Desulfotomaculum aquiferis sp. nov. and Desulfotomaculum profundi sp. nov., isolated from a deep natural gas storage aquifer

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Two novel strictly anaerobic bacteria, strains Bs105T and Bs107T, were isolated from a deep aquifer-derived hydrocarbonoclastic community. The cells were rod-shaped, not motile and had terminal spores. Phylogenetic affiliation and physiological properties revealed that these isolates belong to two novel species of the genus Desulfotomaculum. Optimal growth temperatures for strains Bs105T and Bs107T were 42 and 45 °C, respectively. The estimated G+C content of the genomic DNA was 42.9 and 48.7 mol%. For both strains, the major cellular fatty acid was palmitate (C16:0). Specific carbon fatty acid signatures of Gram-positive bacteria (iso-C17:0) and sulfate-reducing bacteria (C17:0 cyc) were also detected. An insertion was revealed in one of the two 16S rRNA gene copies harboured by strain Bs107T. Similar insertions have previously been highlighted among moderately thermophilic species of the genus Desulfotomaculum. Both strains shared the ability to oxidize aromatic acids (Bs105T: hydroquinone, acetophenone, para-toluic acid, 2-phenylethanol, trans-cinnamic acid, 4-hydroxybenzaldehyde, benzyl alcohol, benzoic acid 4-hydroxybutyl ester; Bs107T: ortho-toluic acid, benzoic acid 4-hydroxybutyl ester). The names Desulfotomaculum aquiferis sp. nov. and Desulfotomaculum profundi sp. nov. are proposed for the type strains Bs105T (=DSM 24088T=JCM 31386T) and Bs107T (=DSM 24093T=JCM 31387T).

