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Taxonomic characterization of *Mogibacterium diversum* sp. nov. and *Mogibacterium neglectum* sp. nov., isolated from human oral cavities

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Novel isolates, strains HM-7, HM-6, HH-31, P9a-h^T and UJB13-d, which were isolated from tongue plaque and necrotic dental pulp, were studied taxonomically and phylogenetically. These organisms were anaerobic, non-spore-forming, Gram-positive, rod-shaped bacteria that were inert in most of the conventional biochemical tests and phenotypically resemble *Mogibacterium* species or asaccharolytic *Eubacterium* species. The G+C contents of the DNAs from the novel isolates ranged from 41 to 42 mol%. DNA–DNA hybridization studies demonstrated that these strains might be assigned to the genus *Mogibacterium* but not to the previously described species. It was also apparent that strain HM-7 belonged to the same species as strains HM-6 and HH-31, and that strains P9a-h^T and UJB13-d belonged to a second species. The levels of DNA–DNA relatedness to asaccharolytic *Eubacterium* species, including *Eubacterium brachy*, *Eubacterium nodatum*, *Eubacterium saphenum* and the more recently proposed *Eubacterium minutum* and *Eubacterium exiguum* (reclassified as *Slackia exigua*), are less than 2%. The results of 16S rDNA sequence comparisons revealed that these organisms represent novel lineages distinct from all previously described species of Gram-positive, rod-shaped bacteria. On the basis of phenotypic characteristics, DNA–DNA hybridization data and phylogenetic analysis with 16S rRNA gene sequence data, new species are proposed, namely *Mogibacterium diversum* (for strains HM-7,HM-6 and HH-31) and *Mogibacterium neglectum* (for strains P9a-h^T and UJB13-d). HM-7^T (= ATCC 700923^T = JCM 11205^T) is the type strain of the former and P9a-h^T (= ATCC 700924^T = JCM 11204^T) is the type strain for the latter.

Keywords: *Mogibacterium diversum* sp. nov., *Mogibacterium neglectum* sp. nov., asaccharolytic anaerobic Gram-positive rod

INTRODUCTION

It has been reported that numerous bacterial strains of asaccharolytic, anaerobic, Gram-positive rods (AAGPRs) have been isolated from human oral cavities. Several bacterial species of AAGPRs have been documented as aetiologic agents of chronic periodontitis (Hill *et al*., 1987; Holdeman *et al*., 1980; Martin *et al*., 1986; Moore *et al*., 1982, 1983, 1985; Slayne & Wade, 1994; Uematsu & Hoshino, 1992; Uematsu *et al*., 1993; Wade *et al*., 1992) on the basis of their frequent isolation from diseased periodontal sites. In addition, antibody titres against some species of AAGPRs in the sera of patients with periodontitis are higher than those of healthy people, suggesting that these bacteria cause immunological reactions in period-

Abbreviation: AAGPRs, asaccharolytic, anaerobic, Gram-positive rods.

The GenBank/EMBL/DDBJ accession numbers for the sequences determined in this paper are AB037874 (*Mogibacterium diversum* HM-7^T = ATCC 700923^T = JCM 11205^T) and AB037875 (*Mogibacterium neglectum* P9a-h^T = ATCC 700924^T = JCM 11204^T).

ontal lesions (Gunsolley *et al*., 1990; Tew *et al*., 1985; Tolo & Jorkjend, 1990; Tolo & Schenck, 1985). Moreover, they have been recognized as pathogens in oral and dental infections, such as advanced caries (Ando & Hoshino, 1990; Edwardsson, 1974; Hoshino, 1985), pulpal infections (Hoshino *et al*., 1992; Sato *et al*., 1993a) and dentoalveolar abscesses (Wade *et al*., 1994), and also in non-oral, clinically significant infections, such as head and neck infections, abdominal infections (Brook & Frazier, 1993), bacteraemia associated with malignancy (Fanstein *et al*., 1989), and genital tract infection associated with intrauterine devices (Brook, 1983).

