Description of *Mogibacterium pumilum* gen. nov., sp. nov. and *Mogibacterium vescum* gen. nov., sp. nov., and reclassification of *Eubacterium timidum* (Holdeman *et al.* 1980) as *Mogibacterium timidum* gen. nov., comb. nov.

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A new genus, *Mogibacterium*, is proposed for anaerobic, non-spore-forming, Gram-positive, rod-shaped bacteria which have been isolated from the periodontal pockets of adult human patients with periodontal disease and infected root canals. The novel isolates, strains D2-18^T, BA11a-f and D5-2^T, were inert in most of the conventional biochemical tests and phenotypically resemble asaccharolytic *Eubacterium* species. The protein profiles of whole cells on SDS-PAGE gels and Western immunoblotting reaction analysis distinguished these organisms from type strains belonging to the previously described *Eubacterium* species. The G+C content of the DNA is 45–46 mol% for *Mogibacterium pumilum* and 46 mol% for *Mogibacterium vescum*. The levels of DNA–DNA relatedness of these new species to other *Eubacterium* species, including *Eubacterium limosum*, *Eubacterium brachy*, *Eubacterium lentum*, *Eubacterium nodatum*, *Eubacterium sapheum*, and the more recently proposed *Eubacterium minutum* and *Eubacterium exiguum* (reclassified as *Slackia exigua*), are less than 2%. The DNA–DNA hybridization value between *M. pumilum* and *M. vescum* was 30%. *Eubacterium timidum* exhibited DNA homologies with *Mogibacterium* species which were low (17 and 18%) but clearly higher than with all the other *Eubacterium* species. Phylogenetic analysis based on 16S rRNA gene sequences revealed that the closest phylogenetic neighbour of *Mogibacterium* species was *E. timidum*, and that these three species represent a novel lineage distinct from the previously described genera of Gram-positive, rod-shaped bacteria. On the basis of phenotypic characteristics and 16S rRNA gene sequence comparisons, it is also proposed that *E. timidum* is transferred to the genus *Mogibacterium* gen. nov. as *Mogibacterium timidum* gen. nov., comb. nov. (type strain ATCC 33093^T).

Keywords: *Mogibacterium pumilum* gen. nov., sp. nov., *Mogibacterium vescum* gen. nov., sp. nov., *Mogibacterium timidum* gen. nov., comb. nov.

INTRODUCTION

Numerous bacterial strains of Gram-positive, non-spore-forming, obligately anaerobic rods belonging to the genus *Eubacterium* have been isolated from human

oral cavities, including dental plaque (Hill *et al.*, 1987), periodontal lesions (Holdeman *et al.*, 1980; Martin *et al.*, 1986; Moore *et al.*, 1982, 1985; Uematsu & Hoshino, 1992; Wade *et al.*, 1992), infected dental pulp (Hoshino *et al.*, 1992; Sato *et al.*, 1993a; Sundqvist, 1976), carious dentine (Ando & Hoshino, 1990; Edwardsson, 1974; Hoshino, 1985) and dento-alveolar abscesses (Wade *et al.*, 1994). Several species of *Eubacterium*, particularly asaccharolytic species,

The GenBank/EMBL/DBJ accession numbers for the 16S rRNA sequences are AB021701 (*Mogibacterium pumilum* D2-18^T = ATCC 700696^T) and AB021702 (*Mogibacterium vescum* D5-2^T = ATCC 700697^T).

are associated with moderate and severe periodontitis and are less frequent in the supragingival or subgingival sites of healthy persons (Moore *et al.*, 1983; Wade *et al.*, 1992). In addition, it has been recognized that antibody titres against *Eubacterium* species in the serum of patients with periodontitis are higher than those of healthy controls, suggesting that these bacteria cause immunological reactions in periodontal lesions (Gunsolley *et al.*, 1990; Slayne & Wade, 1994; Tew *et al.*, 1985; Tolo & Jorkjend, 1990; Tolo & Schenck, 1985). *Eubacterium* strains have also been isolated from non-oral clinically significant infections, such as head and neck infections, abdominal infection (Brook & Frazier, 1993), bacteraemia associated with malignancy (Fanstein *et al.*, 1989) and genital tract infection associated with intrauterine devices (Brook, 1983).