Desulfotomaculum are known to use a wide range of substrates including sugars, organic acids and aromatic compounds. Members of this genus present various types of metabolism of interest such as hydrocarbon biodegradation (Morash et al., 2004; Aullo et al., 2016), chemolithoautotrophy (Daumas et al., 1988; Takai et al., 2001; Imachi et al., 2006) and metal transformation (Newman et al., 1997; Tebo & Obraztsova, 1998; Junier et al., 2009; Otwell et al., 2015). Desulfotomaculum are commonly found in deep subsurface environments such as mines (Baker et al., 2003; Trimarco et al., 2006; Kaksonen et al., 2006), aquifers (Baker et al., 2003, 2009; Giese et al., 2009; Aullo et al., 2016), hot oil-field waters (Nazina & Rozanova, 1978) and hydrothermal vent sediments (Cha et al., 2013; Aüllo et al., 2013). Members of this genus can be the only sulfate-reducers and...
As reported by Aüllo et al. (2016), the BTEX-amended microbial enrichments contained the following basal medium (per liter of distilled water): 0.3 g KH₂PO₄, 0.3 g K₂HPO₄, 0.5 g NH₄Cl, 0.5 g NaCl, 2 g Na₂SO₄, 0.1 g MgCl₂, 6H₂O, 0.06 g CaCl₂, 2H₂O, 1 ml of 0.1 % resazurin and a 1 ml anoxic trace-element solution (Pfennig et al., 1981). The basal medium was sterilized by autoclaving for 20 min at 120 °C and immediately flushed under a stream of O₂-free N₂ gas before being cooled to room temperature. Sterile and anaerobic solutions of 2 g FeCl₃·4H₂O, 2.55 g Na₂S·H₂O and 4 ml of vitamins (Eichler & Pfennig, 1986) were added to the medium separately. The pH was adjusted to pH 8.0 with 10 M KOH. Strain Bs107ᵀ was isolated from a benzene-amended enrichment (10 ppm), while strain Bs105ᵀ originated from a BTEX-amended enrichment containing 10 ppm of each compound (benzene, toluene, ethylbenzene and p-, o-, m-xylene; Sigma-Aldrich). For facilitating the isolation of spore-forming microorganisms, enrichment cultures were pasteurized at 80 °C for 10 min. Several media and conditions were used to separate the two representatives of the genus Desulfotomaculum. For Bs107ᵀ, the enrichment was subcultured several times using the commercial BSR Labège test kit (CFG). After 8 days of incubation, black colonies developed and axenic cultures were obtained by spreading repeatedly on Petri dishes incubated anaerobically. Regarding Bs105ᵀ, the enrichment was subcultured several times with a medium designed to isolate fermentative microorganisms with the following substrates (per liter of distilled water): 15 g biotryptcase (bioMérieux), 10 g yeast extract, 2.5 g glucose, 1 g L-cysteine, 15 g agar and 1 ml of 0.1 % resazurin at pH 7 in an anaerobic glove box (Getinge La Calhene). Several white, irregularly shaped colonies were obtained after 6 days of incubation and repeated serial dilution to obtain pure cultures. Finally, some of the colonies were enriched on the commercial BSR Labège test kit. For routine cultivation and characterization, the following anoxic medium was used (per liter): 0.3 g KH₂PO₄, 0.3 g K₂HPO₄, 1.0 g NH₄Cl, 1.0 g NaCl, 3.0 g Na₂SO₄, 0.1 g KCl, 0.1 g CaCl₂·2H₂O, 0.5 g MgCl₂·6H₂O, 1 ml of trace mineral element solution (Widdel & Pfennig, 1981) and 1 ml of 0.1 % resazurin. For Bs107ᵀ, the addition of 3 µg Na₂SeO₃ 1⁻¹ to the basal medium was required for growth. At room temperature, 10 mg Na-dithionite 1⁻¹, 0.5 g Na₂S·9H₂O·1·1⁻¹, 2 g NaHCO₃ and 10 ml vitamin solution (Balch et al., 1979) were added from sterile stock solutions. The pH was adjusted to pH 7.2 with 10 M NaOH. The Hugute technique (Hugute, 1969) was used throughout the entire culture investigation process. In short, 5 ml aliquots were placed in the Hungate tubes (Dutscher) under a stream of O₂-free N₂:CO₂ (80:20) and the tubes were autoclaved. Purity was checked by phase contrast microscopy (Olympus BX60), by the terminal-RFLP technique (data not shown) and by cloning-sequencing. For Transmission Electron Microscopy (TEM), exponentially grown cells were negatively stained with 1 % sodium phosphotungstic acid (pH 7.2) and were fixed with a mixture of 2 % glutaraldehyde (v/v) and 2.5 % formaldehyde (v/v) in a 0.1 M phosphate-buffered solution at pH 7.6. For Scanning Electron Microscopy (SEM) observations, cells were fixed with a formaldehyde solution (2.5 %) in a saline buffer. For TEM, whole cells were observed using a Hitachi H 600 model electron microscope at an accelerating voltage of 75 kV. For SEM, whole cells were observed using a high-resolution field emission gun-sourced SEM with low vacuum and wet mode (Peltier stage) capability (environmental SEM: FEI XL30 ESEM FEG with Oxford Instruments INCA Energy) and also using a high vacuum electron microscope (Zeiss NTS S660 SEM with Oxford Instruments INCA Energy Plus) fitted with secondary and backscattered electron detectors. Cells of both strains were non-motile rods, Gram-positive-stained, with no visible flagella. Their ability to form spores checked by pasteurization tests performed at 80, 90 and 100 °C for 10 and 20 min and confirmed. The presence of spherical and terminal spores was observed in both strains under starving conditions or under the effects of heat stress. Cells of strain Bs105ᵀ were oval rods, approximately 0.7 µm wide and 2 µm long, while cells of strain Bs107ᵀ were long rods, approximately 0.5 µm wide and 3 µm long (Fig. 1).