Previously, many oral AAGPRs have been classified as members of the genus *Eubacterium*, but this genus 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 diverse organisms (Cheeseman *et al*., 1996; Nakazawa & Hoshino, 1993), and it was inevitable that there was considerable heterogeneity among the species that were placed within it. It now contains many species and groups that are phylogenetically unrelated (Moore & Moore, 1986; Nakazawa & Hoshino, 1994). In recent studies, many oral AAGPR 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, b; Wade *et al*., 1994; Sato *et al*., 1993a). In addition, several novel genera have been proposed for some of the AAGPRs, e.g. *Cryptobacterium*, *Slackia*, *Eggerthella* and *Mogibacterium*, and some species of *Eubacterium* have been transferred to the novel genera according to their phylogenetic characteristics (Nakazawa *et al*., 1999, 2000; Wade *et al*., 1999).

Recently, a study in our laboratory has shown that novel strains of oral AAGPRs, including strains HM-6, HM-7^T, HH-31, P9a-h^T and UJB13-d, can be distinguished by RFLP analysis of 16S rDNA amplified by PCR (Sato *et al*., 1993b). These organisms, isolated from tongue plaque and necrotic dental pulp, are non-spore-forming, grow slowly, and phenotypically resemble members of the genus *Mogibacterium*. However, these strains could not be classified within any of the established bacterial species. In this paper, we report the phenotypic and phylogenetic characteristics of five novel strains and compare them with data on previously described reference species. On the basis of these results, these strains were identified as new species of the genus *Mogibacterium*. *Mogibacterium diversum* is proposed for strains HM-7^T, HM-6 and HH-31, and *Mogibacterium neglectum* is proposed for strains P9a-h^T and UJB13-d.

METHODS

Bacterial strains. The novel strains HM-6, HM-7^T and HH-31 were isolated from human tongue plaque (Hori *et al*., 1999), and strains P9a-h^T and UJB13-d were also isolated from necrotic dental pulp (Sundqvist, 1976; Sato *et al*., 1993b). The type strains of previously established AAGPR species, together with the more recently proposed species, *Eubacterium minutum* (Poco *et al*., 1996b), *Slackia exigua* which was emended from *Eubacterium exiguum* (Poco *et al*., 1996a; Wade *et al*., 1999), *Cryptobacterium curtum* (Nakazawa *et al*., 1999), *Mogibacterium pumilum* and *Mogibacterium vescum* (Nakazawa *et al*., 2000), were included in the present study. *Eubacterium infirmum* and *Eubacterium tardum* (Cheeseman *et al*., 1996), though discussed here, were not examined in this work.

Culture conditions. All of 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 % N_2, 10 % H_2 and 10 % CO_2. The bacterial cells were harvested by centrifugation, washed with 10 mM sodium phosphate-buffered saline (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 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 using the methods described in the *Anaerobe Laboratory Manual* (Holdeman *et al*., 1977). End products produced in peptone/yeast extract/glucose (PYG) broth were assayed by GC, as described previously (Hoshino & Sato, 1986). To examine the enzymic profiles of these organisms, a Rapid ID 32A kit (API System; Montalieu) 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 previously (Laemmli, 1970). Approximately 50 mg (wet weight) 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 was measured by using 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 previously (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 modifications as described previously (Nakazawa & Hoshino, 1993), briefly, by using an immune rabbit antiserum (1:1000) as the first antibody and finally incubating them with goat anti-rabbit immunoglobulin G conjugated with peroxidase (1:1000) as the second antibody; then the colour was developed.

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

DNA–DNA hybridization analysis. DNA–DNA hybridization was performed using the membrane filter method as described previously (Meyer & Schleifer, 1978; Nakazawa & Hoshino, 1994). 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 % (w/v) SDS, 0·02 % (w/v) polyvinylpyrrolidone, 0·02 % (w/v) Ficoll 400, BSA (Fraction V; Sigma) and 1 ml 6× standard saline citrate (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 the homologous DNA.

