Thermoanaerobacter pentosaceus sp. nov., an anaerobic, extremely thermophilic, high ethanol-yielding bacterium isolated from household waste

Ana Faria Tomás, Dimitar Karakashev and Irini Angelidaki

Department of Environmental Engineering, Technical University of Denmark, Miljoevej 113, 2800 Kongens Lyngby, Denmark

An extremely thermophilic, xylanolytic, spore-forming and strictly anaerobic bacterium, strain DTU01\(^T\), was isolated from a continuously stirred tank reactor fed with xylene and household waste. Cells stained Gram-negative and were rod-shaped (0.5–2 \(\mu\)m in length). Spores were terminal with a diameter of approximately 0.5 \(\mu\)m. Optimal growth occurred at 70 °C and pH 7, with a maximum growth rate of 0.1 \(h^{-1}\). DNA G+C content was 34.2 mol%. Strain DTU01\(^T\) could ferment arabinose, cellobiose, fructose, galactose, glucose, lactose, mannitol, mannose, melibiose, pectin, starch, sucrose, xylan, yeast extract and xylene, but not cellulose, Avicel, inositol, inulin, glycerol, rhamnose, acetate, lactate, ethanol, butanol or peptone. Ethanol was the major fermentation product and a maximum yield of 1.39 mol ethanol per mol xylene was achieved when sulfite was added to the cultivation medium. Thiosulfate, but not sulfate, nitrate or nitrite, could be used as electron acceptor. On the basis of 16S rRNA gene sequence similarity, strain DTU01\(^T\) was shown to be closely related to Thermoanaerobacter mathanii A3\(^T\), Thermoanaerobacter italicus Ab9\(^T\) and Thermoanaerobacter thermcopriae JT3-3\(^T\), with 98–99% similarity. Despite this, the physiological and phylogenetic differences (DNA G+C content, substrate utilization, electron acceptors, phylogenetic distance and isolation site) allow for the proposal of strain DTU01\(^T\) as a representative of a novel species within the genus Thermoanaerobacter, for which the name Thermoanaerobacter pentosaceus sp. nov. is proposed, with the type strain DTU01\(^T\) (=DSM 25963\(^T\)=KCTC 4529\(^T\)=VKM B-2752\(^T\)=CECT 8142\(^T\)).

Extremely thermophilic micro-organisms have in the last few decades been the subject of great interest, not only due to their biological and evolutionary significance, but also for their biotechnological potential. One of the major interesting features is their ubiquitous ability to ferment a large range of carbohydrates, including in many cases those that compose feedstock for second-generation bio-ethanol production. In addition to this, a second-generation ethanologenic process employing an extremely thermophilic micro-organism would be less prone to contamination, and have a facilitated downstream product recovery, compared to a mesophilic process (Taylor et al., 2009).

Thermophilic, anaerobic, saccharolytic bacteria are distributed within the genera Thermoanaerobacter, Thermoanaerobacterium and Clostridium. The latest major phylogenetic revision of these genera occurred in 1994, when Collins et al. (1994) following work by Lee et al. (1993) and Rainey et al. (1993) proposed the reorganization of the genus Clostridium and related species, such as strains representing the genus Thermoanaerobacter, into several clusters, based on 16S rRNA gene analysis. This resulted in the regrouping of the included micro-organisms into several clusters, of which cluster V comprised old and newly assigned or combined species of the genus Thermoanaerobacter. Since then, other species have been added to the genus, most of them isolated from natural thermophilic environments, with the exception of Thermoanaerobacter thermcopriae, of which the type strain was isolated from cattle dung compost (Jin et al., 1988). Recently, Verbeke et al. (2011a) have reported difficulties in the classification of new isolates representing the genus Thermoanaerobacter due to the presence of intervening sequences in some of the copies of the 16S rRNA gene in the bacterial genome. Based on these and previous findings regarding interoperonic diversity in Thermoanaerobacter pseudethanolicus and other related species (Fardeau et al., 2004; Pei et al., 2010), Verbeke et al. (2011a) suggest that the sole use of 16S rRNA gene similarity for taxonomic

**Abbreviations:** ANIb, average nucleotide identity by BLAST; DSMZ, German Collection of Microorganisms and Cell Cultures.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DTU01\(^T\) is GU176611.

