Prevotella bergensis sp. nov., isolated from human infections

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Eight strains of anaerobic Gram-negative bacilli isolated from infections of the skin and soft tissues were subjected to a comprehensive range of phenotypic and genotypic tests. 16S rRNA gene sequence analysis revealed the strains to constitute a homogeneous group, distinct from species with validly published names but related to a cluster including *Prevotella buccae*, *Prevotella dentalis* and *Prevotella baroniae*. A novel species, *Prevotella bergensis* sp. nov., is proposed to accommodate these strains. *Prevotella bergensis* is saccharolytic and produces acetic and succinic acids as end products of fermentation. The G + C content of the DNA of the type strain is 48 mol%. The type strain of *Prevotella bergensis* is 94067913^T (=DSM 17361^T=CCUG 51224^T).

Members of the genus *Prevotella* (Shah & Collins, 1990) are Gram-negative, moderately saccharolytic, bile-sensitive organisms that are part of the normal flora of the human mouth and vagina, and the rumen in various mammalian species (Shah *et al.*, 1998). They are also frequently isolated from infections of the mouth and other body sites. In this study, eight strains of anaerobic Gram-negative bacilli isolated from skin and soft-tissue infections in humans were subjected to a range of phenotypic and genotypic tests.

Strains 94044900 and 94067937 were isolated from skin abscesses, strains 94067913^T, 94068344, 95002865 and 95058389 were isolated from soft-tissue abscesses, 94082166 from a wound infection and 95062687 from a decubitus ulcer. *Prevotella buccae* CCUG 15401^T was obtained from the CCUG, *Prevotella dentalis* DSM 3688^T from the DSMZ and *Prevotella baroniae* E9.33^T from our collection (King's College London).

Strains were grown at 37 °C on Fastidious Anaerobe Agar (FAA; LabM) supplemented with 5 % horse blood under

anaerobic conditions (80 % N₂, 10 % H₂, 10 % CO₂) in an anaerobic workstation (Don Whitley Scientific). Colonial morphologies on this medium after 4 days incubation were viewed using a dissecting microscope. Cellular morphology was recorded after Gram-staining of smears prepared from 2-day FAA cultures. Hanging-drop preparations of 18-h cultures of peptone/yeast extract/glucose (PYG) broth were examined under phase-contrast microscopy for cellular motility. Transmission electron microscopy was used to examine the cell-wall ultrastructure of strains 94067913^T and 94068344 as described previously (Downes *et al.*, 2000).

Fermentation tests were performed using pre-reduced, anaerobically sterilized (PRAS) sugars prepared in-house in an anaerobic workstation (Holdeman *et al.*, 1977). Other biochemical tests were performed as described by Holdeman *et al.* (1977) and Jousimies-Somer *et al.* (2002). Bacterial strains were grown in peptone/yeast extract (PY) broth, with and without glucose and short-chain volatile and non-volatile fatty acids, extracted by standard methods and analysed by gas chromatography (Holdeman *et al.*, 1977), using a CP9002 instrument (Chrompak). Enzyme profiles were generated with the Rapid ID 32A anaerobe identification kit (bioMérieux), according to the manufacturer's instructions, using bacteria harvested from blood agar plates (Blood Agar Base no. 2; LabM) supplemented with 5 % horse blood, and performed in triplicate.

Cellular fatty acid composition was determined for all strains as described previously (Sutcliffe, 2000; Downes *et al.*,

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Abbreviation: FAME, fatty acid methyl ester.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *P. bergensis* DSM 17361^T is AY350613.

Electron micrographs of *P. bergensis*, an extended phylogenetic tree and FAME analyses compared with other *Prevotella* species are available as supplementary material in IJSEM Online.

2005). Fatty acid methyl esters (FAMES) were identified by comparison with authentic FAME standards (Sigma). The G+C content of the DNA of strains 94067913^T and 94068344 was estimated by an HPLC method as described previously (Wade *et al.*, 1999). A thermal denaturation method (Huß *et al.*, 1983) was used to determine the extent of DNA–DNA hybridization between strains.

