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Treponema medium sp. nov., Isolated from Human Subgingival Dental Plaque

TOSHIHIKO UMEMOTO,[1]* FUTOSHI NAKAZAWA,[2] ETSURO HOSHINO,[2] KEIJI OKADA,[3] MASAHITO FUKUNAGA,[3] AND ISAMU NAMIKAWA[1]

Department of Oral Microbiology, School of Dentistry, Asahi University, Gifu 501-02,[1] Department of Oral Microbiology, School of Dentistry, Niigata University, Niigata 951,[2] and Department of Molecular Microbiology, Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Fukuyama, Hiroshima 729-02,[3] Japan

A new *Treponema* species, for which we propose the name *Treponema medium*, was isolated from subgingival plaque from an adult with periodontal disease. The morphological characteristics, differential biochemical characteristics, and protein profiles on sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels of this organism are described. The guanine-plus-cytosine content of the DNA of *T. medium* is 51 mol%. The levels of DNA-DNA relatedness of the new species to other *Treponema* species, including *Treponema denticola*, *Treponema vincentii*, *Treponema socranskii*, *Treponema pallidum*, and *Treponema phagedenis*, are less than 30%. A phylogenetic analysis based on 16S rRNA sequences distinguished the new *Treponema* strain from strains belonging to previously described *Treponema* species. The type strain of *T. medium* is strain G7201.

The incidence and numbers of treponemes in the human gingival flora become greater as gingivitis becomes more severe (27). The number of treponemes increases to approximately more than 50% of all bacterial morphotypes in subgingival samples from periodontitis patients (10, 12, 13, 27). Some oral treponemal species have been associated with soft-tissue damage due to proteolytic enzymes or with inflammatory reactions due to antibodies against their specific antigens, and eventually this leads to periodontal tissue destruction in certain types of periodontal disease (12, 18, 21, 36). Simonson et al., for instance, have reported that *Treponema denticola* is linked to the severity of periodontal disease (25). All of the human oral spirochetes mentioned above belong to the genus *Treponema* in the family *Spirochaetaceae*; these bacteria characteristically are tightly coiled organisms that consist of a protoplasmic cylinder (length, approximately 6 to 20 μm; width, 0.1 to 0.5 μm) covered by an envelope (an outer sheath) and periplasmic flagella (axial flagella) which originate subterminally at the ends of the protoplasmic cylinder (26). Choi et al. used a 16S rRNA gene cloning technique to document the qualitative nature of human oral spirochetes and demonstrated that there is great genetic diversity in oral spirochetes, even in a single periodontitis patient (2). Human oral spirochetes, however, have been conventionally grouped into the following three morphotypes: (i) small spirochetes, including *T. denticola*, *Treponema socranskii*, and *Treponema pectinovorum*; (ii) medium-sized spirochetes, such as *Treponema vincentii*; and (iii) large spirochetes which have not been isolated in pure culture (3, 11).

T. denticola, *T. socranskii*, *T. pectinovorum*, *T. vincentii*, *Treponema scoliodontum*, *Treponema macrodentium*, and *Treponema orale* have been described as human oral *Treponema* species, although the last two species have no standing in nomenclature at this time (26–28). *T. denticola* and *T. socranskii* also have been reported to be the most common species in human oral samples (27). Riviere et al. investigated the subgingival distribution of these two spirochetes and a pathogen-related spirochete with different periodontal status and reported that human oral spirochetes were not uniformly distributed within the dentition or around individual teeth (23).

We isolated *Treponema* sp. strain G7201^T (T = type strain), which could not be classified in any previously described species. We examined the phenotypic, serologic, and genetic characteristics of this organism and compared the results with data for reference species.

In this paper we describe the characteristics of this *Treponema* strain and propose a new species, *Treponema medium*, for it on the basis of morphological, biochemical, and immunological characteristics, DNA-DNA hybridization data, and 16S rRNA sequence data.

