Desulfotomaculum peckii sp. nov., a moderately thermophilic member of the genus Desulfotomaculum, isolated from an upflow anaerobic filter treating abattoir wastewaters

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A novel anaerobic thermophilic sulfate-reducing bacterium designated strain LINDBHT1T was isolated from an anaerobic digester treating abattoir wastewaters in Tunisia. Strain LINDBHT1T grew at temperatures between 50 and 65 °C (optimum 55–60 °C), and at pH between 5.9 and 9.2 (optimum pH 6.0–6.8). Strain LINDBHT1T required salt for growth (1–40 g NaCl l⁻¹), with an optimum of 20–30 g l⁻¹. In the presence of sulfate as terminal electron acceptor, strain LINDBHT1T used H₂/CO₂, propanol, butanol and ethanol as carbon and energy sources but fumarate, formate, lactate and pyruvate were not utilized. Butanol was converted to butyrate, while propanol and ethanol were oxidized to propionate and acetate, respectively. Sulfate, sulfite and thiosulfate were utilized as terminal electron acceptors but elemental sulfur, iron (III), fumarate, nitrate and nitrite were not used. The G+C content of the genomic DNA was 44.4 mol%.

Phylogenetic analysis of the small-subunit rRNA gene sequence indicated that strain LINDBHT1T was affiliated to the genus Desulfotomaculum with the type strains of Desulfotomaculum halophilum and Desulfotomaculum alkaliphilum as its closest phylogenetic relatives (about 89 % similarity). This strain represents a novel species of the genus Desulfotomaculum, Desulfotomaculum peckii sp. nov.; the type strain is LINDBHT1T (≡ DSM 23769T ≡ JCM 17209T).

Sulfate-reducing bacteria (SRB) represent the anaerobic micro-organisms that reduce the widest spectrum of different terminal electron acceptors, including inorganic sulfur compounds and various other inorganic and organic compounds (Barton & Fauque, 2009; Fauque et al., 1991; Fauque & Ollivier, 2004; Fauque, 1995; LeGall & Fauque, 1988; Muyzer & Stams, 2008; Rabus et al., 2006). This suggests that their metabolic and ecological function in nature is of great importance. SRB are widely distributed in marine, terrestrial and subterrestrial ecosystems (Barton & Fauque, 2009; Fauque, 1995; Muyzer & Stams, 2008; Ollivier et al., 2007). As of 2011, 65 genera containing 250 species of SRB were known (Fauque & Barton, 2012). There are at least five genera of spore-forming SRB, e.g. Desulfovirgula, Desulfurispora, Desulfotomaculum, Desulfosporinus and Desulfoverruca (Kaksonen et al., 2007a, 2007b; Sass et al., 2004; Spring & Rosenzweig, 2006; Widdel, 2006), found among the Firmicutes.

The genus Desulfotomaculum includes both thermophilic and mesophilic species, all of which are capable of using a large number of substrates (Widdel & Hansen, 1992; Widdel, 2006). According to the phylogenetic data, all Desulfotomaculum species are divided into five subclusters (Stackebrandt et al., 1997; Og & Patel, 2011). Subcluster I (formerly subcluster 5) comprises only two species, Desulfotomaculum halophilum (Tardy-Jacquenod et al., 1998) and Desulfotomaculum alkaliphilum (sequence similarity 92.7 %) (Pikuta et al., 2000). There was only a slight relatedness between these two strains and most of the species of the genus Desulfotomaculum.

In this paper, we report on the isolation and characterization of a novel thermophilic, anaerobic, sulfate-reducing...
strain, designated LINDBHT1T included in subcluster I of the genus *Desulfotomaculum*.

Strain LINDBHT1T was isolated from an upflow anaerobic filter treating abattoir wastewaters in Tunisia (Gannoun et al., 2009). The digester was operated under both mesophilic (37 °C) and thermophilic (55 °C) conditions. A sample was taken during the thermophilic phase of the process in order to isolate sulfate-reducing micro-organisms.

