Treponema denticola (ex Brumpt 1925) sp. nov., nom. rev., and Identification of New Spirochete Isolates from Periodontal Pockets

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Standard growth and isolation methods were used to obtain five new *Treponema* strains in pure culture from deep periodontal pockets. The strains were identified to the species level by various methods, including agglutination, immunofluorescence, a dot blot immunoassay, electron microscopy, gas-liquid chromatography of metabolic volatile fatty acids, and DNA hybridization. Two isolates were strains of *Treponema socranskii*; the other three were strains of "*Treponema denticola*," a species described in 1925 by Brumpt. Because no type strain was designated for this species previously, the name was not included on the Approved Lists of Bacterial Names and has no current nomenclatural standing. We propose that *Treponema denticola* (ex Brumpt) sp. nov., nom. rev. is a valid and distinct species of the genus *Treponema* and designate strain ATCC 35405 as the type strain and strains ATCC 33520 and ATCC 35404 as reference strains. *T. denticola* appears to be the species that is most frequently isolated from periodontal pockets. Unless new isolation and cultivation techniques are introduced, it appears that present technology can yield only isolates belonging to the currently described oral anaerobic spirochete species and that there is little chance of isolating the larger treponemes.

The treponemes have been implicated in the etiology of all forms of periodontal disease by virtue of their proliferation to high numbers in diseased sites (6, 13, 15). Evidence on the quantitative relationship of *Treponema denticola* to the severity of periodontal disease has been presented previously (22, 23). Investigations to elucidate the underlying mechanisms for the virulence of the oral anaerobic treponemes have been performed (5, 7, 18, 21, 27). Not only has *T. denticola* been the spirochete species of choice for laboratory investigations as described above, but it is also the species that is most frequently isolated from subgingival plaque (4, 20).

The species name *T. denticola* is not on the 1980 Approved Lists of Bacterial Names (24) or on any subsequent validation list. Therefore, this name has not been validly published. However, it has been published in *Bergey's Manual of Systematic Bacteriology* (25), this is only "effective publication" according to the *International Code of Nomenclature of Bacteria* (11), and a type strain was not designated. In this study, we characterized five new oral anaerobic spirochetes isolated from periodontal pockets and identified them to species level. On the basis of our data, as well as the previously documented characteristics of the taxon, we propose that *Treponema denticola* (ex Brumpt 1925) sp. nov., nom. rev. (2) is a valid and distinct species of the genus *Treponema* and designate strain ATCC 35405 as the type strain and strains ATCC 33520 and ATCC 35404 as reference strains.

MATERIALS AND METHODS

Isolation of new spirochetes. Treponemes are usually isolated by the following two general methods: (i) membrane filters placed on the surface of agar media and (ii) incorporation of the antibiotic rifampin into media as a selective

agent (25). In our attempt to isolate new oral anaerobic spirochetes, we combined these two methods (3) and used membrane filters of several pore sizes (1.2, 3, and 5 μ m) and rifampin at a final concentration of 4 μ g/ml. Several spirochete media were compared for their ability to support growth of *T. denticola* (data not shown), new oral spirochete (NOS) medium of Leschine and Canale-Parola (12) was the medium of choice and was used throughout this work. Treponemes migrated through the filters and grew in NOS agar as a white, hazy, cottony growth. Such growth was removed with a Pasteur pipette and was inoculated into prerduced NOS agar medium containing rifampin. The resulting growth was checked by dark-field microscopy for purity.

Five axenic cultures were isolated as described above and were designated strains 975a, 975b, 9710, 9715, and 979.

Control spirochete cultures. The following spirochete cultures were used for comparison: *T. denticola* ATCC 35405^T (serotype or strain a^T) (T = type strain), ATCC 35404 (strain c), strain d (4), and ATCC 33520; *Treponema vincentii* ATCC 35580; *Treponema socranskii* subsp. *socranskii* ATCC 35536; and *Treponema phagedenis* Reiter. *T. denticola* serotypes a, c, and d were isolated and characterized in our laboratory (3). All cultures, including the new isolates, were maintained on NOS medium containing 0.3% agar; cells for study were grown in NOS broth.