MATERIALS AND METHODS

Bacterial strains and culture conditions. A host-associated spirochete, strain G7201^T, was isolated from a subgingival plaque sample from a 52-year-old male patient with adult periodontitis by a well-plate method as described by Rosebury and Foley (24). Briefly, subgingival plaque samples were suspended in sterile saline, and the resulting suspension (0.2 ml) was subcutaneously injected into guinea pigs (weight, approximately 250 g). Pus was aseptically removed from swollen lesions on the guinea pigs and then placed in agar wells, each approximately 2 mm wide and 1 cm deep, on 12.5% inactivated horse serum-containing sterility test medium plates (BBL Microbiology Systems, Cockeysville, Md.) in a candle jar. White haze growing out from each well into the agar and away from bacterial contaminants was observed after 10 days of anaerobic incubation at 37°C. Agar plugs at the leading edge of the haze were cut with a sterile glass pipette and were transferred into agar wells in fresh agar plates. In order to obtain a pure culture, the procedure was repeated more than 10 times, and spirochetal colonies were picked from solid sterility test medium supplemented with horse serum (35). Later, TYGVS medium was used as the routine culture medium and an anaerobic chamber (85% N_2, 10% H_2, 5% CO_2) was used for anaerobic conditions (34, 36).

T. denticola ATCC 33520 and ATCC 35405, *T. socranskii* subsp. *buccale* ATCC 35534, *T. socranskii* subsp. *socranskii* ATCC 35536, *T. vincentii* ATCC 35580, *Treponema phagedenis* biotype Reiter, and an avirulent variant, *Treponema pallidum* ATCC 27087, were used as reference organisms in this study. *T. socranskii* was grown anaerobically in modified NOS medium (ATCC medium 1494). The other microorganisms were grown in TYGVS medium at 37°C for 4 to 7 days (29). Each strain was harvested by centrifugation and washed twice in 0.01 M phosphate-buffered saline (pH 7.2).

Electron microscopy. Fresh microorganisms from a 3-day-old culture of strain G7201^T were harvested by centrifugation at 5,000 × *g* for 10 min and were gently washed three times in phosphate-buffered saline and once in distilled water. The resulting bacterial suspension was placed on collodion membrane-coated copper grids. The cells were negatively stained with a 1% phosphotungstic acid solution (pH 7.0) and observed with an electron microscope (model JEM 1200EX; JEOL, Tokyo, Japan). For scanning electron microscopy, the washed bacterial cells were

* Corresponding author. Mailing address: Department of Oral Microbiology, Asahi University School of Dentistry, Hozumi, Gifu-Ken, 501-02 Japan. Phone and fax: (81) 058-329-1423. E-mail: toshiut@hatelecom.or.jp.

also fixed for 30 min at room temperature in a 2.5% glutaraldehyde solution and for 30 min in a 1% osmium tetroxide solution; both of these solutions were buffered at pH 7.2 with 0.1 M cacodylate buffer. The fixed cells were then washed three times with distilled water. Aliquots were placed on the hydrophilic surfaces of GelBond PAG film disks (diameter, 6 mm; FMC BioProducts, Rockland, Maine), which were dried in air, mounted on specimen holders, and observed with a scanning electron microscope (model JSM-356C; JEOL).

Biochemical characterization. The routine tests used for strain G7201^T included tests for acid production from sugars; production of indole; catalase; gas and ammonia; hydrolysis of gelatin, esculin, and hippurate; and nitrate reduction. Sterility test medium and glucose-free thioglycolate medium (BBL) which had been supplemented with 15% inactivated horse serum were used as the basal media. The procedures used to determine biochemical characteristics were similar to the procedures described by Sutter et al. (32). Tests that produced questionable results were repeated.

Production of volatile fatty acids. The short-chain fatty acids in a 7-day-old TYGVS culture were converted to methyl esters and studied by a conventional procedure, using capillary gas chromatography (model GC-8 gas-liquid chromatograph; Shimadzu, Tokyo, Japan), a glass Chromosorb W80/100 column (diameter, 3 mm; length, 2 m; Shinwa Chemical Industries, Kyoto, Japan), and a flame ionization detector under the following operational conditions: column temperature, 160°C (isothermal); injection and detection temperature, 210°C; and nitrogen gas flow rate, 50 ml/min.