The genus *Eubacterium* is distinguished from other genera mainly on the basis of negative metabolic characteristics (Moore & Moore, 1986). Because of its broad definition, the genus has historically acted as a repository for a large number of phenotypically diverse organisms (Cheeseman *et al.*, 1996; Nakazawa & Hoshino, 1993). In addition to their phenotypic heterogeneity, it is reported that the bacterial species belonging to the genus are not phylogenetically homogeneous and several species may be closely related to members of the genus *Clostridium* (Collins *et al.*, 1994; Willems *et al.*, 1996). In recent studies, many oral asaccharolytic isolates have been described, some of which qualify as members of the genus *Eubacterium* but could not be assigned to any of the named species (Cheeseman *et al.*, 1996; Poco *et al.*, 1996a, 1996b; Wade *et al.*, 1994; Sato *et al.*, 1993a).

Recently, we have isolated *Eubacterium*-like strains, D2-18^T, BA11a-f and D5-2^T, from periodontal pockets and infected root canals. However, these strains could not be classified in any of the established *Eubacterium* species. These organisms are asaccharolytic and Gram-positive rods, grow slowly and resemble *Eubacterium timidum* in phenotypic characteristics when they are compared with the previously established asaccharolytic bacterial species. There are nine previously established oral asaccharolytic *Eubacterium* species, namely *E. timidum*, *Eubacterium brachy*, *Eubacterium nodatum*, *Eubacterium lentum*, *Eubacterium saphenum*, *Eubacterium minutum*, *Eubacterium exiguum* (reclassified as *Slackia exigua*), *Eubacterium tardum* and *Eubacterium infirmum*. These species are unreactive in conventional biochemical tests and difficult to culture because of their slow growth even on artificial media designed for fastidious organisms and requirement for strict anaerobiosis (Uematsu & Hoshino, 1992). A recent study has shown that reference strains of oral asaccharolytic *Eubacterium* species, including strains D2-18^T, BA11a-f and D5-2^T, can be distinguished from each other by RFLP analysis of 16S rDNA amplified by PCR (Sato *et al.*, 1993b). To determine the taxonomic position of these novel isolates, we examined their phenotypic and phylogenetic charac-

teristics and compared our results with data for previously described reference species. In this paper, we report phenotypic characteristics, including whole-cell protein profiles and Western immunoblotting reactions, and the results of DNA-DNA hybridization studies, DNA base composition analysis and 16S rRNA gene sequence studies of these organisms. On the basis of these results, we propose a new genus, *Mogibacterium* gen. nov., and new species, *Mogibacterium pumilum* sp. nov. and *Mogibacterium vescum* sp. nov., for these organisms. It is also proposed that *E. timidum* is transferred to the genus *Mogibacterium* gen. nov. as *Mogibacterium timidum* gen. nov., comb. nov.

METHODS

Bacterial strains. The novel strains D2-18^T and D5-2^T were isolated from human periodontal pockets, and the novel strain BA11a-f was isolated from an infected root canal (Sato *et al.*, 1993b; Slayne & Wade, 1994). The type strains of previously established asaccharolytic *Eubacterium* species, together with the more recently proposed species *E. minutum* (Poco *et al.*, 1996b) and *E. exiguum* (Poco *et al.*, 1996a) were included in this study. *E. infirmum* and *E. tardum* (Cheeseman *et al.*, 1996) were not examined in this work, but are discussed.

Culture conditions. All the strains were cultured on brain-heart infusion (BHI)-blood agar plates under strictly anaerobic conditions for 7–10 d in an anaerobic glove box (model AZ-Hard; Hirasawa) containing 80% N₂, 10% H₂ and 10% CO₂. The bacterial cells were harvested by centrifugation, washed with 10 mM PBS (136 mM NaCl in 10 mM sodium phosphate buffer (pH 7.2) and stored at –20 °C until they were used. To ensure strictly anaerobic conditions in the box, the reduction of methyl viologen (at –446 mV) was carefully checked whenever experimental procedures were carried out.

Morphological studies. Cellular morphology was determined by examining cells grown on BHI-blood agar plates. Transmission electron microscopy was used for cell wall characterization. Cultures (48 h) were fixed in a solution of 2.5% (v/v) glutaraldehyde in 0.1 M potassium phosphate buffer (pH 7.4) for 1 h at 4 °C. The cells were centrifuged and washed three times with the same buffer. They were post-fixed with 1% (v/v) osmic acid for 18 h at 4 °C and dehydrated for 10 min each with 50, 75, 90, 95 and 100% (v/v) ethanol. Preparations were washed with 33.3, 66.6 and 100% (v/v) epoxy resin in propylene oxide for 60 min, and then embedded in 100% epoxy resin. After hardening, ultrathin sections were cut with a diamond knife and then stained with uranyl acetate and lead citrate. The thin sections were studied with a Hitachi H-600A electron microscope.