A supplementary figure is available with the online version of this paper.
assignment in this genus ‘may not accurately reflect true evolutionary relationships’.

In this work we report the isolation of a new member of the
genus Thermoanaerobacter that was indirectly isolated from household waste. Household and municipal waste and its
deposition sites, such as landfills or composting facilities, are composed of materials coming from the most varied sources. A few studies have been made on the microbial diversity at these sites, mostly with the purpose of addressing problems that occur in the degradation processes (Partanen
et al., 2010; Pourcher et al., 2001; Westlake et al., 1995),
rather than focusing on isolation of new micro-organisms. Westlake et al. (1995) detected cellulolytic bacteria in a
landfill, and members of the class Clostridia were detected in the thermophilic, poorly aerated late stage of a full-scale
composting facility (Partanen et al., 2010). These findings suggest that industrially relevant species can be found in
such environments. Recent reports of new isolations from anthropogenic habitats include isolates from compost (Yabe
et al., 2011), household waste (Yassin et al., 2009), or animal faeces (Morotomi et al., 2012).

An extreme thermophilic enrichment culture previously
achieved 0.49 g ethanol g xylose
−1 under specific cultivation conditions (Zhao et al., 2010). This culture had been
enriched from a hydrogen-producing reactor, operating at 70
°C and using xylose as the main carbon source (Kongjan
et al., 2009). The inoculum for this reactor had been in
turn obtained from a laboratory scale hydrogen-producing
continuously stirred tank reactor fed with household waste,
with an operating temperature of 70
°C and a hydraulic
retention time of 3 days (Liu et al., 2008).

This high-yielding ethanol-producing culture was used as
the inoculum for the isolation process, which was carried
out following the roll-tube technique (Hungate, 1969;
Macy et al., 1972). A series of dilutions of the enrichment
cultures were transferred into a modified basic anaerobic (BA) medium (Angelidaki et al., 1990): cysteine hydro-
chloride was omitted, 1 g yeast extract l
−1 was added, and
it was supplemented with 11 g Gelrite l
−1 and 2 g MgCl2
l
−1 to ensure that the medium remained solid at the
incubation temperature of 70
°C (Lin & Casida, 1984).
Except where noted, 5 g xylose l
−1 was used as the carbon
source throughout the experiments. Colonies were picked
with sterile Pasteur pipettes, diluted in fresh liquid medium
and transferred to new roll tubes when grown. This
procedure was repeated until only one colony type was
present and then three times more to ensure purity.

After two weeks of incubation, 0.5–1 mm diameter, beige,
flat colonies were visible in the tubes. Cell morphology was
examined using a bright-field microscope (Axioskop; Zeiss).
Photomicrographs were taken with a camera (DFC220;
Leica) connected to the microscope. Cells were rod-shaped
(0.5–2 μm) and appeared singly, in pairs or in chains (Fig.
1a). No active mobility was observed. Gram-negative
staining was observed during both exponential and
stationary growth phases, although the organism was

Fig. 1. Photomicrographs of vegetative cells (a) and sporulating cells
(b) of strain DTU01
T. In (b), spores are stained green. Bars, 5 μm.

phylogenetically Gram-positive (Wiegel & Ljungdahl, 1981). Spore staining was performed according to Schaeffer & Fulton (1933). Sporulation of cells into round, terminal
spores was observed in the stationary phase of growth (Fig.
1b). Sporulating cultures survived autoclaving for 1 h at
70
°C, showing that the spores were heat-resistant.

The variation of OD600 was measured using a Spectronic
20D+ (Thermo Scientific) and correlated with cell dry-
weight using a calibration curve obtained under corres-
ponding cultivation conditions. This method was used
throughout the experiments to detect and quantify growth of
the micro-organism. All batch tests and sampling were
performed in triplicate. The doubling time under optimal
conditions was 3.6 h. For pH range determination exper-
iments, four different buffers were used in the media, at a
final concentration of 50 mM: MES sodium salt, NaHCO3,
Tris (Trizma base) and Na2CO3. The temperature range for
growth at pH 7 was 45–80
°C, with an optimum of 70
°C, and the pH range for growth at 70
°C was 5.36–8.92.
Incubation at temperatures and pH higher than the growth
range resulted in browning of the medium, caused by
caramelization of xylose or the Maillard reaction (Buera
et al., 1987; Hill & Patton, 1947). Strain DTU01
T was
strictly anaerobic, with no detectable growth in aerobic or
microaerophilic conditions.