In order to establish the phospholipid fatty acid profiles, standardization of the biomass of strains Bs105ᵀ and Bs107ᵀ was applied using its physiological age at the point of harvest as recommended in MIDI Technical Note 101 (Sasser, 1990; see www.midi-inc.com). Miller’s method (Miller, 1982), modified by Kuykendall et al. (1988), was used, and the analysis was performed by gas chromatography (Agilent Technologies model 6890N) using the Microbial Identification System (MIDI Sherlock version 6.1; database, TSBA40). The complete fatty acid profiles of strains Bs105ᵀ and Bs107ᵀ grown on pyruvate were compared to those of D. putei, D. hydrothermalis and D. aeromaticum (Table 2). The phospholipid fatty acid patterns mainly consisted of saturated fatty acids.
Table 1. Physical and chemical parameters associated with the water sampled from the underground gas storage aquifer (Paris Basin, France) and analysed at atmospheric pressure

<table>
<thead>
<tr>
<th>Physical parameters</th>
<th>Paris Basin, France</th>
</tr>
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<tr>
<td>Temperature</td>
<td>37 °C</td>
</tr>
<tr>
<td>pH</td>
<td>8.2</td>
</tr>
<tr>
<td>Redox conditions (mV)</td>
<td>From −540 to −700</td>
</tr>
<tr>
<td>Conductivity (µS cm⁻¹, 20 °C)</td>
<td>1902.50</td>
</tr>
<tr>
<td>Sulfide (mg S l⁻¹)</td>
<td>0.2</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Chemical composition (mg l⁻¹)</th>
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<tr>
<td>Ca²⁺</td>
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<td>Mg²⁺</td>
</tr>
<tr>
<td>Na⁺</td>
</tr>
<tr>
<td>K⁺</td>
</tr>
<tr>
<td>NH₄⁺</td>
</tr>
<tr>
<td>Mn²⁺</td>
</tr>
<tr>
<td>Fe²⁺</td>
</tr>
<tr>
<td>CO₃⁻</td>
</tr>
<tr>
<td>HCO₃⁻</td>
</tr>
<tr>
<td>Cl⁻</td>
</tr>
<tr>
<td>SO₄²⁻</td>
</tr>
<tr>
<td>F⁻</td>
</tr>
<tr>
<td>NO₃⁻</td>
</tr>
<tr>
<td>SiO₃²⁻</td>
</tr>
</tbody>
</table>

DNA was extracted from cultures by using a commercial DNA extraction kit (Bio-Rad InstaGene Matrix) according to the manufacturer’s protocol. The bacterial 16S rRNA gene was directly amplified from pure culture with the forward bacterial primer 8F (5′-AGAGTTTGATCCTTGCTGCTAG-3′) (Weisburg et al., 1991) and the reverse universal primer U1492R (5′-GTTACCTTGTTACGACTT-3′) (Lane, 1991). DNA amplicons of appropriate sizes were purified using the GFX DNA and Gel Band purification kit (Amersham Biosciences). To obtain the complete sequence of the 16S rRNA gene, purified PCR products from the two isolates were then cloned using the Topo TA cloning 10F' kit (Invitrogen) in TOP 10 Escherichia coli cells according to the manufacturer’s instructions. Inserts of an appropriate size amplified from ten clones of strain Bs105T and Bs107T were then sequenced by QIAGEN Genomic Services (Hilden, Germany). Alignment of the full sequence was performed using the DIALIGN-TX web server (Morgenstern, 2004) in order to obtain the best alignment. Reference sequences were obtained from the National Center of...
Published data retrieved from the literature for other described species of the genus *Desulfotomaculum* are also indicated. Carbon sources used for cultivation are indicated thereafter in : 1, strain BS105\(^T\) (pyruvate); 2, strain BS107\(^T\) (pyruvate); 3, *D. putei* TH-11\(^T\) (modified MSA medium with lactate and yeast extract); 4, *D. hydrothermale* Lam5\(^T\) (glucose and yeast extract); 5, *D. aeuracutum* 9\(^T\) (pyruvate). Data for reference species were taken from Liu et al. (2008) and Hagenauer et al. (1997). Fatty acids amounting to <1% of the total fatty acids are not shown.

