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Transfer of *Peptococcus indolicus, Peptococcus asaccharolyticus, Peptococcus prevotii*, and *Peptococcus magnus* to the Genus *Peptostreptococcus* and Proposal of *Peptostreptococcus tetradius* sp. nov.

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The guanine-plus-cytosine (G+C) contents of the deoxyribonucleic acids (DNAs) of *Peptococcus asaccharolyticus* ATCC 14963^T (T = type strain), *Peptococcus indolicus* ATCC 29427^T, *Peptococcus prevotii* ATCC 9321^T, and *Peptococcus magnus* ATCC 15794^T ranged from 29 to 34 mol%, whereas the G+C content of the DNA of *Peptococcus niger* ATCC 27731^T, the type species of the genus *Peptococcus*, is 51 mol%. The G+C content of the DNA of *Peptostreptococcus anaerobius* ATCC 27337^T, the type species of the genus *Peptostreptococcus*, was 33 mol%. Thus, the DNA base compositions of *Peptococcus asaccharolyticus, Peptococcus indolicus, Peptococcus prevotii*, and *Peptococcus magnus* resemble the DNA base composition of the type species of the genus *Peptostreptococcus* rather than the DNA base composition of the type species of the genus *Peptococcus*. It is not desirable for the genus *Peptococcus* to include any species whose G+C content is far from the G+C content of the type species of the genus. The levels of DNA-DNA homology between *Peptostreptococcus anaerobius* ATCC 27337^T and the four species of *Peptococcus* with low G+C DNA contents ranged from 23 to 36%. The cellular fatty acid profiles of *Peptococcus asaccharolyticus, Peptococcus indolicus, Peptococcus prevotii*, and *Peptococcus magnus* also resembled the cellular fatty acid profile of the type species of *Peptostreptococcus*. Other biochemical characteristics of these species revealed their close resemblance to *Peptostreptococcus anaerobius*. For these reasons we propose transfer of the four *Peptococcus* species that have low G+C contents to the genus *Peptostreptococcus* as *Peptostreptococcus asaccharolyticus* (Distaso 1912) comb. nov., *Peptostreptococcus indolicus* (Christiansen 1934) comb. nov., *Peptostreptococcus prevotii* (Foubert and Douglas 1948) comb. nov., and *Peptostreptococcus mangus* (Prévot 1933) Smith 1957 comb. rev. A group of organisms previously referred to as "*Gaffkya anaerobia*" (Choukévitch) Prévot is an identifiable *Peptostreptococcus* species based on phenotypic and genotypic characteristics. The name of *Peptostreptococcus tetradius* sp. nov. is proposed for this group of organisms. Strain GIFU 7672 (= ATCC 35098) is designated the type strain. Clinical strains of *P. asaccharolyticus* that were identified by conventional methods were divided into two homology groups and one unclassified group (A-1). DNA-DNA homology between the type strain of *P. prevotii* and clinical strains which had been received as *P. prevotii* ranged from 0 to 73%.

When this work was initiated, the genus *Peptococcus* Kluyver and van Niel 1936 was composed of seven species, including the type species, *Peptococcus niger*. The genus *Peptostreptococcus* Kluyver and van Niel 1936 contained four species, with *Peptostreptococcus anaerobius* as the type species. *Peptococcus* and *Peptostreptococcus*, together with *Coprococcus, Ruminococcus*, and *Sarcina*, are members of the family *Peptococcaceae*. These genera have been separated from each other by cellular arrangement, metabolic end products, and utilization of peptides and carbohydrates (15, 31, 32). However, reliable characteristics for differentiating *Peptococcus* and *Peptostreptococcus* have not been proposed. We recently reported that the guanine-plus-cytosine (G+C) contents of the deoxyribonucleic acids (DNAs) of the type strains of *Peptococcus asaccharolyticus, Peptococcus indolicus, Peptococcus pre-*

votii, *Peptococcus magnus*, and *Staphylococcus saccharolyticus* (*Peptococcus saccharolyticus*) are very different from the G+C content of *Peptococcus niger* DNA, whereas these values are similar to the value for *Peptostreptococcus anaerobius* DNA (14). The G+C contents of strains with characteristics similar to those described for "*Gaffkya anaerobia*" also are similar to the G+C content of *Peptostreptococcus anaerobius*.

This study of the genera *Peptococcus* and *Peptostreptococcus* was based on extensive biochemical characterization of 262 strains of gram-positive anaerobic cocci, chiefly of clinical origin, and the G+C contents and levels of DNA-DNA homology among selected strains. The taxonomic position of "*G. anaerobia*" also was determined.

MATERIALS AND METHODS

Bacterial strains. From about 300 strains of anaerobic gram-positive cocci isolated mostly from human clinical specimens, 65 were selected for this study (Table 1). These 65 strains, which were stored in 10% skim milk at −90°C, were transferred into Gifu anaerobic medium (GAM; Nissui, Tokyo, Japan) broth and then streaked onto GAM plates for discrete colony formation. Cultures were incubated anaerobically for 48 h at 37°C. Working cultures were maintained in GAM broth.

Bacterial identification. All media were prepared anaerobically, kept for 48 h in an anaerobic chamber (85% nitrogen, 5% hydrogen, 10% carbon dioxide) before use, and inoculated under carbon dioxide gas. Carbohydrate fermentation tests and other biochemical tests were performed by the methods described in the *Anaerobe Laboratory Manual*, 4th ed. (14).

For volatile and nonvolatile fatty acid analyses, each strain was grown both in prereduced peptone-yeast extract medium supplemented with 1% glucose (PYG medium) and in GAM medium supplemented with 1% glucose for 1 week at 37°C. For gas-liquid chromatographic analyses, cultures were processed according to the methods described in the *Anaerobe Laboratory VPI Manual*, 4th ed. (14). A Shimadzu model GC-6A gas chromatograph equipped with a flame ionization detector was used. Peptone-yeast extract broth was the basal medium used for the biochemical tests.