Yeast extract was not required, but it stimulated growth.
Using 1 g yeast extract l
−1 as the sole substrate led only to
limited growth. The ability of strain DTU01T to utilize other substrates was determined using BA medium, but with 2 g test substrate \(\text{L}^{-1}\) instead of 5 g xylose \(\text{L}^{-1}\). Utilization was considered positive when variation in OD\(_{600}\) was twice or more than that of control cultures (containing 1 g yeast extract \(\text{L}^{-1}\) as sole carbon source), and weakly positive when the variation was only 10% higher. In the case of insoluble substrates, utilization was assessed by comparing the concentration of metabolic products present in the medium for each substrate with controls supplied only with 1 g yeast extract \(\text{L}^{-1}\) as a carbon source. Strain DTU01T grew on xylose, glucose, arabinose, fructose, galactose, cellobiose, lactose, mannitol, mannose, melibiose, pectin, soluble starch, sucrose and xylan from beechwood; but not on cellulose, Avicel, inulin, inositol, glycerol, rhamnose, acetate, lactate, ethanol, butanol or peptone.

Fermentation end products were determined using standard HPLC and GC techniques as described previously (Zhao et al., 2009). The major metabolic products after 24 h of cultivation, when using 5 g xylose \(\text{L}^{-1}\) as a carbon source, were ethanol, hydrogen, lactate and acetate.

The micro-organism was able to tolerate 4% (w/v) salinity. The use of the electron acceptors \(\text{Na}_2\text{SO}_4\), \(\text{Na}_2\text{SO}_3\), \(\text{Na}_2\text{S}_2\text{O}_3\), \(\text{NaNO}_3\) and \(\text{NaNO}_2\) (20, 5 and 2.5, 20 and 5 mM, respectively) was tested in medium that contained 0.5 g cysteine-HCl \(\text{L}^{-1}\) instead of \(\text{Na}_2\text{S}\) as the reducing agent. It was found that \(\text{NaNO}_2\) inhibited growth, that \(\text{Na}_2\text{SO}_4\) and \(\text{NaNO}_3\) had no effect, and that \(\text{Na}_2\text{SO}_3\) and \(\text{Na}_2\text{S}_2\text{O}_3\) greatly increased the growth rate: after 18 h, the concentration of cells was, respectively, 1.2 and 1.5 times higher than when no electron acceptor was added. Accordingly, sulfide was detected in the medium when sulfite and thiosulfate were used. Interestingly, the use of \(\text{Na}_2\text{S}_2\text{O}_3\) led to a shift of the metabolic pathway to acetate production rather than ethanol: the ethanol:acetate ratio decreased from 4.76 (when no electron acceptor was used) to 0.35. In contrast, the use of \(\text{Na}_2\text{SO}_3\) resulted also in increased ethanol yields. In fact, previously, a yield of 1.28 mol ethanol per mol xylose had been achieved with this isolate (Tomás et al., 2011); this had been the highest ethanol yield from pentoses to be reported for a non-genetically modified strain. With the addition of 2.5 mM \(\text{Na}_2\text{SO}_3\) to a cultivation medium supplied with 5 g xylose \(\text{L}^{-1}\), 1.39 mol ethanol mol xylose \(\text{L}^{-1}\) was obtained, which is 83% of the theoretical ethanol yield from xylose. This result confirms that strain DTU01T is a promising candidate for a thermophilic industrial ethanol producer.

Chemotaxonomic analysis, such as detection of respiratory quinones, peptidoglycan structure, fatty acid composition and polar lipids, were performed by the Identification Service of the German Collection of Microorganisms and Cell Cultures (DSMZ; Braunschweig, Germany). Cells were grown overnight in liquid BA medium as described above, at 70 °C and pH 7, then centrifuged, resuspended in a 10% (w/v) sucrose solution, and freeze-dried, before being sent to DSMZ.