Biochemical characteristics. Biochemical reactions were tested by the methods described by Holdeman *et al.* (1977). End products produced in peptone-yeast extract-glucose (PYG) broth were assayed by gas chromatography as described previously (Hoshino & Sato, 1986). To examine enzymic profiles of these organisms, a Rapid ID 32A kit (API BioMérieux) was used as recommended by the manufacturer, except that preparations were incubated under strictly anaerobic conditions.

Whole-cell protein profiles. Cellular protein profiles were examined by using the SDS-PAGE procedure as described

by Laemmli (1970). Approximately 50 mg (wet wt) whole cells were suspended in 150 µl lysis buffer containing 4% (w/v) SDS, 40% (v/v) glycerol, 10% (v/v) 2-mercaptoethanol and 0.25 M Tris/HCl (pH 6.8). The suspensions were boiled for 10 min and then centrifuged at 12000 *g* for 10 min. The protein concentration of the supernatant were measured with a protein assay kit (Bio-Rad). The supernatants (total protein; 10 µg per lane) were subjected to electrophoresis on a 12.5% polyacrylamide gel by using a model KS-8010 Micro slab gel electrophoresis system (Marysol). After electrophoresis, the gel was stained with Coomassie brilliant blue R-250.

Western immunoblotting analysis. Proteins were transferred from SDS-PAGE gels to a nitrocellulose membrane (pore size 0.45 µm; Bio-Rad) by using the transfer buffer system described by Burnette (1981), in conjunction with the *trans* blot system (Marysol) at a constant current of 350 mA for 4 h with cooling. Membranes were processed with slight modification as described previously (Nakazawa & Hoshino, 1993). Briefly, a rabbit immune antiserum (1:1000) was used as first antibody, goat anti-rabbit IgG conjugated with peroxidase (1:1000) was used as the second antibody and the colour was then developed.

DNA isolation and DNA base composition. DNA was isolated and purified following a modification of the Marmur protocol (Marmur, 1961; Nakazawa & Hoshino, 1994). DNA G + C content was determined by HPLC as described by Katayama-Fujimura *et al.* (1984).

DNA–DNA hybridization analysis. DNA–DNA hybridization was performed by the membrane filter method as described previously (Meyer & Schleifer, 1978; Nakazawa & Hoshino, 1993). Briefly, reference DNA was labelled by using a multiprime DNA labelling kit (Amersham) and [α - 32 P]dCTP and then purified with a Nick column (Pharmacia LKB Biotechnology). About 40 µg unlabelled single-stranded DNA immobilized on each nitrocellulose membrane filter and 0.015 µg labelled reference DNA were reassociated in a solution containing 0.08% SDS, 0.02% (w/v) polyvinylpyrrolidone, 0.02% (w/v) Ficoll 400, BSA (Fraction V; Sigma) and 1 ml 6 × SSC (1 × SSC is 0.15 M NaCl plus 15 mM sodium citrate). After incubation overnight at 60 °C, the filters were washed and dried. The radioactivity was measured with a liquid scintillation counter. Triplicate tests were performed for each assay and the results were normalized to 100% for homologous DNA.

Sequencing of the 16S rRNA gene and phylogenetic analysis. The 16S rRNA gene was amplified by PCR with a nucleotide primer set [5'-AGA GTT TGA TCM TGG CTC AG-3', located at positions 8–27 (*Escherichia coli* numbering) and 3'-TTC AGC ATT GTT CCA TYG GCAT-5', located at positions 1492–1513 (*Escherichia coli* numbering)]. The PCR were performed with Premix Taq (Takara) according to the manufacturer's instructions in a thermal controller (model PTC-100; MJ Research). The DNA fragment obtained by PCR amplification was sequenced by using a Thermo Sequenase Fluorescent Labelled Primer Cycle Sequencing kit (Amersham Japan) and a series of custom-synthesized primers with an ALFexpress DNA sequencer (Pharmacia LKB) according to the manufacturer's instructions. The sequences were aligned by using SEQMAN II of the LASERGENE program (DNASTAR). MEGALIGN of LASERGENE, CLUSTAL W and NJPLOT programs were used to compare sequences and to construct a evolutionary tree by the neighbour-joining method (Saitou & Nei, 1987). Confidence values were assessed by CLUSTAL W with bootstrap analysis.