Ammonia production from amino acids. A filter-sterilized 10% (wt/vol) aqueous amino acid solution was added to peptone-yeast extract broth to make a final concentration of 0.7%. After anaerobic incubation for 1 week, 2 drops of the culture and 4 drops of Nessler solution were placed onto a spot plate. Peptone-yeast extract broth cultures were tested simultaneously as controls. A stronger orange color with the amino acid culture than with the peptone-yeast extract broth culture was considered a positive reaction.

API ZYM tests. Bacterial strains cultured in 20 ml of prereduced GAM broth for 2 days at 37°C were centrifuged, and the sedimented cells were washed once with saline. After recentrifugation, a saline suspension of each strain was prepared to equal the turbidity of a MacFarland no. 5 standard. Enzyme activities were determined after 4 h of incubation at 37°C. Activity scores of 3 to 5 were considered positive, and a score of 2 was considered weakly positive.

Analysis of cellular fatty acids. The cells of 23 strains (see Table 4) grown anaerobically in GAM broth for 24 to 36 h were sedimented by centrifugation and washed twice with distilled water. After whole-cell saponification, the cellular fatty acids were methylated and extracted by the procedure of Moss and Dees (29). The fatty acid methyl esters were analyzed with a gas chromatograph equipped with a fused silica capillary column (25 m by 0.2 mm [inner diameter]) coated with 3% OV-101 (Shimadzu, Kyoto, Japan). The temperature of the column was programmed to increase from 150 to 250°C at a rate of 5°C/min after sample injection. The fatty acid methyl esters were identified by comparing their retention times with those of fatty acid methyl ester standards. Identification was further confirmed by analysis on a polar column (Diacot EW; FFAP; 20 m by 0.28 cm [inner diameter]; Japan Chromatography, Tokyo, Japan) and by gas-liquid chromatography–mass spectrometry (29).

DNA isolation. Each of the 65 selected strains (see Table 2) was incubated anaerobically in 1.8 liter of GAM broth at 37°C for 36 h. The bacterial cells were sedimented by centrifugation and then suspended in a solution containing 0.15 M NaCl and 0.01 M ethylenediaminetetraacetic acid (pH 8.0). Achromopeptidase (Wako Chemical, Osaka, Japan) and lysozyme (Sigma Chemical Co., St. Louis, Mo.) were each added to a final concentration of 2,000 U/ml. The suspension was kept at 35°C until the solution became clear. The organisms resistant to the lytic activity of achromopeptidase and lysozyme were disrupted by shaking with beads (B. Braun, Melsungen, West Germany). After the cells were lysed, sodium dodecyl sulfate (final concentration, 1%) was added, and the solution was held at 60°C for 10 min, after which pronase E (Kaken Chemical, Tokyo, Japan) was added to the solution to a final concentration of 100 μg/ml. The viscosity of the lysate was reduced by sonication (Tomy Seiko, Tokyo, Japan) at a 70% energy setting. Ribonuclease I from bovine pancreas (Miles Laboratories Inc., Elkhart, Ind.) was added to the lysate to a final concentration of a 100 μg/ml, and then the lysate was incubated for 1 h at 35°C. After preliminary extraction with chromatography-grade phenol in 0.15 M NaCl–0.01 M ethylenediaminetetraacetic acid (pH 8.0), the DNA was extracted by the method of Marmur (28).

Determination of the G+C content of the DNA. The thermal melting point of DNA was measured with an automatic recording spectrophotometer (Japan Spectroscopic, Tokyo, Japan) equipped with four sampling chambers and a cell programmer, as described previously (9). Reference DNA from *Escherichia coli* strain K-12 was used for each experimental run. The G+C value was calculated by the equation of Mandel et al. (27). To maintain comparable conditions, the DNAs of strain K-12 and 72 strains of anaerobic cocci were dialyzed in the same 0.5 M saline-trisodium citrate buffer (pH 7.0) before each experimental run.

DNA-DNA homology experiments. The DNA-DNA homology procedures used have been described previously (9). DNA was labeled with [^{3}H]thymidine triphosphate by a nick-translation method (20). After labeling, the reaction mixture was passed through a

column (1 by 7.5 cm) of 100- to 200-mesh Bio-Gel P100 polyacrylamide (Bio-Rad Laboratories, Richmond, Calif.), using 0.1× saline-trisodium citrate buffer (pH 7.0) containing 0.1% sodium dodecyl sulfate. The specific activity of the labeled DNA preparation was about 3×10^5 to 2×10^6 cpm/μg. Membrane homology experiments were performed by the procedure of Gillespie and Spiegelman (12), with minor modifications (9). Reassociation reactions were run in 2× saline-trisodium citrate buffer (pH 7.0) at 55°C for 24 h.

RESULTS AND DISCUSSION

Evidence for the transfer of four species of *Peptococcus* to the genus *Peptostreptococcus*. (i) G+C contents of DNAs and DNA-DNA homology values. The G+C contents of DNAs from the type strains of *Peptococcus asaccharolyticus, Peptococcus indolicus, Peptococcus prevotii*, and *Peptococcus magnus* ranged from 32 to 34 mol%, values close to the value of 33 mol% obtained for the DNA of the type strain of *Peptostreptococcus anaerobius* and far from the value of 50 to 51 mol% obtained for the DNA of the type strain of *Peptococcus niger* (38) (Table 2). The levels of DNA-DNA homology between the type strain of *Peptostreptococcus anaerobius* and the type strains of *Peptococcus asaccharolyticus, Peptococcus indolicus, Peptococcus prevotii*, and *Peptococcus magnus* ranged from 23 to 36%, whereas negligible homology (0 to 6%) was observed between these strains and the type strain of *Peptococcus niger*. These values indicate that *Peptococcus asaccharolyticus, Peptococcus indolicus, Peptococcus prevotii*, and *Peptococcus magnus* are more closely related to *Peptostreptococcus anaerobius*, the type species of the genus *Peptostreptococcus*, than to *Peptococcus niger*, the type species of the genus *Peptococcus*. Therefore, we propose that these organisms be transferred to the genus *Peptostreptococcus* as *Peptostreptococcus asaccharolyticus* (Distaso) comb. nov., *Peptostreptococcus indolicus* (Christiansen) comb. nov., *Peptostreptococcus prevotii* (Foubert and Douglas) comb. nov., and *Peptostreptococcus magnus* (Prévot) Smith 1957 comb. rev.