No respiratory quinones were detected by the two-stage method described by Tindall (1990a, b). This is not unexpected for strictly anaerobic bacteria (Collins & Jones, 1981; Crespo et al., 2012; Kim et al., 2001; Yokoyama et al., 2010) and most new taxonomic descriptions of this type of micro-organism do not include isoprenoid quinone detection in their characterization methods. However, in a study by Yamamoto et al. (1998), menaquinone 7 was found in several thermophilic, anaerobic, Gram-positive bacteria, indicating that perhaps these molecules are more widespread than is currently thought.

Peptidoglycan analysis was performed by subjecting hydrolysate of whole cells of strain DTU01T to TLC on cellulose plates, as described by Schumann (2011). The whole-cell hydrolysate contained meso-diaminopimelic acid as the diagnostic amino acid, indicating that the peptidoglycan type in strain DTU01T is A\(\gamma\) or A\(\gamma'\), a feature shared with other anaerobic thermophiles (Yamamoto et al., 1998).

For determination of the fatty acid profile, fatty acid methyl esters were obtained by saponification and methylation and extracted using the methods of Miller (1982) and Kuykendall et al. (1988) with minor modifications. The mixtures were further separated using the Sherlock Microbial Identification System (MIDI; Microbial ID). The predominant fatty acid was iso-C\(15:0\) (40.39%), followed by other branched acids such as iso-C\(14:0\) 3-OH (15.79%), iso-C\(17:0\) (15.51%), iso-C\(16:0\) (7.42%), anteiso-C\(15:0\) (5.94%) and anteiso-C\(17:0\) (4.25%). Minor components were iso-C\(13:0\) (1.8%), C\(18:0\) (1.44%), C\(14:0\) (1.38%), C\(14:0\) 2-OH (1.35%) and iso-C\(11:0\) (0.59%). No unsaturated acids were detected. iso-Diolic acid, an unusual, very long (C32) dimethyl dicarboxylic acid, which has been identified as the most abundant transmembrane fatty acid in some thermophilic strains (Balk et al., 2009; Jung et al., 1994; Lee et al., 2002), has not been detected in strain DTU01T.

Polar lipids were extracted using a chloroform:methanol: 0.3% aqueous NaCl mixture, 1:2:0.8 (by vol.) (Bligh & Dyer, 1959), and then separated by two-dimensional silica gel TLC, as described by Tindall et al. (2007). The profile of strain DTU01T presented eight resolved lipid spots (Fig. S1, available in IJSEM Online), corresponding to phosphatidylglycerol, diphosphatidylglycerol, two unidentified phospholipids and four unidentified polar lipid classes. Phosphatidylglycerol and diphosphatidylglycerol are commonly found in most prokaryotes (da Costa et al., 2011; Lechevalier & Lechevalier, 1988). The remaining unidentified lipids could correspond to plasmagens and sphingolipids, which are common in anaerobic bacteria (Lechevalier & Lechevalier, 1988).

For colony purity assessment and phylogenetic analysis, DNA was extracted using a QIAmp DNA Stool Mini kit (Qiagen, 51504). PCR-DGGE (denaturing gradient gel electrophoresis) was performed as described by Zhao et al. (2009). The PCR product was purified using a GenElute
PCR DNA Purification kit (NA1020; Sigma) and sent for sequencing at DNA Technology (Risskov, Denmark). Primers used for sequencing were universal primer 1492-r, bacteria specific primer 24-f and a home-designed primer (5'-GGATTAGATACTGAGTGTTCCAG-3'). The identification of phylogenetic neighbours based on 16S rRNA gene sequence similarity was initially carried out by using the BLAST (Altschul et al., 1997) and megablast (Zhang et al., 2000) programs against the database of type strains with validly published prokaryotic names (Chun et al., 2007). The sequences with the highest scores were then selected for the calculation of pairwise sequence similarity using a global alignment algorithm, which was implemented at the EzTaxon server (http://www.eztaxon.org; Chun et al., 2007). Multiple sequence alignment was performed using the program CLUSTAL X 2 (Larkin et al., 2007) and manually edited in MEGA 5 (Tamura et al., 2011), which was also used for phylogenetic analysis. Consensus trees were inferred from 1000 replicates and were reconstructed using the maximum-likelihood method based on the Jukes–Cantor model (Jukes & Cantor, 1969).