Agglutination tests. Cells of each isolate were sedimented by centrifugation at 8,000 $\times$ g for 10 min at 4°C. They were then washed twice with 0.01 M phosphate-buffered saline (pH 7.2). Suspensions containing washed cells of the isolates were standardized to an optical density at 490 nm of 1.0. The rabbit antisera used for testing were prepared as described previously (4). We used antisera to the following spirochetes: *T. vincentii* ATCC 35580, *T. socranskii* ATCC 35536, *T. phagedenis* Reiter, and *T. denticola* ATCC 35405^T (serotype a), ATCC 35404 (serotype c), and serotype d. These antisera were serially diluted twofold in 10 mM Tris buffer (pH 7.2) containing 1% bovine serum albumin and 0.05%

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TABLE 1. Results of agglutination tests performed with oral spirochete isolates

Strain	Highest titer of rabbit antiserum prepared against ^a :					
	<i>T. denticola</i> ATCC 35405 ^T	<i>T. denticola</i> ATCC 35404	<i>T. denticola</i> strain d	<i>T. socranskii</i> ATCC 35536	<i>T. vincentii</i> ATCC 35580	<i>T. phagedenis</i> Reiter
975a	0	0	0	32	0	16
975b	0	0	0	4	0	16
9710	8	16	8	16	2	8
9715	8	16	8	32	0	8
979	8	32	8	0	8	8
Homologous strain	64	64	128	128	32	32

^a Reciprocal of the highest dilution that produced positive slide agglutination.

Tween 20 (TBT buffer). Portions (10 µl) of each dilution of a test antiserum (e.g., anti-*T. vincentii* antiserum) were placed onto different slides, and 10 µl of a cell suspension of a single isolate was added to each of the diluted antiserum preparations. This procedure was repeated with each of the isolates. The cells and antiserum were mixed, and the reaction was scored visually by eye. The reciprocal of the highest dilution that gave positive agglutination was considered the highest titer. Each test antiserum interacting with its homologous cell suspension (e.g., *T. vincentii* and anti-*T. vincentii* antiserum) was used as a positive control, and serially diluted normal rabbit serum was used as the negative control.

Indirect immunofluorescence. Cell suspensions of each isolate were standardized as described above. Portions (10 µl) of a cell suspension of one of the isolates were placed onto six microscopic slides, and the resulting smears were allowed to air dry. The smears were heat fixed and rehydrated with TBT buffer for 15 min, and the TBT buffer was drained off the slides. All of the test antisera were diluted 1:20 in TBT buffer, and 20 µl of each diluted antiserum (e.g., anti-*T. vincentii* or anti-*T. socranskii* antiserum) was added to a smear. The slides were incubated for 20 min at room temperature, after which they were washed three times with TBT buffer and then drained dry. A 20-µl portion of a 1:20 dilution of fluorescein-conjugated goat anti-rabbit immunoglobulin G in TBT buffer was added to each smear, and the slides were incubated for 20 min at room temperature. The smears were washed three times with TBT buffer and once with distilled water. The water was drained from each slide, and 1 drop of mounting fluid (90% glycerol, 10% carbonate buffer; pH 9.0) was placed onto each smear, which was then covered with a coverslip. The coverslips were sealed to the slides with nail varnish. The smears were examined by performing epifluorescence microscopy with a Leitz Dialux microscope at a magnification of ×1,000. This procedure was repeated with each of the isolates. Each test antiserum interaction with the homologous organism (e.g., anti-*T.*

vincentii antiserum and *T. vincentii*) was used as a positive control. Smears of the new isolates or previously characterized oral spirochetes that were treated only with fluorescein-conjugated goat anti-rabbit immunoglobulin G were used as the negative controls.

Dot blot immunoassay. A dot blot apparatus and nitrocellulose membranes were obtained from Bio-Rad Laboratories, Richmond, Calif. A nitrocellulose membrane was soaked in 10 mM Tris-buffered saline (pH 7.2) for 20 min before it was placed into the apparatus.