(ii) Morphological and biochemical characters. Present descriptions of the family *Peptococcaceae* do not allow separation of the genus *Peptococcus* from the genus *Peptostreptococcus*. Chain formation has been cited as a major factor to discriminate between these two genera (15, 32), but this is not always a reliable character. In strains of *Peptostreptococcus anaerobius*, chains usually are present in stained preparations of cultures grown in broth medium, but not in stained preparations made from growth on solid medium. Moreover, strains of species of *Peptococcus* occasionally form chains. Catalase production, which is useful in differentiating staphylococci from streptococci, cannot be used to differentiate peptococci from peptostreptococci because catalase production varies among species, or even among strains of a species, in these genera.

Peptococcus niger does not ferment carbohydrates. Strains of *Peptostreptococcus anaerobius* are weakly saccharolytic, as are strains of *Peptococcus prevotii* and some strains of *Peptococcus magnus* (Table 3). However, carbohydrates are not fermented by strains of *Peptococcus indolicus* and by most strains of *Peptococcus asaccharolyticus*. Even though we have found no usual phenotypic test or morphological characteristic that can differentiate between *Peptococcus* and *Peptostreptococcus*, the validity of separate genera is established by the differences in the G+C contents of the DNAs of the type species of the two genera, and the proposed transfer of the four species to *Peptostreptococcus* is supported by the G+C contents of the species and our DNA-DNA homology results.

(iii) Cellular fatty acid profiles. The cellular fatty acid profiles of GAM cultures of the butyric acid-producing species of *Peptococcus* resembled the profile of the type strain of *Peptostreptococcus anaerobius* (Table 4). The profiles of these cocci incubated in PYG broth were different from the profiles of cultures incubated in GAM broth (data not shown). Differences in medium composition also may account for slight differences between our results and previous results (25). C18:1 was a major cellular fatty acid in cultures in both PYG and GAM. C18:1 aldehyde was detected in most cultures. Therefore, the cellular fatty acid profiles support the inclusion of *Peptococcus asaccharolyticus, Peptococcus indolicus, Peptococcus prevotii*, and *Peptococcus magnus* in the genus *Peptostreptococcus*.

Emended description of the genus *Peptostreptococcus*. (i) History and discussion. Kluyver and van Niel (24) first named the genera *Peptococcus* and *Peptostreptococcus* and assigned them to the families *Micrococcaceae* and *Streptococcaceae*, respectively. Rogosa placed these genera in a new family, the *Peptococcaceae* (32).

Of the seven species of *Peptococcus* on the Approved Lists of Bacterial Names (36), only *Peptococcus niger* will remain after transfer of *Peptococcus asaccharolyticus, Peptococcus indolicus, Peptococcus magnus*, and *Peptococcus prevotii* to the genus *Peptostreptococcus*. On the basis of nucleic acid studies (7, 21, 22), cell wall analyses (35), and biochemical studies (35), Kilpper-Bälz and Schleifer (23) proposed the transfer of *Peptococcus saccharolyticus* to the genus *Staphylococcus*. We found that the cellular fatty acids of *Staphylococcus saccharolyticus* were different from the cellular fatty acids of

TABLE 1. Origins of the 65 selected strains

Species or group	GIFU strain no.	Received as:	Other strain no.	Source of isolation	Received from:[a]
Peptostreptococcus anaerobius	7882	*Peptostreptococcus anaerobius*	ATCC 27337^T	Not recorded	7
Peptostreptococcus asaccharolyticus	7656	*Peptococcus asaccharolyticus*	ATCC 14963^T	Not recorded	7
	3302	*Peptococcus asaccharolyticus*		Skin abscess	3
	7877	*Peptococcus asaccharolyticus*		Vaginal discharge	2
	3306	*Peptococcus asaccharolyticus*		Vaginal discharge	4
	6287	*Peptococcus asaccharolyticus*		Vaginal discharge	4
	3315	*Peptococcus asaccharolyticus*		Vaginal discharge	3
	3290	*Peptococcus asaccharolyticus*		Peritoneal abscess	3
	7951	*Peptococcus asaccharolyticus*		Vaginal discharge	4
	6663	*Peptococcus asaccharolyticus*		Otorrhea	3
	6291	*Peptococcus asaccharolyticus*		Skin abscess	4
	7871	*Peptococcus asaccharolyticus*		Vaginal discharge	2
	7693	*Peptococcus asaccharolyticus*		Vaginal discharge	3
	7681	*Peptococcus asaccharolyticus*		Vaginal discharge	3
	7939	*Peptococcus asaccharolyticus*		Vaginal discharge	4
	7955	*Peptococcus asaccharolyticus*		Vaginal discharge	2
Unclassified group A-1	1244	*Peptococcus asaccharolyticus*		Feces	4
	7662	*Peptococcus asaccharolyticus*		Feces	4
	7872	*Peptococcus asaccharolyticus*		Vaginal discharge	5
Peptostreptococcus indolicus	7848	*Peptococcus indolicus*	ATCC 29427^T	Summer mastitis secretion of heifer	7
	8287	*Peptococcus indolicus*	172 PcR8	Not recorded	6
	8288	*Peptococcus indolicus*	196 PcR3	Not recorded	6
	8296	*Peptococcus indolicus*		Rat feces	4
Peptostreptococcus prevotii	7658	*Peptococcus prevotii*	ATCC 9321^T	Plasma	7
	7789	*Peptococcus prevotii*		Vaginal discharge	4
	7732	*Peptococcus prevotii*		Skin abscess	3
	7794	*Peptococcus prevotii*		Vaginal discharge	4
	7678	*Peptococcus prevotii*		Discharge from peritonitis	3
	7695	*Peptococcus prevotii*		Vaginal discharge	3
	7954	*Peptococcus prevotii*		Ovarial abscess	2
Peptostreptococcus tetradius	7672^T	*"Gaffkya anaerobia"*		Vaginal discharge	4
	3282	*"Gaffkya anaerobia"*		Otorrhea	3
	3298	*"Gaffkya anaerobia"*		Vaginal discharge	2
	3270	*"Gaffkya anaerobia"*		Vaginal discharge	3
	7617	*"Gaffkya anaerobia"*		Vaginal discharge	4
Unclassified group P-1	7668	*Peptococcus prevotii*		Lacrimal sac	1
	7878	*Peptococcus prevotii*		Discharge from endometritis	3