Phylogenetic and similarity sequence analysis of the 16S rRNA gene sequence of strain DTU01T revealed that it was affiliated to the genus *Thermoanaerobacter* of the family *Thermoanaerobacteraceae* (phylum ‘Firmicutes’) (Fig. 2). The calculation of pairwise sequence similarity values revealed that the closest relatives with validly published names were the two subspecies of *Thermoanaerobacter mathranii* (99.4%; Carlier et al., 2006; Larsen et al., 1997), *Thermoanaerobacter italicus* Ab9T (98.4%; Kozianowski et al., 1997) and *Thermoanaerobacter thermocopriae* JT3-3T (97.8%; Jin et al., 1988). Similarity with the remaining members of the genus *Thermoanaerobacter* was between 92.3 and 95.9%. All species of the genus *Caldanaerobacter* had 90.4–92.3% 16S rRNA gene similarity with strain DTU01T, while similarity with other bacteria remained below 89.9%. *Thermoanaerobacter mathranii* A3T and *Thermoanaerobacter italicus* Ab9T were ordered from the (DSMZ) and initially cultivated using the recommended media. For comparison purposes, both strains were cultivated in the same medium as for strain DTU01T.

All the closest relatives of strain DTU01T are thermophilic, anaerobic microbes that can ferment a wide range of substrates and produce ethanol as one of the products of mixed-acid fermentation, along with lactate, acetate, CO₂ and hydrogen. Despite this, a few morphological and physiological differences remain: *Thermoanaerobacter mathranii* A3T is not able to ferment pectin, and it can stain Gram-positive, while strain DTU01T always stains Gram-negative. In addition to this, it can use sulfate and thiosulfate as an electron acceptor, while DTU01T can use thiosulfate, but not sulfate. *Thermoanaerobacter italicus* Ab9T, despite not having the highest 16S rRNA gene similarity with DTU01T, shares more features and is placed on the same branch in the generated phylogenetic tree (Fig. 2). The substrate utilization profile is very similar, except for mannitol and the fact that while *Thermoanaerobacter italicus* Ab9T is able to degrade pectin and inulin efficiently; growth of strain DTU01T on pectin could only be classified as weakly positive. As for *Thermoanaerobacter thermocopriae* JT3-3T, this strain has a 10 °C lower temperature optimum and a distinct substrate profile.

Cells of strain DTU01T are generally smaller (0.5–2 μm) than cells of *Thermoanaerobacter mathranii* A3T, *Thermoanaerobacter italicus* Ab9T and *Thermoanaerobacter thermocopriae* JT3-3T. In addition to this, strain DTU01T was isolated from a reactor inoculated with household waste, while the others were isolated from thermophilic, natural environments such as an alkaline hot spring in the case of *Thermoanaerobacter mathranii* and a thermal spa in the case of *Thermoanaerobacter italicus* Ab9T. Strain DTU01T yields a higher amount of ethanol from xylose than any other wild-type, thermophilic anaerobe: 1.39 mol ethanol mol xylose⁻¹, which corresponds to 83% of the theoretical yield. When cultivated under the same conditions, strains DTU01T, *Thermoanaerobacter mathranii* A3T and *Thermoanaerobacter italicus* Ab9T exhibit distinct fermentation product profiles, and strain DTU01T remains the one with the highest ethanol yield on xylose. Finally, *Thermoanaerobacter mathranii* has a generation time that is almost twice as fast as that of strain DTU01T and *Thermoanaerobacter italicus*. A summary of the morphological and physiological differences between these closely related strains is reported in Table 1.

