Pedobacter tournemirensis sp. nov., isolated from a fault water sample of a deep Toarcian argillite layer

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A Gram-negative bacterium, designated TF5-37.2-LB10T, was isolated from subsurface water of the Toarcian geological layer of Tournemire, France. Cells were non-motile straight rods that formed cream to light pink colonies on 10-fold diluted LB agar. Strain TF5-37.2-LB10T contained menaquinone 7 and its major fatty acids were iso-C15:0 summed feature 3 (iso-C15:0 2-OH and/or C16:1ω7c), iso-C17:0 3-OH and iso-C17:1ω9c. The G+C content of the genomic DNA was 46 mol%. Phylogenetic analysis of the 16S rRNA gene sequence placed strain TF5-37.2-LB10T within the genus Pedobacter, family Sphingobacteriaceae. Pedobacter composti TR6-06T and Pedobacter oryzae DSM 19973T were the closest phylogenetic relatives (93.5 and 93.3 % 16S rRNA gene sequence similarity, respectively). On the basis of 16S rRNA gene sequence comparison and physiological and biochemical characteristics, strain TF5-37.2-LB10T represents a novel species of the genus Pedobacter, for which the name Pedobacter tournemirensis sp. nov. is proposed. The type strain is TF5-37.2-LB10T (=DSM 23085T=CIP 110085T=MOLA 820T).

The genus Pedobacter (comprising the type species Pedobacter heparinus as well as Pedobacter piscium, Pedobacter africanus and Pedobacter saltans) was described by Steyn et al. (1998) as a new genus of the newly proposed family Sphingobacteriaceae in the phylum Bacteroidetes. Since then, the number of Pedobacter species has greatly increased and, at the time of writing, this genus encompasses 35 species with validly published names (www.bacterio.cict.fr). Members of the genus have been isolated from different environments such as soils and compost (Yoon et al., 2006), freshwater (An et al., 2007; Shivaji et al., 2005), glacier ice (An et al., 2009; Muurholm et al., 2007) and activated sludge (Steyn et al., 1998). All members of the genus Pedobacter are Gram-negative rods with menaquinone 7 (MK-7) as the major respiratory quinone and iso-C15:0 summed feature 3 (iso-C15:0 2-OH and/or C16:1ω7c) and iso-C17:0 3-OH as the major fatty acids. All strains tested contain sphingolipids (Steyn et al., 1998). In this paper, a strain isolated from water circulating in a fault inside a deep geological argillite layer and sharing 93.5 % 16S rRNA gene sequence similarity with Pedobacter composti TR6-06T, the nearest relative, is characterized as a novel species belonging to the genus Pedobacter.

A water sample was collected from a borehole intersecting a fracture geodic cavity in the Toarcian argillite at Tournemire, Aveyron, France (43° 58’ 40” N, 3° 00’ 38” E) (Beaucaire et al., 2008). A subsample was incubated in 10-fold diluted LB broth (Sigma) at 37 °C. An aliquot of the enrichment culture was spread on 10-fold diluted LB agar and incubated at 37 °C for 2 days. Colonies were picked and subsequently purified three times. Of these colonies, an isolate forming a cream colony was selected and designated strain TF5-37.2-LB10T.

The 16S rRNA gene of strain TF5-37.2-LB10T was amplified with primers 8F and 1525R according to Basso et al. (2005). Sequencing and phylogenetic analysis were performed according to Urios et al. (2006). Briefly, sequences were analysed for nearest neighbours using BLAST (Altschul et al., 1997) on the GenBank database. Alignments and similarity levels were obtained using CLUSTAL X (Thompson et al., 1994). Phylogenetic reconstructions were produced using the neighbour-joining method (Felsenstein, 1981) and maximum-likelihood (Felsenstein, 1985) methods. Bootstrap values were determined according to Felsenstein (1985) with 1000 replicates. Strain TF5-37.2-LB10T was phylogenetically affiliated to the genus Pedobacter in the family Sphingobacteriaceae (Fig. 1). Sequence similarity was determined using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net; Kim et al., 2012). The closest relatives were P. composti TR6-06T (Lee et al., 2009) and Pedobacter oryzae DSM 19973T (Jeon et al., 2009) with 93.5 and 93.3 %
Fig. 1. Neighbour-joining phylogenetic tree showing the positions of strain TF5-37.2-LB10\(^T\) and members of the genus *Pedobacter* and other genera in the family *Sphingobacteriaceae*. Bootstrap values (>70\%) based on 1000 replicates are shown at branch nodes. *Balneola vulgaris* DSM 17893\(^T\) was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.
16S rRNA gene sequence similarity, respectively. The G+C content of strain TF5-37.2-LB10\(^T\) was determined by the Identification Service of the DSMZ (Braunschweig, Germany) using HPLC according to Mesbah et al. (1989). The G+C content of the genomic DNA of strain TF5-37.2-LB10\(^T\) was 46 mol%, a value significantly higher than those reported for \(P.\) composti LMG 23490\(^T\) (41.9 mol%) and \(P.\) oryzae DSM 19973\(^T\) (37.7 mol%).