Blocking solution TBT buffer was applied to each well and allowed to drain by gravity filtration. A 25-µl portion of cells of an isolate (optical density at 620 nm, 0.2) was then added to each well and allowed to settle by gravity filtration. Bovine serum albumin blocking solution (100 µl) was added, and after this solution was drained from the apparatus, three washes were done, each with 200 µl of 10 mM Tris buffer (pH 7.2) containing 0.05% Tween 20 (TT buffer) with a vacuum applied. Starting with antiserum diluted 1:80, two-fold sequential dilutions were made in TBT buffer (e.g., anti-*T. vincentii* antiserum). Portions (30-µl) of each dilution of the specific antiserum (e.g., anti-*T. vincentii* antiserum) were added to different wells. After the antibody solution had drained completely, the preparations were washed three times with TT buffer with a gentle vacuum. A 30-µl portion of a 1:1,000 dilution of goat anti-rabbit immunoglobulin G alkaline phosphatase was added to each well, and filtration was carried out by gravity. The reaction wells were washed with TT buffer by using a gentle vacuum.

The membrane was then removed and placed into a substrate solution containing 15 mg of 5-bromo-4-chloro-3-indoyl phosphate, 30 mg of nitroblue tetrazolium, and 100 ml of 10 mM Tris buffer (pH 9.5). Each of the isolates was tested in the same way with each of the test antisera. The negative control consisted of cells that interacted with the goat anti-rabbit immunoglobulin G alkaline phosphate conjugate and substrate. The color development solution was

TABLE 2. Indirect immunofluorescence reactions of oral spirochete isolates

Strain	Reaction with antiserum prepared against ^a :					
	<i>T. denticola</i> ATCC 35405 ^T	<i>T. denticola</i> ATCC 35404	<i>T. denticola</i> strain d	<i>T. socranskii</i> ATCC 35536	<i>T. vincentii</i> ATCC 35580	<i>T. phagedenis</i> Reiter
975a	0	0	0	+++	++	+++
975b	0	0	+	+++	+	++
9710	+	++	++	+++	+	++
9715	+	+++	+	++	++	+++
979	+	++	+++	++	+	++
Homologous strain	+++	+++	++++	+++	+++	+++

^a The levels of immunofluorescence ranged from ++++ (very strong) to 0 (none).

FIG. 1. Dot blot analysis of isolates performed with anti-*T. socranskii* antiserum. Control 1 was a preparation without anti-*T. socranskii* antiserum. Control 2 was a preparation without anti-*T. socranskii* antiserum and cells.

agitated gently for 5 min in the dark. The nitrocellulose membrane was removed and rinsed with distilled water to stop the reaction.

Electron microscopy. Phosphate-buffered saline-washed cells were suspended in distilled water to an optical density at 620 nm of 1.0; then 1 drop of 2% phosphotungstic acid stain and 1 drop of cells were mixed together in a petri dish. A copper grid was placed on this mixture. After 15 s the grid was removed, and the excess liquid was absorbed with a piece of bibulous paper. The negatively stained specimens were examined with a Phillips model 300 electron microscope. The cell length, diameter, wavelength, and amplitude and the number of axial fibrils were determined for each new isolate as described previously (3).

Production of volatile metabolic fatty acids. Cells of the

new spirochete isolates, as well as cells of *T. denticola* strains a^T, c, and d, *T. vincentii*, and *T. socranskii*, were grown in NOS broth without supplemental fatty acids. At the end of the exponential growth phase, the cells were centrifuged, and the resulting supernatants were used for an analysis of volatile fatty acids. The procedures used for extraction and determination of the volatile metabolic fatty acids were the procedures of the Anaerobe Laboratory at Virginia Polytechnic Institute and State University (8). A 2.5- μ l portion of an ether extract was injected into a column (15% silicone GE SE-52 on Chromosorb W, AW, DMCS; 100/120 mesh; Applied Science Laboratories, Inc., State College, Pa.) for detection. The apparatus used was a Hewlett-Packard model 402 high-efficiency gas chromatograph. Volatile fatty acid standards were obtained from a Capco AnaBac standard solution (International Scientific of Canada, Ottawa, Canada).

