Atopobium deltae sp. nov., isolated from the blood of a patient with Fournier’s gangrene

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A Gram-stain-positive, obligately anaerobic, short rod, designated strain HHRM1715T, was isolated from the blood of a patient with Fournier’s gangrene, complicated by sepsis. On the basis of 16S rRNA gene sequence analysis, strain HHRM1715T was shown to belong to the genus Atopobium and was most closely related to Atopobium minutum (95% similarity). The results of 16S rRNA gene-based phylogenetic analysis, cellular fatty acid analysis and differential biochemical tests, showed that strain HHRM1715T represented a novel species of the genus Atopobium. We therefore describe Atopobium deltae sp. nov. with HHRM1715T (=LMG 27987T=CCUG 65171T) as the type strain and propose an emended description of the genus Atopobium with regard to the DNA G+C content.

The genus Atopobium was introduced by Collins & Wallbanks (1992) to reclassify species formerly designated Lactobacillus minutus (Hauduroy et al., 1937), Lactobacillus rimae (Olsen et al., 1991) and Streptococcus parvulus (Weinberg et al., 1937). In 1999, Eubacterium fossor (Bailey & Love, 1986) and a newly described species, Atopobium vaginae (Rodriguez Jovita et al., 1999), were added to the genus (Kageyama et al., 1999).

At the time of writing, the genus comprises five species, i.e. A. vaginae (type strain CCUG 38953T), A. rimae (ATCC 49626T), A. parvulum (ATCC 33793T), A. fossor (NCTC 11919T) and A. minutum (ATCC 33267T), with the last being the type species (Collins & Wallbanks, 1992), and is placed together with the genus Olsenella under the newly proposed family Atopobiaceae (Gupta et al., 2013). Olsenella umbonata (Kraatz et al., 2011) is the closest phylogenetic relative of the genus.

Here, we report on the isolation of a strain, HHRM1715T, from the blood of a patient with Fournier’s gangrene, complicated with sepsis. Sequencing of the 16S rRNA gene, fatty acid analysis, matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS analysis and biochemical tests showed that this isolate represented a novel species of the genus Atopobium, which we propose to name Atopobium deltae sp. nov.

Strain HHRM1715T was isolated from the blood of a patient with Fournier’s gangrene, complicated by sepsis (Oyaert et al., 2014). After incubation of two anaerobic blood culture bottles (BACTEC FX, Becton Dickinson) for 83 h at 35 °C, the blood culture bottles yielded short, rod-shaped, Gram-stain-positive, catalase-negative bacteria. Neither a commercially available Rapid ID 32A test (bioMérieux), producing a presumptive identification of a member of the genus Bacillus with poor probability (53.1%), nor MALDI-TOF MS (database MBT-BDAL-5627) (Bruker), giving an identification score of 3.317 for Sphingobium chlorophenolicum, resulted in identification of the strain.

Subsequently, the nearly complete 16S rRNA gene was sequenced as described by Cools et al. (2013) (1368 bases) and compared with the publicly available bacterial 16S rRNA gene sequences deposited in GenBank using the Basic Local Alignment Search Tool (BLAST) software (www.ncbi.nlm.nih.gov/Blast cgi), showing highest similarity with the 16S rRNA gene sequence of A. minutum of only 95% (strains DSM 20586T and CIP 110250). Because it is generally accepted that a similarity score of <97% with a nearest neighbour is indicative of a novel species (Petti, 2007), further testing was performed to confirm that strain HHRM1715T represents a novel species.

Pure cultures of strain HHRM1715T grew well on CDC anaerobe agar +5% sheep blood, Schaedler agar with vitamin K1 +5% sheep blood, tryptic soy agar +5% sheep blood, Columbia agar +5% sheep blood (all from Becton Dickinson) and Schaedler agar +5% sheep blood...
(bioMérieux). Because growth was not observed in the presence of oxygen, nor under micro-aerophilic conditions (GasPak EZ Campy Container System Sachets, Becton Dickinson), but only under anaerobic atmosphere (10% H2, 5% CO2 in N2) (BugBox, LedTechno) after 2–5 days at 35–37 °C, the strain can be considered strictly anaerobic.

The strain, examined after 3–5 days of incubation, grew as non-haemolytic pinpoint translucent colonies with smooth and shiny ‘wet’ surfaces, which were slightly raised and convex (Fig. 1). Dark field microscopy and light microscopy after Gram staining were used to investigate the cell morphology. Cells were found to be Gram-stain-positive and non-haemolytic pinpoint translucent colonies with smooth and convex (Fig. 1). Dark field microscopy and light microscopy after Gram staining were used to investigate the cell morphology. Cells were found to be Gram-stain-positive short rods, 1.0 × 1.2–1.5 μm in size, non-motile and non-sporulating, occurring most often singly or in pairs.

To clarify the taxonomic position of strain HHRM1715T, phylogenetic analysis based on the 16S rRNA gene sequence and chemotaxonomic analysis based on MALDI-TOF MS spectra were performed. For the chemotaxonomic analysis, the complete 16S rRNA gene sequences of the type strains of the species belonging to the class Coriobacteriia were retrieved (http://www.ncbi.nlm.nih.gov/nucleotide/).