For enrichment and isolation, the basal medium contained (per litre of distilled water) 0.3 g KH2PO4, 0.3 g K2HPO4, 1 g NH4Cl, 20 g NaCl, 0.1 g KCl, 0.1 g CaCl2 .2H2O, 0.5 g L-cysteine HCl, 0.1 g yeast extract (Difco), 20 mM thiosulphate, 1 ml oligo-element solution (Widdel & Pfennig, 1981) and 1 ml 0.1 % resazurin (Sigma). The pH was adjusted to 7.2 with a 10 M KOH solution. The medium was boiled under a stream of O2-free nitrogen and cooled to room temperature. Aliquots of 5 ml were dispensed into Hungate tubes, degassed under N2 : CO2 (80 : 20 %, v/v) and subsequently sterilized by autoclaving at 120 °C for 20 min. Prior to inoculation, 0.1 ml 10 % (w/v) NaHCO3, 0.1 ml 2 % (w/v) Na2S.9H2O and 3 g MgCl2 .6H2O from sterile stock solutions were injected into the tubes. Finally, the medium was supplemented with 20 mM acetate and H2/CO2 at 2 bar as substrate. The Hungate technique (Hungate, 1969) was used throughout the study.

A 0.5 ml aliquot of the sample was inoculated into Hungate tubes that were subsequently incubated at 60 °C. To obtain pure cultures, the enrichment was subcultured several times under the same growth conditions prior to isolation. For isolation, the culture was serially diluted tenfold in roll tubes (Miller & Wolin, 1974) containing the same culture medium supplemented with 2.5 % agar (w/v). Several colonies developed after incubation at 60 °C and were picked separately. The process of serial dilution was repeated several times until the isolates were deemed to be axenic. Several strains were isolated; their morphologies and metabolic profiles were similar and the same phylogenetic inference was obtained for all of them. One strain, designated LINDBHT1T, was selected and used for further metabolic and physiological characterization.

Methods for purification of the DNA, PCR amplification and sequencing of the 16S rRNA gene have been described previously (Ben Dhia Thabet et al., 2004). The partial sequences generated were assembled using BioEdit v. 5.0.9. (Hall, 1999) and the consensus sequence of 1502 nt was corrected manually for errors. The sequence was compared with available sequences in GenBank (version 178) using a BLAST search (Altschul et al., 1990). The consensus sequence was then manually adjusted to confirm to the 16S rRNA secondary structure model (Winker & Woese, 1991). Nucleotide ambiguities were omitted and evolutionary distances were calculated using the Jukes and Cantor option (Jukes & Cantor, 1969). Phylogenetic trees were constructed with the TREECON program using the neighbour-joining (Fig. 1) (Saitou & Nei, 1987), maximum-parsimony and maximum-likelihood algorithms. Tree topology was evaluated by a bootstrap analysis using 1000 resamplings of the sequences (Felsenstein, 1985).

On the basis of 16S rRNA gene sequence analysis, strain LINDBHT1T was affiliated to the genus *Desulfotomaculum*. Its closest phylogenetic relatives were *Desulfotomaculum halophilum* isolated from oil production facilities (Tardy-Jacquenod et al., 1998) and *Desulfotomaculum alkalophilum* isolated from cow/pig manure (Pikuta et al., 2000) with a sequence similarity of 89 %.

The Gram reaction was performed with heat-fixed liquid cultures stained with the Difco kit reagents. Cellular morphology and purity of the strain were assessed under an Optiphot (Nikon) phase-contrast microscope. For transmission electron microscopy studies cells were negatively stained with sodium phosphotungstate as previously described (Fardeau et al., 1997). The presence of spores was assessed (i) by microscopic observation of cultures and (ii) by pasteurization tests performed at 80, 90 and 100 °C for 10 and 20 min.

Growth experiments were performed in duplicate, using Hungate tubes containing the above-mentioned basal medium. The pH of the medium was adjusted to pH 4–9 (at 0.5 pH unit intervals) by injecting aliquots of anaerobic stock solutions of 1 M HCl, 10 % NaHCO3 or Na2CO3 into the Hungate tubes. The pH of the medium was verified after autoclaving. Water baths were used for incubating bacterial cultures at 30–70 °C (at 5 °C intervals). To assess the requirement of and tolerance to NaCl, this salt (0–150 g l−1 at 10 g l−1 intervals) was weighed directly into the tubes before the medium was dispensed. The strain was subcultured at least twice under the same experimental conditions before the growth rates were determined. Glucose, fructose, succinate, fumarate, butyrate, propanol, butanol, ethanol, formate, malate, peptone, Casamino acids, formate, formate + acetate, acetate, lactate and pyruvate were tested as electron donors. Each substrate was added to the basal medium at a final concentration of 20 mM. H2 : CO2 (80 : 20) alone or in the presence of acetate (2 mM) as a carbon source was also tested at 2 bar. The effect of yeast extract (Panreac), biotrypticase (Panreac) and Widdel’s vitamins (Widdel & Pfennig, 1981) on growth was tested with and without substrate added. Elemental sulfur (1 % w/v), sodium sulfate (20 mM), sodium thiosulfate (20 mM), sodium sulfite (2 mM), sodium nitrate (10 mM) and sodium nitrite (2 mM), were tested as terminal electron acceptors. H2S production was determined photometrically as colloidal CuS by using the method of Cord-Ruwisch (1985). Bacterial growth was monitored by measuring the increase in OD580 by insertion of Hungate tubes into the cuvette holder of a spectrophotometer (Cary 50, Varian). End products of metabolism were measured by HPLC and gas chromatography of the gases released after two weeks of incubation at 60 °C (Fardeau et al., 2000). The presence of desulfoviridin and c-type cytochromes was tested in the crude bacterial extract following the method described by Postgate (1959).
Cells of strain LINDBHT\textsuperscript{T} were non-motile, spore-forming rods, the spore position is central to subterminal (Fig. 2a, b). The cells stained Gram-positive. The cell wall had a typical Gram-positive structure in the form of a thin electron-dense layer (Fig. 2c).