Nucleotide sequence accession numbers. The accession numbers of the 16S rRNAs sequences of reference strains were: *Acetobacterium bakii*, X96960; *Clostridium acetobutylicum*, X78073; *Clostridium acidurici*, M59084; '*Clostridium aminobutyricum*', X76161; *Clostridium beijerinckii*, X68180; *Clostridium bifermentans*, X73437; *Clostridium difficile*, X73450; *Clostridium felsineum*, X77851; *Clostridium formicoaceticum*, X77836; *Clostridium ghoni*, X73451; *Clostridium glycolicum*, X76750; *Clostridium innocuum*, M23732; *Clostridium irregularis*, X73447; *Clostridium litorale*, X77845; *Clostridium paradoxum*, Z69934; *Clostridium propionicum*, X77841; *Clostridium purinolyticum*, M60491; *Clostridium sordellii*, M59105; *Clostridium villosum* (reclassified as *Filifactor villosus*), X73452; *Eubacterium barkeri*, M23927; *Eubacterium biforme*, M59230; *Eubacterium brachy*, Z36272; *Eubacterium callanderi*, X96961; *Eubacterium cellulosolvens*, L34613; *Eubacterium infirmum*, U13039; *Eubacterium limosum*, M59120; *Eubacterium minutum*, AJ005636; *Eubacterium nodatum*, U13041; *Eubacterium sapenum*, U65987; *Eubacterium tardum*, U13037; *Eubacterium tenue*, M59118; '*Eubacterium thermomarinus*', L10086; *Eubacterium timidum*, Z36296; *Peptostreptococcus anaerobius*, L04168; *Peptostreptococcus asaccharolyticus*, D14138; and *Peptostreptococcus micros*, D14143.

RESULTS AND DISCUSSION

Colony and cell morphology

Colonies visible by eye of novel strains D2-18^T and BA11a-f, and D5-2^T (classified as *M. pumilum* and *M. vescum*, respectively, on the basis of the results given below) appeared on BHI-blood agar after 2 d incubation. After 4–6 d, the colonies were circular, convex, translucent, had a smooth surface and were about 0.4–0.6 mm in diameter. Even after prolonged incubation, the colonies were less than 1 mm in diameter. No haemolysis occurred around colonies of these strains on BHI-blood agar plates. The strains were non-spore, non-motile, short, rod-shaped organisms, occurring mostly as single cells, but sometimes in pairs, short chains or clumps. The growing cells were 0.2–0.3 µm × 1.0–1.5 µm in size and Gram-positive, but sometimes cells in older cultures were Gram-negative. The organisms are very difficult to culture in broth media because of their poor growth with or without carbohydrates even on fastidious artificial media, and their requirement for strict anaerobiosis, both of which are characteristics typical of oral asaccharolytic *Eubacterium* species.

Electron micrographs of ultrathin sections of *M. pumilum* D2-18^T and *M. vescum* D5-2^T revealed a cell wall architecture typical of Gram-positive bacteria, including a plasma membrane layer, thin peptidoglycan layer in the middle and a thicker outer layer (Fig. 1).

Phenotypic analysis

These organisms were asaccharolytic because they did not ferment glucose and other carbohydrates, and were inert in most of the conventional biochemical

Fig. 1. Transmission electron microphotographs of thin sections of cells of *Mogibacterium* species showing the Gram-positive cell wall. The cell wall (CW) and cytoplasmic membrane (CM) are indicated. (a) *Mogibacterium pumilum* D2-18^T (= ATCC 700696^T); (b) *Mogibacterium vescum* D5-2^T (= ATCC 700697^T). Bars, 100 nm.