DNA extraction, PCR and sequencing of 16S rRNA genes were performed as described previously (Downes *et al.*, 2005). Sequences were connected using the BioEdit program (Hall, 2004) and identified by BLAST interrogation of the GenBank database (Altschul *et al.*, 1990). Related sequences were aligned using BioEdit and further phylogenetic analysis was performed using the PHYLIP suite of programs (Felsenstein, 1993). Specifically, a distance matrix was constructed using the Jukes–Cantor algorithm by means of DNADIST, and NEIGHBOR was used to construct phylogenetic trees which were viewed using TreeView (Page, 1996).

The eight strains were obligately anaerobic, non-motile, Gram-negative bacilli which were 0.7–0.8 µm wide by 0.8–6 µm long. After 4 days incubation on FAA plates, colonies were 0.6–0.8 mm in diameter, circular, entire, convex, opaque and grey to off-white in colour. Transmission electron microscopic examination of ultrathin sections through the cells of strains 94067913^T and 94068344 showed the presence of a typical Gram-negative cell wall composed of a thin peptidoglycan layer surrounded by an outer membrane. In addition, a capsule was clearly visible around the cells of both strains (micrographs included as Supplementary Fig. S1 in IJSEM Online).

Growth of all strains in PY broth produced a moderately turbid suspension (2 to 3+ on a scale of 0 to 4+). Growth was enhanced (3 to 4+) by the addition of 1% fermentable carbohydrates. Strains were saccharolytic and major amounts of acetic and succinic acids and a trace of isovaleric acid were produced as end products of metabolism in PYG. All strains hydrolysed aesculin but arginine, gelatin and urea were not hydrolysed. Indole and catalase were not produced and nitrate was not reduced. There was no growth in 20% bile. All strains were positive for β-galactosidase, α-glucosidase, α-arabinosidase, alkaline phosphatase, leucyl glycine arylamidase and alanine arylamidase in the Rapid ID 32A identification panel; reactions for β-glucosidase, β-glucuronidase, β-N-acetyl-glucosaminidase, mannose fermentation, arginine arylamidase and glutamyl glutamic acid arylamidase were weak and variable. The G+C content of the DNA of strains 94067913^T and 94068344 was 48 mol%.

Phylogenetic analysis of the 16S rRNA gene sequence of strain 94067913^T revealed this organism to be most closely related to species belonging to the genus *Prevotella* (Fig. 1). A fuller phylogenetic tree is available as Supplementary Fig. S2 in IJSEM Online. The most closely related species were *Prevotella dentalis* (90.2% sequence identity), *P. baroniae* (89.1%) and *P. buccae* (86.7%). The 5'-end of the 16S rRNA gene was sequenced for the remaining seven strains

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequence comparisons over 1412 aligned bases showing the relationship between *Prevotella bergensis* sp. nov. and related species. The tree was constructed using the neighbour-joining method following distance analysis of aligned sequences. Numbers represent bootstrap values for each branch based on data for 100 trees. Accession numbers for 16S rRNA gene sequences are given in parentheses for each strain. Bar, 0.1 nucleotide substitutions per site.

and showed ≥98.8% sequence identity to strain 94067913^T over 485 unambiguously aligned bases.

DNA–DNA hybridization between strains 94067913^T and 94068344 was determined to be 85%. Relatedness between strain 94067913^T and *P. buccae* CCUG 15401^T, *P. dentalis* DSM 3688^T and *P. baroniae* E9.33^T was estimated at 16, 15 and 4%, respectively.

Cellular FAME results for the strains are available in Supplementary Table S1 in IJSEM Online. The non-hydroxy FAME profiles were consistent with those previously described for the genus *Prevotella* (Downes *et al.*, 2005; Moore *et al.*, 1994; Sakamoto *et al.*, 2004), with methyl-branched fatty acids predominating (notably anteiso-C15:0). As noted previously (Downes *et al.*, 2005), the use of acid-catalysed methanolysis in the FAME preparation may have prevented the recovery of hydroxy fatty acids. Rather than revise the description of the FAME composition of the genus *Prevotella*, we reemphasize our previous conclusion (Downes *et al.*, 2005) that a comprehensive, standardized survey of FAME composition in the genus *Prevotella* is now warranted. Most significantly, in the present context, the FAME profiles for the strains described herein were similar to those previously reported for *P. baroniae*, consistent with the close relationship of these species deduced from the phylogenetic analysis, although iso-C14:0 was noted to be increased at the expense of iso- and anteiso-C17:0 FAMES (see Supplementary Table S1).

