Treponema socranskii sp. nov., *Treponema socranskii* subsp. *socranskii* subsp. nov., *Treponema socranskii* subsp. *buccale* subsp. nov., and *Treponema socranskii* subsp. *paredis* subsp. nov. Isolated from the Human Periodontia

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A new species, *Treponema socranskii*, and three new subspecies, *T. socranskii* subsp. *socranskii*, *T. socranskii* subsp. *buccale*, and *T. socranskii* subsp. *paredis*, which were isolated from supragingival and subgingival samples from patients with periodontitis and from patients with experimental gingivitis, are described. These organisms are treponemes that ferment carbohydrates and require rumen fluid or short-chain volatile fatty acids for growth. Fermentable carbohydrates are required as an energy source. The major products of fermentation are acetic, lactic, and succinic acids. Trace amounts of formic acid are also produced. The level of deoxyribonucleic acid-deoxyribonucleic acid homology within each subspecies is greater than 80%, whereas the level of homology between subspecies is about 60%. The average guanine-plus-cytosine content of the deoxyribonucleic acid is 51 ± 1 mol%. The type strain of *T. socranskii* is strain ATCC 35536 (= VPI DR56BR1116); the type strain of *T. socranskii* subsp. *buccale* is strain ATCC 35534 (= VPI D2B8); and the type strain *T. socranskii* subsp. *paredis* is strain ATCC 35535 (= VPI D46CPE1).

Treponemes have long been observed in the oral cavities of humans. In the microbial flora in severe, moderate, and juvenile periodontitis and in experimental gingivitis, the predominant treponemes isolated ferment carbohydrates and require a medium supplemented with volatile fatty acids. These isolates are unlike *Treponema denticola* and *Treponema vincentii*, which do not produce acid pH values in media containing carbohydrates and require a serum-supplemented medium for growth. They are also unlike any other previously described species of the genus *Treponema*. One of these new isolates, *Treponema pectinovorum* (16), has been described recently. The purpose of this report is to describe the characteristics of another species of these treponemes and propose an appropriate name for this organism.

MATERIALS AND METHODS

Sites sampled. Treponemes were isolated from subgingival and residual supragingival samples from patients with moderate, severe, or juvenile periodontitis, from adult patients with experimental gingivitis (7, 16), and from patients with healthy gingivae. The disease categories and the sampling methods used have been described previously (10-12, 16).

Samples. Samples were taken with Morse scalers fitted with nickel-plated size 00 detachable tips (10-12, 16). The scaler tips containing samples were placed in tubes of prerduced chopped meat-saline diluent containing small glass beads (10-12, 16). The tubes were flushed with oxygen-free CO₂. Bacterial cells were dispersed by shaking the tubes on a Vortex mixer for 5 to 10 s. Serial 10-fold dilutions of each sample were made in 0.9-ml portions of gelatin-salts dilution medium (3) and cultured on duplicate plates of reduced isolation media and in prerduced selective treponeme isolation broth. The inoculated plates were immediately placed back into GasPak (BBL Microbiology Systems, Cockeysville, Md.) jars as described previously (11, 16).

Isolation media. Oral treponeme isolation (OTI) agar con-

taining rumen fluid, serum, fresh yeast autolysate, and thiamine pyrophosphate and the methods used for isolation of oral treponemes have been described previously (11, 16). An additional isolation method was employed, in which rifampin was used as a selective agent (6, 17). Rifampin (2 µg/ml) and polymyxin (800 U/ml) were added to OTI broth containing 0.16% agar. The rifampin-polymyxin isolation medium was inoculated with dilutions of the samples, and these preparations were incubated at 37°C for 2 weeks. The cultures were checked for growth of treponemes every 5 to 7 days by dark-field microscopy.

Colonies of oral treponemes were picked from OTI agar medium pour bottle plates as described previously (16) and inoculated into OTI broth. In addition, OTI medium containing 1.3% agar in petri dishes that had been prerduced by incubation for 2 days in a GasPak jar were streaked with cultures and immediately placed in GasPak jars that were flushed with CO₂. The jars were sealed, evacuated, and filled three times with a gas mixture containing 10% CO₂ and 90% hydrogen. The cultures were incubated at 37°C for 2 weeks. All cultures in OTI broth containing dimethyl sulfoxide were stored at -85°C or in liquid nitrogen.