Table 1. Biochemical characteristics of *Mogibacterium* and *Eubacterium* species

Taxon	End products from PYG*	Arginine hydrolysis	Nitrate reduction
<i>Mogibacterium pumilum</i> D2-18 ^T	phe-a	–	–
<i>Mogibacterium pumilum</i> BA11a-f	phe-a	–	–
<i>Mogibacterium vescum</i> D5-2 ^T	phe-a	–	–
<i>Mogibacterium (Eubacterium) timidum</i> ATCC 33093 ^T	phe-a	–	–
<i>Eubacterium exiguum</i> (<i>Slakia exigua</i>) ATCC 700122 ^T	–	+	–
<i>Eubacterium lentum</i> (<i>Eggerthella lenta</i>) ATCC 25559 ^T	–	+	+
<i>Eubacterium minutum</i> ATCC 700079 ^T	B or b	–	–
<i>Eubacterium nodatum</i> ATCC 33099 ^T	a, B	+	–
<i>Eubacterium saphenum</i> ATCC 49989 ^T	a, b	–	–
<i>Eubacterium brachy</i> ATCC 33089 ^T	ib, ic, iv, phe-p	–	–

* a, Acetate; b, butyrate; ib, isobutyrate; ic, isocaproate; iv, isovalerate; phe-a, phenyl acetate; phe-p, phenyl propionate. Capital letters represent an amount of product equal or more than 10 mM and small letters represent an amount less than 10 mM.

tests. They were nitrate-reduction-negative and did not hydrolyse aesculin, starch, arginine or gelatin. They also did not produce indole, ammonia, urease or catalase. They produced phenyl acetate as the sole metabolic end product in PYG medium, as assayed by gas chromatography (Table 1). Among the previously described oral asaccharolytic *Eubacterium* species, they phenotypically resembled *E. timidum*, which also produces phenyl acetate as an end product. They are phenotypically distinct from the acetate- and butyrate-producing asaccharolytic *Eubacterium* species (Table 1) such as *E. brachy*, *E. nodatum*, *E. saphenum*, *E. minutum*, *E. tardum* and *E. infirmum* (Cheeseman *et al.*, 1996). The enzymic activities of these strains showed a profile (API code 0000020000) including a positive reaction in the aminopeptidase and proline arylamidase tests, which was the same as that reported by Cheeseman *et al.* (1996) for *E. timidum*. The previously described oral asaccharolytic *Eubacterium* species, including the recently proposed species, *E. minutum*, *E. exiguum*, *E. tardum* and *E. infirmum*, each showed quite typical enzymic profiles (Cheeseman *et al.*, 1996; Poco *et al.*, 1996a, b).

In the whole-cell protein profiles examined by SDS-PAGE, *M. pumilum* D2-18^T had a major band in the region of about 30 kDa, whilst *M. vescum* D5-2^T had major bands of about 55, 45 and 30 kDa (data not shown). These oral asaccharolytic bacteria, including *Eubacterium* species examined in the present study, namely *E. exiguum*, *E. lentum*, *E. brachy*, *E. nodatum*, *E. timidum*, *E. minutum*, *E. saphenum*, *M. vescum* and *M. pumilum*, each showed typical protein profiles and no major bands were common, indicating great heterogeneity in the whole-cell protein components. Western immunoblotting reactions with rabbit antisera revealed that antigens of *M. pumilum* and *M.*

vescum did not react with the antisera against the reference oral asaccharolytic species, whilst antigens from the previously described species were recognized by their respective antisera (data not shown). These results clearly showed that *M. pumilum* and *M. vescum* could be distinguished from the reference species by their protein components, including the structural proteins of the whole bacterial cells, and by their immunological reactions. These data are also consistent with the previously published data of Nakazawa & Hoshino (1993).

Genotypic analysis

As previously reported, the DNA G+C contents of oral asaccharolytic *Eubacterium* species range from 38 to 62 mol % (Nakazawa & Hoshino, 1994; Poco *et al.*, 1996a, b). The G+C contents of *M. pumilum* strains D2-18^T and BA11a-f, and *M. vescum* strain D5-2^T were 46, 45 and 46 mol %, respectively (Table 2), which are similar to *E. saphenum* and *E. tardum* (Cheeseman *et al.*, 1996; Uematsu *et al.*, 1993) but are clearly distinguishable from the other *Eubacterium* species, *E. brachy*, *E. nodatum*, *E. minutum*, *E. lentum*, *E. timidum*, *E. exiguum*, *E. infirmum* and *E. limosum*, which is the type species of the genus *Eubacterium* (Table 2).

