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

Olga O. Glazunova,1 Thierry Launay,2 Didier Raoul1 and Véronique Roux1

1Laboratoire de Bactériologie–Virologie, Hôpital de la Timone, CNRS UMR 6020, IFR48, 264 rue Saint-Pierre, 13385 Marseille Cedex 05, France
2Service de Chirurgie Générale, Clinique Vert-Coteau, 41 rue Brandis, 13005 Marseille, France

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 salivae* (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 obligate 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 pallens* (Kononen et al., 1998), *Prevotella shahii*, *Prevotella salivae* and *Prevotella multi-formis* (Sakamoto et al., 2004, 2005), *Prevotella marshii* and *Prevotella baroniae* (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 have also 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 % CO₂. 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) equivalent to 20 % (v/v) bile. Antibiotic resistance to 5 μg glucose agar plates supplemented with 2 % (w/v) dehydrated gall salt (bile sac powder; MP Biomedicals) were compared with those of ATCC 35310<sup>T</sup>, P. timonensis sp. nov. and related species. The phenotypic characteristics of strain 4401737<sup>T</sup> were determined 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 (bioMérieux) were used to characterize the bacterial strains. A 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.

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 Prevotella. 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>18:2</sub>ω6,9c/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 Prevotella 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>18:1</sub>ω9c (Moore et al., 1994; Sakamoto et al., 2004). The presence of C<sub>18:2</sub>ω6,9c 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 Prevotella. However, unexpected fatty acid contents have already been reported for other representatives of the genus Prevotella (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; 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-

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Temperature range for growth is 25–37°C. 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.

**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 72 h growth on sheep blood agar, surface colonies are circular, white–greyish, smooth, shiny 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, rods are 0.8–1.4 μm in length, 0.3–0.5 μm in diameter and occur singly (as observed by TEM). Catalase-negative. Ferments glucose, lactose and maltose. Tests positive for gelatin hydrolysis using an API 20A strip. With an API 20A strip, tests negative for indole formation, urease activity, aesculin hydrolysis and fermentation of mannitol, sucrose, salicin, xylose, arabinose, glycerol, cellobiose, mannose, melezitose, raffinose, sorbitol, rhamnose and trehalose. Using API ID 32A tests, positive for activities of alkaline phosphatase, β-galactosidase, α-glucosidase, N-acetyl-β-glucosaminidase, x-fucosidase, arginine arylamidase, leucyl glycine arylamidase and alanine arylamidase.

**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).

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The type strain, 4401737T (=CIP 108522T=CCUG 50105T), was isolated from a human breast abscess.

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**References**


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