TABLE 1—*Continued*

Species or group	GIFU strain no.	Received as:	Other strain no.	Source of isolation	Received from:[a]
Peptostreptococcus magnus	7629	*Peptococcus magnus*	ATCC 15794^T	Not recorded	7
	7726	*Peptococcus magnus*		Vaginal discharge	3
	7795	*Peptococcus magnus*		Vaginal discharge	2
	7943	*Peptococcus magnus*		Vaginal discharge	2
	7711	*Peptococcus magnus*		Discharge from endometritis	3
	7724	*Peptococcus magnus*		Otorrhea	3
	7624	*Peptococcus magnus*		Vaginal discharge	3
	7785	*Peptococcus magnus*		Vaginal discharge	4
	7655	*Peptococcus magnus*		Vaginal discharge	3
	7651	*Peptococcus magnus*		Skin abscess	3
	7735	*Peptococcus magnus*		Skin abscess	5
	7792	*Peptococcus magnus*		Vaginal discharge	4
	7661	*Peptococcus magnus*		Lung abscess	4
	7723	*Peptococcus magnus*		Ovarial abscess	3
	7677	*Peptococcus magnus*		Skin abscess	3
	7957	*Peptococcus magnus*		Vaginal discharge	4
	7881	*Peptococcus magnus*	ATCC 14955	Draining sinus	7
	7700	*Peptococcus magnus*		Vaginal discharge	4
	7716	*Peptococcus magnus*		Skin abscess	2
	7654	*Peptococcus magnus*		Ovarial abscess	4
	7690	*Peptococcus magnus*		Blood culture	4
	7644	*Peptococcus magnus*		Skin abscess	3
	7679	*Peptococcus magnus*		Vaginal discharge	3
Peptostreptococcus micros	7824	*Peptostreptococcus micros*	VPI 5464^T	Prulent pleuresy	8
Peptostreptococcus productus	7707	*Peptostreptococcus productus*	ATCC 27340^T	Septicemia	7
Staphylococcus saccharolyticus	7633	*Peptococcus saccharolyticus*	ATCC 14953^T	Plasma	7
Streptococcus parvulus	7866	*Peptostreptococcus parvulus*	VPI 0546^T	Not recorded	8
Peptococcus niger	7850	*Peptococcus niger*	ATCC 27731^T	Umbilicus	7

[a] Sources: 1, Niigata University Hospital, Niigata, Japan; 2, Tajimi Municipal Hospital, Tajimi, Japan; 3, Jundendo University Hospital, Tokyo, Japan; 4, Gifu University, Gifu, Japan; 5, Nagoya City Hospital, Nagoya, Japan; 6, Swedish University of Agricultural Science, Uppsala, Sweden; 7, American Type Culture Collection, Rockville, Md.; 8, Virginia Polytechnic Institute and State University, Blacksburg, Va.

species of peptostreptococci (Table 4), and strains of *Staphylococcus saccharolyticus* were sensitive to both lysozyme and achromopeptidase (11).

Cato et al. (4) have shown that *Peptococcus glycinophilis* (2) is a later synonym of *Peptostreptococcus micros*. The DNAs of the type strains of these two species show 84% homology (4).

(ii) *Peptostreptococcus* Kluyver and van Niel 1980. Cells are spherical to ovoid and 0.3 to 1.8 μm in diameter and occur in pairs, masses, tetrads, or chains. Gram-positive. Strictly anaerobic. Usually nonhemolytic on sheep or rabbit blood agar. A few strains are hemolytic. Colonies are pinpoint to 2 mm in diameter, circular, smooth, and translucent. Peptone and amino acids can be used as major energy sources. Metabolic end products from PYG broth vary among species. Acidic products may include any of the following: acetic, butyric, isocaproic, lactic, propionic, isobutyric, and isovaleric acids. Catalase variable. Gas production in peptone-yeast extract and peptone-yeast extract-glucose media is variable. The saccharolytic activity of most species is negative or only weakly positive. Tween 80 enhances fermentation of carbohydrates by some species.

Isolated from female genital tracts, feces, blood, and abscesses of various sites.

The G+C content of the DNA ranges from 28 to 34 mol%, as determined by thermal denaturation (T_m), except for *Peptostreptococcus productus*, whose G+C content is 45 mol% (T_m).

The type species is *Peptostreptococcus anaerobius* (Natvig 1905) Kluyver and van Niel 1936.