With the fast-paced advance of genetic technologies, the ‘gold standard’ for bacterial species definition that is DNA–DNA hybridization (Richter & Rosselló-Móra, 2009) is slowly being left behind in favour of newer methods. Whole-genome sequence similarity has been shown to be correlated with DNA–DNA hybridization (Goris et al., 2007; Konstantinidis & Tiedje, 2005), and so has similarity of gene sequences other than the 16S rRNA gene (Verbeke et al., 2011b; Zeigler, 2003). With this in mind, and in order to further clarify the phylogenetic position of strain DTU01T regarding its closest relatives, total genomic DNA was sent to BGI (China) for determination of the DNA G+C content and sequencing. A draft genome (in 143 contigs), with a DNA G+C content of 34.2 mol% was obtained. This sequence was used to determine the average nucleotide identity by BLAST (ANIb) between strains DTU01T, *Thermoanaerobacter mathranii* subsp. *mathranii* A3T, *Thermoanaerobacter italicus* Ab9T and other reference strains representing the genus *Thermoanaerobacter* whose genome sequences are available from GenBank and the Joint Genome Institute (JGI; http://img.jgi.doe.gov/cgi-bin/w/main.cgi), by using the in silico DNA–DNA hybridization method of the Jspecies software (http://www.imedea.uib-csic.es/jspecies/about.html; Richter & Rosselló-Móra, 2009). *Thermoanaerobacterium thermosaccharolyticum* DSM 571T was used as a phylogenetically related outgroup for comparison purposes.

Previously, Verbeke et al. (2011a) conducted a similar analysis among species of the genus *Thermoanaerobacter*.
Since the parameters and genomic data used were the same, and for a better overview, the results of all the calculations are presented in Table 2. If the recommended species-delineating cut-off value of 95% ANIb is taken into account, then *Thermoanaerobacter mathranii* A3\(^T\), *Thermoanaerobacter italicus* Ab9\(^T\) and strain DTU01\(^T\) should be considered as the same species. This had already been pointed out by Verbeke et al. (2011a), based not only...
on ANIb values, but also on several other well-described and validated genome-relatedness predicting models. However, this is not the only case where micro-organisms that have been classified as different species are shown to have above-threshold similarity values (and vice-versa). Such is the case of infectious disease-related species like Escherichia coli and Shigella flexneri, or the peculiar case of the species of the genus Bordetella (Konstantinidis & Tiedje, 2005).

Different subspecies and strains with similar genotypic and/or phenotypic properties are often assigned to a single species when they are isolated from the same, or a very similar, habitat (Fardeau et al., 2004; Verbeke et al., 2011a). However, this is not the case with strain DTU01\textsuperscript{T} and its closest relatives Thermoanaerobacter mathranii subsp. mathranii A3\textsuperscript{T} (Larsen et al., 1997); 3, Thermoanaerobacter italicus Ab9\textsuperscript{T} (Kozianowski et al., 1997); 4, Thermoanaerobacter thermocopriae JT3-3\textsuperscript{T} (Jin et al., 1988). +, Positive; −, negative; NR, not reported; + w, weakly positive. Doubling time, substrate utilization, fermentation products and electron acceptor data from this study for all strains, except strain 4. All strains produce spores and are able to grow with galactose.