### Table 2. Fatty acid composition in strains BS105\(^T\) and BS107\(^T\)

<table>
<thead>
<tr>
<th>Fatty acid</th>
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<th>4</th>
<th>5</th>
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<td>Straight-chain saturated</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14:0</td>
<td>5.3</td>
<td>2.3</td>
<td>8.8</td>
<td>–</td>
<td>1.5</td>
</tr>
<tr>
<td>C15:0</td>
<td>–</td>
<td>–</td>
<td>3.3</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C16:0</td>
<td>29.0</td>
<td>28.0</td>
<td>20.7</td>
<td>10.7</td>
<td>12.1</td>
</tr>
<tr>
<td>C17:0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.0</td>
<td>–</td>
</tr>
<tr>
<td>C18:0</td>
<td>1.7</td>
<td>4.3</td>
<td>1.0</td>
<td>1.9</td>
<td>2.1</td>
</tr>
<tr>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>iso-C15:0</td>
<td>8.0</td>
<td>6.0</td>
<td>15.4</td>
<td>15.7</td>
<td>7.7</td>
</tr>
<tr>
<td>iso-C16:0</td>
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<td>–</td>
<td>1.8</td>
<td>–</td>
<td>–</td>
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<td>iso-C17:0</td>
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<td>6.6</td>
<td>5.2</td>
<td>35.5</td>
<td>6.5</td>
</tr>
<tr>
<td>anteiso-C15:0</td>
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<td>–</td>
<td>1.6</td>
<td>2.0</td>
<td>1.2</td>
</tr>
<tr>
<td>anteiso-C17:0</td>
<td>1.3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4.3</td>
</tr>
<tr>
<td>Monounsaturated</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:1ω9c</td>
<td>5.5</td>
<td>5.5</td>
<td>4.4</td>
<td>3.6</td>
<td>16.0</td>
</tr>
<tr>
<td>C16:1ω7c</td>
<td>16.9</td>
<td>15.7</td>
<td>13.3</td>
<td>1.3</td>
<td>5.2</td>
</tr>
<tr>
<td>C18:1ω5c</td>
<td>1.3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C18:1ω9c</td>
<td>1.2</td>
<td>2.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C18:1ω7c</td>
<td>4.2</td>
<td>6.1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>iso-C17:1ω9c</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>iso-C17:1ω7c</td>
<td>–</td>
<td>–</td>
<td>12.1</td>
<td>13.2</td>
<td>–</td>
</tr>
<tr>
<td>iso-C17:1anteiso-C17:1 B</td>
<td>3.8</td>
<td>6.8</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cyclopropyls</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C17:0vc</td>
<td>–</td>
<td>1.5</td>
<td>–</td>
<td>5.8</td>
<td>–</td>
</tr>
</tbody>
</table>

Biotechnology Information (NCBI) nucleotide database including the GenBank, EMBL and DDBJ databases. Pairwise evolutionary distances based on partial sequences of 16S rRNA genes were computed according to the Jukes & Cantor (1969) method using MEGA 6.06 software (Tamura et al., 2013). Comparison of the 16S rRNA gene sequences showed phylogenetic affiliation of both strains to the *Desulfotomaculum* subcluster Ia as defined by Stackebrandt et al. (1997). Nevertheless, both strains were phylogenetically distinct, exhibiting a low level of similarity between them (94.3% identity; Fig. 2). The closest affiliation of strain BS105\(^T\) (96% identity) was with an environmental clone retrieved from a deep subsurface aquifer in South Africa (AY604051). This strain also shared high similarities with *Desulfotomaculum* and environmental sequences from sites contaminated with heavy metals (DRU95951) or hydrocarbons (JN038217). On the other hand, strain BS107\(^T\) had the anaerobic bacterium Kupl, isolated from an alkaline environment, and two uncultured bacteria originating from deep environments, as its closest phylogenetic relatives. DNA for measuring G+C content was extracted and purified by chromatography on hydroxyapatite using the procedure of Cashion et al. (1977). As for the other members of the *Desulfotomaculum* subcluster Ia, strains BS105\(^T\) and BS107\(^T\) had a low DNA G+C content (42.9% for strain BS105\(^T\) and 48.7% for strain BS107\(^T\); Table 3) as determined by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany), using the method of Mesbah et al. (1989).