Biochemical and cultural tests. The procedures and media used to characterize the strains have been described previously (3, 11, 16). Tests that produced questionable results were repeated. The oxygen-free gas mixture used was composed of 90% nitrogen and 10% carbon dioxide. A carbohydrate was considered fermented if the final pH of the culture was 6.0 or below.

Agglutination test. Antisera were prepared in rabbits injected with whole washed treponeme cells in Freund complete adjuvant. For the slide agglutination test, cells from a 4- to 5-day-old culture grown in 10 ml of OTI broth were sedimented, washed once in saline, and suspended in either 0.5 or 1 ml of saline. The slide agglutination test was performed by adding 2 drops of antigen and 1 to 2 drops of antiserum to the Diagluto slide system (Beckman Diagnostics, Fullerton, Calif.).

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TABLE 1. Isolation of *T. socranskii* subsp. *socranskii* (group A), *T. socranskii* subsp. *buccale* (group A), and *T. socranskii* subsp. *paredis* (group K)

Disease classification	No. of sites sampled	No. of sites positive for treponemes	% Of sites positive for treponemes	% Occurrence of <i>T. socranskii</i> (all three subspecies) ^a	% Occurrence of <i>T. socranskii</i> subsp. <i>paredis</i> ^b
Moderate, supragingival	22	18	82	80	2
Moderate, subgingival	34	30	88	89	9
Severe, supragingival	38	24	63	42	4
Severe, subgingival	41	39	95	65	3
Juvenile diseased sites, supragingival	25	18	72	65	2
Juvenile diseased sites, subgingival	30	24	80	74	0
Juvenile healthy sites, supragingival	7	2	28	50	0
Juvenile healthy sites, subgingival	7	3	52	33	0
Experimental gingivitis	96	34	35	91	12
Healthy, supragingival	17	2	12	100	0
Healthy, subgingival	7	2	12	100	0

^a Occurrence is the percentage of sites positive for treponemes that contain all three subspecies of *T. socranskii* (homology groups A1, A2, and K). *T. socranskii* subsp. *socranskii* and *T. socranskii* subsp. *buccale* cannot be differentiated at present by phenotypic tests.

^b Occurrence is the percentage of sites positive for treponemes that contain *T. socranskii* subsp. *paredis* (homology group K).

Metabolic acids. Peptone-yeast extract (PY)-glucose-rumen fluid-serum broth cultures were acidified with a few drops of 50% H₂SO₄. A 1-ml portion of an acidified culture was pipetted into a type CE-1001 Clin-Elute column (Analytichem International Inc., Harbor City, Calif.). The fatty acids were eluted from the column with three 3-ml portions of ethyl ether. The acids were extracted from the combined ether fractions by adding 1 ml of 0.2 N NaOH. The ether was discarded, and 20 μ l of the aqueous sample was loaded onto a high-performance liquid chromatograph (16) equipped with a type HPX-87H organic acid column (Bio-Rad Laboratories, Richmond, Calif.). The eluting solvent was 0.013 N H₂SO₄ containing 5% (final concentration) acetonitrile. The solvent was pumped through the column at a rate of 0.8 ml/min with a Waters Associates model M-45 pump. Fatty acids were detected at 214 nm by using a Bio-Rad Laboratories model 1305 variable-wavelength detector and a Hewlett-Packard model 3380A integrator-recorder.

DNA extraction and homology studies. For deoxyribonucleic acid (DNA) extraction, treponemal strains were grown in 2.5-liter amounts of broth prepared as described previously (1). This medium contained mineral salts, 1% (wt/vol) pepticase (BBL), 0.5% yeast extract (Difco Laboratories, Detroit, Mich.), 1% brain heart infusion broth (Difco), 1% glucose, 0.05% cysteine, 0.01% heme, 30% (vol/vol) rumen fluid, and 0.05 M potassium phosphate buffer (pH 7.0). At the time of inoculation, 20 ml of sterile 10% NaHCO₃ and about 100 ml of inoculum were added to each flask. The cultures were incubated at 37°C with gentle shaking until maximum growth occurred (usually about 7 days). For strains that did not grow well, the medium was supplemented with about 250 ml of sterile inactivated rabbit serum.