Microscopic observations showed that cells of strain TF5-37.2-LB10\(^T\) were non-motile rods. Gliding motility was not observed, neither by microscopic examination of the edge of a young colony on R2A medium nor by the hanging drop technique (Bernardet et al., 2002). The Ryu KOH reaction (Powers, 1995) led to immediate cell lysis, which was confirmed by microscopy. This positive reaction indicated that the strain was Gram-negative.

Growth conditions of strain TF5-37.2-LB10\(^T\), \(P.\) composti LMG 23490\(^T\) and \(P.\) oryzae DSM 19973\(^T\) were tested on 10-fold diluted LB agar (Jeon et al., 2009) with 1–5 % NaCl (at 0.5 % intervals), at pH 5.0–10.0 (at 1 pH unit intervals).

### Table 1. Differential characteristics of strain TF5-37.2-LB10\(^T\) and its closest phylogenetic neighbours in the genus *Pedobacter*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature range (°C)</td>
<td>12–37</td>
<td>15–37</td>
<td>15–35</td>
</tr>
<tr>
<td>pH range</td>
<td>6.0–9.0</td>
<td>5.0–8.0</td>
<td>6.0–8.0</td>
</tr>
<tr>
<td>NaCl range (%)</td>
<td>0–2</td>
<td>0–1</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Utilization of (API 50CH and GN2 MicroPlate):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycerol</td>
<td>–</td>
<td>W</td>
<td>W</td>
</tr>
<tr>
<td>D-Arabinose</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mannitol</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Xyitol</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Methyl β-D-xyloside</td>
<td>–</td>
<td>W</td>
<td>W</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>+</td>
<td>–</td>
<td>(+)</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+</td>
<td>–</td>
<td>(+)</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Methyl α-D-mannoside</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Arbutin</td>
<td>–</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Inulin</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Starch</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Glycogen</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gentiose</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Turanose</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Trehalose</td>
<td>+</td>
<td>–</td>
<td>(+)</td>
</tr>
<tr>
<td>Melibiose</td>
<td>+</td>
<td>(+)</td>
<td>–</td>
</tr>
<tr>
<td>Salicin</td>
<td>–</td>
<td>–</td>
<td>(+)</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Enzyme activities (API ZYM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trypsin</td>
<td>–</td>
<td>–</td>
<td>w</td>
</tr>
<tr>
<td>α-Chymotrypsin</td>
<td>–</td>
<td>–</td>
<td>w</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>+</td>
<td>+</td>
<td>w</td>
</tr>
<tr>
<td>Naphthol-AS-BI-phosphohydrolase</td>
<td>+</td>
<td>–</td>
<td>w</td>
</tr>
<tr>
<td>α-Galactosidase</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>+</td>
<td>W</td>
<td>+</td>
</tr>
<tr>
<td>N-Acetyl-β-glucosaminidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>46.0</td>
<td>41.9</td>
<td>37.7</td>
</tr>
</tbody>
</table>
Table 2. Fatty acid content of strain TF5-37.2-LB10\textsuperscript{T} and its closest phylogenetic neighbours in the genus Pedobacter