Sequencing of the 16S rRNA gene and phylogenetic analysis. The 16S rRNA gene was amplified by a 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 PCRs 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) and a series of custom-synthesized primers with an ALFexpress DNA sequencer (Pharmacia LKB) according to the manufacturer's instructions. The sequences which we determined were aligned by using SEQMAN II of the LASERGENE program (DNASTAR). MEGALIGN of LASERGENE, CLUSTAL W and the NJPLOT program were used to compare sequences and to construct an evolutionary tree for the neighbour-joining method (Saito & Nei, 1987). Also, confidence values were assessed by CLUSTAL W with bootstrap analysis.

RESULTS AND DISCUSSION

Morphological characteristics

The novel strains HM-7^T, HM-6 and HH-31 (classified as *M. diversum* on the basis of the results given below), and P9a-h^T and UJB13-d (classified as *M. neglectum* on the basis of the results given below) were non-sporing, non-motile, short, rod-shaped organisms occurring mostly as single cells but sometimes in pairs, short chains or clumps. Growing cells are 0·3–0·5 μm × 1·0–1·5 μm in size, very difficult to culture in broth media because of their poor growth with or without carbohydrates, even on fastidious artificial media, and have a requirement for strict anaerobiosis; these are characteristics typical of oral AAGPR species.

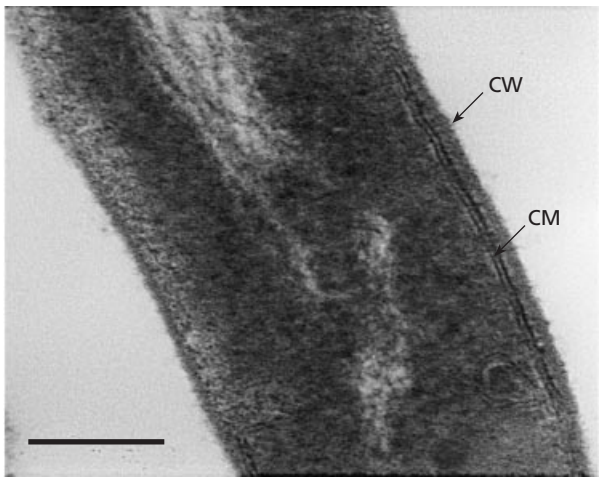

Fig. 1. Transmission electron microphotograph of a thin section of cell of *Mogibacterium diversum* HM-7^T (= ATCC 700923^T = JCM 11205^T) showing the Gram-positive cell wall. Bar, 100 nm. The cell wall (CW) and cytoplasmic membrane (CM) are indicated.

Electron micrographs of ultrathin sections of *M. diversum* HM-7^T (Fig. 1) and *M. neglectum* P9a-h^T (not shown) revealed a cell wall architecture typical of Gram-positive bacteria: a plasma membrane layer, a thin peptidoglycan layer in the middle, and a thicker outer layer. Pili- or flagella-like structures were not found.

All of the organisms formed tiny colonies on BHI-blood agar plates: the colonies were about 0·3–0·6 mm in diameter, circular, convex, translucent and had a smooth surface. 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.

Phenotypic analysis

The novel five organisms were asaccharolytic (they did not ferment glucose and other carbohydrates) and were negative in most of the conventional biochemical tests (nitrate reduction, hydrolysis of arginine, aesculin, starch and gelatin). They also did not produce indole, ammonia, urease and catalase. These strains produced phenylacetate as a sole metabolic end product in PYG medium, as assayed by GC (Table 1). Phenotypically, they resembled *Mogibacterium* species (Nakazawa *et al*., 2000), which also produced phenylacetate as an end product, among the previously described oral AAGPR species. They are pheno-