DNA homology. Cells of the various strains of spirochetes were harvested by centrifugation (4°C) and $15,000 \times g$ for 15 min as soon as the cultures reached their maximum density. DNAs were extracted from the cells of all of the strains by using the procedure described by Marmur (16). The DNA concentration obtained for each treponeme strain was determined by measuring A_{260} with a spectrophotometer, using the following relationship: $1.0 \text{ U of } A_{260} = 50 \mu\text{g}$ of double-stranded DNA per ml.

A 1- μ g portion of DNA from each known species was denatured by heating it for 10 min at 95°C and then cooling it on ice immediately. Labelling of this DNA with tritiated thymidine 5'-triphosphate was carried out by using a Random Primed DNA Labelling Kit (catalog no. 1004 760; Molecular Biology Boehringer Mannheim, Mannheim, Germany) or the Random Primers DNA Labeling System (catalog no. 8187SA; Bethesda Research Laboratories, Gaithersburg, Md.) following the instructions of the manufacturers.

Labelled DNA was separated from unincorporated deoxynucleotide triphosphates with Nick columns (catalog no. 17-0855-01; Pharmacia LKB, Biotechnology AB, Uppsala, Sweden), which were used according to the directions of the

FIG. 2. Whole cells of isolate 9710 used to determine cellular characteristics. Bar = 2 μ m.

FIG. 3. Isolate 9715 at two magnifications used to determine endoflagellar arrangement. The arrows indicate endoflagella. (A) Bar = 0.5 μm . (B) Bar = 0.1 μm .

supplier. The labelled DNA was concentrated by ethanol precipitation. The average specific activity of the labelled DNAs was 3.2×10^6 cpm/ μg .

DNA hybridization assays were performed as described by Johnson (10). A 30- μg portion of unlabelled DNA (or 30 μg of salmon sperm DNA as a control) was mixed with 0.02 to 0.05 μg of labelled DNA in a solution containing 0.4 M sodium chloride and 0.4 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 7.0) in a total volume of 120 μl . The samples were denatured by heating them in a boiling water bath for 10 min and then incubated at 37°C for 20 to 24 h. A 1-ml portion of S1 assay buffer (5 mM sodium acetate, 0.3 M sodium chloride, 0.5 mM zinc chloride; pH 4.6) was added to each reaction mixture, and then 225 U of S1 nuclease was added. The reaction mixtures were incubated at 25°C for 1 h. A 2-ml portion of ice-cold 10% (wt/vol) trichloroacetic acid was then added to each sample together with 25 μg of carrier DNA. The precipitated DNA was collected on a Whatman glass fiber filter, washed with 10% trichloroacetic acid, dried, and counted with an LKB model 1215 Rackbeta II liquid scintillation counter. The percentages of homology were calculated as follows: [(counts per minute of hybrid formed with test DNA – counts per minute of hybrid formed with salmon sperm DNA)/(counts per minute of hybrid formed with homologous

DNA – counts per minute of hybrid formed with salmon sperm DNA)] $\times 100$.

RESULTS AND DISCUSSION

Serological tests. Various serological tests were performed to determine whether the new oral anaerobic spirochete isolates had antigenic determinants in common with previously described strains and species of spirochetes.

Table 1 shows the agglutination reactions of the new oral spirochete isolates when they were mixed with rabbit antisera prepared against previously described spirochete strains and species. Strains 975a and 975b did not agglutinate at all with antisera against three strains of *T. denticola* and *T. vincentii*. Strains 9710, 9715, and 979 agglutinated with antisera against *T. denticola* strains a^T, c, and d. In addition, strains 9710 and 9715 agglutinated to quite high titers with antiserum against *T. socranskii*, while strain 979 agglutinated with antiserum against *T. vincentii* but not with antiserum against *T. socranskii*. Of interest was the observation that all of the oral spirochetes cross-agglutinated with antiserum prepared against *T. phagedenis*.