SDS-PAGE and Western immunoblotting analysis. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was performed by the method of Laemmli (9) with a MicroSlab model KS-8010 gel electrophoresis system (Marysol, Tokyo, Japan). Whole bacterial cells of each strain were heated in solubilizing buffer (0.125 N Tris buffer [pH 6.8] containing 4% [wt/vol] SDS, 20% [vol/vol] glycerol, and 5% [vol/vol] 2-mercaptoethanol) for 10 min at 100°C. After centrifugation at 10,000 × *g* for 15 min, the protein concentration of the supernatant was determined with a protein assay kit (Bio-Rad Laboratories, Richmond, Calif.). Supernatant (10 μg) from each bacterial strain was applied to an SDS–10% polyacrylamide gel. Electrophoresis was performed at a constant current of 20 mA at room temperature. After electrophoresis, the gel was stained with Coomassie brilliant blue R-250 or used for Western transfer. Western transfer of the antigens from the gel to a nitrocellulose membrane was performed as described previously (19, 34). An immunoblotting analysis was carried out by using goat anti-rabbit immunoglobulin G conjugated with peroxidase as the second antibody as described previously (19). It has been reported that a polypeptide is a main antigen of strain G7201^T (17). Therefore, a polyclonal rabbit antiserum against the 44-kDa polypeptide was used in the immunoblotting analysis as the first antibody.

DNA isolation. Bacterial cells (wet weight, 1 g) were resuspended in 5 ml of saline-EDTA buffer (0.15 M NaCl, 0.1 M EDTA; pH 8.0). After 15 ml of 0.1 M Tris buffer (pH 9.0) containing 1% (wt/vol) SDS was added, the preparation was incubated at 60°C until the solution became clear. DNA was isolated from the resulting cell lysate by the method of Marmur (14), with slight modifications (20). The DNA concentrations of the preparations were determined spectrophotometrically by determining the A_{260}-to-A_{280} ratio.

DNA base composition. The guanine-plus-cytosine (G+C) content of DNA was estimated by high-performance liquid chromatography (HPLC) (8). Each DNA preparation was dissolved in distilled water to a concentration of 1 mg/ml, and then the solution was heated at 100°C for 5 min. After rapid cooling in an ice bath, 10 μl of the resulting denatured DNA solution was reacted with 10 μl of a nuclease P1 (Yamasa Shoyu Co., Chiba, Japan) solution (2 U/ml of 40 mM sodium acetate buffer containing 0.2 mM $ZnCl_2$) as recommended by the manufacturer. The hydrolysate and a standard solution containing equal concentrations of four deoxyribonucleotide monophosphates (dAMP, dCMP, dGMP, and dTMP) were subjected separately to HPLC by using a Hitachi model L-6200 apparatus and a type 3013-N column (4 by 150 mm). The column was eluted with a mobile phase containing 30 mM KCl, 10 mM KH_2PO_4, and 10% CH_3CN (pH 3.5). The deoxyribonucleotide monophosphates were detected with a Hitachi model L-4200 apparatus at 267 nm.

DNA-DNA hybridization. The extent of DNA-DNA reassociation was determined by the membrane filter method described by Meyer and Schleifer (15), with a slight modification (20). Triplicate tests were run for each assay, and the results were normalized to 100% for the homologous DNA-DNA hybridization experiment.

Sequencing of the 16S rRNA gene. A nucleotide primer set (5′-GCT GGC AGT GCG TCT TAA GCA TGC-3′, located at positions 35 to 58 [*Escherichia coli* numbering], and 5′-GTG ACG GGC GGT GTG TAC AAG GCC C-3′, located at positions 1408 to 1384) was synthesized and used for PCR amplification. PCR amplification was performed as described previously (4). The DNA fragment obtained by PCR amplification was sequenced by using a Thermo Sequenase labeled primer cycle sequencing kit (Amersham Japan, Osaka, Japan) and a series of custom-synthesized primers (5). Phylogenetic analysis was performed by using the DNASTAR program (DNASTAR, Inc., Madison, Wis.), and nucleotide sequence pair similarity values for the sequences were calculated by using the CLUSTAL V method (6). Confidence intervals were assessed by performing a CLUSTAL V bootstrap analysis.

Nucleotide sequence accession numbers. The 16S rRNA gene sequences of *Treponema* strains determined in this study have been assigned DDBJ, EMBL, and GenBank accession numbers D85437 (strain G7201^T) and D85438 (*T. denticola* 33520). Previously published sequences were also used in this study; these sequences included the sequences of *Spirochaeta zuelzerae* (accession number M34265), *T. pallidum* (M34266), *T. phagedenis* (M57739), *Treponema saccharophilum* (M71238), *Treponema succinifaciens* (M577381), *Treponema rumen* CA (M59294), *Treponema bryantii* (M57737), *Treponema pectinovorum* (M71237), *Borrelia burgdorferi* (M88329), and *Leptospira interrogans* (X17547) (22).