The levels of DNA relatedness estimated by quantitative DNA–DNA hybridization studies with the membrane filter method are listed in Table 2. The DNA of *M. pumilum* strain D2-18^T exhibited a very high level of reassociation with the DNA of the strain BA11a-f (97%), clearly suggesting that they belong to the same bacterial species. On the other hand, *M. vescum* strain D5-2^T showed 31 % DNA reassociation with *M. pumilum* strain D2-18^T. Moreover, these

Table 2. DNA base compositions and levels of DNA relatedness

Source of DNA	G + C content (mol %)	% Homology with labelled DNA from:*		
		D2-18 ^T	D5-2 ^T	33093 ^T
<i>Mogibacterium pumilum</i> D2-18 ^T	46	100		
<i>Mogibacterium pumilum</i> BA11a-f	45	97		
<i>Mogibacterium vescum</i> D5-2 ^T	46	31	100	
<i>Mogibacterium (Eubacterium) timidum</i> ATCC 33093 ^T	50	17	18	100
<i>Eubacterium exiguum (Slackia exigua)</i> ATCC 700122 ^T	60	2	3	2
<i>Eubacterium minutum</i> ATCC 700079 ^T	38–40	2	3	2
<i>Eubacterium nodatum</i> ATCC 33099 ^T	41	2	2	2
<i>Eubacterium lentum (Eggerthella lenta)</i> ATCC 25559 ^T	62	1	2	3
<i>Eubacterium brachy</i> ATCC 33089 ^T	39	2	2	2
<i>Eubacterium sapenum</i> ATCC 49989 ^T	45	2	2	1
<i>Eubacterium limosum</i> ATCC 8486 ^T	50	2	1	1

* All values were normalized to 100 % for the homologous reactions.

Fig. 2. Evolutionary tree based on 16S rRNA gene sequence comparisons, showing the phylogenetic positions of *Mogibacterium pumilum* (D2-18^T = ATCC 700696^T) and *Mogibacterium vescum* (D5-2^T = ATCC 700697^T). The dendrogram was created by using the neighbour-joining method. The numbers on the tree indicate percentage bootstrap values calculated from 1000 replications. Nucleotide sequence accession numbers are given in the text.

Mogibacterium species showed very low levels (< 2 %) of DNA reassociation with the oral asaccharolytic *Eubacterium* species, including the type species of the genus *Eubacterium*, *E. limosum*, except *E. timidum* (Table 2). The DNA–DNA hybridization values between *Mogibacterium* species and *E. timidum* were 17 and 18 %, which were clearly higher than all the other *Eubacterium* species. Johnson (1984) has proposed that groups of bacteria with intragroup DNA homology of 80–90 % and intergroup homology between 60 and 70 % could be considered different subspecies within a species. Johnson (1984) also stated that strains showing homology between 20 and 60 % could be considered closely related species. These criteria, taken with the DNA–DNA hybridization data in the present study, suggest that these novel strains may be assigned to a genus different from the genus *Eubacterium*, and strain D2-18^T may belong to the same species as strain BA11a-f whilst strain D5-2^T belongs to a second species. These DNA homology data also suggest that *E. timidum* may be related to *Mogibacterium* species.

Phylogenetic analysis

The 16S rRNA gene sequences of *M. pumilum* strain D2-18^T (length, 1470 bases) and *M. vescum* strain D5-2^T (length, 1477 bases) were determined in order to assess the phylogenetic positions of the organisms. The sequences were also compared with those of related Gram-positive bacteria which were selected from GenBank database by the BLAST and FASTA algorithms. The database search revealed that *M. pumilum* D2-18^T and *M. vescum* D5-2^T belong to the *Clostridium* subphylum of the Gram-positive bacteria and that their 16S rRNA sequences are most similar to those of members of *Clostridium* cluster XI (Collins *et al.*,

Table 3. Levels of 16S rDNA sequence similarity of *Mogibacterium* species and *Eubacterium limosum* to other *Eubacterium* species and other members of subcluster XI-A