High-molecular-weight DNA was isolated by the method of Marmur (4, 8). The preparations were stored at -20°C in 0.1 $\times$ SSC-1 mM HEPES (pH 7.0) (SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0; HEPES is sodium *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonate). Thermal melting points were used to determine the guanine-plus-cytosine (G+C) values of the DNA preparations (4, 9). For use in the homology experiments, the DNA preparations were fragmented by passage three times through a French pressure cell at 16,000 lb/in². The DNA preparations were

then denatured by heating in a boiling water bath for 5 min. After cooling in an ice water bath, the DNA preparations were centrifuged at 12,000 $\times$ *g* for 15 min to remove particulate debris. The concentration of each preparation was then adjusted to 0.4 mg/ml, and the preparations were stored at -20°C until they were used either for labeling or as unlabeled DNA in the homology experiments. Labeled DNA was prepared by iodination (14). The specific activities of the labeled preparations ranged from 2 $\times$ 10⁶ to 4 $\times$ 10⁶ cpm/ μ g. DNA homology values were determined by using the S1 nuclease procedure (4). The reassociation vials each contained 10 μ l of labeled DNA (0.01 to 0.03 μ g), 50 μ l of unlabeled DNA (20 μ g), 25 μ l of 5.28 M NaCl-1 mM HEPES (pH 7.0), and 25 μ l of dionized formamide. This produced a sodium ion concentration equivalent to 6 $\times$ SSC and a formamide concentration of 23%. The vials were incubated for 24 h, at 57°C, which is 25°C below the melting temperature of the native DNA in this buffer system and resulted in a *C*₀*t* value of 47 mol $\cdot$ s/liter.

Source of strains. A total of 31 strains of treponemes were used in this study; these organisms were isolated from subgingival and residual supragingival samples taken from either the mesial or distal tooth surfaces of patients with moderate, severe, or juvenile periodontitis and from patients with experimental gingivitis.

RESULTS AND DISCUSSION

A total of 20 patients with moderate periodontal disease, 20 patients with juvenile periodontal disease, 21 patients with severe periodontal disease, 4 adults with experimental gingivitis, and 12 subjects with healthy gingivae were studied. From these 77 patients, 1,359 treponeme isolates were cultured from 332 sites. Approximately 834 of the treponeme isolates from these sites were divided on the basis of similarity of phenotypic patterns into two distinct groups (groups A and K). These groups represented the most frequently isolated organisms. The percentages of sites positive for treponemes by either culture or dark-field microscopic examination and the occurrence of group A and K treponemes in each of the sites that contained treponemes are shown in Table 1.

DNA homology. The G+C values and DNA homology

TABLE 2. DNA homology of *T. socranskii* strains

Strain	G+C content (mol %)	% Homology with labeled DNA from:					
		<i>T. socranskii</i> subsp. <i>socranskii</i> DR56BR116 ^T	<i>T. socranskii</i> subsp. <i>buccale</i> strains			<i>T. socranskii</i> subsp. <i>paredis</i> strains	
			D2B8 ^{Ta}	D11A1	D40DPE1	D46CPE1 ^T	E2M1
Group A1		(85 ± 5.2) ^b	(61 ± 3)	(66 ± 3)	(56 ± 2)	(57 ± 4)	(56 ± 3)
DR56BR116 ^T	52	100	60	66	58	60	56
D43BR1		94	63	64	56	60	59
D20B3		92	61	64	56	53	54
D37B2		87	60	66	55	54	56
D20A2		86	67	69	58	53	56
D30B3		85	57	65	53	53	51
D55AR1		84	64	70	60	68	62
D25D1		83	61	64	54	56	55
D43AR5		83	57	63	55	60	53
D43DR3		82	62	67	55	55	52
DH56CR1	51	82	57	66	52	56	55
D39BR1		74	68	74	59	56	59
Group A2		(59 ± 10)	(86 ± 3)	(81 ± 3)	(82 ± 4)	(65 ± 6)	(67 ± 3)
D2B8 ^T		50	100	80	75	53	65
D11A1		66	86	100	82	67	72
D40DPE1	50	60	84	80	100	63	68
D34B4		47	90	79	81	58	66
D22A5	50	62	89	80	87	65	66
D40DPE2		50	86	81	91	68	64
D4A4	50	61	85	79	80	66	64
D7B4		48	83	78	79	68	64
D28D2V		64	82	84	81	66	70
D36FEP4		83	91	88	81	77	68
Group K		(49 ± 4)	(63 ± 1)	(63 ± 3)	(59 ± 3)	(82 ± 5)	(83 ± 4)
D46CPE1 ^T	51	41	63	57	55	100	88
D28C3	50	53	63	63	61	87	80
D43AR1-3		54	65	67	60	84	85
D16A1		46	64	65	57	76	79
E2M1		53	64	62	62	77	100
E2F5	50	49	63			91	47
D71CR4	50	52	64			82	45
D51BR5	51	47	62			76	35
E2αI-1		52	66				