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>C\textsubscript{15:0}</td>
<td>–</td>
<td>6.0</td>
<td>9.5</td>
</tr>
<tr>
<td>C\textsubscript{16:0}</td>
<td>1.3</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>C\textsubscript{15:0} 2-OH</td>
<td>–</td>
<td>–</td>
<td>1.1</td>
</tr>
<tr>
<td>C\textsubscript{16:0} 3-OH</td>
<td>Tr</td>
<td>1.1</td>
<td>Tr</td>
</tr>
<tr>
<td>C\textsubscript{17:0} 2-OH</td>
<td>–</td>
<td>–</td>
<td>4.1</td>
</tr>
<tr>
<td>iso-C\textsubscript{13:0} 1.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>iso-C\textsubscript{14:0}</td>
<td>–</td>
<td>–</td>
<td>1.4</td>
</tr>
<tr>
<td>iso-C\textsubscript{15:0}</td>
<td>45.6</td>
<td>31.7</td>
<td>10.7</td>
</tr>
<tr>
<td>iso-C\textsubscript{15:1} F</td>
<td>1.5</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>iso-C\textsubscript{16:0}</td>
<td>–</td>
<td>9.5</td>
<td>–</td>
</tr>
<tr>
<td>iso-C\textsubscript{16:1}</td>
<td>–</td>
<td>5.1</td>
<td>–</td>
</tr>
<tr>
<td>iso-C\textsubscript{16:1} 3-OH</td>
<td>–</td>
<td>1.9</td>
<td>–</td>
</tr>
<tr>
<td>iso-C\textsubscript{15:0} 2-OH</td>
<td>2.8</td>
<td>5.2</td>
<td>1.1</td>
</tr>
<tr>
<td>iso-C\textsubscript{16:0} 3-OH</td>
<td>–</td>
<td>3.6</td>
<td>7.2</td>
</tr>
<tr>
<td>iso-C\textsubscript{17:0} 2-OH</td>
<td>10.5</td>
<td>12.5</td>
<td>5.9</td>
</tr>
<tr>
<td>antteiso-C\textsubscript{15:0}</td>
<td>–</td>
<td>8.9</td>
<td>10.1</td>
</tr>
<tr>
<td>C\textsubscript{15:1} 10:6c</td>
<td>–</td>
<td>3.8</td>
<td>6.6</td>
</tr>
<tr>
<td>C\textsubscript{16:1} 10:6c</td>
<td>1.8</td>
<td>Tr</td>
<td>Tr</td>
</tr>
<tr>
<td>C\textsubscript{17:1} 10:9c</td>
<td>–</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>iso-C\textsubscript{17:1} 10:9c</td>
<td>–</td>
<td>2.7</td>
<td>–</td>
</tr>
<tr>
<td>antteiso-C\textsubscript{17:1} 10:9c</td>
<td>–</td>
<td>1.6</td>
<td></td>
</tr>
</tbody>
</table>

Summed features*

| 1   | 2.7 | –   |
| 3   | 18.9 | 11.8 | 10.8 |
| 4   | 2.0 | –   |
| C\textsubscript{16:0} 10-methyl | –   | 2.1 | –   |
| ECL 13.565 | –   | 1.3 | –   |

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 1 consisted of iso-C\textsubscript{15:1} and/or C\textsubscript{13:0} 3-OH. Summed feature 3 consisted of iso-C\textsubscript{15:0} 2-OH and/or C\textsubscript{16:1} 10:7c. Summed feature 4 consisted of iso-C\textsubscript{17:1} and/or antteiso-C\textsubscript{17:1}.

Using MES, PIPES, AMPSO or MOPS buffers (Sigma) and at 4, 12, 20, 25, 30, 37, 42 and 50 °C. Growth occurred at 12–37 °C (optimum, 30 °C), with <2 % NaCl (optimum, 0–1 %) and at pH 6.0–9.0 (optimum, pH 7.0). Anaerobic growth was assessed on TSA under anaerobic conditions (N\textsubscript{2}/H\textsubscript{2}/CO\textsubscript{2}, 90:5:5) at 25 °C. No growth was observed after 10 days. Catalase activity was determined by bubble production in 3 % (v/v) H\textsubscript{2}O\textsubscript{2}. Oxidase activity was tested by using oxidase reagent (bioMérieux). The production of flexirubin-type pigments was investigated according to Bernardet \textit{et al.} (2002).

The ability of strain TF5-37.2-LB10\textsuperscript{T} and the two reference strains to utilize different substrates was investigated using the API 50 CH and API 20 NE kits (bioMérieux) and the GN2 MicroPlate system (Biolog), according to the manufacturers’ instructions. Starch, aesculin, casein and Tween 80 hydrolysis were tested on agar according to Lányi (1987). H\textsubscript{2}S production was investigated using H\textsubscript{2}S test strips (Sigma). Enzymic activities were investigated using the API ZYM kit (bioMérieux), according to the manufacturer’s instructions. A comparison of the phenotypic characteristics of strain TF5-37.2-LB10\textsuperscript{T} and its closest relatives is presented in Table 1, and other phenotypic characteristics of strain TF5-37.2-LB10\textsuperscript{T} are given in the species description.