Table 1. Comparison of biochemical characteristics and enzymic profiles

Bacterial taxon	End products from peptone/yeast extract/glucose broth*	Arginine hydrolysis	Nitrate reduction
Mogibacterium diversum			
HM-7^T (= ATCC 700923^T)	phe-a	−	−
HM-6	phe-a	−	−
HH-31	phe-a	−	−
Mogibacterium neglectum			
P9a-h^T (= ATCC 700924^T)	phe-a	−	−
UJB13-d	phe-a	−	−
Mogibacterium pumilum ATCC 700696^T	phe-a	−	−
Mogibacterium vescum ATCC 700697^T	phe-a	−	−
Mogibacterium timidum ATCC 33093^T (basonym *Eubacterium timidum*)	phe-a	−	−
Slackia exigua ATCC 700122^T (basonym *Eubacterium exiguum*)	−	+	−
Cryptobacterium curtum ATCC 700683^T	−	+	−
Eggerthella lenta ATCC 25559^T (basonym *Eubacterium lentum*)	−	+	+
Eubacterium minutum ATCC 700079^T	B or b	−	−
Eubacterium nodatum ATCC 33099^T	a, B	+	−
Eubacterium saphenum ATCC 49989^T	a, b		−
Eubacterium brachy ATCC 33089^T	ib, ic, iv, phe-p	−	−

* a: Acetate, b: butyrate, ib: isobutyrate, ic: isocaproate, iv: isovalerate, phe-a: phenylacetate, phe-p: phenylpropionate. Upper-case letters indicate $\geqq$ 10 mM product, and lower-case letters indicate $<$ 10 mM product.

typically distinct from the acetate- and butyrate-producing AAGPR species (Table 1) such as *Eubacterium brachy*, *Eubacterium nodatum*, *Eubacterium saphenum*, *Eubacterium minutum* and *Eubacterium infirmum* (Cheeseman *et al.*, 1996).

The enzymic profiles of these strains (API code, 0000000000) in the API Rapid ID 32A kit were distinct from those of the established *Mogibacterium* species exhibiting positive reactions (API code, 0000020000) for aminopeptidase and proline arylamidase (Cheeseman *et al.*, 1996; Nakazawa *et al.*, 2000). The previously described oral AAGPR species, including *C. curtum*, *S. exigua*, *Eggerthella lenta*, *Eubacterium brachy*, *Eubacterium nodatum*, *Eubacterium minutum* and *Eubacterium infirmum* showed quite typical enzymic profiles (Poco *et al.*, 1996a, b).

In the whole-cell protein profiles examined using SDS-PAGE, *M. diversum* HM-7^T had major bands in the region of 60, 38 and 36 kDa, whereas *M. neglectum* P9a-h^T had major bands of about 70, 60, 38 and 36 kDa in 10% gel (data not shown). These oral AAGPR bacteria, including previously proposed species, 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 strain HM-7^T and P9a-h^T did not react with the antisera against the previously established oral AAGPR species, while antigens from the previously described species were recognized by their respective antisera (data not shown). These results clearly showed that these novel strains 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.

Genotypic analysis

It was reported previously that the DNA G+C contents of oral AAGPRs, including the more recently proposed species, *M. pumilum*, *M. vescum* and *C. curtum*, range from 38 to 62 mol% (Nakazawa & Hoshino, 1994; Nakazawa *et al.*, 2000; Poco *et al.*, 1996a, b). The G+C content of DNA from novel strains HM-7^T, HM-6, HH-31, P9a-h^T and UJB13-d was 42 mol%, as determined by HPLC (Table 2); this is similar to the G+C content for *Eubacterium nodatum* (41 mol%), but they are clearly distinguishable from the other AAGPR species and *Eubacterium limosum* (50 mol%), which is the type species of the genus *Eubacterium*.