**Table 1.** Phenotypic comparison of strain DTU01\textsuperscript{T} and the type strains of its closest relatives

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rRNA gene similarity (%) to strain DTU01\textsuperscript{T}</td>
<td>–</td>
<td>99.4</td>
<td>98.4</td>
<td>97.8</td>
</tr>
<tr>
<td>DNA G+C (mol%)</td>
<td>34.2</td>
<td>37</td>
<td>34.4</td>
<td>37.2</td>
</tr>
<tr>
<td>Cell size (μm)</td>
<td>0.5–2</td>
<td>0.7–3.9</td>
<td>0.40–0.75 × 2–6</td>
<td>0.4–0.7 × 2–8</td>
</tr>
<tr>
<td>Motility</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>NR</td>
</tr>
<tr>
<td>Doubling time (h)</td>
<td>3.6</td>
<td>1.9</td>
<td>3.6</td>
<td>NR</td>
</tr>
<tr>
<td>Fermentation products (mol xylose mol\textsuperscript{−1})</td>
<td>Ethanol, 0.46; acetate, 0.2; lactate, 0.1; hydrogen, 0.45</td>
<td>Ethanol, 0.29; acetate, 0.39; lactate, 0.14; hydrogen, 0.70</td>
<td>Ethanol, 0.41; acetate, 0.06; lactate, 0.34; hydrogen, 0.08</td>
<td>Ethanol, acetate, lactate, hydrogen</td>
</tr>
<tr>
<td>Temperature (°C):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Optimum</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>60</td>
</tr>
<tr>
<td>Range</td>
<td>50–80</td>
<td>58–78</td>
<td>45–78</td>
<td>47–74</td>
</tr>
<tr>
<td>Growth with:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+ W</td>
</tr>
<tr>
<td>Inulin</td>
<td>–</td>
<td>–</td>
<td>+ W</td>
<td>+ W</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>+</td>
<td>+ W</td>
<td>–</td>
</tr>
<tr>
<td>Melibiose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Starch</td>
<td>+</td>
<td>+</td>
<td>+ W</td>
<td>+ W</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Pectin</td>
<td>+ W</td>
<td>–</td>
<td>+</td>
<td>NR</td>
</tr>
<tr>
<td>Electron acceptor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfate</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>NR</td>
</tr>
<tr>
<td>Thiosulfate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NR</td>
</tr>
<tr>
<td>Source</td>
<td>Household waste (Denmark)</td>
<td>Slightly alkaline hot spring (Iceland)</td>
<td>Thermal spa (Italy)</td>
<td>Cattle dung compost (Japan)</td>
</tr>
</tbody>
</table>

Different subspecies and strains with similar genotypic and/or phenotypic properties are often assigned to a single species when they are isolated from the same, or a very similar, habitat (Fardeau et al., 2004; Verbeke et al., 2011a). However, this is not the case with strain DTU01\textsuperscript{T} and its closest relatives Thermoanaerobacter mathranii subsp. mathranii A3\textsuperscript{T} and Thermoanaerobacter italicus Ab9\textsuperscript{T}. Instead, it is more likely to be the product of adaptive evolution of sporulating, mesophilic strains present in the initial inoculum (Liu et al., 2008). It is clear that a new definition of bacterial species needs to be agreed upon; one that accounts for genomic, phenotypic and environmental factors, and that makes use of the most recent advances in genetic and identification tools. Until then, and in order to maintain coherence with the existing classifications within the genus Thermoanaerobacter, on the basis of physiological, chemotaxonomic, phylogenetic and environmental characteristics of strain DTU01\textsuperscript{T}, we propose strain DTU01\textsuperscript{T} to be assigned to a novel species of the genus Thermoanaerobacter, within the family Thermoanaerobacteraceae, order Thermoanaerobacterales, class Clostridia, phylum ‘Firmicutes’.

**Description of Thermoanaerobacter pentosaceus sp. nov.**

*Thermoanaerobacter pentosaceus* (pen.to.sa’ce.us N.L. neut. n. pentosum a pentose sugar; L. masc. suff. -aceus suffix used with the sense of belonging to; N.L. masc. adj. pentosaceus relating to a pentose).

Cells are spore forming rods (0.5–2 μm in length), non-motile, and stain Gram-negative. They can appear singly, in pairs or short chains. Spores are terminal, round and approximately 0.5 μm in diameter. Strictly anaerobic, extremely thermophilic, neutrophilic, growing optimally at 70 °C (and within 50–80 °C) and at pH 7 (no growth at pH 6.5).
Table 2. ANI<sub>b</sub> between strain DTU01<sup>T</sup> and selected reference strains, determined using Jspecies (Richter & Rosselló-Móra, 2009)

Strains: 1, *Thermoanaerobacter* sp. X513; 2, *Thermoanaerobacterium thermosaccharolyticum* DSM 571<sup>T</sup>; 3, *Thermoanaerobacter italicus* Ab9<sup>T</sup>; 4, *Thermoanaerobacter tengcongensis* MB4<sup>T</sup>; 5, *Thermoanaerobacter* sp. X514; 6, *Thermoanaerobacter wiegelii* Rt8B1<sup>T</sup>; 7, DTU01<sup>T</sup>; 8, *Thermoanaerobacter pseudethanolicus* ATCC 33223<sup>T</sup>; 9, *Thermoanaerobacter mathranii* subsp. mathranii A3<sup>T</sup>; 10, *Thermoanaerobacter brockii* subsp. finnii AKO-1<sup>T</sup>. Numbers in bold type represent values above the recommended cut-off value of 95% to determine species identity.

