Prevotella timonensis sp. nov., isolated from a human breast abscess

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Gram-negative anaerobic rods were isolated from a human breast abscess. Based on genotypic and phenotypic characteristics, the novel strain belonged to the genus Prevotella. Phylogenetic analysis based on 16S rRNA gene sequence comparisons showed that it was closely related to Prevotella buccalis (94 % 16S rRNA gene sequence similarity), Prevotella saliva (90 %) and Prevotella oris (89.1 %). The major cellular fatty acid was C14 : 0 (19.5 %). The new isolate represents a novel species in the genus Prevotella, for which the name Prevotella timonensis sp. nov. is proposed. The type strain is strain 4401737T (= CIP 108522T = CCUG 50105T).

The creation of the genus Prevotella was proposed by Shah & Collins (1990) to characterize 'the moderately saccharolytic, predominantly oral Bacteroides species'. Identification at the species level among this group of obligatory anaerobic Gram-negative rods remains problematic due to the lack of characteristic phenotypic and biochemical traits. 16S rRNA gene sequence analysis has improved this situation and the number of recognized Prevotella species has increased over the last few years. Prevotella pullens (Kononen et al., 1998), Prevotella shahii, Prevotella saliva and Prevotella multiformis (Sakamoto et al., 2004, 2005), Prevotella marshii and Prevotella baronii (Downes et al., 2005) have been described recently. Prevotella species have been isolated from the oral cavity, the upper respiratory tract and the urogenital tract in humans. Some species, such as Prevotella ruminicola and the closely related species Prevotella brevis, Prevotella bryantii and Prevotella albensis, have been recovered from the rumen and hindgut of many mammalian species (Avgustin et al., 1997). Species of the genus Prevotella are considered to be part of the normal flora, but sometimes they can induce disease. Thus, bacteraemia and sepsis caused by representatives of the genus Prevotella have led to liver and spleen abscesses (Brook & Frazier, 1998), appendix abscess (Paneri et al., 2002), cervical abscess, meningitis (Frat et al., 2004) and also have provoked endocarditis in an immunocompromised patient (Dominguez-Castellano et al., 2001). Recently, Prevotella intermedia and Prevotella nigrescens were characterized by specific 16S rRNA gene PCR amplification from artery samples of patients suffering from vascular disease (Fiehn et al., 2005; Iwai et al., 2005). DNA of Prevotella species was PCR amplified and identified after cloning from samples of endocardial infections (Rolph et al., 2001) and from the bacterial microbiota in the human stomach (Bik et al., 2006). Genomic identification of these bacteria will allow a better understanding of their involvement in pathology.

In this report, we describe a novel species belonging to the genus Prevotella isolated from a human breast abscess.

A 40-year-old woman underwent breast abscess puncture. Blood sample analysis revealed anaemia (haemoglobin, 109 g l$^{-1}$) and the erythrocyte sedimentation rate was recorded as 32 mm h$^{-1}$. Liquid from the punctured abscess was cultured and two different Gram-negative bacteria were isolated. The first isolate was identified as Prevotella disiens using API 20A strips (bioMérieux). However, a doubtful identification was obtained for the second isolate, strain 4401737T. 16S rRNA gene sequence determination was performed. This confirmed that strain 4401737T was a member of the genus Prevotella and was possibly a representative of a novel species. The antimicrobial susceptibility of strain 4401737T was determined according to the National Committee for Clinical Laboratory Standards (NCCLS) criteria. Strain 4401737T showed intermediate susceptibility to penicillin G, but was susceptible to Augmentin, cefotetan, imipenem, metronidazole and vancomycin (30 μg ml$^{-1}$).