Bacterial species	Percentage similarity with:			
	<i>M. pumilum</i>	<i>M. vescum</i>	<i>M. timidum</i>	<i>E. limosum</i>
<i>Mogibacterium pumilum</i>	100.0			
<i>Mogibacterium vescum</i>	95.8	100.0		
<i>Mogibacterium (Eubacterium) timidum</i>	92.7	92.3	100.0	
<i>Eubacterium limosum</i>	72.0	71.9	70.2	100.0
<i>Eubacterium infirmum</i>	83.9	84.3	82.5	73.0
<i>Eubacterium saphenum</i>	83.5	83.5	83.5	76.1
<i>Eubacterium brachy</i>	83.3	82.9	83.8	70.9
<i>Eubacterium minutum</i>	79.8	79.9	80.1	74.0
<i>Eubacterium nodatum</i>	79.6	78.7	78.6	74.2
<i>Eubacterium tardum</i>	75.8	78.2	79.1	72.6
' <i>Eubacterium thermomarinus</i> '	74.3	74.3	72.9	78.6
<i>Eubacterium tenue</i>	72.4	73.0	71.0	73.8
<i>Eubacterium callanderi</i>	71.0	71.1	71.0	97.6
<i>Eubacterium barkeri</i>	70.7	71.0	69.9	91.7
<i>Eubacterium cellulosolvens</i>	68.4	68.6	67.7	73.3
<i>Eubacterium bifforme</i>	65.1	65.6	65.7	64.9
' <i>Clostridium aminobutyricum</i> '	84.5	84.9	83.1	74.4

1994). A phylogenetic analysis was performed including all taxa from cluster XI, other *Eubacterium* species and representatives of other clostridial clusters defined by Collins *et al.* (1994). On the basis of the sequence similarity matrix data, an evolutionary tree was constructed by MEGALIGN using the neighbour-joining method (Saitou & Nei, 1987) and is shown in Fig. 2.

Our analysis indicates that the cluster XI may be divided into two subclusters, XI-A and XI-B, and that *M. pumilum* and *M. vescum* are members of the subcluster XI-A (Fig. 2). Although oral asaccharolytic *Eubacterium* species examined in the present study also belong to the subcluster XI-A, *M. pumilum* and *M. vescum* could be distinguished from these species. The closest phylogenetic neighbour of *Mogibacterium* species is *E. timidum*, and these three species form a branch in the phylogenetic tree. These three species had no close phylogenetic affinities with the other members of the subcluster XI-A (sequence similarities < 84.9%; sequence divergence values > 12.2%) when compared directly with these taxa (Table 3). These data are consistent with the results of DNA–DNA hybridization and biochemical characterization studies (Tables 1 and 2). Our phylogenetic analysis based on 16S rRNA gene sequences indicates that *M. pumilum*, *M. vescum* and *E. timidum* are not closely related to not only other asaccharolytic *Eubacterium* but also to other taxa established previously, which is consistent with the creation of a new genus and species. These data agree with previous results obtained by RFLP analysis of 16S rDNA from oral asaccharolytic *Eubacterium* species (Sato *et al.*, 1993b).

E. timidum showed 16S rRNA sequence similarities of 92.7 and 92.3% to the sequences of *M. pumilum* and *M. vescum*, respectively (Table 3). These similarities may be considered low for the same genus, but there is no strict guideline on the level of similarity for genera. According to the topology of the phylogenetic tree and DNA–DNA hybridization data, together with the phenotypic similarity, it seems quite reasonable to transfer *E. timidum* to *Mogibacterium*.

On the other hand, *E. limosum*, which is the type species of the genus *Eubacterium*, showed very low levels of sequence similarity (< 78.6%) to other *Eubacterium* species except *E. callanderi* (97.6%) and *E. barkeri* (91.6%), as shown in Table 3. These similarity data and the phylogenetic tree (Fig. 2) indicate that the genus *Eubacterium* may be incoherent and *E. limosum* is remotely related to other *Eubacterium* species phylogenetically, as reported by several authors (Cheeseman *et al.*, 1996; Nakazawa & Hoshino, 1993, 1994; Wade *et al.*, 1994). The results also suggest that some of the other *Eubacterium* species, especially asaccharolytic ones, i.e. *E. saphenum*, *E. infirmum*, *E. brachy*, *E. nodatum*, *E. tardum* and *E. minutum*, may need reassignment to different genera, which is in agreement with our previous study (Nakazawa & Hoshino, 1994). In fact, *E. exiguum* and *E. lentum* were recently reclassified in new genera, *Slakia exigua* and *Eggerthella lenta*, respectively (Wade *et al.*, 1999).