^a T = type strain.^b The values in parentheses are means ± standard deviations for groups.

results for 31 representative strains are shown in Table 2. An average value of 51 ± 1 mol% G+C was obtained for the organisms tested. There was substantial DNA homology among all of the organisms tested, although subgroups were readily differentiated. Strains of treponeme phenotypic group A formed two clusters (groups A1 and A2) that had an average intragroup homology value of more than 80% and an intergroup homology value of about 60%. A third cluster with similar relationships to groups A1 and A2 was formed with strains belonging to treponeme phenotypic group K.

Labeled DNA from homology cluster A1 strain DR56BR6 had very low levels of homology with *T. denticola* strain FM (3%), *T. vincentii* strain N-9 (10%), and *Treponema phagedenis* strain Reiter (3%). This was expected because of the large difference in G+C contents between these organisms (36 to 44 mol%) and *Treponema socranskii* (51 mol%).

Because of the high level of intergroup DNA homology among strains of phenotypic groups A and K, we consider these organisms to be members of a single species and regard each of the homology groups as a subspecies.

Genus. The isolates which we studied belong to the genus *Treponema* because they are anaerobic host-associated spirochetes that have between 1 to 10 periplasmic flagella. For these strains we propose the new species and subspecies described below.

***Treponema socranskii* sp. nov.** *Treponema socranskii* (so.cran.ski.i. N.L. gen. n. *socranskii* named for Sigmund S. Socransky, Forsyth Dental Center) (homology groups A1, A2, and K) is an obligately anaerobic motile helically coiled treponeme (Fig. 1). Electron photomicrographs of cultures in the logarithmic phase of growth show that there is one periplasmic flagellum originating from each end of the cell. The periplasmic flagella overlap in the center region of the cell, giving a "1-2-1" relationship.

The cells are 6 to 15 µm long and 0.16 to 0.18 µm wide. They have tapered ends with a slight bend or "hook" at one or both ends of the cell. The cells form coccoid bodies in the late stationary growth phase. Cells in broth cultures have both rotational and translational movement. Serpentine movement of cells can be seen by dark-field microscopy of cultures grown in a semisolid medium.

Colonies. Colonies usually appear in OTI agar in 7 to 10 days. The colonies grow into the agar, are white and translucent, and often have slightly denser centers. The edges of the colonies may be entire or irregular. The colonies spread and become larger after extended incubation.

Cultural and growth characteristics. *T. socranskii* grows only in media containing a fermentable carbohydrate and either rumen fluid (20 to 30%) or a mixture of short-chain fatty acids (3, 16). Serum is not required. The optimum

FIG. 1. Dark-field photomicrographs of wet-mount preparations of *T. socranskii* subsp. *socranskii* (a), *T. socranskii* subsp. *buccale* (b), and *T. socranskii* subsp. *paredis* (c). Bar = 10 μ m.

growth temperature is 37°C. There is only slight growth at 25 or 42°C. Broth cultures become turbid with a slightly granular sediment in 3 to 4 days.

Biochemical reactions and fermentation products. The phenotypic characteristics of representative strains of *T. socranskii* are shown in Table 3. The majority of the strains ferment glucose, with a final pH ranging from 5.1 to 5.9. A few strains belonging to *T. socranskii* subsp. *buccale* do not produce a pH below 6.0 when they are grown in glucose-containing broth. These cultures have a pH range of 6.2 to 6.7.

Inulin, lactose, melizitose, melibiose, cellobiose, salicin, D-sorbitol, glycerol, amygdalin, adonitol, dulcitol, i-erythritol, inositol, and D-mannitol are not fermented, as indicated by only a slight change in the pH of the medium. Hydrogen sulfide is produced in SIM medium (BBL) supplemented with rumen fluid by all but 1 of 32 strains tested.

None of the strains studied produces catalase, peroxidase, indole, or acetylmethylcarbinol. Esculin is not hydrolyzed.