Surface colonies on sheep blood agar (bioMérieux) were circular, white–greyish, smooth, shiny, non-pigmented and 1–2 mm in diameter after 72 h. Growth and haemolytic activity were tested at 37 °C on Columbia agar with 5 % sheep blood. Growth was tested in anaerobic and microaerophilic atmospheres which were created using GENbag anaer and GENbag microaer incubation systems (bioMérieux), respectively. Growth was also tested in the presence of air or 5 % CO$_2$. Growth was tested at different temperatures (25, 30, 37 and 45 °C). Optimum growth of
strain 4401737<sup>T</sup> was obtained at 37 °C, but growth occurred between 25 and 37 °C. Strain 4401737<sup>T</sup> was strictly anaerobic and non-haemolytic.

The size and ultrastructure of cells of the novel strain were determined by transmission electron microscopy (TEM). Cells were grown in liquid tryptase soy broth (TSB; Becton Dickinson) medium for 48 h, collected by pipetting and stained with 1 % (w/v) phosphotungstic acid. Samples were examined on an electron microscope (Morgagni 268D; Philips) at an operating voltage of 60 kV. The rods were 0.8–1.4 μm long and 0.3–0.5 μm wide and usually occurred singly.

Catalase activity was negative as determined by the ID colour catalase test kit (bioMérieux). Bile resistance was tested by growing the bacteria on peptone/yeast extract/glucose agar plates supplemented with 2 % (w/v) dehydrated gall salt (bile sac powder; MP Biomedicals) and tested by growing the bacteria on peptone/yeast extract/glucose agar plates supplemented with 2 % (w/v) dehydrated gall salt (bile sac powder; MP Biomedicals) equivalent to 20 % (v/v) bile. Antibiotic resistance to 5 μg vancomycin ml<sup>−1</sup>, 1000 μg kanamycin ml<sup>−1</sup> and 10 μg colistin ml<sup>−1</sup> was tested in thioglycollate with resazurin broth (bioMérieux). An anaerobic atmosphere was created by the addition of 2 ml paraffin oil. Strain 4401737<sup>T</sup> was bile sensitive and resistant to vancomycin, colistin and kanamycin.

Commercially available API 20A, rapid API 32A and API 50CH strips (bioMe´rieux) were used to characterize the strains: 1, strain 4401737<sup>T</sup>; 2, <i>P. timonensis</i> ATCC 33269<sup>T</sup> which were identified as the most closely related species to strain 4401737<sup>T</sup> when 16S rRNA gene sequences were compared with sequences deposited in the GenBank database by using the BLAST program through the NCBI server. Gene sequences were aligned using the multisequence alignment program CLUSTAL_X (1.8). Phylogenetic relationships with closely related species were determined by using MEGA version 2.1 (Kumar et al., 2001). Distance matrices were determined following the assumptions described by Kimura (1980) and were used to elaborate the dendrogram using the neighbour-

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<th>1</th>
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<td>Raffinose</td>
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<td>Major cellular fatty acids</td>
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<td>ND</td>
<td>C&lt;sub&gt;18:1ω9c&lt;/sub&gt;</td>
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Preparation and determination of cellular fatty acids were carried out by following the procedures given for the Sherlock Microbial Identification System (MIDI). The fatty acid content of strain 4401737<sup>T</sup> was significantly different to that of other members of the genus <i>Prevotella</i>. The predominant cellular fatty acids were C<sub>14:0</sub> (19.5 %), C<sub>16:0</sub> (15.3 %), iso-C<sub>14:0</sub> (14 %) and C<sub>18:1ω6</sub>9c/C<sub>18:0</sub> (16 %; fatty acids could not be separated by the MIDI system). It has been reported previously that the major fatty acids in the genus <i>Prevotella</i> are anteiso-C<sub>15:0</sub>, iso-C<sub>15:0</sub>, iso-C<sub>17:0</sub> 3-OH, C<sub>16:0</sub> and C<sub>18:1ω9c</sub> (Moore et al., 1994; Sakamoto et al., 2004). The presence of C<sub>18:1ω9c</sub> and the absence of iso-C<sub>17:0</sub> 3-OH which were noted for our novel isolate are not usual features for species of the genus <i>Prevotella</i>. However, unexpected fatty acid contents have already been reported for other representatives of the genus <i>Prevotella</i> (Sakamoto et al., 2004; Willems & Collins, 1995).