The levels of DNA relatedness estimated by quantitative DNA–DNA hybridization studies with the membrane filter method are listed in Table 2. The

Table 2. DNA base compositions and levels of DNA relatedness to oral asaccharolytic anaerobic Gram-positive rods

Source of DNA	G+C content (mol %)	Per cent homology with labelled DNA from:*				
		HM-7^T	HM-6	HH-31	P9a-h^T	UJB13-d
Mogibacterium diversum						
HM-7^T (= ATCC 700923^T)	42	100				
HM-6	41	106	100			
HH-31	42	76	78	100		
Mogibacterium neglectum						
P9a-h^T (= ATCC 700924^T)	42	34	30	35	100	
UJB13-d	42	34	38	34	96	100
Mogibacterium pumilum ATCC 700696^T	46	38	37	38	39	29
Mogibacterium vescum ATCC 700697^T	46	30	26	23	29	26
Mogibacterium timidum ATCC 33093^T (basonym *Eubacterium timidum*)	50	16	16	16	18	17
Eubacterium nodatum ATCC 33099^T	41	2	1	1	1	1
Eubacterium saphenum ATCC 49989^T	45	2	1	2	3	2
Eubacterium brachy ATCC 33089^T	39	1	1	2	3	2

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

reassociations of DNA among *M. diversum* strains HM-7^T, HM-6 and HH-31, and those between *M. neglectum* strains P9a-h^T and UJB13-d, were at a high level (76–106 %), clearly suggesting that they belong to the same bacterial species, respectively. On the other hand, these novel *Mogibacterium* species, *M. diversum* and *M. neglectum*, showed 23–39 % DNA reassociation with type strains of the previously proposed *Mogibacterium* species, such as *M. pumilum* and *M. vescum*. Moreover, these novel *Mogibacterium* species showed very low levels (< 3 %) of DNA reassociation with the other AAGPR species, except *Mogibacterium timidum* (Table 2). The DNA–DNA hybridization values between novel *Mogibacterium* species and *M. timidum* were between 16 and 18 %, and were clearly higher than those for all the other AAGPR 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 has also stated that strains showing homology of between 20 and 60 % could be closely related species.

These criteria, in the context of the DNA–DNA hybridization results in the present study, suggest that these novel strains could be assigned to two different species in the genus *Mogibacterium*, that strains HM-7^T, HM-6 and HH-31 might belong to the same species as *M. diversum*, and that strains P9a-h^T and UJB13-d might belong to a second species (*M. neglectum*).

Phylogenetic analysis

The 16S rRNA gene sequences of *M. diversum* strain HM-7^T (length, 1455 bases) and *M. neglectum* strain P9a-h^T (length, 1478 bases) were determined in order to assess the phylogenetic positions. The sequences were compared with those of oral AAGPRs and related Gram-positive bacteria selected from GenBank database by the BLAST and FASTA algorithms. On the basis of the sequence similarity matrix data, an evolutionary tree (Fig. 2) was constructed by MEGALIGN, using the neighbour-joining method (Saito & Nei, 1987). Our phylogenetic analysis revealed that *M. diversum* HM-7^T and *M. neglectum* P9a-h^T belong to subcluster XI-A (Nakazawa *et al.*, 2000) in *Clostridium* cluster XI (Collins *et al.*, 1994), and that their 16S rRNA sequences are most similar to those of the previously established *Mogibacterium* species. Although oral asaccharolytic *Eubacterium* species examined in the present study also belong to subcluster XI-A, *M. diversum* and *M. neglectum* could be distinguished from these *Eubacterium* species. The closest phylogenetic neighbour of these novel species is *M. vescum*, and these species, together with *M. pumilum* and *M. timidum*, are found to form a branch in the phylogenetic tree. However, these novel species showed distinct lines exhibiting specific phylogenetic association with the established *Mogibacterium* species (sequence similarities, < 96·2 %) when compared directly with these taxa (data not shown). 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 sequence indicates that *M. diversum* and *M. neglectum* are not closely related either to other asaccharolytic eubacteria, or to other taxa established previously, which is consistent with the creation of new species, respectively. These data agreed with previous results obtained by RFLP analysis of 16S rDNA from oral asaccharolytic *Eubacterium* species (Sato *et al.*, 1993b).