The results of the indirect immunofluorescence reactions for the oral isolates are shown in Table 2. Again, strains 975a and 975b did not exhibit any fluorescence with antisera

TABLE 3. Cellular characteristics of new oral spirochete isolates

Isolate	Cell length (μm)	Cell diam (μm)	Wavelength (μm)	Amplitude (μm)	No. of endoflagella
975a	5.45 ± 0.07^a	0.15 ± 0.01	0.71 ± 0.01	0.30 ± 0.05	2
975b	6.8 ± 0.28	0.20 ± 0.01	0.79 ± 0.01	0.35 ± 0.04	2
9710	9.13 ± 2.18	0.20 ± 0.04	0.96 ± 0.03	0.32 ± 0.03	4
9715	7.05 ± 0.64	0.22 ± 0.03	1.06 ± 0.15	0.42 ± 0.02	4
979	6.60 ± 0.26	0.21 ± 0.04	1.25 ± 0.28	0.60 ± 0.14	4

^a Average $\pm$ standard deviation. Averages were calculated from the data for 5 to 10 cells in negatively stained electron micrographs.

FIG. 4. Volatile fatty acid profiles of known oral treponemas and oral isolates. *T. denticola* produces acetic acid (a) and a trace of formic acid (f); *T. socranskii* produces only acetic acid; and *T. vincentii* produces both acetic acid and propionic acid (p). The volatile fatty acid production profile of isolate 979 is similar to that of *T. denticola*, while the profile of isolate 975a is the same as that of *T. socranskii*.

prepared against the three strains of *T. denticola*, although strain 975b showed weak immunofluorescence with antiserum against *T. denticola* strain d. However, both strain 975a and strain 975b showed very strong fluorescence when they were reacted with antiserum against *T. socranskii*. Strains 9710, 9715, and 979 reacted with antisera prepared against all of the previously described spirochetes tested, including *T. phagedenis*.

The dot immunoblotting results obtained when antiserum prepared against *T. socranskii* was used are shown in Fig. 1. A darker blot indicates a positive reaction between the cells and an exposed antiserum. Figure 1 shows that strains 975a and 975b cross-reacted with antiserum prepared against *T. socranskii*. The other three strains cross-reacted with antisera prepared against *T. denticola* strains a^T, c, and d (data not shown). The serological results from agglutination, immunofluorescence, and dot blot assays (Tables 1 and 2 and

Fig. 1) show quite clearly that isolates 975a and 975b are probably not members of *T. denticola* but may be members of *T. socranskii*. Similarly, isolates 9710, 9715, and 979 may be members of *T. denticola* but certainly are not members of *T. socranskii*. These findings were evident despite some cross-reactions with polyclonal antibodies, especially with polyclonal antibodies against *T. phagedenis*.

Electron microscopy. Figure 2 shows whole cells of isolate 9710 and is typical of the micrographs used for measuring cell dimensions, wavelengths, and amplitudes. Figure 3 shows isolate 9715 at two magnifications as an example of how endoflagellum numbers were determined for each spirochete isolate. Morphological characterization data for the negatively stained new oral spirochetes are shown in Table 3. The cellular wavelengths of isolates 975a and 975b (average, 0.75 μm) are shorter than the cellular wavelengths of isolates 9710, 9715, and 979 (average, 1.09 μm). Isolates 975a

TABLE 4. Levels of homology among *Treponema* strains and new oral spirochete isolates

Strain	% Homology with labelled DNA from:		
	<i>T. denticola</i> ATCC 33520	<i>T. socranskii</i> ATCC 35536	<i>T. vincentii</i> ATCC 35580
<i>T. denticola</i> ATCC 33520	100	7	2
<i>T. denticola</i> ATCC 35405 ^T	125	0	0
<i>T. denticola</i> ATCC 35404	66	10	0
<i>T. vincentii</i> ATCC 35580	13	27	100
<i>T. socranskii</i> ATCC 35536	3	100	0
<i>T. phagedenis</i> Reiter	0	14	0
975a	3	84	0
975b	5	90	0
979	101	12	0
9710	86	24	8
9715	103	17	0

and 975b had a 1-2-1 endoflagellum arrangement, while the other three isolates had a 2-4-2 arrangement.