In addition, the saccharolytic species *Peptostreptococcus parvulus* and *Peptostreptococcus productus* have DNAs with G+C contents

TABLE 2. DNA-DNA homologies among the 65 selected strains of anaerobic cocci

Competitive DNA from:	G+C content (mol%)	% Homology with labeled DNA from:							
		Peptostreptococcus asaccharolyticus		Unclassified group A-1 strain GIFU 1244	*Peptostreptococcus prevotii* ATCC 9321^T	*Peptostreptococcus tetradius* GIFU 7672^T	*Peptostreptococcus magnus*		*Peptostreptococcus anaerobius* ATCC 27337^T
		ATCC 14963^T	GIFU 6663				ATCC 15794^T	ATCC 14955	
Peptostreptococcus anaerobius ATCC 27337^T	33	26	NT[a]	NT	25	20	NT	23	100
Peptostreptococcus asaccharolyticus homology group 1									
ATCC 14963^T	32	100	58	56	35	23	NT	NT	25
GIFU 3302	33	107	NT	NT	30	NT	NT	NT	31
GIFU 7877	33	102	NT	NT	23	NT	NT	NT	NT
GIFU 3306	33	101	46	NT	28	NT	NT	NT	NT
GIFU 6287	34	100	NT	NT	22	NT	NT	NT	NT
GIFU 3315	32	99	NT	NT	23	NT	NT	NT	NT
GIFU 3290	33	97	NT	NT	23	NT	NT	NT	NT
GIFU 7951	33	97	NT	NT	20	NT	NT	NT	NT
Peptostreptococcus asaccharolyticus homology group 2									
GIFU 6663	33	52	100	NT	14	NT	NT	NT	NT
GIFU 6291	34	58	103	28	27	NT	NT	NT	NT
GIFU 7871	32	34	96	NT	27	NT	NT	NT	NT
GIFU 7693	34	40	89	NT	27	NT	NT	NT	NT
GIFU 7681	34	67	75	NT	30	29	NT	NT	34
GIFU 7939	34	32	71	NT	21	NT	NT	NT	NT
GIFU 7955	33	53	65	NT	27	NT	NT	NT	NT
Unclassified group A-1									
GIFU 1244	30	48	22	100	44	NT	NT	NT	NT
GIFU 7662	31	47	24	90	32	NT	NT	NT	NT
GIFU 7872	31	33	25	76	34	NT	NT	NT	NT
Peptostreptococcus indolicus ATCC 29427^T	34	15	NT	NT	20	NT	NT	NT	NT
GIFU 8287	33	34	NT	NT	33	NT	NT	NT	27
GIFU 8288	33	27	NT	NT	22	NT	NT	NT	31
GIFU 8296	33	23	NT	NT	37	NT	NT	NT	NT
Peptostreptococcus prevotii									
ATCC 9321^T	33	46	NT	NT	100	28	NT	NT	23
GIFU 7789	30	44	21	NT	73	31	NT	NT	NT
GIFU 7732	29	NT	28	NT	73	NT	NT	NT	NT
GIFU 7794	30	40	NT	NT	71	25	NT	NT	36
GIFU 7678	30	35	31	NT	37	39	NT	15	NT

GIFU 7695	31	42	25	NT	35	35	NT	NT	NT
GIFU 7954	33	21	NT	NT	35	NT	NT	NT	NT
Peptostreptococcus tetradius									
GIFU 7672^T	30	37	NT	NT	24	100	NT	NT	27
GIFU 3282	32	43	NT	NT	24	96	NT	NT	NT
GIFU 3298	32	40	NT	NT	32	95	NT	NT	NT
GIFU 3270	32	37	NT	NT	22	76	NT	NT	NT
GIFU 7617	32	40	NT	NT	27	73	NT	27	31
Unclassified group P-1									
GIFU 7668	24	13	NT	NT	3	NT	NT	NT	12
GIFU 7878	24	2	NT	NT	0	NT	NT	NT	NT
Peptostreptococcus magnus									
ATCC 15794^T	32	NT	NT	NT	NT	NT	100	95	36
GIFU 7726	32	NT	NT	NT	NT	NT	100	94	NT
GIFU 7795	32	NT	NT	NT	NT	NT	100	97	NT
GIFU 7943	33	NT	NT	NT	NT	NT	99	91	NT
GIFU 7711	33	NT	NT	NT	NT	NT	99	83	27
GIFU 7724	32	NT	NT	NT	NT	NT	98	97	NT
GIFU 7624	33	NT	NT	NT	NT	NT	98	85	NT
GIFU 7785	33	NT	NT	NT	NT	NT	98	83	NT
GIFU 7655	33	NT	NT	NT	NT	NT	98	82	NT
GIFU 7651	33	NT	NT	NT	NT	NT	98	82	NT
GIFU 7735	32	NT	NT	NT	NT	NT	98	78	NT
GIFU 7792	32	NT	NT	NT	NT	NT	97	77	NT
GIFU 7661	34	NT	NT	NT	NT	NT	85	76	NT
GIFU 7723	32	NT	NT	NT	NT	NT	79	85	NT
GIFU 7677	33	NT	NT	NT	NT	NT	79	72	NT
GIFU 7957	33	NT	NT	NT	NT	NT	70	68	NT
ATCC 14955	32	NT	NT	NT	NT	26	100	100	36
GIFU 7700	33	NT	NT	NT	NT	NT	93	99	NT
GIFU 7716	33	NT	NT	NT	NT	NT	90	98	NT
GIFU 7654	33	NT	NT	NT	NT	NT	97	83	NT
GIFU 7690	33	NT	NT	NT	NT	NT	77	74	NT
GIFU 7644	33	NT	NT	NT	NT	NT	71	73	NT
GIFU 7679	34	NT	NT	NT	NT	NT	67	74	NT
Peptostreptococcus micros VPI 5464^T	28	NT	NT	NT	NT	NT	NT	18	21
Staphylococcus saccharolyticus ATCC 14953^T	31	4	NT	NT	4	NT	13	NT	NT
Peptostreptococcus productus ATCC 27340^T	45	NT	NT	NT	NT	NT	NT	NT	1
Streptococcus parvulus VPI 0546^T	44	NT	NT	NT	NT	NT	NT	NT	0
Peptococcus niger ATCC 27731^T	51	6	NT	NT	2	1	0	NT	NT

[a] NT, Not tested.