As shown in Fig. S1 (available in the online Supplementary Material), analysis of 16S rRNA gene sequences of strain BS107\(^T\) revealed the intragenomic macroheterogeneity of two 16S rRNA gene copies as already described in *D. australicum* (Patel et al., 1992), *D. kuznetsovii*, *D. thermonbenzoicum* TSB\(^T\), *D. thermoacetoxidans* CAMZ\(^T\), *Desulfotomaculum* sp. DSM 7475 and *D. luciae* SLT\(^T\) (Tourova et al., 2001) in particular and more recently, in strain UFO1, a fermentative Firmicute (*Veillonellaceae*) isolated from a subsurface environment (Ray et al., 2010). A short insertion sequence (110 nt long) was present in helix 6 of the 16S rRNA at the 5’ terminal region of the gene corresponding to *E. coli* nucleotide positions 69–180. A comparison of 16S rRNA gene copies from strain BS107\(^T\) (copies with and without inserts) revealed a difference of 3.2% between the two copies of the 16S rRNA gene, which is lower than the heterogeneity found in 16S rRNA gene copies of *D. kuznetsovii*, evaluated at 8% (Tourova et al., 2001; Acinas et al., 2004). As observed for the 100bp insertion sequence from strain UFO1 (Ray et al., 2010), the secondary structure predicted using the RNAfold Web Server software (Hofacker et al., 1994; Gruber et al., 2008) showed the formation of an energetically favourable stem loop structure with a minimum free energy of −62.53 kcal mol\(^{-1}\), as compared to −63.33 kcal mol\(^{-1}\) for the non-insert gene (Fig. S2). The insertion of a few nucleotides into the gene sequence encoding the 16S rRNA gene is usual, but insertions exceeding a hundred nucleotides are much rarer (Tourova et al., 2001). Several authors have questioned the impact of such insertions on micro-organisms. Firstly, these inserts do not appear to be random in the 16S rRNA gene as they are carried out inside the hypervariable helix 6, producing a longer version of it (Patel et al., 1992; Tourova et al., 2001; Villemur et al., 2007; Ray et al., 2010). In the case of *Clostridium paradoxum*, this superlong insertion seems silent (Rainey et al., 1996). Since helix 6 seems to play a key role in the structure of the ribosome, we can assume that there is a slight structural difference in the ribosome impacted by the insertion (Patel et al., 1992). Several authors have hypothesized the presence of divergent copies of rRNA [with or without insert(s)] as a possible adaptation to improve fitness under different temperature conditions (López-López et al., 2007; Ray et al., 2010). These insertions in helix 6 of the 16S rRNA are recurrent among the members of the order *Clostridiales* (Case et al., 2007; Villemur et al., 2007; Ray et al., 2010). These inserts have probably originated from

\[ \text{4332} \]
horizontal gene transfer. Specific comparison of the insertion region with other insertion sequences showed an unexpected affiliation with moderately thermophilic organisms belonging to the family Peptococcaceae (Desulfotomaculum, Desulfurispora and Desulfitibacter), in particular with an environmental sequence related to the genus Desulfotomaculum (accession no. HE672493) from alkaline groundwater at 130 m depth (Fig. S3). Unlike D. kuznetsovii (Tourova et al., 2001), the heterogeneity of the 16S rRNA gene sequence has very little impact on the positioning of Bs107T within the phylogeny of the genus Desulfotomaculum (Fig. 2).