RESULTS

Morphological characteristics. The optimum temperature for growth of *T. medium* G7201^T was 37°C. The cells were between 5 and 16 μm long and about 0.2 to 0.3 μm wide under the culture conditions employed. Each spirochetal cell had relatively blunt ends and five to seven periplasmic flagella that originated subterminally at the ends of the protoplasmic cylinder (Fig. 1). In addition, electron microscopic observation of negatively stained cells revealed that this microorganism had intracytoplasmic tubules (formerly designated fine fibrils) within the cytoplasmic cylinder (Fig. 1). We observed these tubules, each of which was approximately 10 nm in diameter, just under the two electron-dense layers of the protoplasmic cylinder (33). Possession of cytoplasmic tubules by spirochetes is one of the important criteria for classification in the genus *Treponema* (7, 16).

Biochemical characteristics. *T. medium* G7201^T fermented glucose, fructose, maltose, mannose, galactose, sucrose, ribose, trehalose, inulin, salicin, and raffinose, but did not ferment mannitol, arabinose, sorbitol, adonitol, xylose, or lactose. This organism also produced ammonia and hydrogen sulfate, but not indole, and hydrolyzed esculin and hippuric acid, but not gelatin. This strain was nitrate reduction negative. The volatile fatty acids produced in conventional TYGVS medium containing 0.1% glucose by strain G7201^T were detected by gas-liquid chromatography. This strain produced predominantly acetic acid and *n*-butyric acid, as well as traces of *n*-valeric acid. The phenotypic characteristics of *T. medium* G7201^T are shown in Table 1.

SDS-PAGE protein profiles and Western immunoblotting reactions. The protein profile of *T. medium* had major protein bands in the region between about 80 and 30 kDa and seemed to be different from the protein profiles of previously described *Treponema* species, including *T. denticola*, *T. vincentii*, *T. socranskii*, *T. phagedenis*, and *T. pallidum* (Fig. 2). Western immunoblotting assays in which anti-rabbit immunoglobulin G was used as a conjugate revealed that anti-44-kDa protein serum, which was an antiserum against a major 44-kDa antigen of *T. medium* G7201^T, did not react with the antigens from *T. denticola*, *T. vincentii*, *T. socranskii*, *T. phagedenis*, and *T. pallidum* (data not shown). These results demonstrated that *T. medium* could be distinguished from the reference species by its protein components, including the structural proteins of whole bacterial cells, and by its immunological reactions.

G+C contents. As reported previously (22), the G+C contents of *Treponema* species range widely, from 36 to 54 mol%, as determined by the thermal melting method. The G+C content of *T. medium* G7201^T was 51 mol%, as determined by HPLC in the present study. This value is almost the same as the G+C content of *T. socranskii*, which has three subspecies (50 to 52 mol%) (27).

DNA-DNA hybridization. The DNA of *T. medium* exhibited very low levels of reassociation with the DNAs of the other *Treponema* species examined in the present study, as shown in Table 2. The data in Table 2 revealed that *T. medium* was completely distinct from the other *Treponema* species. The quantitative DNA-DNA hybridization studies performed by the filter membrane method also showed that the labeled DNA