TABLE 3. Biochemical characters of the 65 selected strains of anaerobic cocci

Organism	Metabolic end product(s) from PYG[a]	Indole	Coagulase	Nitrate reduction	Propionate from lactate	Gas from PYG	Gelatin liquefaction	Catalase	Urease	Starch hydrolysis	Esculin hydrolysis
Peptostreptococcus anaerobius ATCC 27337^T	IC, A, iv, ib	−[b]	−	−	−	+	−	−	−	−	−
Peptostreptococcus asaccharolyticus homology group 1											
ATCC 14963^T	B, a, p	+	−	−	−	+	−	+	−	−	−
GIFU 3302	B, A	+	−	−	−	+	−	−	−	−	−
GIFU 7877	B, A	+	−	−	−	+	−	−	−	−	−
GIFU 3306	B, A	+	−	−	−	+	−	−	−	−	−
GIFU 6287	B, A, p	+	−	−	−	+	−	+	−	−	−
GIFU 3315	B, A	+	−	−	−	+	−	−	−	−	−
GIFU 3290	B, A (p, l)	+	−	−	−	+	−	−	−	−	−
GIFU 7951	B, A	+	−	−	−	+	−	+	−	−	−
Peptostreptococcus asaccharolyticus homology group 2											
GIFU 6663	B, A, p	+	−	−	−	+	−	+	−	−	−
GIFU 6291	B, A, p	+	−	−	−	+	−	+	−	−	−
GIFU 7871	B, A, p	+	−	−	−	+	−	−	−	−	−
GIFU 7693	B, A, l	+	−	−	−	+	−	−	−	−	−
GIFU 7681	B, A	+	NT	−	NT	+	−	−	−	−	−
GIFU 7939	B, A (p, l)	+	−	−	−	+	−	−	−	−	−
GIFU 7955	B, A, iv, ib	+	NT	−	NT	+	−	+	−	−	w
Unclassified group A-1											
GIFU 1244	B, l, a, p	+	−	−	−	+	−	−	−	−	−
GIFU 7662	B, L, a	+	−	−	−	+	−	+	−	−	−
GIFU 7872	B, L, a, p	+	−	−	−	+	−	+	−	−	−
Peptostreptococcus indolicus											
ATCC 29427^T	B, A, p	+	+	+	+	+	−	−	−	−	−
GIFU 8287	B, A, p	+	+	+	+	+	−	−	−	−	−
GIFU 8288	B, A, p	+	+	+	+	+	−	−	−	−	−
GIFU 8296	B, A, p	+	+	+	+	+	−	−	−	−	−
Peptostreptococcus prevotii											
ATCC 9321^T	B, a, p	−	−	−	−	+	−	+	+	−	−
GIFU 7789	B, A, p	−	−	−	−	+	−	−	−	−	−
GIFU 7732	B, a (l)	−	NT	−	NT	+	−	−	−	−	−
GIFU 7794	B, l, a, p	−	NT	−	NT	+	−	+	−	−	w
GIFU 7678	B, a, l	−	NT	−	NT	+	−	−	−	−	−
GIFU 7695	B, a, p	−	NT	−	NT	+	−	+	−	−	−
GIFU 7954	B, a, p	−	−	−	−	+	−	+	−	−	−
Peptostreptococcus tetradius											
GIFU 7672^T	B, L, a	−	−	−	−	+	−	−	+	−	−

of 44 to 45 mol% (T_m) (3, 10, 33). These G+C values are somewhat higher than the values for other species of *Peptostreptococcus*. Recently, *Peptostreptococcus parvulus* has been transferred to the genus *Streptococcus* (3), but the taxonomic position of *Peptostreptococcus productus* has not yet been evaluated in detail.

Descriptions of four species of the genus *Peptostreptococcus* Kluyver and van Niel 1980. (i) *Peptostreptococcus asaccharolyticus* (Distaso 1912) comb. nov. emend. Cells are 0.5 to 1.6 μm in diameter and occur in masses or pairs. Colonies range from 1 to 2 mm in diameter on sheep blood agar. In PYG broth, all strains produce butyric acid, and some strains occasionally produce acetic, propionic, or lactic acid; none produces propionate from lactate. Most strains produce ammonia from glutamate, threonine, and serine, but not from glycine. Most strains fail to produce acid from carbohydrates. Nitrate is not reduced, and coagulase is not produced.

Other characteristics of this species are given in Tables 3 and 4.

May be pathogenic.

Isolated from normal and pathological genital secretions, normal feces, purulent pleurisy, skin, and brain abscesses (34).

The G+C content of the DNA is 30 to 34 mol% (T_m).

TABLE 3—*Continued*

Ammonia from glutamate	Ammonia from glycine	Carbohydrate fermentation					API ZYM tests						
		Fructose	Maltose	Mannose	Sucrose	Glucose	Alkaline phosphatase	Esterase (C-4)	Esterase lipase (C-8)	Leucine arylamidase	β-Galactosidase	β-Glucuronidase	α-Glucosidase
−	−	−	w	w	w	w	−	+	w	−	−	−	+
+	−	−	−	−	−	−	−	−	−	−	−	−	−
+	−	−	−	−	−	−	−	−	−	−	−	−	−
+	−	−	−	−	−	−	−	−	−	−	−	−	−
+	−	−	−	−	−	−	−	−	−	−	−	−	−
+	−	−	−	−	−	−	−	−	−	−	−	−	−
+	−	−	−	−	−	−	−	−	−	−	−	−	−
+	−	−	−	−	−	−	−	−	−	−	−	−	−
+	−	−	−	−	−	−	w	−	w	−	−	−	−
+	−	−	−	−	−	−	w	−	w	−	−	−	−
+	−	−	−	−	−	−	−	−	−	−	−	−	−
+	−	−	−	−	−	+	w	−	w	−	−	−	−
+	−	−	−	−	−	−	−	−	−	−	−	−	−
+	−	−	−	−	−	−	w	−	−	−	−	−	−
+	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	+	+	w	w	−	−	−	−
−	−	−	−	−	−	+	+	−	−	−	−	−	−
−	−	−	−	−	−	+	+	−	w	−	−	−	−
+	−	−	−	−	−	−	+	−	−	−	−	−	−
+	−	−	−	−	−	−	+	−	−	−	−	−	−
+	−	−	−	−	−	−	+	−	−	−	−	−	−
+	−	−	−	−	−	−	+	−	−	−	−	−	−
−	−	w	−	w	w	w	+	+	+	−	+	+	+
−	−	w	w	w	−	w	+	−	−	w	−	−	−
−	−	−	w	−	−	+	+	−	−	w	−	−	−
−	−	−	w	−	−	w	+	−	−	w	−	−	−
−	−	−	w	w	−	+	+	−	−	w	−	−	−
−	−	−	+	w	w	+	+	−	−	w	−	−	−
−	−	−	+	w	−	+	+	−	−	w	−	−	−
−	−	+	+	+	+	+	−	−	w	w	−	+	w

Continued on p. 692 and 693.