These species were included because recently, Gupta et al. (2013) proposed to divide the class Coriobacteriia into two orders, the order Eggerthellales, hosting the single family Eggerthellaceae and the order Coriobacteriales, containing the families Coriobacteriaceae and Atopobiacae. The sequences were aligned with the 16S rRNA gene sequence of strain HHRM1715T using the online CLUSTAL Omega Software (http://www.ebi.ac.uk/Tools/msa/clustalo) with default parameters. Evolutionary distances were calculated by Kimura’s two-parameter model (Kimura, 1980) using the MEGA 5.2.2 software package (http://www.megasoftware.net) (Tamura et al., 2013) and a phylogenetic tree was reconstructed using the maximum-likelihood method (Felsenstein, 1981), with Bifidobacterium bifidum as the outgroup. The tree is presented in Fig. 2. A similar tree topology was obtained using the neighbour-joining method (Saitou & Nei, 1987) (tree not shown). Phylogenetic analysis indicated that strain HHRM1715T formed a distinct phylogenetic lineage within the genus Atopobium, most closely related to the lineage comprising A. fossor and A. minutum.

For the chemotaxonomic analysis, first, reference spectra of HHRM1715T and A. fossor DSM 15642T (which is lacking in the Bruker database MBT-BDAL-5627) were created as suggested by the manufacturer. For the creation of a reference spectrum, a few colonies were used for an ethanol/formic acid extraction, after which 1 μl of supernatant was spotted eight times on a polished steel target plate. After drying, spots were covered with 1 μl matrix solution (saturated solution of ω-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid), and 24 spectra were generated for each strain from three independent measurements. Spectra were acquired using the Bruker Daltonik’s FlexControl version 3.0 software and analysed over a mass range of 2000–20000 Da. The 24 raw spectra obtained were processed (smoothing, normalization, baseline subtraction and peak picking) using the FlexAnalysis software (Bruker Daltonik). After checking the spectra manually for inconsistencies, a reference main spectrum was created for the two strains from 20 individual spectra using the MALDI Biotyper software 3.0. A dendrogram was made from the created reference spectra and the reference spectra of the other species of the genus Atopobium present in the Bruker library (Fig. 3). The dendrogram confirmed the tree topology obtained for the genus Atopobium on the basis of 16S rRNA gene sequencing.

To assess the DNA G+C content of strain HHRM1715T, DNA was extracted according to the method described by Gevers et al. (2001) and enzymically degraded into nucleosides followed by HPLC separation (Mesbah et al., 1989). The genomic DNA G+C content was found to be 50.3 mol%, which is outside the range of values reported for the members of the genus Atopobium, i.e. 39–45 mol% (A. parvulum, 39 mol%; A. fossor, A. minutum, A. vaginae, 44 mol%; and A. rimae, 45 mol%) (Lawson, 2012) (Table 1). However, in their emended description of A. parvulum, Cato et al. (1983) reported the DNA G+C content of the type strain ATCC 33793T to be 46 mol%, which is in disagreement with the DNA G+C content of 39 mol% later reported for the same type strain (VPI 0546T=ATCC 33793T) by Olsen et al. (1991). Based on the full genome sequence of A. parvulum IPP 1246T (=ATCC 33793T), Copeland et al. (2009) reported the DNA G+C content to be 45.7 mol%. Likewise, based on the analysis of the complete genome sequence, the DNA G+C content of A. rimae ATCC 49626T was found to be 49.3 mol% (Gupta et al., 2013), in disagreement with the 45 mol% as determined by Olsen et al. (1991) for the same strain. Taken together, these data indicate that, based on the species currently belonging to the genus, the range of the
DNA G+C content of the species of the genus *Atopobium* is 44–49.3 mol%, instead of 39.0–45.0 mol%. Including the novel species described here, the DNA G+C content ranges between 44 and 50.3 mol%.

The cellular fatty acid composition of strain HHRM1715T was determined at the Laboratory of Microbiology (LM-UGent, Ghent, Belgium) by GC after growing the bacteria for 48 h at 35 °C on LMG medium no. 267 (http://lmg.ugent.be), under anaerobic conditions. Inoculation and harvesting of the cells, extraction and analysis were performed according to the recommendations of the commercial identification system MIDI (Microbial Identification System), except that cells were harvested from five complete plates to obtain a sufficient concentration of fatty acids in the extract. The predominant fatty acids were C16:0 (33.3 %), C18:1ω9c (27.7 %) and C18:0 (11.9 %) (Table 1). Rapid ID 32A test (bioMérieux) was performed at the LM-UGent according to the manufacturer’s instructions. Characteristics differentiating strain HHRM1715T from other species of the genus *Atopobium* are summarized in Table 1.

Minimum inhibitory concentrations for penicillin, meropenem, metronidazole, clindamycin, vancomycin and cefotaxime were determined and described by Oyaert et al. (2014).