<table>
<thead>
<tr>
<th>Strain</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>–</td>
<td>70.05</td>
<td>91.63</td>
<td>76.85</td>
<td>100</td>
<td>93.66</td>
<td>91.29</td>
<td>97.77</td>
<td>91.46</td>
</tr>
<tr>
<td>2</td>
<td>69.93</td>
<td>–</td>
<td>69.55</td>
<td>68.00</td>
<td>69.93</td>
<td>69.63</td>
<td>70.10</td>
<td>69.58</td>
<td>69.66</td>
</tr>
<tr>
<td>3</td>
<td>91.97</td>
<td>69.64</td>
<td>–</td>
<td>76.46</td>
<td>91.97</td>
<td>90.71</td>
<td>98.15</td>
<td>92.01</td>
<td>97.94</td>
</tr>
<tr>
<td>4</td>
<td>76.97</td>
<td>67.77</td>
<td>76.31</td>
<td>–</td>
<td>76.96</td>
<td>77.75</td>
<td>76.55</td>
<td>77.30</td>
<td>76.48</td>
</tr>
<tr>
<td>5</td>
<td>99.98</td>
<td>69.99</td>
<td>91.63</td>
<td>76.91</td>
<td>–</td>
<td>93.53</td>
<td>91.29</td>
<td>97.74</td>
<td>91.38</td>
</tr>
<tr>
<td>6</td>
<td>93.68</td>
<td>69.99</td>
<td>90.42</td>
<td>77.48</td>
<td>93.68</td>
<td>–</td>
<td>90.47</td>
<td>93.92</td>
<td>90.37</td>
</tr>
<tr>
<td>7</td>
<td>91.45</td>
<td>70.22</td>
<td>98.02</td>
<td>76.41</td>
<td>91.45</td>
<td>90.54</td>
<td>–</td>
<td>91.61</td>
<td>98.31</td>
</tr>
<tr>
<td>8</td>
<td>97.84</td>
<td>69.89</td>
<td>91.83</td>
<td>77.17</td>
<td>97.84</td>
<td>93.98</td>
<td>91.75</td>
<td>–</td>
<td>91.54</td>
</tr>
<tr>
<td>9</td>
<td>91.67</td>
<td>69.67</td>
<td>98.04</td>
<td>76.51</td>
<td>91.68</td>
<td>90.59</td>
<td>98.53</td>
<td>91.64</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>97.99</td>
<td>69.83</td>
<td>76.96</td>
<td>97.99</td>
<td>93.97</td>
<td>91.67</td>
<td>99.99</td>
<td>91.44</td>
<td>–</td>
</tr>
</tbody>
</table>

below pH 5.36 or above pH 8.5). Can tolerate 4% (w/v) NaCl. Can use arabinose, cellobiose, fructose, galactose, glucose, lactose, mannitol, mannose, melibiose, pectin, starch, sucrose, xylan, yeast extract and xylose, but not cellulose, Avice, inositol, inulin, glycerol, rhamnose, acetate, lactate, ethanol, butanol or peptone, as carbon and energy source. Sulfite and thiosulfate can be used as terminal electron acceptor, but not sulfate, nitrite or nitrate. The doubling time under optimal conditions is 6.6 h.

The type strain, DTU01<sup>T</sup> (=DSM 25963<sup>T</sup>=KCTC 4529<sup>T</sup>=VKM B-2752<sup>T</sup>=CECT 8142<sup>T</sup>), was isolated from a thermophilic reactor fed with household solid waste from a biogas plant in Grindsted, Denmark. The DNA G+C content of the type strain is 34.2 mol%.

Acknowledgements
This work was supported by Fundação para a Ciência e Tecnologia (FCT), Portugal, through grant SFRH/BD/43863/2008.

References
Thermoanaerobacter pentosaceus sp. nov.


Verbeke, T. J., Sparling, R., Hill, J. E., Links, M. G., Levin, D. B. & Dumonceaux, T. J. (2011b). Predicting relatedness of bacterial genomes using the chaperonin-60 universal target (cpx60 UT);