The type strain is strain ATCC 14963.

Indole production by *Peptostreptococcus asaccharolyticus* and *Peptostreptococcus indolicus* is a key character which differentiates these species from other butyrate-producing peptostreptococci (Table 5).

In addition, the levels of DNA-DNA homology among 18 strains of *Peptostreptococcus asaccharolyticus*, which were identified according to the characteristics listed in the *Anaerobe Laboratory Manual*, 4th ed. (14), separated these strains into three homology groups (Table 2). Group 1 contained the type strain. The levels of DNA-DNA homology between the type strain *Peptostreptococcus asaccharolyticus* and strains of group 2 were 32 to 58%. Attempts to separate group 2 strains from group 1 strains biochemically were not successful. Some group 2 strains were misidentified as *Peptostreptococcus prevotii* because they were indole negative initially; however, these strains produced indole after successive transfers in GAM broth. They also produced ammonia from glutamate. Homology group 3 (unclassified group A-1 [Table 2]) consisted of three strains. These strains had slightly lower G+C values than the members of the other groups of *Peptostreptococcus asaccharolyticus*, produced little or no ammonia

TABLE 3. Biochemical characters of the 65 selected strains of anaerobic cocci—*Continued*

Organism	Metabolic end product(s) from PYG[a]	Indole	Coagulase	Nitrate reduction	Propionate from lactate	Gas from PYG	Gelatin liquefaction	Catalase	Urease	Starch hydrolysis	Esculin hydrolysis
GIFU 3282	B, L, a	–	–	–	–	+	–	+	+	–	–
GIFU 3298	B, L, a	–	–	–	–	+	–	+	+	–	–
GIFU 3270	B, L	–	–	–	–	+	–	–	+	–	w
GIFU 7617	B, L	–	–	–	–	+	–	–	+	–	w
Unclassified group P-1											
GIFU 7668	B, A, l	–	NT	–	NT	+	–	–	–	–	–
GIFU 7878	B, A, iv	–	NT	–	NT	+	–	–	–	–	–
Peptostreptococcus magnus											
ATCC 15794^T	A	–	–	–	–	–	+	+	–	–	–
GIFU 7726	A	–	–	–	NT	–	+	+	–	–	–
GIFU 7795	A	–	NT	–	NT	–	+	+	–	–	–
GIFU 7943	A	–	–	–	–	–	+	+	–	–	–
GIFU 7711	A	–	NT	–	NT	–	+	+	–	–	–
GIFU 7724	A	–	–	–	–	–	+	–	–	–	–
GIFU 7624	A	–	NT	–	NT	–	+	–	–	–	–
GIFU 7785	A	–	–	–	NT	–	+	–	–	–	–
GIFU 7655	A	–	NT	–	NT	–	+	+	–	–	–
GIFU 7651	A	–	–	–	–	–	+	+	–	–	–
GIFU 7735	A	–	NT	–	NT	–	+	+	–	–	–
GIFU 7792	A	–	–	–	–	–	+	+	–	–	–
GIFU 7661	A	–	–	–	NT	–	+	+	–	–	–
GIFU 7723	A	–	–	–	–	–	+	–	–	–	–
GIFU 7677	A	–	NT	–	NT	–	+	+	–	–	–
GIFU 7957	A	–	NT	–	NT	–	+	+	–	–	–
ATCC 14955	A	–	–	–	–	–	–	+	–	–	–
GIFU 7700	A	–	NT	–	NT	–	–	+	–	–	–
GIFU 7716	A	–	–	–	NT	–	–	+	–	–	–
GIFU 7654	A	–	NT	–	NT	–	–	+	–	–	–
GIFU 7690	A	–	NT	–	NT	–	–	+	–	–	–
GIFU 7644	A	–	NT	–	NT	–	–	–	–	–	–
GIFU 7679	A	–	NT	–	NT	–	–	+	–	–	–
Peptostreptococcus micros VPI 5464^T	A, s, l	–	–	–	–	–	–	–	–	–	–
Staphylococcus saccharolyticus ATCC 14953^T	A	–	–	+	–	+	–	+	+	–	–
Peptostreptococcus productus ATCC 27340^T	A, s	–	–	–	–	+	–	–	–	–	+
Streptococcus parvulus VPI 0546^T	L, a	–	–	–	–	–	–	–	–	–	+
Peptococcus niger ATCC 27731^T	B, C, iv, a	–	–	–	–	w	–	–	–	–	–

[a] a and A, Acetic acid; C, caproic acid; B, butyric acid; l and L, lactic acid; ib, isobutyric acid; iv, isovaleric acid; IC, isocaproic acid; p, propionic acid; s, succinic acid. The capital letters indicate major products, and the lower-case letters indicate minor products. The products in parentheses are occasional products.

[b] +, Positive; –, negative; w, weakly positive; NT, not tested.

from glutamate, and produced acid from glucose (Table 3) and alkaline phosphatase in GAM broth. These biochemical characteristics clearly separate the group 3 strains from the strains of the other two homology groups.