Bacterial DNA was extracted using the FastDNA Kit (BIO 101; Illkirch) as recommended by the manufacturer. PCR amplification of the 16S rRNA gene was performed using the universal primer pair fD1 and rp2 (Weisburg et al., 1991). PCR products were purified using MultiScreen PCR (Millipore) and sequencing reactions were carried out using a DNA sequencing kit (BigDye Terminator Cycle Sequencing v2.0 Ready Reactions; PE Biosystems) according to the manufacturer’s instructions. Sequencing products were purified and electrophoresis was performed with a 3100 Genetic Analyzer (Applied Biosystems). The sequences obtained for strain 4401737<sup>T</sup> were compared with sequences deposited in the GenBank database by using the BLAST program through the NCBI server. Gene sequences were aligned using the multisequence alignment program CLUSTAL_X (1.8). Phylogenetic relationships with closely related species were determined by using MEGA version 2.1 (Kumar et al., 2001). Distance matrices were determined following the assumptions described by Kimura (1980) and were used to elaborate the dendrogram using the neighbour-
Description of *Prevotella timonensis* sp. nov.

*Prevotella timonensis* (ti.mo.n.en’sis. N.L. fem. adj. timon-ensis from the name Hôpital de la Timone, the hospital in Marseille, France, from where the type strain was isolated).

Cells are obligately anaerobic, non-pigmented, non-spore-forming, non-motile, Gram-negative straight rods. Growth occurs on sheep blood agar and in TSB liquid medium. Growth is inhibited by 20 % (w/v) bile. After 48 h growth in TSB medium, rods are 0.8–1.4 μm in length, 0.3–0.5 μm in diameter and up to 1–2 mm in diameter. Temperature range for growth is 25–37°C, with an optimum at 37°C. After 48 h growth in TSB medium, colonies are circular, white–greyish, smooth, shiny and up to 1–2 mm in diameter.

Growth is inhibited by 20 % (w/v) bile. After 72 h growth on sheep blood agar and in TSB liquid medium, colonies are circular, white–greyish, smooth, shiny and up to 1–2 mm in diameter. Blood sheep agar, surface colonies are circular, white–greyish, smooth, shiny and up to 1–2 mm in diameter.

*Prevotella timonensis* sp. nov. is a member of the genus *Prevotella* (Fig. 1). Strain 4401737 T was closely related to *P. buccalis* (94 % 16S rRNA gene sequence similarity), *P. salivae* (90 %) and *P. oris* (89.1 %). These percentages of similarity were low (<97 %), confirming that strain 4401737 T represents a novel species.

Based on the results described above, we propose that strain 4401737 T represents a novel species, *Prevotella timonensis* sp. nov.

**Fig. 1.** Phylogenetic tree of representatives of the genus *Prevotella* inferred from 16S rRNA gene sequence comparisons (1451 nt fragment). Numbers at nodes are the proportions of 100 resamplings that support the topology shown. *Bacteroides tectus* JCM 10003 T was used as the outgroup. Bar, 0.02 nucleotide changes per nucleotide position.

**References**


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<th>Prevotella timonensis ATCC 51261 T (AJ006535)</th>
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<td>P. ruminicola ATCC 191818 T (L16482)</td>
<td>Bacteroides tectus JCM 10003 T (AB200228)</td>
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Joining method (Saitou & Nei, 1987). The maximum parsimony algorithm was also used to infer phylogenetic relationships. A bootstrap analysis (bootstrap values were obtained for a consensus tree based on 100 randomly generated trees) was performed to investigate the stability of the trees obtained. The tree topology was the same with both methods. Phylogenetic analysis demonstrated that strain 4401737 T is a member of the genus *Prevotella* (Fig. 1). Strain 4401737 T was closely related to *P. buccalis* (94 % 16S rRNA gene sequence similarity), *P. salivae* (90 %) and *P. oris* (89.1 %). These percentages of similarity were low (<97 %), confirming that strain 4401737 T represents a novel species.