The ability of both strains Bs105T and Bs107T to grow with different energy sources (20 mM) and terminal electron acceptors was determined by measuring optical density (580 nm) in Hungate tubes containing 10 ml basal medium. Growth was measured by inserting tubes directly into a Cary 50 model Scan spectrophotometer (Varian). The end products of metabolism were measured as described previously (Haouari et al., 2008), using HPLC
Table 3. Characteristics of strains Bs105<sup>T</sup> and Bs107<sup>T</sup> compared with those of other species of the Desulfotomaculum genus

Reference strains: 1, strain Bs105<sup>T</sup>; 2, strain Bs107<sup>T</sup>; 3, D. putei TH-11<sup>T</sup>; 4, D. hydrothermale Lam5<sup>T</sup>; 5, D. varum RH04-3<sup>T</sup>; 6, D. aeronactium 9<sup>T</sup>; 7, D. reducens MI-1<sup>T</sup>. Data for reference species were taken from Liu et al. (1997), Haouari et al. (2008), Ogg & Patel (2011), Hagenauer et al. (1997), Tebo & Obraztsova (1998) and Visser et al. (2016). +, positive; −, negative; +/−, weakly positive; *, with acetate; ND, no data.

<table>
<thead>
<tr>
<th>Characteristic</th>
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<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tbody>
<tr>
<td>DNA G+C content (mol%)</td>
<td>42.9</td>
<td>48.7</td>
<td>47.1</td>
<td>46.8</td>
<td>52.4</td>
<td>43.8</td>
<td>42.3</td>
</tr>
<tr>
<td>Gram stain</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>pH range (optimum)</td>
<td>6.2–8.0</td>
<td>6.3–7.8</td>
<td>6–7.9</td>
<td>5.8–8.2</td>
<td>5.0–8.5</td>
<td>6.0–9.0</td>
<td>+</td>
</tr>
<tr>
<td>Temperature range (optimum)</td>
<td>30–45°C</td>
<td>30–60°C</td>
<td>40–65°C</td>
<td>40–60°C</td>
<td>37–55°C</td>
<td>20–42°C</td>
<td>(7.0–7.2)</td>
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<tr>
<td>NaCl range (optimum)</td>
<td>0–2.5%</td>
<td>0–0.5%</td>
<td>&lt;2%</td>
<td>0–1.5%</td>
<td>0–1%</td>
<td>0–2.5%</td>
<td>(0–2%)</td>
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<tr>
<td>Spore location</td>
<td>Terminal</td>
<td>Terminal</td>
<td>Paracentral</td>
<td>Terminal</td>
<td>Subterminal to terminal</td>
<td>Subterminal to central</td>
<td>Subterminal to terminal</td>
</tr>
<tr>
<td>Sulfate</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<td>Sulfite</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>ND</td>
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<td>Thiosulfate</td>
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<td>+</td>
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<tr>
<td>Formate</td>
<td>−</td>
<td>+</td>
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<td>+</td>
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<td>−</td>
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after 4 weeks of incubation at optimal temperature and pH. Dissolved sulfide was determined photometrically as colloidal CuS using the method described by Cord-Ruwisch (1985). Optimal growth condition measurements (NaCl, temperature, pH) were all carried out in duplicate and repeated four times with pyruvate (20 mM) as the energy source and sulfate (20 mM) as the electron acceptor. Vitamins were required for growth, but not yeast extract or tryptone. Growth was tested at temperatures ranging from 20 to 60 °C, at NaCl concentrations from 0 to 4% and at pH from 5.5 to 9.0. NaCl was weighed directly in the tubes prior to dispensing the medium. Strain Bs105<sup>T</sup> grew at temperatures from 30 to 45 °C (optimum 42 °C), in the pH range from 6.2 to 8.0 (optimum 7.4) and at NaCl concentrations up to 2.5% (optimum 2%) (Table 3). Strain Bs107<sup>T</sup> grew at temperatures from

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30 to 60 °C (optimum 45 °C), in the pH range from 6.3–7.8 (optimum 7.2) and with up to 0.5% NaCl (optimum in the absence of NaCl). Because of the ability of strain Bs107 to grow at up to 60 °C, the latter should be considered as a moderate thermophile. Under optimal conditions, the doubling time for Bs105 and Bs107 were 8.3 h and 7.2 h, respectively. Neither strain used oxygen, nitrite, nitrite, elemental sulfur nor iron (III) oxide as terminal electron acceptors. Both strains used sulfate, sulfite and thiosulfate as terminal electron acceptors. Strain Bs107 may use sulfite at a concentration of up to 20 mM.