Volatile fatty acids produced. The metabolic volatile fatty acid profiles of previously described oral spirochete species are shown in Fig. 4. As Fig. 4 shows, *T. denticola* produced acetic acid and a relatively small amount of formic acid in spent NOS broth; *T. socranskii* produced only acetic acid; and *T. vincentii* produced acetic acid and propionic acid. Figure 4 also shows the volatile fatty acid profiles of isolates 975a and 979. Isolate 975a produced only acetic acid; the profile of isolate 975b was similar (data not shown). Isolate 979 produced acetic acid and a trace of formic acid; this profile was shared by isolates 9710 and 9715.

DNA homologies. The DNA homology results for the isolates and previously described treponema species are shown in Table 4. Substantial levels of homology were observed when isolates 975a and 975b were compared with *T. socranskii*. Isolates 9710, 9715, and 979 exhibited substantial levels of homology with *T. denticola* ATCC 33520.

Identification of new oral anaerobic spirochete isolates. The accumulated data from the characterization studies of the new oral spirochete isolates showed that isolates 975a and 975b are strains of *T. socranskii*, while isolates 979, 9710, and 9715 are strains of *T. denticola*. The results of the serological tests supported this conclusion even though the agglutination and immunofluorescence data were not clear because of cross-reactions. It should be noted that antiserum against *T. phagedenis* reacted with all of the oral spirochetes tested in agglutination and immunofluorescence reactions. This species is not an oral spirochete. (*T. phagedenis* is found in ulcers on human external genitalia, and there has been a report of an isolate from a case of primary syphilis in a human.) Five antigens of *T. phagedenis* cross-reacted with antibodies in syphilitic sera (25). In addition, monoclonal antibody C2-1, which was produced by immunization with *Treponema pallidum*, cross-reacted with *T. denticola* strains, *T. vincentii*, *T. pallidum*, and *T. phagedenis*, as well as *Leptospira* and *Borrelia* strains (19). These findings support the hypothesis that common antigenic determinants are shared by *Treponema* species. Therefore, it was not unusual that Riviere et al. (19) were able to identify a periopathogen related to *T. pallidum*. It follows that the cross-reactions of anti-*T. phagedenis* antiserum with the oral spirochetes which we observed is not surprising. The results of Paster et al. (17) lend credence to the concept that there are cross-reactions on a molecular basis; these authors performed a 16S rRNA sequence analysis of *T. pallidum*, *T. phagedenis*,

and *T. denticola* and showed that these species exhibit an average level of interspecies similarity of 89.9%.

Electron microscopic data supported the identification described above to the extent that the cellular wavelengths of isolates 975a and 975b are shorter than the cellular wavelengths of the other three new isolates and isolates 975a and 975b have a 1-2-1 endoflagellar arrangement. Isolates 979, 9710, and 9715 have a 2-4-2 endoflagellar arrangement, which is the dominant arrangement among *T. denticola* strains (1, 26). However, the use of endoflagellar arrangement as a definitive criterion in classification is questionable since widely different flagellar arrangements have been reported for *T. denticola*. For instance, 3-6-3 and 5-10-5 endoflagellar arrangements have been documented for *T. denticola* strains (3, 14).

However, the comparative data from the analysis of volatile fatty acids in fermented stale medium for the various oral anaerobic spirochete isolates showed clearly that isolates 975a and 975b are *T. socranskii* strains (which produce only acetic acid), while isolates 9710, 9715, and 979 are *T. denticola* strains (which produce acetic acid and a trace of formic acid).