Based on phylogenetic, chemotaxonomic, biochemical and cellular fatty acid analysis, we propose that HHRM1715T represents a novel species, *Atopobium deltae* sp. nov.
Fig. 3. MALDI-TOF MS dendrogram showing the relationships between strain HHRM1715T and species of the genus *Atopobium*. The distance matrix and dendrogram were calculated with the Biotyper software 2.0; distance levels above 500 are uninformative (Sauer et al., 2008).

Table 1. Differential characteristics among members of the genus *Atopobium*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>50.3</td>
<td>43–46</td>
<td>44</td>
<td>45.7*</td>
<td>49.3†</td>
<td>44</td>
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<tr>
<td>Predominant fatty acids (%)</td>
<td></td>
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<tr>
<td>C16:0</td>
<td>33.3</td>
<td>ND</td>
<td>/</td>
<td>0.8</td>
<td>1.1</td>
<td>34.8</td>
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<tr>
<td>C18:1ω9c</td>
<td>27.7</td>
<td>ND</td>
<td>40.0</td>
<td>38.2</td>
<td>32.5</td>
<td>25.4</td>
</tr>
<tr>
<td>C18:1ω9c DMA</td>
<td>/</td>
<td>ND</td>
<td>24.7</td>
<td>24.1</td>
<td>30.1</td>
<td>/</td>
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<tr>
<td>C18:0</td>
<td>11.9</td>
<td>ND</td>
<td>0.8</td>
<td>0.6</td>
<td>/</td>
<td>17.5</td>
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<tr>
<td>Acidification of D-mannose</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>−</td>
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<td>Enzymic activities</td>
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<tr>
<td>Alanine arylamidase</td>
<td>+</td>
<td>ND</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<td>Arginine dihydrolase</td>
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<td>ND</td>
<td>+</td>
<td>−</td>
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<td>ND</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<td>ND</td>
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<td>β-Galactosidase</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Leucine arylamidase</td>
<td>+</td>
<td>ND</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<tr>
<td>Proline arylamidase</td>
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<td>−</td>
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<td>+</td>
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<td>Pyroglutamic acid arylamidase</td>
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<td>ND</td>
<td>V</td>
<td>+</td>
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<tr>
<td>Glycine arylamidase</td>
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<td>ND</td>
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<td>+</td>
<td>−</td>
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<tr>
<td>Serine arylamidase</td>
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<td>+</td>
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<td>+</td>
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<td>ND</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>−</td>
</tr>
</tbody>
</table>

*Copeland et al. (2009), based on full genome sequencing of the type strain, reported a DNA G+C content of 46 mol%, whereas Olsen et al. (1991) reported 39 mol%.
†Gupta et al. (2013), based on full genome sequencing of the type strain, reported a DNA G+C content of 49.3 mol%, whereas Olsen et al. (1991) reported 45 mol% for the type strain.
Emended description of the genus *Atopobium* Collins & Wallbanks 1992

The description remains as given by Collins & Wallbanks (1992) with the following emendation. DNA G+C content is 44–50.3 mol%.

Description of *Atopobium deltae* sp. nov.

*Atopobium deltae* (del’tae. N.L. fem. gen. n. deltae of Delta, referring to the Delta Hospital in Roeselare, Belgium, the hospital where the type strain was isolated).

Cells are Gram-stain-positive short rods, 1.0×1.2–1.5 μm in size, non-motile and non-sporulating, occurring most often singly or in pairs. Colonies are non-haemolytic pinpoint translucent with a smooth and shiny 'wet' surface, slightly raised and convex. Grows strictly anaerobically on tryptic soy agar +5 % sheep blood, Schaedler agar +5 % sheep blood and Columbia agar +5 % sheep blood after 2–5 days. Susceptible to clindamycin, meropenem, penicillin and vancomycin; resistant to metronidazole. Produces arginine dihydrolase, arginine arylamidase, proline arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, leucine arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase and serine arylamidase, but not urease, α-galactosidase, β-galactosidase, β-galactosidase 6-phosphate, α-glucosidase, β-glucosidase, α-arabinosidase, β-glucuronidase, N-acetylβ-glucosaminidase, glutamic acid decarboxylase, α-fucosidase, alkaline phosphatase, pyrogallanic acid arylamidase or glutamyl glutamic acid arylamidase. Acid is produced from D-mannose. Nitrate is not reduced. Indole is not produced. Gelatin and aesculin are not hydrolysed. The predominant fatty acids are C<sub>16:0</sub>, C<sub>18:1</sub>ω9c and C<sub>18:0</sub>.

The type strain is HHRM1715<sup>T</sup> (=LMG 27987<sup>T</sup> =CCUG 65711<sup>T</sup>), isolated from the blood of a patient with Fournier's gangrene. The DNA G+C content of the type strain is 50.3 mol%.

Acknowledgements

Funding was provided by AZ Delta, Roeselare, Belgium. The funders had no role in study design, data collection and analysis, or preparation of the manuscript. The authors wish to thank Ms. Jelle De Medts (Department of Microbiology, Immunology and Clinical Chemistry, Faculty of Medicine and Health Sciences, Ghent University, De Pintelaan 185, Ghent 9000, Belgium) for her contribution in providing photographs of *Atopobium delae* HHRM1715<sup>T</sup>.

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