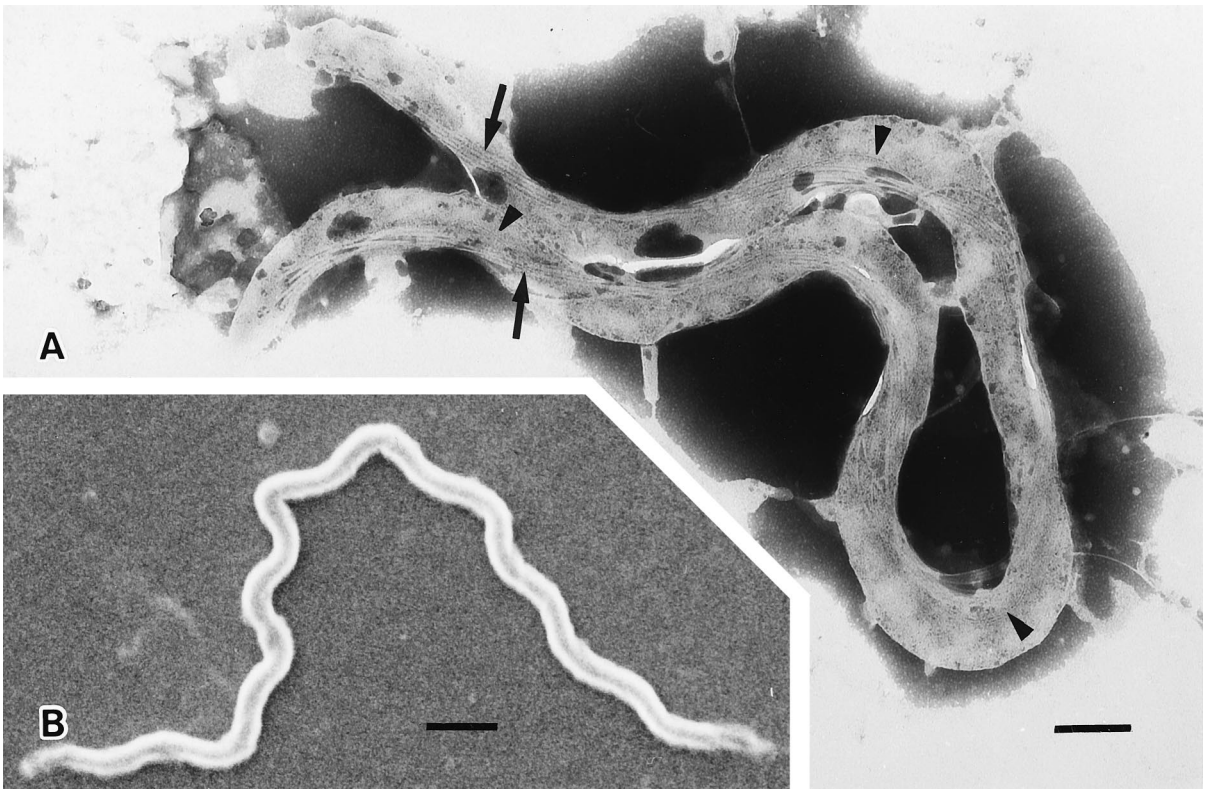

FIG. 1. Electron micrographs showing typical morphology of strain G7201^T. (A) Negatively stained cell. Periplasmic flagella (arrows) originating subterminally at the ends of the cell and intracytoplasmic tubules (arrowheads) in the cytoplasmic cylinder are visible, but the outer envelope was lost during sample preparation. Bar = 250 nm. (B) Scanning electron micrograph. Bar = 500 nm.

probe from *T. denticola* ATCC 33520 was reasonably homologous (level of homology, 95%) with DNA from *T. denticola* ATCC 35405, which was consistent with the results of Chan et al. (1). This observation supports the validity of the data obtained for the species studied.

Sequence and phylogenetic analysis. We cloned the 16S rRNA genes of *T. denticola* ATCC 33520 and *T. medium* G7201^T, and the nucleotide sequences (length, 1,381 bases) were determined in order to quantitatively assess the phylogenetic relationships to strains belonging to other treponemal species, including *Treponema bryantii*, *T. pallidum*, *T. pectinovorum*, *T. phagedenis*, *T. rumen*, *T. succinifaciens*, *T. saccharophilum*, and *Spirochaeta zuelzerae*, and two other pathogenic spirochetes, the agent of Lyme disease (*Borrelia burgdorferi*) and *Leptospira interrogans* (which is associated with Weil's disease). The *T. medium* 16S rRNA gene sequence (length without PCR primer sequences, 1,381 bases) was aligned and compared with the sequences of other treponemes by using the DNASTAR program.

The nucleotide sequence pair similarity values for the sequences are shown in Table 3, and a neighbor-joining phylogenetic tree constructed on the basis of the data in the sequence similarity matrix is shown in Fig. 3. The phylogenetic analysis placed strain G7201^T in a coherent cluster of *Treponema* species, and *T. medium* G7201^T, *T. phagedenis*, *T. denticola*, and *T. pallidum* belonged to a group different from the human oral organism *T. pectinovorum*. The tree in Fig. 3 clearly shows that strain G7201^T is not closely related to the other organisms in the cluster and that this strain seems to have diverged at the species level.