The isolate required yeast extract for growth, which could not be replaced by biotrypticase or Widdel’s vitamins (Widdel & Pfennig, 1981). In the presence of sulfate, propanol, butanol, ethanol, H\textsubscript{2} : CO\textsubscript{2} and H\textsubscript{2} : CO\textsubscript{2} + acetate are used as electron donors, but glucose, fructose, succinate, fumarate, butyrate, formate, malate, peptone, Casamino acids, formate, formate + acetate, acetate, lactate and pyruvate are not used. Sodium sulfate, sodium thiosulfate and sodium sulfite are used as terminal electron acceptors.
but fumarate, iron (III), elemental sulfur, sodium nitrate and sodium nitrite are not used. Propanol, butanol and ethanol are transformed to propionate, butyrate and acetate, respectively with H$_2$S production. No desulfoviridin, or c-type cytochromes were detected in the crude bacterial extract, but a sulfite reductase was observed with an absorption peak at 580 nm. This sirohemoprotein is similar to the dissimilatory high-spin bisulfite reductase, P582, characteristic of the genera Desulfotomaculum and Desulfofoporusinus (Fauque and Barton, 2012).

For fatty acids analysis, the biomass of strain LINDBHT1$^T$ was standardized for its physiological age at the point of harvest according to Technical Note 101 of MIDI (http://www.microbialid.com/PDF/TechNote_101.pdf). Fatty acids were extracted using the method of Miller (1982) with the modifications of Kuykendall et al. (1988) and analysed by GC (model 6890N, Agilent Technologies) at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany, using the Microbial Identification System (MIDI, Sherlock Version 6.1; database, TSBA40). DNA was isolated and purified by chromatography on hydroxyapatite using the procedure of Cashion et al. (1977) and the G+C content was determined at the DSMZ by using HPLC as described by Membah et al. (1989).

The fatty acid profile of strain LINDBHT1$^T$ is composed only of four fatty acids [C$_{16:0}$ (39.1 %), C$_{18:0}$ (31.4 %), iso-C$_{15:0}$ (15.7 %) and a summed feature consisting of iso-C$_{17:1}$ I/anteiso-C$_{17:1}$ B (13.8 %)] (Table S1 available in IJSEM Online). The fatty acid composition of Desulfotomaculum halophilum has not been published (Tardy-Jacquenod et al., 1998) The fatty acid profile of Desulfotomaculum alkaliphilum is very different from that of strain LINDBHT1$^T$ [due to absence of iso-C$_{15:0}$ and the low amount of C$_{18:0}$ (5.6 %)] (Pikuta et al., 2000).

Phylogenetic analysis of 16S rRNA sequence comprising 1524 nt indicated that the LINDBHT1$^T$ is a member of the family Peptococcaceae, and was most closely related to Desulfotomaculum halophilum (similarity value 89.1%) and Desulfotomaculum alkaliphilum (similarity value 88.5%) of the genus Desulfotomaculum cluster If defined previously (Stackebrandt et al., 1997; Ogg & Patel, 2011). Compared with the sequences of members of other subclusters, the sequence of strain LINDBHT1$^T$ showed the high numbers of common signatures nucleotides with subcluster If (32 out of 42 signature nucleotides versus 18 with subcluster Ia, 15 with subcluster Ib, 23 with subcluster Ic, 21 with subcluster Id and 26 with subcluster Ie) defined by Ogg & Patel (2011) confirming its phylogenetic placement as a member of this cluster (Table S2). The 16S rRNA gene sequence of LINDBHT1 has been deposited in GenBank under accession number JX861507.

