Dental caries is one of the commonest bacterial diseases of man and results from the dissolution of tooth enamel by acids produced by bacterial members of the dental plaque biofilm (Bowden & Edwardsson, 1994). Streptococcus mutans is the organism principally implicated in the pathogenesis of the disease, although many other bacterial species are capable of playing a similar pathogenic role in producing acid from fermentable carbohydrates and being tolerant of acidic conditions (Beighton, 2005). If carious lesions are not treated, the destruction of a tooth can continue into the dentine. Obligate anaerobes predominate in carious dentine and proteolytic activity may be important in the progression of the lesion as it approaches the pulp, where the bacteria can utilize proteins leaking into the dentine (Chhour et al., 2005). Members of the genus Propionibacterium are commonly detected in carious dentine (Aas et al., 2008; Chhour et al., 2005; Edwardsson, 1974; Hoshino, 1985), with Propionibacterium acnes being the species most commonly reported.

In a combined cultural and molecular analysis of the microbiota in dentinal caries, we found that an as-yet unnamed taxon was the dominant Propionibacterium taxon present and was one of only three taxa at species level to be found in every lesion (Munson et al., 2004). The aim of the present study was to perform a genetic and phenotypic characterization of three strains that are representative of this unnamed Propionibacterium taxon.

Three strains of anaerobic, pleomorphic, Gram-positive-staining bacilli, which were isolated from human carious dentine, were subjected to a comprehensive range of phenotypic and genotypic tests and were found to comprise a homogeneous group. The strains were saccharolytic and produced acetic and propionic acids in large amounts, and succinic acid in moderate amounts, as the end products of fermentation. 16S rRNA gene and RpoB protein sequence analyses revealed that the strains constituted a novel group within the genus Propionibacterium, most closely related to Propionibacterium australiensis but sharing only 8% DNA–DNA relatedness with the type strain of that species. Therefore, a novel species, Propionibacterium acidifaciens sp. nov., is proposed to accommodate these strains. The DNA G+C content of the type strain is 70 mol%. The type strain is C3M_31T (=DSM 21887T =CCUG 57100T).

Strains C3M_31T, C2A_34 and C5A_34 were isolated from the dentine of carious lesions in three subjects, as described previously (Munson et al., 2004). Strains were grown at 37°C on fastidious anaerobe agar (FAA; LabM) supplemented with 5% horse blood in an anaerobic workstation (Don Whitley Scientific) under anaerobic conditions (80% N2/10% H2/10% CO2). Colonial morphologies of 6-day-old FAA plate cultures were viewed by means of a dissecting microscope. The morphology of Gram-stained cells harvested from 2-day-old FAA plate cultures was determined by light microscopy at ×1000 magnification. Cell motility in hanging-drop preparations of 18 h cultures from peptone-yeast extract-glucose (PYG) broth was determined by examination using phase-contrast microscopy at ×400 magnification.

Biochemical and physiological tests were performed using standard methods (Holdeman et al., 1977; Jousimies-Somer et al., 2002). Fermentation tests were performed using prerduced, anaerobically sterilized sugars prepared in an anaerobic workstation (Holdeman et al., 1977). Bile sensitivity was determined by growth on tryptone soy agar (TSA; Oxoid) with and without 2% oxgall (Sigma) (equivalent to 20% bile). Susceptibility to special-potency antibiotic discs containing vancomycin (5 µg), kanamycin (1 mg) and colistin (10 µg) was determined on FAA (Jousimies-Somer et al., 2002). Presence and amounts of volatile and non-volatile fatty acids produced as metabolic end products in 7-day-old PYG broth cultures were determined by gas chromatography (Holdeman et al., 1977). Enzyme profiles were generated with the Rapid ID 32A anaerobe identification kit (bioMérieux), according to the manufacturer’s instructions, using bacteria harvested from 2-day-old Columbia agar plates (LabM) supplemented with 5% horse blood.

Propionibacterium acidifaciens sp. nov., isolated from the human mouth

Julia Downes and William G. Wade

King’s College London Dental Institute at Guy’s, King’s College and St Thomas’ Hospital, Infection Research Group, London SE1 9RT, UK
The G+C content of the DNA of strain C3M_31T was determined by an HPLC method as described previously (Wade et al., 1999). The 16S rRNA genes of the strains were sequenced as described previously (Downes et al., 2005). Sequences were assembled with the BioEdit program (Hall, 2004) and their closest relatives were determined by BLAST interrogation of the GenBank database (Altschul et al., 1990). Sequences were aligned using CLUSTAL W within BioEdit and phylogenetic trees were constructed in MEGA version 4 (Tamura et al., 2007) using the neighbour-joining method from distance matrices prepared with the Jukes–Cantor correction. Pairwise estimations of DNA–DNA relatedness were performed using a thermal denaturation method (Huš et al., 1983). Primers that were specific for part of the rpoB gene were designed from an alignment of rpoB sequences from actinobacterial genome sequences, as detailed in Table 1. The primers were Prop_rpoB_551F, 5'-GACCTTCATYATCAACGCAGC-3', and Prop_rpoB_1489R, 5'-CACATVCRCGGTATG-3', and were predicted to give an amplicon of around 950 bases. The amplified genes were sequenced and translated and a dendrogram based on the amino acid sequences was constructed in MEGA with the neighbour-joining method using the Poisson correction.