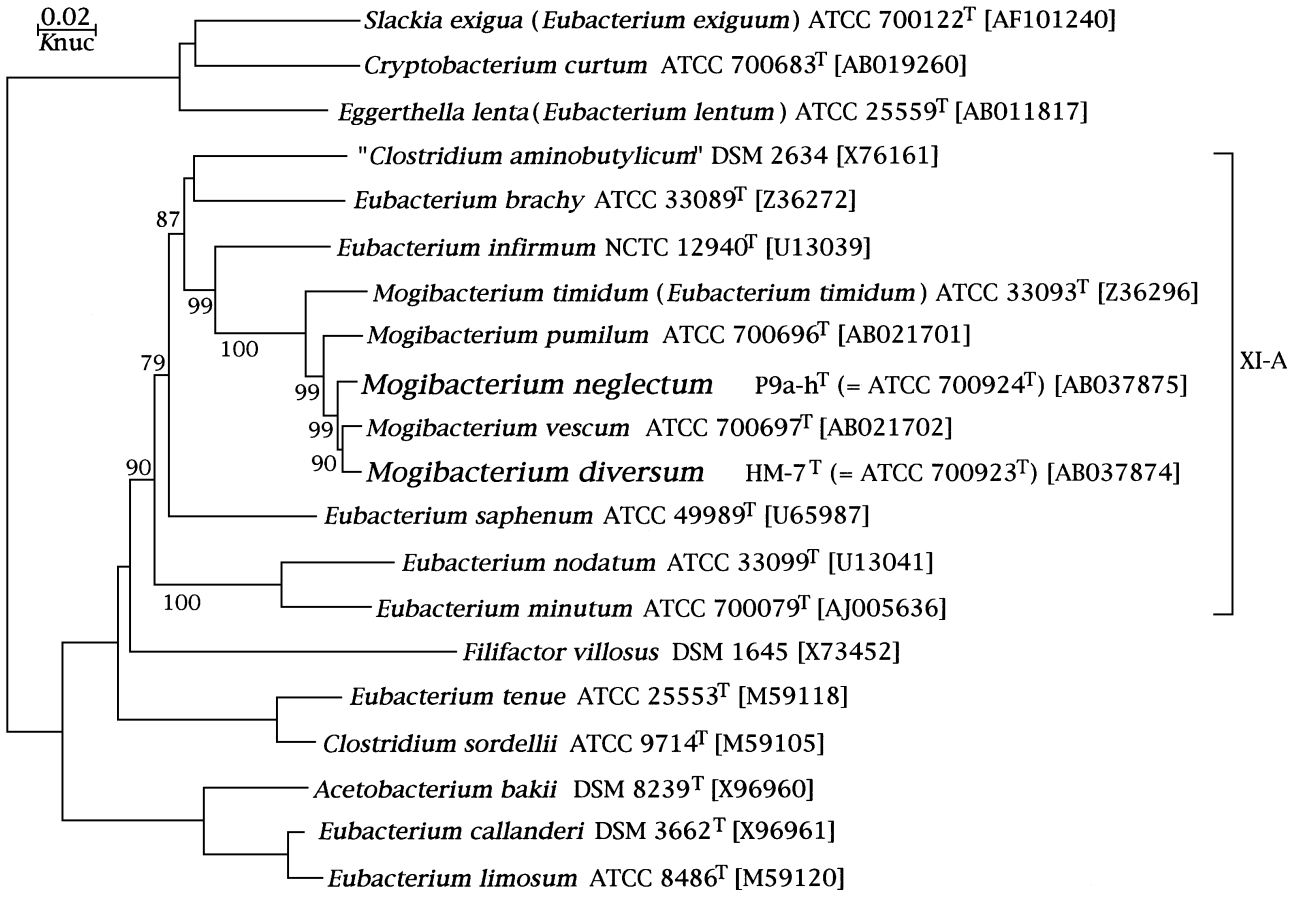

Fig. 2. Evolutionary tree based on a 16S rRNA gene sequence comparison, showing the phylogenetic position of *M. diversum* HM-7^T (= ATCC 700923^T = JCM 11205^T) and *Mogibacterium neglectum* P9a-h^T (= ATCC 700924^T = JCM 11204^T). The dendrogram was created by using the neighbour-joining method. The numbers on the tree indicate bootstrap values (percentages) calculated from 1000 replications. The nucleotide sequence accession numbers are given in parentheses.

