Prevotella nanceiensis sp. nov., isolated from human clinical samples

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Three strains of anaerobic, non-pigmented, Gram-negative bacilli isolated from various human clinical samples were characterized in terms of phenotypic and genotypic tests, including sequence analysis of 16S rRNA and rpoB genes. The strains were most closely related to the type strains of Prevotella marshii and Prevotella shahii on the basis of both 16S rRNA (89.8 and 89.0 % identity, respectively) and rpoB gene sequences (83.1 and 82.8 % identity, respectively). Phylogenetic analysis showed that the isolates constituted a robust homogeneous group distinct from known species in the genus Prevotella. The rrn skeleton (as determined by PFGE) and the DNA G+C content, determined to be 39.4 mol% for strain LBN 293T, distinguished the novel isolates from the type strains of P. marshii and P. shahii. The three strains were saccharolytic and produced acetic, lactic and succinic acids as major metabolic end products. Polyphasic investigations supported the proposal of a novel species, Prevotella nanceiensis sp. nov., with LBN 293T (=AIP 261.03T =CIP 108993T =CCUG 54409T) as the type strain.

The genus Prevotella includes strictly anaerobic, Gram-negative, moderately saccharolytic, bile-sensitive rods that formerly belonged to the genus Bacteroides (Shah & Collins, 1990). These bacteria, which are part of the human oral, intestinal and urogenital flora, may be involved in various infections of the head and neck, lower respiratory tract, central nervous system, abdominal and female genital tract and in bacteraemia (Jousimies-Somer et al., 2002). The use of molecular methods, such as 16S rRNA gene sequencing, for the identification of anaerobic bacteria has revealed that a significant proportion of strains did not correspond to species with validly published names (Jousimies-Somer, 1997; Jousimies-Somer et al., 2003) and has led to a great expansion of the genus Prevotella, with the description of ten novel species within the last 3 years. In this study, three strains of anaerobic Gram-negative rods, isolated from different human clinical samples and affiliated to the genus Prevotella by presumptive identification tests, were subjected to a comprehensive range of phenotypic, genotypic, genomic and phylogenetic tests.

Strains LBN 293T and LBN 297 were both recovered in pure culture. Strain LBN 293T was isolated in 2003 from blood cultures of a 78-year-old man (Mory et al., 2005) and strain LBN 297 was isolated in 2004 from lung abscess pus from a 67-year-old man. Strain LBN 298 was isolated in 2005 from a broncho-alveolar lavage fluid from a 66-year-old woman. The broncho-alveolar lavage fluid also contained Pseudomonas aeruginosa and a coagulase-negative Staphylococcus sp. Strains were grown at 36 °C on Brucella agar supplemented with 5 % sheep blood, haemin...
and vitamin K₁ (BBA) in an anaerobic chamber (Concept 1000; Ruskinn).

DNA was extracted by using the QIAamp DNA Mini kit (Qiagen). The 16S rRNA gene was amplified by PCR and sequenced as described previously (Carlier et al., 2004). A 374 bp fragment of the gene rpoB was amplified using the primer pair Prev3250F (5'-AACCCGTGGGTGTGCC-3') and Prev3623R (5'-AGIGCCCAAACCTCCATCTCC-3') (Berger et al., 2005). Nucleotide sequences were analysed by using SeqScape software (version 2.5; Applied Biosystems). The sequences were compared to those deposited in the GenBank and Ribosomal Database Project II databases using the BLAST program (Altschul et al., 1997) and Seqmatch program (Cole et al., 2007), respectively. The isolates displayed the highest 16S rRNA gene sequence similarity to members of the genus Prevotella. Maximum similarity (99.4 %) was observed with the sequence of Prevotella sp. oral clone BI027, obtained from human subgingival plaque (Paster et al., 2001). The 16S rRNA gene sequences were aligned against sequences of all known Prevotella type strains and that of Prevotella oral clone BI027 using the DIALIGN program (Morgenstern, 2002). The distance matrix constructed using the Similarity table program of the PHYLIP package (Felsenstein, 1993) showed that strains LBN 293ᵀ, LBN 297 and LBN 298 shared more than 99.6 % of their 16S rRNA gene nucleotide positions. The best sequence matches with strains of species with validly published names were obtained with Prevotella marshii E9.34ᵀ and Prevotella shahii JCM 12083ᵀ, but the similarity levels (89.8 and 89.0 %, respectively) were relatively low. The three isolates displayed 100 % identity in rpoB gene sequences. Maximum similarity was again observed with the sequences of P. marshii E9.34ᵀ and P. shahii JCM 12083ᵀ (83.1 and 82.8 %, respectively). Altogether, the sequencing results suggested that the three strains belonged to a new taxon. This prompted us to investigate the taxonomic position of these strains by a polyphasic approach.

Evolutionary trees were reconstructed using the PHYLIP suite of programs (Felsenstein, 1993) by maximum-parsimony (Kluge & Farris, 1969) and by neighbour-joining (algorithm F84 for substitution model) (Saitou & Nei, 1987; Kishino & Hasegawa, 1989). The robustness of the nodes was evaluated by 1000 bootstrap replications using SEQBOOT and CONSENSE programs (Felsenstein, 1993). The maximum-likelihood (ML) tree was reconstructed using phyML software with GTR (gamma distribution and invariable sites) as the substitution model and 100 bootstrap replications (Guindon & Gascuel, 2003). The 16S rRNA gene-based ML phylogenetic tree is shown in Fig. 1. Nodes labelled by asterisks were found by all three
The strains were obligately anaerobic, non-spore-forming, (Fig. 2). Shahii. P. marshii. skeleton distinguished the novel strains from their most P. marshii. skeletons of the three isolates were studied in comparison used as molecular size marker. Sizes are indicated in kilobases. DSM 16973T. Lane Sc, 1998; Marchandin et al. 3.09 Mb in size, similar to the P. marshii. shown). The chromosome of the novel strains was 3.02 to 2218 2003a; Jumas-Bilak et al. (39–60 mol%) (Willems & Collins, 1995; Avgúštín et al., 1997) and was clearly distinct from the DNA G+C contents determined for the most closely related species, P. shahii (51.0 mol%; Downes et al., 2005) and P. shahii (44.3 mol%; Sakamoto et al., 2004).