Bs105 grew on lactate (10 mM), fumarate (10 mM), pyruvate (10 mM), ethanol (10 mM), propanol (10 mM), H₂/acetate as carbon source (1 bar/10 mM), hydroquinone (20 mM), acetoephone (20 mM), p-toluic acid (20 mM), 2-phenylethanol (20 mM), trans-cinnamic acid (20 mM), 4-hydroxybenzaldehyde (20 mM), benzy alcohol (20 mM) and benzoic acid 4-hydroxybutyl ester (20 mM) under sulfate-reducing conditions (Tables 3 and 4). Optimum growth was obtained in the presence of propanol or pyruvate as energy sources. No growth was observed for Bs105 on acetate (10 mM), citrate (5 mM), formate/acetate as carbon source (20 mM/2 mM), formate (10 mM), malate (10 mM), succinate (10 mM), butyrate (5 mM), propionate (10 mM), glycerol (10 mM), methanol (10 mM), butanol (10 mM), pentanol (10 mM), fructose (5 mM), glucose (5 mM), lactose (5 mM), mannose (5 mM), peptone (0.5 g l⁻¹), casamino acids (0.5 g l⁻¹), acetone (5 mM), yeast extract (0.5 g l⁻¹), styrene (20 mM), resorcinol (20 mM), catechol (20 mM), benzy alcohol (20 mM), o-toluic acid (20 mM), 3-phenylethanol (20 mM) or benzoic acid butyl ester (20 mM). Bs107 grew on formate/acetate as carbon source (20 mM/2 mM), formate (10 mM), fumarate (10 mM), pyruvate (10 mM), glycerol (10 mM), ethanol (10 mM), butanol (10 mM), H₂/CO₂ (80/20, 2 bars), H₂/acetate as carbon source (1 bar/10 mM), o-toluic acid (20 mM) and benzoic acid 4-hydroxybutyl ester (20 mM) under sulfate-reducing conditions. It is noteworthy that in contrast to strain Bs105, strain Bs107 was also able to carry out lithotrophic growth using hydrogen as electron donor. For this strain, no growth was observed with acetate (10 mM), lactate (10 mM), malate (10 mM), succinate (10 mM), butyrate (5 mM), propionate (10 mM), methanol (10 mM), pentanol (10 mM), propanol (10 mM), lactate (5 mM), glucose (5 mM), fructose (5 mM), mannose (5 mM), peptone (0.5 g l⁻¹), casamino acids (0.5 g l⁻¹), styrene (20 mM), resorcinol (20 mM), hydroquinone (20 mM), catechol (20 mM), acetoephone (20 mM), p-toluic acid (20 mM), 2-phenylethanol (20 mM), 3-phenylethanol (20 mM), trans-cinnamic acid (20 mM), 4-hydroxybenzaldehyde (20 mM), benzylic acid (20 mM), benzoic acid butyl ester (20 mM) or benzy alcohol (20 mM).

Bs105 and Bs107 were able to ferment fumarate (10 mM) as shown in Table 3. Only Bs107 fermented pyruvate (10 mM). No growth on sugars and alcohol was observed under fermentative conditions.