Proposal of *T. denticola* (ex Brumpt) sp. nov., nom. rev. Research on *T. denticola* has been very active in recent years (see above), and thus this species occupies a place of importance in the field of periodontal microbiology. Therefore, it was very surprising that no type strain has been designated previously for this species and that its name was not included on the Approved Lists of Bacterial Names (24) or any subsequent validation list. Hence, the name *T. denticola* has no current nomenclatural standing. For this reason we propose that *Treponema denticola* (ex Brumpt) sp. nov., nom. rev. is a valid and distinct species of the genus *Treponema* and designate strain ATCC 35405 as the type strain and strains ATCC 33520 (9) and ATCC 35404 as reference strains. Some characteristics which differentiate *T. denticola* from other *Treponema* species that are cultivable and do not ferment carbohydrates are shown in Table 5. These characteristics are described in *Bergey's Manual of Systematic Bacteriology* (25). Two species that were isolated relatively recently from oral cavities, *Treponema pectinovorum* and *T. socranskii*, are not included in Table 5 because they were not included in *Bergey's Manual*. Furthermore, *T. pectinovorum* ferments pectin, polygalacturonic acid, galacturonic acid, and glucuronic acid, and *T. socranskii* ferments glucose. Figure 4 also shows that *T. denticola* produces a trace of formate and no propionate and that *T. vincentii* produces acetate plus propionate but no butyrate.

T. denticola is a small to intermediate spirochete (20). Even though a 2-4-2 endoflagellar arrangement is common, *T. denticola* strains with higher numbers of axial filaments have been found (3, 14). The additional description of the species given below is from *Bergey's Manual* (25). The organism moves with a jerky but fairly rapid motion. It grows well in peptone-yeast extract-serum medium under anaerobic conditions. The organism is primarily an amino acid fermenter and does not use the glycolytic pathway as a major source of energy. It grows at a pH range of 6.5 to 8.0 and a temperature range of 30 to 42°C. The species is found in the oral cavities of humans and chimpanzees, usually in deposits at the junctures between and teeth and gums. The G+C content of the DNA is 37-39 mol% (as determined by the thermal denaturation method).

The type strain, *T. denticola* ATCC 35405, was first described by Cheng et al. (4) and was called serovar a before it was deposited in the American Type Culture Collection

TABLE 5. Differential characteristics of the cultivable *Treponema* species that do not ferment carbohydrates^a

Species	Cell length (µm)	Wavelength (µm)	Amplitude (µm)	Amino acids fermented	Final pH of glucose broth	Hippurate hydrolysis	Pyruvate utilization	H ₂ S production	Produces acetate plus a trace of propionate	Produces acetate and butyrate	Produces only acetate	G+C content of DNA (mol%)	Requires thiamine pyrophosphate	Phosphatase
<i>T. denticola</i>	6-16	0.9	0.15	+	6.7	-	+	+	+	-	-	37-38	+	+
<i>T. vincentii</i>	5-16	1.3	0.2-0.3	+	6.9-7.2	ND ^b	-	+	-	+	-	ND	+	-
<i>T. scroloodontium</i>	6-16	ND	ND	+	6.9-7.1	-	-	-	ND	ND	ND	ND	+	ND
<i>T. refringens</i>	5-16	1.8	0.2-0.3	+	6.5-6.7	+	+	+	-	-	+	39-43	-	-
<i>T. minutum</i>	9-12	1.3	0.2	+	6.5-6.7	+	+	+	-	-	+	37	-	-

^a Data from reference 25.^b ND, no data available.

and acquired an accession number. It exhibits 76% DNA homology with *T. denticola* ATCC 33520 and 82% DNA homology with *T. denticola* ATCC 35404, both of which we selected as reference strains. *T. denticola* ATCC 35405^T is susceptible to the antimicrobial agents spiramycin, metronidazole, tetracycline, penicillin G, and streptomycin but is highly resistant to rifampin (the minimum inhibitory concentration is 50 µg/ml). Its cell dimensions are as follows: length, 7.74 ± 0.94 µm; diameter, 0.20 ± 0.02 µm; wavelength, 1.23 ± 0.15 µm; and amplitude, 0.50 ± 0.05 µm. It has 10 axial fibrils and therefore has a 5-10-5 endoflagellar arrangement.

As noted above, *T. denticola* is the oral spirochete species that is isolated most frequently from subgingival plaque. Despite our renewed concerted efforts to isolate new oral spirochete species, the majority of our new isolates were *T. denticola* strains. Unless new isolation and cultivation techniques are developed, it appears that present procedures can yield only isolates belonging to previously described species and that there is little chance of obtaining larger treponemes.

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