At the present time, we have no information on the distribution or incidence of these bacteria in human mouths. Studies of cell wall composition and anti-

microbial susceptibility, which may be helpful for description of *Mogibacterium* species, will be investigated in our ongoing future research. On the basis of biochemical characteristics, SDS-PAGE and Western immunoblotting analyses, G+C content determination, DNA–DNA hybridization data and phylogenetic analysis with 16S rRNA gene sequence data, we propose a new genus, *Mogibacterium*, and new species, *M. pumilum* for strain D2-18^T and BA11a-f, and *M. vescu*m for strain D5-2^T. It is also proposed that *Eubacterium timidum* is transferred to the genus *Mogibacterium* gen. nov. as *Mogibacterium timidum* gen. nov., comb. nov. The taxonomic description below summarizes the properties of these species.

Description of *Mogibacterium* gen. nov.

Mogibacterium (Mogi.bac.te'ri.um. Gr. n. *mogi* difficult; Gr. n. *bakterion* a small rod; M.L. neut. n. *Mogibacterium* a difficult-to-culture, rod-shaped bacterium).

Cells are Gram-positive and rod-shaped. No spores are formed. Non-motile. Strictly anaerobic and asaccharolytic. Growth in broth media is very poor. Catalase-negative. Metabolic end product in PYG medium is phenyl acetate. The DNA base composition is 45–46 mol% G+C. Members of the genus *Mogibacterium* can be distinguished from each other by 16S rRNA gene sequences and DNA homology values. The type species is *Mogibacterium pumilum*.

Description of *Mogibacterium pumilum* sp. nov.

Mogibacterium pumilum (pu.mi'lum. L. adj. *pumilum* small or tiny, referring to the tiny colonies formed by this organism).

Cells are short, Gram-positive rods, obligately anaerobic, non-motile and non-sporing. Individual cells are about 0.2–0.3 by 1.0 µm, occurring singly, in short chains or in clumps. On BHI-blood agar plates they form minute colonies < 1 mm in diameter even after prolonged incubation in an anaerobic glove box, which are circular, convex and translucent. Growth in PY broth media is very poor with or without carbohydrates. No haemolysis occurs around colonies on BHI-blood agar plates. The cells are inert in most biochemical tests. Starch and aesculin are not hydrolysed and nitrate is not reduced. No liquefaction of gelatin occurs. Indole, urease and catalase tests are also negative. Ammonia is not produced from arginine. All strains are nonfermentative and do not utilize adonitol, amygdalin, arabinose, cellobiose, erythritol, aesculin, fructose, galactose, glucose, glycogen, inositol, lactose, maltose, mannitol, mannose, melezitose, melibiose, rhamnose, ribose, salicin, sorbitol, starch, sucrose, trehalose or xylose. They produce phenyl acetate (about 3 mM) as the sole metabolic end product in PY or PYG broth. The G+C content is 45–46 mol%. The type strain is D2-18^T (=

ATCC 700696^T). Isolated from human periodontal pockets.

Description of *Mogibacterium vescu*m sp. nov.

*Mogibacterium vescu*m (ves'cum. L. adj. *vescum* weak, referring to the poor growth of this organism).

Cells are short, Gram-positive rods, obligately anaerobic, non-motile and non-sporing. Individual cells are about 0.2–0.3 by 1.5 µm, occurring singly or in clumps. On BHI-blood agar plates they form minute colonies < 1 mm in diameter even after prolonged incubation in an anaerobic glove box, which are circular, convex and translucent. Growth in broth media is poor with or without carbohydrates. No haemolysis occurs around colonies on BHI-blood agar plates. The cells are nonfermentative and do not utilize adonitol, amygdalin, arabinose, cellobiose, erythritol, aesculin, fructose, galactose, glucose, glycogen, inositol, lactose, maltose, mannitol, mannose, melezitose, melibiose, rhamnose, ribose, salicin, sorbitol, starch, sucrose, trehalose or xylose. Catalase, urease and indole are not produced, and ammonia is not produced from arginine. They produce phenyl acetate (4 mM) as the sole metabolic end product in PY or PYG broth. The G+C content is 46 mol%. This strain can be distinguished from *Mogibacterium pumilum* according to the almost complete sequence of the 16S rRNA gene and DNA relatedness values. The type strain is D5-2^T (= ATCC 700697^T). Isolated from human periodontal pockets.

Description of *Mogibacterium timidum* (*Eubacterium timidum* Holdeman *et al.* 1980) comb. nov.

M. timidum (ti'mi.dum. L. neut. adj. *timidum* fearful, timid, referring to the slight or slow growth in clumps).

Characteristics of the species are as previously described by Holdeman *et al.* (1980). The type strain is ATCC 33093^T.

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