Hydrogen gas is not detected by gas chromatography of samples taken from the atmosphere above the broth medium in rubber-stoppered tubes.

The major acid fermentation products of all strains grown in PY-glucose-rumen fluid broth are acetic, lactic, and succinic acids. Trace amounts of formic acid occasionally can be found. The strains produce an average of 8 mM lactic acid (range, 3 to 16 mM), 6.5 mM acetic acid (range, 2.6 to 10.4 mM), and 3 mM succinic acid (range, 1 to 6.5 mM).

Habitat. *T. socranskii* is associated with human gingival crevices and gingival sulci of patients with periodontal disease and experimental gingivitis and in people with healthy gingivae. It is the most frequently isolated treponeme and is usually the most numerous organism among the cultivable treponemes in either supragingival or subgingival samples. *T. socranskii* subsp. *socranskii* and *T. socranskii* subsp. *buccale* are more common in these sites than *T. socranskii* subsp. *paredis*.

G+C content. The G+C content of the DNA is 50.5 ± 2

TABLE 3. Biochemical reactions of selected strains of subspecies of *T. socranskii*

Characteristic	<i>T. socranskii</i> subsp. <i>socranskii</i>		<i>T. socranskii</i> subsp. <i>buccale</i>		<i>T. socranskii</i> subsp. <i>paredis</i>	
	Strain ATCC 35536 ^T (= VPI DR56BRII16 ^T)	11 Other strains	Strain ATCC 35534 ^T (= VPI D2B8 ^T)	9 Other strains	Strain ATCC 35535 ^T (= VPI D46CPE1 ^T)	9 Other strains
H ₂ S	+	91 ^a	+	100	+	100
Gelatin hydrolysis	—	36	—	11	+	44
Acid from:						
Arabinose	+	100	+	100	—	0
Dextrin	+	91	+	78	+	78
Fructose	+	100	+	100	+	100
Galactose	+	100	—	67	+	78
Glucose	+	100	+	78	+	100
Glycogen	+	100	+	78	+	100
Maltose	+	100	+	78	+	89
Mannose	+	82	—	56	+	100
Mucin	—	18	—	56	—	22
Pectin	+	45	—	44	+	44
Raffinose	+	55	—	33	—	44
Rhamnose	+	100	+	67	—	0
Ribose	+	100	+	89	+	100
Starch	+	100	+	89	+	100
Sucrose	+	100	+	89	+	100
Trehalose	+	91	+	89	—	22
Xylose	+	100	+	100	—	44

^a Percentage of strains positive.

mol% (thermal denaturation method) for selected strains and 52 mol% (thermal denaturation method) for the type strain.

Type strain. The type strain is strain ATCC 35536 (= VPI DR56BRIII6), which was isolated from a subgingival sample from a patient with severe periodontal disease.

Subspecies. Treponemes in homology group A1 are designated *Treponema socranskii* subsp. *socranskii* subsp. nov. (so.cran'ski.i. N.L. gen. n. *socranskii* named for S. S. Socransky).

The phenotypic characteristics of *T. socranskii* subsp. *socranskii* type strain ATCC 35536 (= VPI DR56BRIII6) are shown in Table 3; other characteristics are the same as those of the species.

Treponemes in homology group A2 are designated *Treponema socranskii* subsp. *buccale* subsp. nov. (buc.ca'le. L. N. *bucca* cheek; N.L. neu. adj. *buccale* pertaining to a cheek). Phenotypic characteristics of this subspecies are shown in Table 3. Other characteristics of the subspecies are the same as those of the species.

The type strain of *T. socranskii* subsp. *buccale* is strain ATCC 35534 (= VPI D2B8), which was isolated from a subgingival sample from a patient with moderate periodontal disease.

