining the initial tree topology.

Cells of strain T3/55T were inspected microscopically with an Axioskop optical microscope (Zeiss). Gram staining was performed according to published procedures (Johnson et al., 1995). The motility of strain T3/55T was observed by light microscopy. Endospore formation was assessed using light microscopy and recultivability after pasteurization (15 min, 80 °C). Colony morphology was observed using cells grown on GS2 agar plates for 4 days at 55 °C. For high-resolution field emission scanning electron microscopy, preparations of freshly harvested cells were fixed with 2.5 % glutaraldehyde in 75 mM cacodylate buffer containing 25 mM NaCl and 2 mM MgCl$_2$ (pH 7.0). Drops of the fixed samples were placed onto carbon-coated glass slides, covered with a coverslip, and rapidly frozen with liquid nitrogen. The coverslip was removed with a razor blade, and the glass slide was immediately post-fixed with 2.5 % (w/v) glutardialdehyde in fixative buffer, washed, post-fixed with 1 % osmium tetroxide, washed with buffer and then with distilled water, dehydrated in a graded series of acetone solutions, and then critical-point dried after transfer to liquid CO$_2$. Specimens were mounted on stubs, coated with 3 nm...
platinum using a magnetron sputter coater, and then examined with a Zeiss Auriga scanning electron microscope operated at 1 kV (Wanner et al., 1989).

The presence of catalase was determined by dropping 10 % (v/v) H$_2$O$_2$ on top of bacterial cells on a microscope slide. Biochemical tests were performed using the EnteroPluri-Test (Liofilchem) in accordance with the manufacturer’s instructions. Casein hydrolysis was tested with GS2 agar plates containing 0.25 % casein. Optimal growth temperature and pH were determined by measuring the cell protein yield of growing cultures in GS2 medium containing 0.2 % (w/v) Avicel after incubation for 6 days (Reveneau et al., 2003). The cell protein yield was determined after recovering bacterial cells by centrifugation, washing the pellets twice with isotonic NaCl, and boiling in 1 % (w/v) CHAPS detergent containing 0.3 M NaCl for 20 min. Total protein was measured using a Bradford Protein Assay kit (Thermo Scientific) with BSA as standard. All data points represent the analysis of at least three replicates.

Tolerance of salinity was tested in GS2 medium supplemented with 0.2–2.2 % (w/v) NaCl and boiling in 1 % (w/v) CHAPS detergent containing 0.3 M NaCl for 20 min. Total protein was measured using a Bradford Protein Assay kit (Thermo Scientific) with BSA as standard. All data points represent the analysis of at least three replicates.

To assess the production of volatile acids and alcohols, strain T3/55$^T$ was cultured for 10 days in GS2 medium supplemented with various carbohydrates at 0.5 % (w/v) (obtained from Sigma Aldrich). Positive growth was assessed visually by comparing the turbidity of the medium with a non-inoculated control of the same substrate. To confirm positive results, the isolates were transferred into medium with the same substrate. The cell protein yield of every growth reaction was measured as described above. For each substrate, two technical replicates and a control without inoculation were included. The volatile acid and alcohol content was measured with a gas chromatography system (GC-2010; Shimadzu) equipped with a flame-ionization detector. The samples were separated using a temperature gradient from 70 to 260 °C with a Phase-Stabilwax-DA capillary column (Shimadzu) in accordance with the manufacturer’s protocol. Hydrogen gas quantification was not possible due to the residual hydrogen in the gas atmosphere used for maintenance of anaerobiosis during media preparation.

To determine hydrolytic activity of secreted proteins, all newly isolated strains were grown in 50 ml GS2 medium with filter paper as the sole carbon source until substrate depletion. The bacterial culture was centrifuged (5000 g, 30 min, 4 °C), and the clarified culture supernatant was incubated overnight at 4 °C with a 60 % saturated (NH$_4$)$_2$SO$_4$ solution. The precipitated protein was harvested by centrifugation (5000 g, 30 min, 4 °C), dissolved in 0.1 M MOPS buffer (pH 6.5), and concentrated to 0.2–0.7 mg ml$^{-1}$. As described above, total protein was measured with a Bradford Protein Assay kit (Thermo Scientific). Aliquots (15 µl) of the protein solutions (containing 0.3–0.5 µg protein µl$^{-1}$) were added to substrate solution (250 µl) containing 0.5 % (w/v) Avicel and 10× reaction buffer (50 µl) and diluted with water to a total volume of 500 µl. All hydrolytic reactions, which were performed in triplicate, were incubated at 60 °C for 24 h. The samples were analysed for the presence of reducing sugar using the DNSA assay with glucose for assay standardization (Miller, 1959). The DNA G+C content of the microbial genome was determined by DNA extraction and subsequent whole genome sequencing, which was established on the Illumina MiSeq system (Koeck and others, unpublished results). Analysis and interpretation of the genome sequence was performed with GenDB (Meyer et al., 2003).