It is noteworthy that none of the asaccharolytic *Eubacterium* species examined in this study, i.e. *Eubacterium brachy*, *Eubacterium infirmum*, *Eubacterium saphenum*, *Eubacterium nodatum* and *Eubacterium minutum*, showed close phylogenetic affinity with *Eubacterium limosum*, which is the type species of the genus *Eubacterium*, in the evolutionary tree. These results indicate that the genus *Eubacterium* may be incoherent and that these oral asaccharolytic *Eubacterium* species may need reassignment to different genera, as suggested by several authors (Cheeseman *et al.*, 1996; Nakazawa & Hoshino, 1993, 1994; Wade *et al.*, 1994).

The two new species proposed in this study are all inert in most biochemical tests, and cannot easily be distinguished phenotypically from the other species in the genus *Mogibacterium*. In the guidelines for the proposal of new species (Wayne *et al.*, 1987), it was recommended that a distinct genospecies that could not be differentiated from another genospecies on the basis of any known phenotypic property not be named until it could be differentiated by some phenotypic property. However, at present there are already three valid species, i.e. *M. pumilum*, *M. vescum* and *M. timidum*, which cannot easily be distinguished phenotypically. The two new species are clearly distinct from the other species in the genus on the basis of DNA–DNA homologies and 16S rDNA sequence similarities (important properties for bacteria that are unresponsive in most biochemical tests).

At present, we have no information on the distribution or incidence of these bacteria in human mouths. Studies of cell wall composition and antimicrobial susceptibility, which may be helpful for description of these novel *Mogibacterium* species, will be reported in the course of our ongoing 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 the new species *M. diversum*, for strains HM-7^T, HM-6 and HH-31, and *M. neglectum*, for strains P9a-h^T and UJB13-d, in the

genus *Mogibacterium*. The taxonomic description below summarizes the properties of these species.

Description of *Mogibacterium diversum* sp. nov.

Mogibacterium diversum (di.ver′sum. L. adj. *diversum* diverse, referring to the novel lineage formed by this organisms in the evolutionary tree).

Cells are short, Gram-positive rods, obligately anaerobic, non-motile and non-sporing. Individual cells are about 0·2–0·3 × 1·0 μm, occurring singly in short chains or in clumps. On BHI-blood agar plates, they form minute colonies that are less than 1 mm in diameter, circular, convex and translucent even after prolonged incubation in an anaerobic glove box. 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 non-fermentative 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 phenylacetate (about 2 mM) as a sole metabolic end product in peptone/yeast extract (PY) or PYG broth. The G+C content of the DNA is 42 mol%. The type strain is strain HM-7^T (= ATCC 700923^T = JCM 11205^T). Isolated from human tongue plaque.

Description of *Mogibacterium neglectum* sp. nov.

Mogibacterium neglectum (neg.lect′um. L. adj. *neglectum* neglected, referring to the poor growth and tiny colonies that caused this organism to be neglected scientifically for a long time).

Cells are short, Gram-positive rods that are obligately anaerobic, non-motile and non-sporing. Individual cells are about 0·2–0·3 × 1·5 μm, occurring singly or in clumps. On BHI-blood agar plates, they form minute colonies that are less than 1 mm in diameter, circular, convex and translucent even after prolonged incubation in an anaerobic glove box. Growth in broth media is poor with or without carbohydrates. No haemolysis occurs around colonies on BHI-blood agar plates. The cells are non-fermentative 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. Phenylacetate (2 mM) is produced as a sole metabolic end product in PY or PYG broth. The G+C content of the DNA is 41–42 mol%. According to the almost complete sequence of the 16S rRNA gene and the DNA relatedness, the strain can be distinguished from *M. pumilum*, *M. vescum* and *M. diversum*. The type strain is strain P9a-h^T (= ATCC 700924^T = JCM 11204^T). Isolated from human necrotic dental pulp.

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