### Table 4. Aromatic compound (20 mM) utilization by strain Bs105 and strain Bs107

<table>
<thead>
<tr>
<th>Aromatic compound</th>
<th>Bs105</th>
<th>Bs107</th>
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<tbody>
<tr>
<td>Hydroquinone</td>
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</tr>
<tr>
<td>Acetophenone</td>
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<td>−</td>
</tr>
<tr>
<td>ortho-Toluic acid</td>
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<tr>
<td>para-Toluic acid</td>
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</tr>
<tr>
<td>2-Phenylenol</td>
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<td>+</td>
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<tr>
<td>trans-Cinnamic acid</td>
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<tr>
<td>4-Hydroxybenzaldehyde</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Benzoic acid 4-hydroxybutyl ester</td>
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</table>

While *Desulfotomaculum* have been detected in various anaerobic hydrocarbon-degrading enrichments, only one of them has been described as a hydrocarbonoclastic species (Morasc et al., 2004). Interestingly, *Desulfotomaculum* seem to be key players in microbial communities able to degrade contaminants such as biphenyl (Selesi et al., 2009), and monoaromatic hydrocarbons from shallow (Morasc et al., 2004; Taubert et al., 2012) or deep aquifers (Basso et al., 2009; Berlendis et al., 2010; Aüllo et al., 2016). Strains Bs105 and Bs107 were isolated from a bacterial community capable of degrading ethylbenzene, toluene and benzene (Aüllo et al., 2016) wherein previous molecular ecology investigations highlighted the presence of only two species after successive enrichments. Here we provide evidence that both strains isolated corresponding to clone Bc105 (KF591690) and clone Bc107 (KF591689) retrieved from the original microbial community degrading BTEX were able to oxidize aromatics. Because of the lengthy incubation time required to obtain the degradation of monoaromatic hydrocarbons (100 to 270 days for 100 ppm of hydrocarbons), the isolation procedure was carried out in rich media to enhance the biomass and repeated several times in order to separate these two representatives of the *Desulfotomaculum*. We unsuccessfully conducted hydrocarbon degradation tests by varying the culture conditions. However, we hypothesize that the capacity to form endospores can help *Desulfotomaculum* to remain key species after drastic changes in the environment such as massive natural gas injection and withdrawal inside deep geological storage aquifers. Because of the metabolic capacities of this genus, *Desulfotomaculum* spp. could play a major ecological role in bioremediation of contaminated (hydrocarbons or metals) deep environments, and in a general way further investigations would be needed to assess whether they might play a key role in the deep carbon cycle in subterranean environments as previously suggested (Detmers et al., 2004; Aüllo et al., 2013).
Description of Desulfotomaculum aquiferis sp. nov.

Desulfotomaculum aquiferis (a.qui.fe’ris. N.L. gen. n. aquiferis of an aquifer, where the type strain was isolated).

This species is an anaerobic, terminal-spore-forming bacterium (no flagellum observed) in the form of an oval rod (long oval rod during exponential growth; length of 2 μm and width of 0.7 μm). This strain is not motile and optimal growth is obtained at 42 °C (30–45 °C), pH 7.4 (6.2–8.0) and with 2% NaCl (0–2.5%). Growth requires vitamins and occurs on ethanol, propanol, fumarate, pyruvate, lactate and H2/acetate as carbon sources. Aromatic acids such as hydroquinone, acetophenone, trans-cinnamic acid, 4-hydroxybenzaldehyde, benzyl alcohol, benzoic acid 4-hydroxybutyl ester, 2-phenylethanol and p-toluic acid are used under sulfate-reducing conditions. Sulfate, thiosulfate and sulfite can be used as electron acceptors. Fumarate is fermented. The cellulosic fatty acids consist mainly of C16:0 and C16:1ω7c. Desulfoviridin is not detected. The DNA G+C content of the type strain is 42.9 %. The Genbank/EMBL/DDBJ accession number for the 16S rRNA gene is KF591693. The type strain, Bs107T (=DSM 240883T=JCM 31386T), is isolated from a deep aquifer formation (–760 m) near Paris (France).

The type strain, Bs107T (=DSM 240933T=JCM 31387T), is isolated from a deep aquifer formation (–760 m) near Paris (France).

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References