DISCUSSION

The differential characteristics of human oral treponemes, including *T. medium* G7201^T, are compared in Table 1. When *T. medium* is compared morphologically with previously described human oral spirochetes, it can be distinguished from *T. denticola*, *T. pectinovorum*, *T. socranskii*, *T. macrodentium*, and *T. orale* by the number of periplasmic flagella, although the cell lengths and cell widths of these organisms are similar. The medium-sized human oral treponeme *T. vincentii* has four to six periplasmic flagella but is not able to ferment carbohydrates (26), suggesting that the saccharolytic organism *T. medium* G7201^T, which has five to seven periplasmic flagella, can be distinguished from *T. vincentii*. The G+C content and SDS-PAGE protein profile data also support this distinction. In addition, *T. medium* exhibits only 28% DNA homology with *T. vincentii*, indicating that *T. medium* is different from *T. vincentii*.

Although the number of periplasmic flagella and the G+C content of *T. scoliodontum* have not been reported, this small asaccharolytic species is different from saccharolytic strain G7201^T. *T. phagedenis*, *Treponema refringens*, and *Treponema minutum* are found in human genitalia. *T. phagedenis* has three to eight periplasmic flagella and a G+C content of 38 to 39 mol%, *T. refringens* has two to four periplasmic flagella and a G+C content of 39 to 43 mol%, and *T. minutum* has two or three periplasmic flagella and a G+C content of 37 mol% (26), suggesting that strain G7201^T (with five to seven periplasmic flagella and a G+C content of 51 mol%) can be readily distinguished from these human genitalia-inhabiting species. Phy-

TABLE 1. Differential characteristics of human oral *Treponema* species[a]

Characteristic	*T. medium*	*T. denticola*	*T. pectinovorum*	*T. scoliodontum*	*T. socranskii*	*T. vincentii*	*T. macrodentium*	*T. orale*
Cell length (μm)	6–15	6–16	7–15	6–16	6–15	5–16	5–16	6–16
Cell width (μm)	0.20–0.30	0.15–0.20	0.28–0.30	0.15–0.20	0.16–0.18	0.20–0.25	0.10–0.25	0.15–0.25
No. of periplasmic flagella	5–7	2–3	2	NR[b]	1	4–6	1	1
Fermentation of:								
Glucose	+	−	−	−	+	−	+	−
Fructose	+	−	−	−	+	−	+	−
Lactose	−	−	−	−	−	−	−	−
Maltose	+	−	−	−	+	−	+	−
Mannitol	−	−	−	−	NR	−	−	−
Mannose	+	−	−	−	+	−	+	−
Galactose	+	−	−	−	+	−	+	−
Starch	ND[c]	−	−	−	NR	−	+	−
Sucrose	+	−	−	−	+	−	+	−
Ribose	+	−	−	−	+	−	+	−
Xylose	−	−	−	−	+	−	+	−
Hydrolysis of:								
Esculin	+	+	−	−	−	—[d]	NR	+
Gelatin	−	+	−	−	—[d]	−	−	+
Production of:								
Ammonia	+	+	+	−	+	+	−	−
Indole	−	—[d]	−	−	−	+	−	−
Hydrogen sulfate	+	+	−	+	+	+	+	+
Fatty acids produced[e]	A, B, v	A, l, s, p, f	A, F, p, l	A, f, p, l, s	A, L, S, f	A, B, l, s	A, F, L	A, p
G+C content (mol%)	51	37–38	39	NR	50–52	44	39	37

[a] Data for all species except *T. medium* are from references 15, 24, 25, and 27.
[b] NR, not reported.
[c] ND, not determined.
[d] Some strains were positive, and some were negative.
[e] A, acetic acid; B, *n*-butyric acid; F and f, fumaric acid; L and l, lactic acid; S and s, succinic acid; p, propionic acid; v, *n*-valeric acid. Uppercase letters indicate major fatty acids, and lowercase letters indicate minor fatty acids.

logenetic data distinguish *T. medium* from *T. phagedenis* as well as *T. denticola* (Fig. 3). *T. medium* also seems to be distinguished from the culturable and fermentative organisms *T. bryantii*, *T. succinifaciens*, and *T. saccharophilum* on the basis of the lower G+C contents of the latter organisms (approximately 36 mol%) and the fact that the latter organisms have one or two periplasmic flagella (26). *Treponema innocens* and *Treponema hyodysenteriae*, both of which have relatively short cells and G+C contents of 25.7 to 25.9 mol% and were initially isolated from swine intestinal materials, have been reclassified in the genus *Serpulina* (30, 31). *T. denticola* (G+C content, 37 to 38 mol%) and *T. pallidum* (G+C content, 52 to 53.7 mol%), however, are quite different (22). In addition, *T. socranskii*, a saccharolytic hydrogen sulfate producer with a G+C content of 50 to 52 mol%, differs from *T. pectinovorum*, an asaccharolytic organism that does not produce hydrogen sulfate and has a G+C content of 39 mol% (Table 1).

