Mitsuokella dentalis sp. nov. from Dental Root Canals

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On the basis of phenetic and chemotaxonomic criteria a new nonmotile anaerobic gram-negative rod-shaped bacterium from dental root canals is described. The new species has characteristics in common with species of the genus Bacteroides, but there are also many differences. According to a detailed biochemical analysis the new species is closer to the genus Mitsuokella than to Bacteroides. We propose a new species, Mitsuokella dentalis. The type strain of M. dentalis is strain DSM 3688.

The genus Bacteroides is a large group of anaerobic nonmotile gram-negative rod-shaped bacteria (6). There are several features which are common to most species, such as main metabolic end products, resistance to kanamycin (which is uncommon among other gram-negative anaerobes), and the presence of respiratory quinones in the cells (1, 6, 12). Also, the cellular and colonial characteristics of species in the genus Bacteroides are different from those of other anaerobic gram-negative genera (6). Yet, in several aspects the genus Bacteroides is quite heterogeneous. The deoxyribonucleic acid (DNA) base composition ranges from 28 to 61 mol% guanine plus cytosine (G+C), and some species are asaccharolytic while others ferment a variety of sugars (6). Studies of the composition of enzymes, major long-chain fatty acids, lipids, and menaquinones have also revealed considerable differences (1–4, 9, 12, 13). It has been suggested that the genus Bacteroides should be restricted to only those species which (i) produce major amounts of succinic acid together with substantial levels of acetic acid and occasionally low levels of other short-chain acids; (ii) contain malate dehydrogenase and glutamate dehydrogenase; (iii) have a DNA base composition within the approximate limiting range of 40 to 52 mol% G+C; (iv) contain sphingolipids and menaquinones; and (v) possess major amounts of straight-chain saturated, iso- and anteiso-methyl branched-chain fatty acids (3, 4, 9–12). On the basis of the description given above, the following four new genera have been suggested recently: Rikenella microfusus (previously Bacteroides microfusus), Mitsuokella multiacidus (previously Bacteroides multiacidus), Megamonas hypermegas (previously Bacteroides hypermegas), and Sebaldella termiditis (previously Bacteroides termiditis) (2, 3, 7, 8, 10, 11).

We isolated from root canals of three individuals three anaerobic gram-negative nonmotile rod-shaped bacteria which could not be identified as any known species (5). These three strains, strains ES2772, ES2645, and KR11, had a few characteristics typical of the genus Bacteroides. The organisms were resistant to kanamycin, their metabolic end products were acetic and succinic acids, as for most saccharolytic Bacteroides spp., and their appearance in Gram stains resembled the appearance of many Bacteroides spp. However, a more profound analysis of biochemical, phenetic, and ultrastructural features clearly showed that ES2772, ES2645, and KR11 cannot be members of the genus Bacteroides. These isolates had a high DNA base composition compared with Bacteroides spp., ranging from 56 to 60 mol% G+C, and they lacked respiratory quinones. Both thin sections and negatively stained specimens revealed prominent fimbriae as a constant finding when cells were studied by electron microscopy. DNA homology studies with other species having high G+C contents (Bacteroides capillosus, Mitsuokella multiacidus, and Rikenella microfusus) showed a level of relatedness of only 20 to 30%. The results of biochemical studies with the three strains showed that they are more similar to members of the genus Mitsuokella than to Bacteroides spp. Therefore, we concluded that strains ES2772, ES2645, and KR11 represent a new species in the genus Mitsuokella, for which we propose the name Mitsuokella dentalis (den.ta’lis. Lat. fem. adj. dentalis pertaining to the teeth).

Description of Mitsuokella dentalis sp. nov. The description given below is based upon our study of strains ES2772, KR11, and ES2645 (5). Nonmotile, nonsporeforming, gram-negative rods approximately 0.7 μm wide by 1 to 2 μm long. Cells from agar are blunt-ended, oval rods which occur singly. Thinly sectioned cells studied by electron microscopy show an outer membrane structure typical of gram-negative bacteria. Numerous fimbriae and a thick capsule-like zone around the cell are present. For ultrastructural details see reference 5.

Obligately anaerobic. Cells grow better on blood agar media with hemolysed blood than in liquid media, where growth is poor. No growth on kanamycin-vancomycin-laked blood agar.

Colonies after 3 days of incubation on enriched horse blood agar (hemolysed blood) are 1 to 2 mm in diameter, convex, irregular in shape, translucent, wet, and mucoid. The colonies have a water drop appearance, which is a distinctive characteristic of this species. α-Hemolysis on horse and sheep blood agar plates is usually seen after 7 days of incubation.

Ferments arabinose, cellobiose, fructose, galactose, glucose, lactose, maltose, mannose, and raffinose in prereduced anaerobically sterilized peptone-yeast extract broth. Melibi-
ose and sucrose are weakly fermented (5.5 < pH < 5.7); erythritol, mannitol melezitose, rhamnose, salicin, and xylose are not fermented. As determined by using the API 20A system the fermentation reactions are negative. Gelatin and starch are not hydrolyzed. Indole negative. The esculin hydrolysis test is positive when a chromogenic enzyme is present, and glutamate dehydrogenase is present only in minor amounts. α-Arabinosidase, α-galactosidase, α-glucosidase, β-galactosidase, β-glucosidase, β-N-acetyl-glucosaminidase, alkaline phosphatase, and alanine-aminopeptidase are present. Hydrolyzes indoxyl acetate, a-aminopeptidase, nine, and glycine aminopeptidases and pyroglutamic acid dehydrogenase is present, and glutamate dehydrogenase is present only in minor amounts. α-Arabinosidase, α-galactosidase, α-glucosidase, β-galactosidase, β-glucosidase, β-N-acetyl-glucosaminidase, alkaline phosphatase, and alanine-aminopeptidase are present. Hydrolyzes indoxyl acetate, α-Fucosidase, α-mannosidase, and β-xiosidase are absent. Leucine, proline, tyrosine, arginine, histidine, phenylalanine, and glycine aminopeptidases and pyroglutamic acid arylamidase are not produced. Arginase is not utilized. Resistant to kanamycin and colistin, while vancomycin is inhibitory at concentrations above 5 μg/ml. Susceptible to erythromycin, penicillin, rifampin, and metronidazole. Sensitive to oxgall and brilliant green.

The cell wall peptidoglycan is based upon meso-diaminopimelic acid; glycine, alanine, glutamic acid, and aspartic acid are present. Hydroxylated and nonhydroxylated long-chain fatty acid methyl esters are present in whole-cell methanolsylates. The major hydroxylated fatty acid is 3-hydroxy-hexadecanoic acid (3-OH-C16:0). The predominant fatty acids are hexadecanoic (C16:0) and 12-methyltridecanoic (iso-C14:0) acids. The G+C content of the DNA ranges from 56 to 60 mol%, as determined by the melting temperature method.

Isolated from human dental root canals. The type strain is strain ES2772 (= DSM 3688).

Characteristics of the new species and related taxa which are useful in identification is summarized in Table 1. Asaccharolytic genera and species are included because of the possibility that fermentation reactions of *Mitsuokella dentalis* are negative if only commercial test systems are used. *Mitsuokella dentalis* is readily identified as a separate species from saccharolytic Bacteroides spp. by its translucent, water drop-like colonies on solid media, its slower growth, and its negative starch hydrolysis reaction.

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**LITERATURE CITED**