The results of the phenotypic tests are given in the species description. The three strains were anaerobic, non-motile, non-spor-forming, Gram-positive-staining bacilli that were 0.7–0.8 μm wide by 1.2–4 μm long. Cells were pleomorphic with straight, slightly curved or club-shaped forms, arranged singly, in pairs or in short chains with some branched diphtheroidal arrangements. After anaerobic incubation for 6 days on FAA plates, colonies were 0.7–1.1 mm in diameter, circular, entire, high-convex to dome-shaped, smooth, shiny, white to pale cream and opaque with a solid non-translucent internal appearance when viewed under a plate microscope. There was no detectable growth on FAA plates incubated in air with 5% CO₂. The strains grew on TSA with 20% bile and the amount of growth was similar to that on TSA without bile, although the size of the colonies was reduced in the presence of bile. Strains were sensitive to vancomycin and kanamycin but resistant to colistin. There was good growth of all strains in peptone-yeast extract broth (3% + on a scale of 0–4 +). Growth was enhanced markedly (4 +) by the addition of 1% fermentable carbohydrates.

Strains were saccharolytic and produced acid from fructose, glucose, lactose, maltose, mannitol, mannose and sucrose, resulting in a final pH of 4.37–4.79. Fermentation of melibiose, raffinose, rhamnose and ribose produced a final pH of 5.07–5.60. L-Arabinose, cellobiose, melezitose and salicin were not fermented (pH 6.06–6.72).
The DNA G+C content of strain C3M_31T was 70 mol%. The phenotypic characteristics that distinguish these strains from the type strains of related Propionibacterium species are shown in Table 2. All three strains gave positive reactions in the Rapid ID 32A panel for β-galactosidase, β-galactosidase, α-glucosidase, arginine arylamidase, proline arylamidase, phenylalanine arylamidase, leucine arylamidase, tyrosine arylamidase and fermentation of mannose and raffinose, resulting in a profile of 4506 0331 00.

The almost-complete sequences of the 16S rRNA genes of all three strains were obtained. Over 1442 unambiguously aligned bases, the sequences of strain C2A_34 and C5A_34 differed from that of C3M_31T by one and two bases, respectively. Phylogenetic analysis of the 16S rRNA gene sequence of strain C3M_31T revealed that this organism belonged to the genus Propionibacterium (Fig. 1), with 99.5% sequence identity to subgingival plaque strain Propionibacterium sp. oral strain FMA5. The most closely related type strain was Propionibacterium australiense 98A072T, with which strain C3M_31T shared 97.7% sequence identity. However, the DNA–DNA relatedness value between strain C3M_31T and P. australiense CCUG 46075T was 8 ± 4%, indicating that the group of strains under study constitutes a novel species. The phylogenetic distinctiveness of C3M_31T was confirmed by RpoB protein sequence analysis with species from within the phylum Actinobacteria, which showed strain C3M_31T to be most closely related to Propionibacterium acnes KPA171202 (Fig. 2). Partial rpoB gene sequences for strains C3M_31T and C5A_34 were identical over 789 bases.

The strains constitute a homogeneous group and are clearly distinct from all species of the genus Propionibacterium with validly published names. We therefore propose the name Propionibacterium acidifaciens sp. nov.

**Description of Propionibacterium acidifaciens sp. nov.**

Propionibacterium acidifaciens (a.ci.di фа’ci.ens. N.L. n. acidum acid; L. v. facio to produce; N.L. part. adj. acidificiens acid-producing).

The description is based on three strains isolated from the human oral cavity. Cells are anaerobic, non-spore-forming, non-motile, pleomorphic, Gram-positive-staining bacilli (0.7–0.8×1.2–4 μm). After incubation for 6 days on FAA plates, colonies are 0.7–1.1 mm in diameter, circular, entire, high-convex to dome-shaped, white to pale cream and opaque with a solid non-translucent internal appearance. Growth in broth media produces a moderate turbidity that is markedly enhanced by the addition of fermentable carbohydrates. Cells are saccharolytic and ferment fructose, glucose, lactose, maltose, mannitol, mannose, melibiose, raffinose, ribose and sucrose; L-arabinose, cellobiose, melezitose and salicin are not fermented. Large amounts of acetic and propionic acids and moderate amounts of succinic acid are produced as the end products of metabolism in peptone-yeast extract-glucose broth. Arginine, aesculin, gelatin and urea are not hydrolysed. Indole and catalase are not produced. Nitrate is not reduced. There is less growth with 20% bile. The Rapid ID 32A profile is 4506 0331 00. The DNA G+C content of the type strain is 70 mol%.

The type strain, C3M_31T (＝DSM 21887T＝CCUG 57100T), was isolated from carious lesions in the human mouth.

![Fig. 1. Phylogenetic tree, based on 16S rRNA gene sequence comparisons of 1321 aligned bases and constructed using the neighbour-joining method, showing the relationship between strains of Propionibacterium acidifaciens sp. nov. and related Propionibacterium species. Percentages at nodes are levels of bootstrap support based on 500 resamplings. Bar, 2 substitutions per 100 nucleotide positions.](image1)

![Fig. 2. Phylogenetic tree, based on RpoB amino acid sequence comparisons over 202 aligned amino acids and constructed using the neighbour-joining method, showing the relationship between strains of P. acidifaciens sp. nov. and related species. Percentages at nodes represent bootstrap values for each branch based on data for 500 trees. Bar, 2 substitutions per 100 amino acid positions.](image2)
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