Large-scale chromosome structure analysis was previously described as a sensitive indicator of phylogenetic relationships between bacteria (Liu et al., 1999; Marchandin et al., 2003a; Jumas-Bilak et al., 2005). Chromosome size and rrrn skeletons of the three isolates were studied in comparison with P. marshii DSM 16973T and P. shahii DSM 15611T using PFGE, as described previously (Jumas-Bilak et al., 1998; Marchandin et al., 2003a, b). All three strains studied possess a unique and circular chromosome (data not shown). The chromosome of the novel strains was 3.02 to 3.09 Mb in size, similar to the P. marshii chromosome (2.95 Mb) but clearly distinct from the P. shahii chromosome (4.01 Mb). I-Ceu1 profiles showed that all the strains tested possess four rrrn operons (Fig. 2). The rrrn skeleton distinguished the novel strains from their most closely related phylogenetic neighbours, P. marshii and P. shahii, each species exhibiting a specific I-Ceu1 profile (Fig. 2).

The strains were obligately anaerobic, non-spore-forming, non-motile, Gram-negative coccoid and short rods. After 4 days incubation on BBA, colonies were 0.5–1 mm in diameter, circular, entire, slightly convex and smooth, white, non-pigmented and surrounded by a β-haemolysis zone. For examination of the cell-wall ultrastructure of strain LBN 293T, cells were prepared as described previously (Carlier et al., 2004) and electron photomicrographs were taken with a JEOL 1010 transmission electron microscope operating at 80 kV. Rods, 0.4–0.5 × 0.5–1.0 μm in size, were observed. Transmission electron microscopic examination of ultrathin sections of strain LBN 293T showed the presence of a typical Gram-negative cell wall composed of a thin peptidoglycan layer surrounded by an outer membrane (see Supplementary Fig. S1 in IJSEM Online).

All strains were susceptible to bile (1 mg tablet) and resistant to kanamycin (1 mg disc), vancomycin (5 μg tablet) and colistin (10 μg tablet).

Biochemical tests were performed in triplicate using the API 20A anaerobe identification kit (bioMérieux) as recommended by the manufacturer. Xylan fermentation was determined in trypticase/yeast extract broth as recommended by Holdeman et al. (1977). Metabolic end products were assayed by quantitative gas chromatography as described previously (Carlier, 1985). Enzyme profiles were generated with the Rapid ID 32A anaerobe identification kit (bioMérieux), according to the manufacturer's instructions, and performed in triplicate. The results of these tests are given in the species description.

Characteristics summarized in Table 1 for the novel strains and related Prevotella species showed that the novel strains were phenotypically very similar to Prevotella buccalis and Prevotella veroralis. However, the novel strains could be distinguished from P. buccalis by the absence of arginine arylamidase production and from P. veroralis by the absence of fermentation of xylan.

The cellular fatty acid composition of strain LBN 293T was determined by using the MIDI Microbial Identification System at the Identification Service of the DSMZ (Supplementary Table S1). Analysis of cellular fatty acids showed that strain LBN 293T had a low similarity index (0.106) to the Prevotella loescheii entry found in MIDI library version 3.8. The high level of summed feature 11 and smaller amount of C18:1ω9c are differential characteristics between strain LBN 293T and closely related species.

On the basis of the above-mentioned findings, we propose that strains LBN 293T, LBN 297 and LBN 298 should be classified within a novel species of the genus Prevotella, Prevotella nanceiensis sp. nov.

**Description of Prevotella nanceiensis sp. nov.**

Prevotella nanceiensis (nan.ce.i.en’s.is. N.L. fem. adj. nanceiensis pertaining to Nanceiwm, the old name of Nancy, the French city where the strains supporting the description of the species were isolated).
Cells are obligately anaerobic, non-spore-forming, non-motile, Gram-negative coccoid and short rods (0.4–0.5 × 1.0 μm). After 4 days incubation on BBA, colonies are 0.5–1 mm in diameter, circular, entire, slightly convex and smooth, white, non-pigmented and surrounded by a β-haemolysis zone. Growth is inhibited in the presence of bile. Strains are saccharolytic and ferment glucose, lactose, maltose, mannose, raffinose and sucrose. Acid glucosidase are variable among strains. All strains are negative for the remaining 16 tests, resulting in a Rapid ID 32A profile of 47+/1-+/+/> 440222. The fatty acid profile predominantly comprises iso-C₁₅:₀ anteiso-C₁₅:₀ C₁₆:₀ C₁₆:₀ 3- OH, C₁₈:₀ anteiso-C₁₉:₀ 12c and summed feature 11 (iso-C₁₇:₀ 3- OH and/or C₁₈:₂ dimethylacetal). Chromosomal genomic size ranges from 3.02 to 3.09 Mb, with four rRN operon copies. The DNA G+C content of the type strain is 39.4 mol%.

The type strain is LBN 293<sup>T</sup> (=AIP 261.03<sup>T</sup> =CIP 108993<sup>T</sup> =CCUG 54409<sup>T</sup>), isolated from a blood culture. Two other strains were recovered from clinical respiratory samples.

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