Comparative analysis of the 16S rRNA sequences of individual bacteria has been widely used to reveal phylogenetic relationships among spirochetes. *T. denticola*, *T. phagedenis*, and *T. pallidum* form a phylogenetic cluster, and *T. socranskii* and *T. pectinovorum* are closely related in another cluster (22). We determined the 16S rRNA gene sequence of strain G7201^T and aligned and compared this sequence with the sequences of

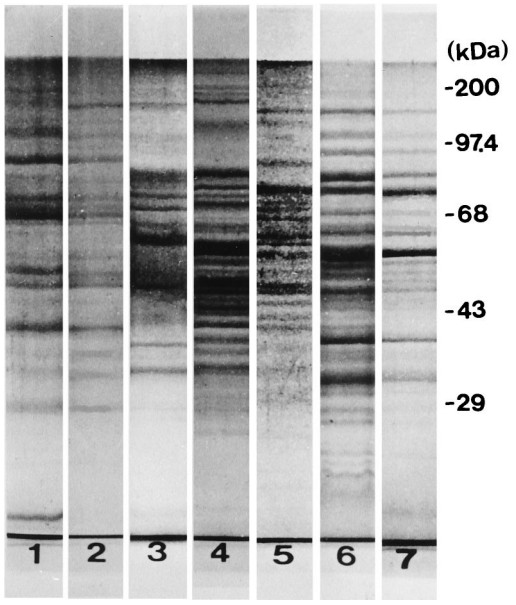

FIG. 2. Coomassie brilliant blue-stained polypeptide patterns of whole cells of seven treponemes on an SDS-polyacrylamide gel. Lane 1, *T. pallidum* ATCC 27087; lane 2, *T. phagedenis* biotype Reiter; lane 3, strain G7201^T; lane 4, *T. vincentii* ATCC 35580; lane 5, *T. socranskii* subsp. *buccale* ATCC 35534; lane 6, *T. denticola* ATCC 35405; lane 7, *T. denticola* ATCC 33520. The positions of molecular weight standards are shown on the right.

TABLE 2. Levels of DNA relatedness between strain G7201^T and strains of other *Treponema* species

Source of unlabeled DNA	% Homology with labeled DNA from strain G7201^T
T. medium G7201^T	100
T. denticola ATCC 33520	7
T. denticola ATCC 35405	8
T. vincentii ATCC 35580	28
T. socranskii subsp. *socranskii* ATCC 35536	30
T. phagedenis biotype Reiter	5
T. pallidum ATCC 27087	5

TABLE 3. 16S rRNA sequence similarity matrix for members of the genus *Treponema* and related organisms

Organism	% 16S rRNA similarity												
	T. medium	*T. rumen* CA	*T. succinifaciens*	*T. saccharophilum*	*T. bryantii*	*T. pectinovorum*	*T. phagedenis*	*T. denticola*	*S. zuelzerae*	*T. pallidum*	*B. burgdorferi*	*L. interrogans*	*E. coli*
T. medium	100	76.0	72.7	78.4	79.2	78.5	88.1	88.0	84.2	84.5	73.5	66.8	67.2
T. rumen CA		100	80.1	81.7	80.0	78.5	76.4	78.1	75.8	77.9	69.6	66.5	65.3
T. succinifaciens			100	75.1	78.4	75.3	73.6	74.8	71.9	74.9	64.9	62.5	61.4
T. saccharophilum				100	77.6	80.5	78.1	79.4	76.3	77.4	69.2	63.1	64.5
T. bryantii					100	82.4	78.9	79.5	77.5	77.8	71.8	63.6	65.2
T. pectinovorum						100	77.9	79.2	76.6	76.1	69.0	61.3	61.8
T. phagedenis							100	90.1	84.2	87.1	72.2	64.9	65.1
T. denticola								100	84.6	86.6	73.7	68.8	65.4
S. zuelzerae									100	80.9	71.0	64.3	62.7
T. pallidum										100	74.4	67.1	65.8
B. burgdorferi											100	56.9	66.5
L. interrogans												100	68.2
E. coli													100

other spirochetes by using the DNASTAR program. A neighbor-joining phylogenetic tree constructed on the basis of the sequence similarity matrix data is shown in Fig. 3. According to this tree, *Treponema* strains could be divided into two major clusters. Strain G7201^T belonged to a cluster that included *T. phagedenis*, *T. denticola*, *S. zuelzerae*, and *T. pallidum*. Our phylogenetic analysis based on 16S rRNA gene sequences revealed that strain G7201^T diverged at the species level, which is consistent with the creation of a new species.

