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 salivae* (90 %) and *Prevotella oris* (89.1 %). The major cellular fatty acid was $C_{14:0}$ (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 4401737^T (=CIP 108522^T=CCUG 50105^T).

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 pallens* (Kononen *et al.*, 1998), *Prevotella shahii*, *Prevotella salivae* and *Prevotella multiformis* (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 endodontic 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 4401737^T. 16S rRNA gene sequence determination was performed. This confirmed that strain 4401737^T was a member of the genus *Prevotella* and was possibly a representative of a novel species. The antimicrobial susceptibility of strain 4401737^T was determined according to the National Committee for Clinical Laboratory Standards (NCCLS) criteria. Strain 4401737^T 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

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Prevotella timonensis* strain 4401737^T is DQ518919.

strain 4401737^T was obtained at 37 °C, but growth occurred between 25 and 37 °C. Strain 4401737^T 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 trypticase 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 vancomycin ml^{-1}, 1000 μg kanamycin ml^{-1} and 10 μg colistin ml^{-1} was tested in thioglycollate with resazurin broth (bioMérieux). An anaerobic atmosphere was created by the addition of 2 ml paraffin oil. Strain 4401737^T 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 biochemical properties of strain 4401737^T according to the manufacturer's instructions. Incubation was performed at 37 °C. The results of these tests are given in the species description. The phenotypic characteristics of strain 4401737^T were compared with those of *Prevotella buccalis* ATCC 35310^T, *P. shahii* EHS11^T and *Prevotella oralis* ATCC 33269^T which were identified as the most closely related species to strain 4401737^T when 16S rRNA gene sequences were analysed. The results are presented in Table 1.

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^T was significantly different to that of other members of the genus *Prevotella*. The predominant cellular fatty acids were $C_{14:0}$ (19.5 %), $C_{16:0}$ (15.3 %), iso-$C_{14:0}$ (14 %) and $C_{18:2}\omega6,9c/C_{18:0}$ (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-$C_{15:0}$, iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, $C_{16:0}$ and $C_{18:1}\omega9c$ (Moore *et al.*, 1994; Sakamoto *et al.*, 2004). The presence of $C_{18:2}\omega6,9c$ and the absence of iso-$C_{17:0}$ 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 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^T 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-

Table 1. Differential characteristics of *P. timonensis* sp. nov. and related *Prevotella* species

Strains: 1, strain 4401737^T; 2, *P. buccalis* ATCC 35310^T; 3, *P. shahii* EHS11^T (data from Sakamoto *et al.*, 2004); 4, *P. oralis* ATCC 33269^T (Holdeman *et al.*, 1984). None of the strains produce pigment. +, Positive; −, negative; W, weakly positive; ND, not determined.

Characteristic	1	2	3	4
Aesculin hydrolysis	W	+	−	+
α-Galactosidase	W	+	W	ND
β-Glucosidase	−	+	−	ND
Fermentation of:				
Mannose	−	+	+	+
Raffinose	−	+	+	+
Arginine arylamidase	+	+	−	ND
Glutamyl glutamic acid arylamidase	−	+	−	ND
Major cellular fatty acids	$C_{14:0}$, $C_{16:0}$, $C_{18:2}\omega6,9c/C_{18:0}$	ND	$C_{18:1}\omega9c$, $C_{16:0}$, $C_{16:0}$ 3-OH	ND

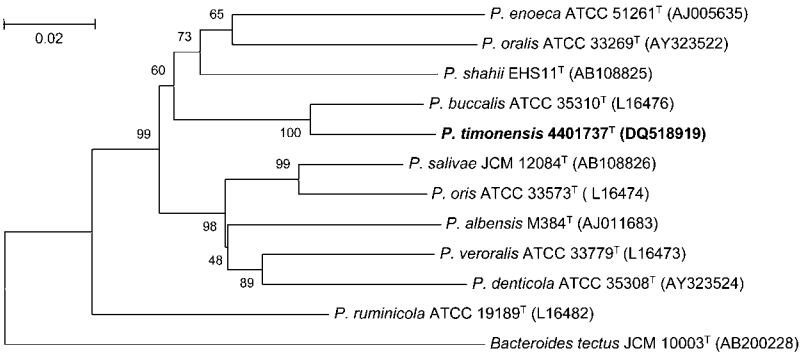

Fig. 1. Phylogenetic tree of representatives of the genus *Prevotella* inferred from 16S rRNA gene sequence comparisons (1451 nt fragment). Numbers at nodes are the proportions of 100 resamplings that support the topology shown. *Bacteroides tectus* JCM 10003^T was used as the outgroup. Bar, 0.02 nucleotide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

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.

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

Description of *Prevotella timonensis* sp. nov.

Prevotella timonensis (ti.mo.n.en′sis. N.L. fem. adj. *timonensis* 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 72 h growth on blood sheep 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, α-fucosidase, arginine arylamidase, leucyl glycine arylamidase and alanine arylamidase. Activities of β-galactosidase 6-phosphate and α-galactosidase are weakly positive. Negative reaction for indole production, reduction of nitrates and fermentation of mannose and raffinose. No activity is detected for glutamic acid decarboxylase, arginine dihydrolase, β-glucosidase, α-arabinosidase, β-glucuronidase, proline arylamidase, phenylalanine arylamidase, leucine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, glycine arylamidase, histidine arylamidase, glutamyl glutamic acid arylamidase or serine arylamidase. Using an API 50CH strip, only reactions for D-ribose, D-tagatose, potassium 5-ketogluconate and aesculin (iron citrate) are positive after 1 week of cultivation. The fatty acid profile is characterized by the predominance of $C_{14:0}$ (19.5 %), followed by $C_{16:0}$ (15.3 %), iso-$C_{14:0}$ (14 %) and a mixture of $C_{18:2}\omega6,9c$ and $C_{18:0}$ (16 %).

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

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