The strains studied here constitute a homogeneous group and are clearly distinct from any species with validly published names. Although the level of 16S rRNA gene sequence identity with related strains is relatively low, this is true for the majority of species of *Prevotella*, which form a loose phylogenetic cluster. There are no phenotypic characteristics

Table 1. Phenotypic characteristics that differentiate *Prevotella bergensis* sp. nov. from other *Prevotella* species

Species: 1, *P. buccae*; 2, *P. dentalis*; 3, *P. oris*; 4, *P. salivae*; 5, *P. bergensis*; 6, *P. oralis*; 7, *P. baroniae*; 8, *P. buccalis*; 9, *P. loescheii*; 10, *P. veroralis*; 11, *P. multiformis*; 12, *P. denticola*; 13, *P. melaninogenica*; 14, *P. shahii*; 15, *P. oulorum*; 16, *P. enoea*; 17, *P. bivia*; 18, *P. tanneriae*; 19, *P. intermedia*; 20, *P. nigrescens*; 21, *P. corporis*; 22, *P. marshii*; 23, *P. pallens*; 24, *P. disiens*. w, Weak; v, variable.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Pigment*	–	–	–	–	–	–	–	–	+	–	–	–	+	+	–	–	–	+	+	+	+	–	+	–
Fermentation of:																								
Arabinose	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cellobiose	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	v	–	–	–	–	–	–
Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	v	+	v	v	+	+	v	–	–
Raffinose	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	–	–	v	v	+	–	–	+	–
Salicin	+	–	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sucrose	+	w	+	+	–	+	+	+	+	+	+	+	+	+	+	–	–	v	+	+	–	–	+	–
Indole production	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	+	–
Aesculin hydrolysis	+	+	+	+	+	+	+	+	+	+	–	+	v	–	+	v	–	–	–	–	–	–	–	–
Gelatin liquefaction	+	–	v	–	–	v	w	–	+	v	+	+	+	+	–	+	+	+	+	+	+	+	+	+

*Pigmentation on blood agar may take up to 14 days and varies from tan to brown to black depending on the species.

among *Prevotella* species which would support the subdivision of the genus. We therefore propose the name *Prevotella bergensis* sp. nov. for this group of strains. Phenotypic characteristics that differentiate *P. bergensis* from other *Prevotella* species are shown in Table 1.

Description of *Prevotella bergensis* sp. nov.

Prevotella bergensis (berg.en'sis. N.L. fem. adj. *bergensis* referring to Bergen, the Norwegian city where the first strains were isolated).

The description is based on eight strains isolated from human skin and soft-tissue abscesses. Biochemical results were identical for all strains studied. Cells are obligately anaerobic, non-motile, Gram-negative bacilli (0.7–0.8 µm × 0.8–6 µm). After 4 days incubation on FAA plates, colonies are 0.6–0.8 mm in diameter, circular, entire, convex, opaque and grey to off-white in colour. Growth in broth media produces a moderate turbidity which is enhanced by the addition of fermentable carbohydrates. Cells are saccharolytic and ferment arabinose, cellobiose, fructose, glucose, lactose, maltose, mannose, rhamnose, salicin and xylose; mannitol and trehalose are fermented variably; melezitose, melibiose, raffinose, ribose, sorbitol and sucrose are not fermented. Major amounts of acetic and succinic acids and a trace of isovaleric acid are produced as end products of metabolism in PYG broth. The non-hydroxylated fatty acid profile is predominately iso-C14:0, iso-C15:0 and anteiso-C15:0. Aesculin is hydrolysed; arginine, gelatin and urea are not hydrolysed. Indole and catalase are not produced and nitrate is not reduced. There is no growth in 20 % bile. The G+C content of the DNA of the type strain is 48 mol%.

The type strain is 94067913^T (=DSM 17361^T=CCUG 51224^T). The type and other strains reported here were isolated from infections of the skin and soft-tissue abscesses.

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