As a representative of the whole group of isolates, strain T3/55$^T$ was further characterized. The cells were Gram-stain-positive, and a control without inoculation was included. The volatile acid and alcohol content was measured with a gas chromatography system (GC-2010; Shimadzu) equipped with a flame-ionization detector. The samples were separated using a temperature gradient from 70 to 260 °C with a Phase-Stabilwax-DA capillary column (Shimadzu) in accordance with the manufacturer’s protocol. Hydrogen gas quantification was not possible due to the residual hydrogen in the gas atmosphere used for maintenance of anaerobiosis during media preparation.

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**Table 1. Phenotypic characteristics that differentiate strain T3/55\(^T\) from phylogenetically related species**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain 1</th>
<th>Strain 2</th>
<th>Strain 3</th>
<th>Strain 4</th>
<th>Strain 5</th>
<th>Strain 6</th>
<th>Strain 7</th>
<th>Strain 8</th>
<th>Strain 9</th>
<th>Strain 10</th>
<th>Strain 11</th>
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</thead>
<tbody>
<tr>
<td>Isolation source</td>
<td>Biogas plant</td>
<td>Hot water well</td>
<td>Soil</td>
<td>Methanogenic digester</td>
<td>Sewage sludge</td>
<td>Forest soil</td>
<td>Intestinal tract of pigs</td>
<td>Sheep rumen</td>
<td>Methanogenic digester</td>
<td>Sheep rumen</td>
<td>Forest soil</td>
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<tr>
<td>Temp. for growth ((^{\circ})C)</td>
<td>55</td>
<td>37</td>
<td>30</td>
<td>37</td>
<td>37</td>
<td>37</td>
<td>39–42</td>
<td>30–38</td>
<td>35–42</td>
<td>35</td>
<td>37</td>
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<tr>
<td>pH for growth</td>
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<td>7.0–7.5</td>
<td>7.0</td>
<td>7.5</td>
<td>8.0–8.5</td>
<td>6.8–7.2</td>
<td>6.8</td>
<td>7.0</td>
<td>6.5–7</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Presence of catalase</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Gram reaction</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
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<td>Flagella</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Motility</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Utilization of:</td>
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<tr>
<td>Galactose</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Arabinose</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>–</td>
<td>w</td>
<td>w</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Xylan</td>
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<td>+</td>
<td>ND</td>
<td>–</td>
<td>ND</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
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<td>+</td>
<td>–</td>
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<td>+</td>
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<td>w</td>
<td>–</td>
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<td>+</td>
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<td>+</td>
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<td>Succrose</td>
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<td>–</td>
<td>+</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>37</td>
<td>33</td>
<td>41</td>
<td>40</td>
<td>28</td>
<td>36</td>
<td>38</td>
<td>42</td>
<td>28</td>
<td>39</td>
<td>41</td>
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<tr>
<td>16S rRNA gene sequence similarity (%)</td>
<td>100</td>
<td>93.9</td>
<td>92.7</td>
<td>92.3</td>
<td>91.5</td>
<td>90.5</td>
<td>91.1</td>
<td>91.5</td>
<td>91.2</td>
<td>90.1</td>
<td>90.6</td>
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</table>

\(\ast\) Filter paper (Whatman No. 1).
Examination of strain T3/55\textsuperscript{T} for the following properties summarized in Table 1 and in the species description. The detailed results of substrate utilization, growth conditions (O\textsubscript{2} concentrations >4 %).

The detailed results of substrate utilization, growth condition analysis and other phenotypic characteristics are summarized in Table 1 and in the species description. Examination of strain T3/55\textsuperscript{T} for the following properties yielded a negative result: sulfate reduction, indole production, catalase expression, lactose fermentation, decarboxylation of ornithine and lysine, dulcitol fermentation, deamination of phenylalanine and degradation of citrate. The major cellular fatty acids (>1 %) detected in the dried cell material of strain T3/55\textsuperscript{T} were 16:0 dimethyl acetal (DMA), 16:0 fatty acid methyl ester (FAME) and 16:0 aldehyde (ALDE) (Table S1).