It should be noted that the levels of reassociation between the DNA of *T. medium* and the DNAs of *T. denticola* and *T. pallidum*, which is the type species of the genus *Treponema*, were less than 10% (Table 2). Our DNA-DNA homology data showed that strain G7201^T was similar in distance from *T. socranskii* and *T. vincentii* (levels of homology, approximately 30%). This finding strongly supports the proposal that *T. medium* should be considered a new species which is isolated from human dental cavities.

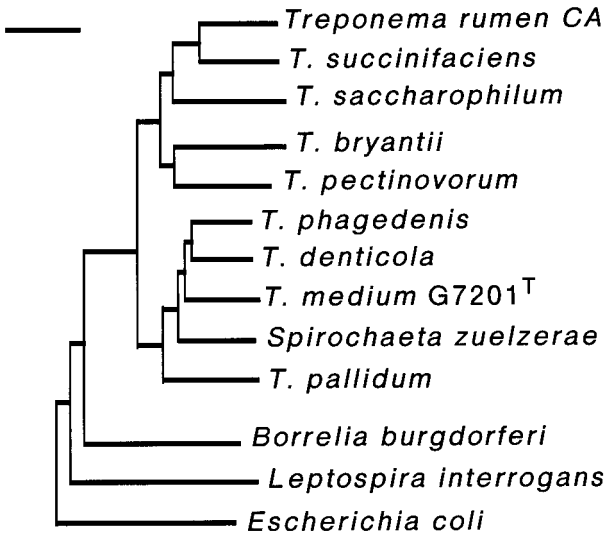

FIG. 3. Phylogenetic tree for spirochetes. The phylogenetic tree was constructed as described in the text by using 16S rRNA gene sequences. Bar = 5% difference between sequences, as determined by measuring the lengths of the horizontal lines connecting species. The 16S rRNA gene sequence of *Escherichia coli* served as an outgroup.

At present, we have no information on the distribution or incidence of this species in human mouths. An immunological survey is in progress; in this survey, subgingival plaque samples from clinically classified patients and polyclonal antibodies against species-specific surface antigens from this microorganism are being used. The pathogenicity of oral spirochete strain G7201^T is not known at present.

On the basis of morphological and biochemical characteristics, the results of SDS-PAGE and a Western immunoblotting analysis, DNA-DNA hybridization data, and 16S rRNA sequence data, we propose a new species, *T. medium*, for strain G7201^T. The taxonomic description below summarizes the properties of this species.

Description of *Treponema medium* sp. nov. *Treponema medium* (me′di.um. L. adj. *medium*, medium, referring to the cell size) is a gram-negative, anaerobic, motile, helically coiled, medium-sized treponeme. The cells are 5 to 16 μm long and 0.2 to 0.3 μm wide. They have cytoplasmic tubules and five to seven periplasmic flagella (axial flagella) that originate subterminally at each end. The cells in broth cultures exhibit rotational and translational movement. The optimum growth temperature is 37°C, and colonies on agar plates are white and translucent. *T. medium* ferments glucose, fructose, maltose, mannose, galactose, sucrose, ribose, trehalose, inulin, salicin, and raffinose. This organism also produces ammonia and hydrogen sulfate and hydrolyzes esculin and hippuric acid. The major acid products of strain G7201^T grown in TYGVS medium containing 0.1% glucose are acetic acid and *n*-butyric acid. A trace of *n*-valeric acid is also found. Isolated from subgingival plaque of patients with adult periodontitis. The G+C content is 51 mol%. The type strain of *T. medium* is strain G7201.

Phenotypic characteristics, DNA-DNA hybridization data, G+C content data, and 16S rRNA gene sequence data indicate that human oral spirochete strain G7201^T should be identified as member of a novel species, *T. medium.*

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