The patterns of phenotypic reactions in Table 3 show that *T. socranskii* subsp. *socranskii* (homology group A1) cannot be readily differentiated from *T. socranskii* subsp. *buccale* (homology group A2). However, when we used a slide agglutination test with washed cells as the antigen, 11 of 12 *T. socranskii* subsp. *socranskii* strains agglutinated antisera against the type strain (strain ATCC 35536) and strain VPI D43BR1. These antisera did not agglutinate any strain of either *T. socranskii* subsp. *buccale* or *T. socranskii* subsp. *paredis*. Of 10 *T. socranskii* subsp. *buccale* strains, 7 agglutinated antisera against the type strain (strain ATCC 35534) and strains VPI D11A1 and VPI D40DPE1 of *T. socranskii* subsp. *buccale*. These antisera did not agglutinate any strain of either *T. socranskii* subsp. *socranskii* or *T. socranskii* subsp. *buccale*. Antiserum against the type strain of *T. socranskii* subsp. *paredis* (strain ATCC 35535) was agglutinated by seven of nine *T. socranskii* subsp. *paredis* strains but not by any strain of either *T. socranskii* subsp. *socranskii* or *T. socranskii* subsp. *buccale*.

Treponemes in homology group K are designated *Treponema socranskii* subsp. *paredis* subsp. nov. (pa.re'dis. Gr. n. *pares* cheek; N.L. gen. n. *paredis* of a cheek). Phenotypic characteristics of this subspecies are shown in Table 3. Arabinose and rhamnose are not fermented. Other characteristics of this subspecies are the same as those of the species.

The type strain of *T. socranskii* subsp. *paredis* is strain ATCC 35535 (= VPI D46CPE1), which was isolated from a supragingival sample from a patient with severe periodontal disease.

T. socranskii subsp. *paredis* can be easily separated from *T. socranskii* subsp. *socranskii* and *T. socranskii* subsp. *buccale* by the inability of *T. socranskii* subsp. *paredis* to ferment L-arabinose and rhamnose (Table 3).

Comparison with other treponeme species. *T. socranskii* does not conform to the description of any previously described species. *T. denticola*, *Treponema refringens*, *Treponema minutum*, and *T. phagedenis* all require serum and long-chain fatty acids for growth and have G+C contents ranging from 36 to 39 mol% (15). *T. vincentii* also requires serum and has a G+C content of 44 mol%. *Treponema hyodysenteriae* and *Treponema innocens* have G+C contents of 25 mol% (5). Three species (*Treponema bryantii*

[18], *Treponema succinifaciens* [2], and *T. pectinovorum* [16] that require short-chain fatty acids for growth have G+C contents of 36 to 39 mol%. In contrast, *T. socranskii* has G+C contents ranging from 50 to 52 mol%, which eliminates any similarity to the above-mentioned species. Mink (R. W. Mink, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, C160, p. 263) described a treponeme isolated from a patient with periodontal disease which had one periplasmic flagellum, fermented glucose and fructose but not mannitol and sucrose, and did not produce indole. The acid end products were acetic, lactic, and succinic acids. This organism had a G+C content of 48 to 49 mol% and might be *T. socranskii*; however, not enough characteristics were given, and it also grew in a medium containing serum. *T. socranskii* requires short-chain fatty acids and does not grow in a medium containing only serum. Treponemes have been reported that have G+C contents of 41 to 46 mol%. A large rumen organism that ferments pectin but not glucose has been described (13, 19, 20). However, one strain, designated strain DA, was reported to have 32 or more periplasmic flagella and cells measuring 0.6 by 15 μ m (13). Other strains isolated from bovine rumina (13, 21) and the oral organism *T. pectinovorum* (16) require pectin for growth, whereas *T. socranskii* does not. The oral isolates described by Weber and Canale-Parola (F. H. Weber and E. Canale-Parola, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, I113, p. 158) require pectin for growth and have G+C contents of 39 mol%.

In previous publications on the bacteriology of experimental gingivitis and severe and moderate periodontal disease (10–12), the treponemes designated groups A and A1 are *T. socranskii* (either *T. socranskii* subsp. *socranskii* or *T. socranskii* subsp. *buccale*), whereas the organisms designated treponeme group K are *T. socranskii* subsp. *paredis*. Organisms designated treponeme group J are *T. pectinovorum* (16).

Three strains identified as treponeme group D also were studied. They had DNA G+C contents of 50 to 51 mol% (thermal denaturation method) and showed 51 to 54% homology to clusters A1, A2, and K. One group D strain had about 25% homology to another group D strain, indicating that there may be two or more additional subspecies of *T. socranskii*. Strains of treponeme group D are H₂S negative and ferment arabinose, fructose, galactose, glucose, mannose, ribose, sucrose, trehalose, xylose, and rhamnose. Some strains ferment raffinose. Dextrin, glycogen, maltose, starch, trehalose, pectin, and mucin are not fermented.

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