The results of the 16S rRNA-based analyses clearly identify biogas plant isolate T3/55\textsuperscript{T} as a member of the family Lachnospiraceae. The position of the respective branch within the edges of the radiation representing the taxa assigned to the family in the consensus tree in Fig. S1 indicates no closer relationship to any of them. Fig. 2 shows an extract of the comprehensive consensus tree (Fig. S1). The 86.2–93.9 % range of overall 16S rRNA gene sequence similarity between strain T3/55\textsuperscript{T} and the other taxa of the family Lachnospiraceae support the situation. The highest overall 16S rRNA gene sequence similarity (93.9 %) is shared by the strains T3/55 and Mobili
talea sibirica DSM 26468\textsuperscript{T} (Podosokorskaya et al., 2014). According to the present and previous (Ludwig et al., 2009) 16S rRNA gene based phylogenetic analyses, a number of the type strains of species with validly published names of the genera Acti
vibrio, Anaerobacter, Bacteroides, Blautia, Clostridium, Desulfo
omaculum, Eubacterium, Fusobacterium, Howardella, Lactobacillus, Lactonifactor and Ruminococcus – currently classified in families other than Lachnospiraceae – are found in the radiation of branches representing members of the latter family. For some of them, rooting in the periphery of the Herbinix gen. nov. branch is supported by the majority of treeing analyses (Fig. S1). Their affiliations, however, are clearly below the genus level (Table 1). Thus, the phylogenetic analysis and the moderate identity scores justify the description of a novel genus and species, Herbinix hemicellulosilytica gen. nov., sp. nov., with isolate T3/55\textsuperscript{T} as the representative type strain.

There are also several phenotypic characteristics that separate strain T3/55\textsuperscript{T} from phylogenetically related species. In particular, the optimal growth temperature of closely related strains such as M. sibirica DSM 26468\textsuperscript{T} is mesophilic, whereas the new isolate showed optimal growth at 55 °C. T3/55\textsuperscript{T} also had a significantly lower DNA G+C content (36.6 mol%) than Clostridium xylanovorans DSM 12503\textsuperscript{T} (40 mol%) and Clostridium jejune DSM 15929\textsuperscript{T} (41 mol%). Fatty acid content profiling also clearly differentiated the novel isolate from its relatives (Table 1). The

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**Fig. 2.** 16S rRNA gene sequence based unrooted dendrogram for type strains from Table 1, moderately related to strain T3/55\textsuperscript{T}. The figure shows an extract of a comprehensive consensus tree generated from multiple treeing analyses (generated as described in Methods) including only alignment positions invariant in at least 50 % of all included 16S rRNA gene sequences representing members of the family Lachnospiraceae. Bar, 10 % estimated base changes.
major cellular fatty acid of *M. sibirica* DSM 26468<sup>T</sup> for example is 16:0 (Podosokorskaya et al., 2014).

Strain T3/55<sup>T</sup> hydrolysed various cellulosic substrates (Whatman filter paper No. 1, PASC, CM-cellulose). Additionally, the hemicellulolytic activity of culture supernatants was tested using several hemicellulosic substrates, each supplied at 1 % (w/v) (Table S1). High hemicellulolytic activity of T3/55<sup>T</sup> could be detected on xylose. Hemicellulolytic activity of T3/55<sup>T</sup> culture supernatant proteins on crystalline cellulose (Avicel) was present but 11-fold lower than that of *C. thermocellum* and 3.75-fold lower than that of *C. stercorarium* (data not shown).

### Description of *Herbinix* gen. nov.

*Herbinix* (Her’bi.nix. L. fem. n. *herba* plant; L. fem. n. *nix* snow; N.L. fem. n. *herbinix* snow-white of plants, pertaining to incomplete degradation of plant cellulose fibres to white powder resembling a snow-like appearance).

Cells are Gram-stain-positive, irregularly shaped rods with rounded, sometimes cigar-shaped ends. No spores are formed. Strictly anaerobic and no growth is observed under aerobic conditions. The catalase reaction is negative. Indole-negative and saccharolytic. Capable of hydrolysing urea but not casein. Glucose, fructose, mannose, arabinose, but not pectin and small amounts of propionic acid. The major metabolic end-products are ethanol, acetic acid, and small amounts of propionic acid. The type strain is T3/55<sup>T</sup> (=DSM 29228<sup>T</sup> = CECT 8801<sup>T</sup>), isolated from a thermophilic biogas plant in Viersen, Germany. The G+C content of genomic DNA of the type strain is 36.6 mol%.

### Acknowledgements

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### References


Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S. & other authors...