Moreover, phylogenetic analysis demonstrated affiliation of strain LINDBHT1$^T$ with representatives of the genus Desulfotomaculum. Strain LINDBHT1$^T$ had Desulfotomaculum halophilum and Desulfotomaculum alkaliphilum as its closest phylogenetic relatives with about 89 % similarity. LINDBHT1$^T$ has a G+C content of 44.4 mol%. The G+C content of species of the genus Desulfotomaculum is 37.5–57.0 mol% (Tardy-Jacquenod et al., 1998). Desulfotomaculum halophilum is a mesophilic, sulfate reducer which has a G+C content of 56.3 mol% and is phenotypically different from our isolate. Desulfotomaculum alkaliphilum is a thermophilic, alkaliphilic bacterium, with a DNA G+C content of 40.9 mol% (Table 1).

Strain LINDBHT1$^T$ is sufficiently different from Desulfotomaculum halophilum and Desulfotomaculum alkaliphilum to be classified as a representative of a novel species of the genus Desulfotomaculum.

**Description of Desulfotomaculum peckii sp. nov.**

Desulfotomaculum peckii [peck’i.i. N.L. gen. masc. n. peckii of Peck, named in memory and in honour of the biochemist Professor Harry D. Peck, Jr (Department of

**Table 1.** Differential characteristics of strain LINDBHT1$^T$ and the two phylogenetically most-closely related strains: *Desulfotomaculum halophilum* and *Desulfotomaculum alkaliphilum*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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</thead>
<tbody>
<tr>
<td>Gram stain</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Temperature (°C) (optimum)</td>
<td>50–65 (55–60)</td>
<td>30–40 (35)</td>
<td>30–58 (50–55)</td>
</tr>
<tr>
<td>pH (optimum)</td>
<td>5.9–9.2 (6–6.8)</td>
<td>6.9–8.0 (7.3)</td>
<td>8.0–9.15 (8.6–8.7)</td>
</tr>
<tr>
<td>NaCl (%) (optimum)</td>
<td>0.1–4 (2–3)</td>
<td>1–14 (4–6)</td>
<td>0.1–5 (0.1–1)</td>
</tr>
<tr>
<td>Motility</td>
<td>–</td>
<td>+ (polar flagellum)</td>
<td>–</td>
</tr>
<tr>
<td>Morphology</td>
<td>Rod-shaped</td>
<td>Straight to curved rods</td>
<td>Vibrioid or curved rod</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>44.4</td>
<td>56.3</td>
<td>40.9</td>
</tr>
<tr>
<td>Main substrates used</td>
<td>H$_2$/CO$_2$, propanol, butanol and ethanol</td>
<td>H$_2$/CO$_2$ + acetate, lactate, pyruvate, malate, formate + acetate, butanol and ethanol</td>
<td>H$_2$/CO$_2$ + acetate, lactate, pyruvate formate and ethanol</td>
</tr>
<tr>
<td>End products of fermentation</td>
<td>Propionate, butyrate, acetate</td>
<td>Acetate</td>
<td>Acetate</td>
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Biochemistry, University of Georgia, Athens, USA), for his great contribution to our knowledge of the biochemistry and physiology of SRB (mainly the discovery of oxidative phosphorylation in anaerobic micro-organisms in 1960)].

Cells are non-motile, spore-forming slightly curved rods, the spore position is central to subterminal. Staining Gram-positive, cells are approximately 2–5 μm in length and 1 μm in diameter and occur singly, in pairs or in chains. The major fatty acids were C_{16:0} (39.1 %), C_{18:0} (31.4 %), iso-C_{15:0} (15.7 %) and a summed feature consisting of iso-C_{17:1}^f anteiso-C_{17:1} B (13.8 %). The DNA G+C content of the type strain of the type species is 44.4 mol%.

Strictly anaerobic. The incubation time is about 2–3 days for cells in a good state. Yeast extract is required for growth. Propanol, butanol, ethanol, H_2:CO_2 and acetate + H_2:CO_2 are used as electron donors, but glucose, fructose, succinate, fumarate, butyrate, formate, malate, peptone, Casamino acids, formate + acetate, acetate, lactate and pyruvate are not utilized. Propanol, butanol and ethanol are transformed into propionate, butyrate and acetate, respectively with H_2S production. Sodium sulfate, sodium thiosulfate, sodium sulfite are used as terminal electron acceptors but fumarate, elemental sulfur, iron (III), sodium nitrate and sodium nitrite are not utilized.

The type strain, LINDBHTT (=DSM 23769T=JCM 17209T) was isolated from an upflow anaerobic filter treating abattoir wastewaters in Tunisia.

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References


Desulfotomaculum peckii sp. nov.