The fatty acid methyl ester composition of strain TF5-37.2-LB10\textsuperscript{T}, \textit{P. composti} LMG 23490\textsuperscript{T} and \textit{P. oryzae} DSM 19973\textsuperscript{T} was determined by the Identification Service of the DSMZ. Biomass was harvested from cells grown for 3 days on TSA at 25 °C. Fatty acids were saponified, methylated and extracted using the standard protocol of the Sherlock Microbial Identification System version 4.5 (MIDI). The fatty acids were analysed by GC (6890N; Agilent) and identified using the TSBA40 database of the Microbial Identification System. The major fatty acids of strain TF5-37.2-LB10\textsuperscript{T} were iso-C\textsubscript{15:0} (45.6 %), summed feature 3 (iso-C\textsubscript{15:0} 2-OH and/or C\textsubscript{16:1} 10:7c 18.9 %), iso-C\textsubscript{17:0} 3-OH (10.5 %) and iso-C\textsubscript{17:1} 10:9c (10.1 %), which were in accordance with its closest phylogenetic neighbours (Table 2). However, the isolate could be distinguished from the two reference strains by the presence/absence or proportions of minor fatty acids. Analysis of the respiratory quinones of strain TF5-37.2-LB10\textsuperscript{T} was also carried out by the Identification Service of the DSMZ and Dr Brian Tindall. Respiratory quinones were extracted using methanol/hexane (Tindall, 1990a, b) followed by phase separation into hexane, and were then separated by TLC on silica gel (no. 805 023; Macherey-Nagel), using hexane/tert-butyl methyl ether (9:1, v/v) as the solvent. UV-absorbing bands corresponding to the different quinone classes were further analysed by HPLC using methanol/heptane (9:1, v/v) as the eluent. Strain TF5-37.2-LB10\textsuperscript{T} contained MK-7, which was in line with all other members of the family \textit{Sphingobacteriaceae}.

Chemotaxonomic data and phylogenetic inference indicate that strain TF5-37.2-LB10\textsuperscript{T} belongs to the genus \textit{Pedobacter}. Nevertheless, it can be differentiated from its closest neighbours on the basis of several phenotypic characteristics and DNA G+C content. Consequently, a novel species is proposed to accommodate strain TF5-37.2-LB10\textsuperscript{T} with the name \textit{Pedobacter tournemirensis} sp. nov.

Description of \textit{Pedobacter tournemirensis} sp. nov.

\textit{Pedobacter tournemirensis} (tour.ne.mi.ren’sis N. L. masc. adj. tournemirensis of or belonging to Tournemire, a village in Aveyron, France).

Cells are strictly aerobic, non-motile straight rods, approximately 1.5–2.0 μm in length and 0.5 μm in width,
and form cream to light pink circular colonies with regular edges on TSA and 10-fold diluted LB agar. Growth occurs at 12–37 °C (optimum, 30 °C), at pH 6.0–9.0 (optimum, pH 7.0) and with 0–2 % NaCl (optimum, 0–1 %). Oxidase- and catalase-positive. Flexirubin pigments are not produced (KOH test negative). The following compounds in the API 50 CH kit and the GN2 MicroPlate system are utilized: D-arabinose, L-arabinose, D-xylene, D-galactose, D-glucose, D-fructose, mannose, rhamnose, methyl α-D-glucoside, N-acetylglucosamine, amygdalin, ascelin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, raffinose, starch, glycerol, gentiobiose, turanose, α-cyclodextrin, dextrin and lactulose; all other substrates are not utilized. With the API 20NE kit, ascelin hydrolysis and β-galactosidase activity are positive, but all other reactions are negative, including nitrate reduction, indole production, urease and gelatinase. On agar, starch and ascelin are hydrolysed, but casesin and Tween 80 are not. H₂S is not produced. With the API ZYM kit, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase and N-acetyl-β-D-glucosaminidase activities are present; other enzyme activities revealed by the API ZYM kit are absent. The only respiratory quinone is MK-7 and the major fatty acids are iso-C₁₅ : 0, summed feature 3 (iso-C₁₅ : 0 2-OH and/or C₁₆ : 0ω7c), iso-C₁₇ : 0 3-OH and iso-C₁₇ : 0ω9c.

The type strain, TF5-37.2-LB10T (=DSM 23085T =CIP 110085T =MOLA 820T), was isolated from subsurface water of the Toarcian geological layer at Tournemire, France. The DNA G+C content of the type strain is 46 mol%.

Acknowledgements

The project was funded by the French Institute of Radioprotection and Nuclear Safety (IRSN). D. Pellegrini and F. Marsal from IRSN and the whole team of the Tournemire experimental site are gratefully acknowledged. We are grateful to the BIO2MAR platform (http://bio2mar.obs-banyuls.fr) for providing technical help and support. We thank the Associate Editor in charge of this manuscript for his constructive comments.

References