In a study of indole-positive anaerobic cocci by Huss et al. (17), five strains of *Peptococcus asaccharolyticus* were used. These authors referred to *Peptostreptococcus asaccharolyticus* ATCC 14963^T (T = type strain) as "*Peptococcus aerogenes*" and to the four other strains as *Peptococcus asaccharaolyticus*. The G+C content of the DNA of the type strain was 31 mol%, and the G+C contents of the four other strains ranged from 36 to 38 mol%. The levels of DNA-DNA homology between the type strain and the four other strains were less than 25%.

(ii) *Peptostreptococcus indolicus* (Christiansen 1934) comb. nov. Cells are 0.7 to 1.6 μm in diameter and occur in masses or in pairs. Colonies on sheep blood agar are 1 to 2 mm in diameter, circular, and smooth. All strains produce propionate from lactate, coagulase, and indole, reduce nitrate to nitrite, and produce

TABLE 3—*Continued*

Ammonia from glutamate	Ammonia from glycine	Carbohydrate fermentation					API ZYM tests						
		Fructose	Maltose	Mannose	Sucrose	Glucose	Alkaline phosphatase	Esterase (C-4)	Esterase lipase (C-8)	Leucine arylamidase	β-Galactosidase	β-Glucuronidase	α-Glucosidase
−	−	+	+	+	+	+	−	−	−	−	−	+	w
−	−	+	+	+	+	+	−	−	−	−	−	+	w
−	−	+	+	+	+	+	−	−	−	−	−	+	w
−	−	+	+	+	+	+	−	−	−	−	−	+	w
+	−	−	−	−	−	−	−	−	−	−	−	−	−
+	−	−	−	−	−	−	−	−	−	−	−	−	−
−	+	w	−	−	−	−	+	+	w	+	−	−	−
−	+	w	−	−	−	w	+	+	w	+	−	−	−
−	+	−	−	−	−	−	w	+	w	+	−	−	−
−	+	+	−	−	−	−	+	+	w	+	−	−	−
−	+	−	−	−	−	−	w	+	w	+	−	−	−
−	+	+	−	−	−	w	+	+	w	+	−	−	−
−	+	−	−	−	−	−	w	w	−	w	−	−	−
−	+	w	−	−	−	−	+	+	w	+	−	−	−
−	+	−	−	−	−	w	w	+	w	+	−	−	−
−	+	+	−	−	−	−	w	w	w	+	−	−	−
−	+	+	−	−	−	−	w	+	w	+	−	−	−
−	+	+	−	−	−	−	w	w	w	+	−	−	−
−	+	+	−	−	−	−	w	+	+	+	−	−	−
−	+	+	−	−	−	−	+	w	w	+	−	−	−
−	+	−	−	−	−	−	w	+	w	+	−	−	−
−	+	−	−	−	−	−	w	+	w	+	−	−	−
−	+	w	−	−	−	−	w	+	+	w	−	−	−
−	+	w	−	−	−	−	w	+	+	+	−	−	−
−	+	w	−	−	−	−	w	w	−	+	−	−	−
−	+	w	−	−	−	−	w	+	+	w	−	−	−
−	+	w	−	−	−	−	w	+	+	w	−	−	−
−	+	w	−	−	−	−	w	+	+	+	−	−	−
−	w	−	−	−	−	−	+	w	+	+	−	−	−
−	−	+	−	+	−	+	−	+	+	−	−	−	−
−	−	+	+	+	+	+	−	+	+	−	+	−	+
−	−	+	+	+	−	+	−	−	−	−	−	−	−
−	−	−	−	−	−	−	NT	NT	NT	NT	NT	NT	NT

butyric acid in PYG broth. Occasionally, some strains also produce acetic and propionic acids in PYG broth. All strains produce ammonia from glutamate, threonine, and serine, but not from glycine. All strains fail to produce acid from carbohydrates.

Other characteristics of the species are given in Tables 3 and 4 and by Høi-Sorenson (13) and other authors (6, 17).

May be pathogenic for animals.

Isolated from cattle, swine, and rats. Isolation of *Peptostreptococcus indolicus* from a human clinical specimen has been reported (1).

The G+C content of the DNA is 32 to 33 mol% (T_m).

The type strain is strain ATCC 29427.

Coagulase production, nitrate reduction, and propionate production from lactate are key characters for separating this species from *Peptostreptococcus asaccharolyticus* (Table 5). An occasional strain does not produce coagulase (37) or reduce nitrate (14).

(iii) *Peptostreptococcus prevotii* (Foubert and Douglas 1948) comb. nov. emend. Cells are 0.7 to 1.8 μm in diameter and occur in pairs, tetrads, or short chains. Hemolytic strains occasionally are observed on horse blood agar. Colonies range from 0.5 to 2 mm in diameter on sheep blood agar. All strains fail to produce coagulase or indole and to reduce nitrate. Many strains

TABLE 4. Major cellular fatty acids and aldehyde of 23 strains of anaerobic cocci

Organism	Major fatty acids and aldehyde (% of total acids)[a]				
	C15:Br	C16:0	C17:1	C18:1	C18:1 aldehyde
Peptostreptococcus anaerobius ATCC 27337^T	0	16	T[b]	65	12
Peptostreptococcus asaccharolyticus					
ATCC 14963^T	0	T	0	74	21
GIFU 7955	0	T	T	75	20
GIFU 6663	0	T	T	70	25
Unclassified group A-1 strain GIFU 1244	0	T	0	74	21
Peptostreptococcus indolicus					
ATCC 29428^T	0	1	0	87	7
GIFU 8288	0	2	0	88	5
GIFU 8296	0	2	T	85	5
Peptostreptococcus prevotii					
ATCC 9321^T	0	T	0	61	35
GIFU 7794	0	T	T	60	39
GIFU 7954	0	T	T	73	26
Unclassified group P-1 strain GIFU 7668	0	3	T	72	25
Peptostreptococcus tetradius					
GIFU 7672^T	0	T	T	85	T
GIFU 3284	0	1	0	82	6
GIFU 7617	0	T	0	88	7
Peptostreptococcus magnus					
ATCC 15794^T	0	20	0	62	12
GIFU 7624	0	T	0	74	21
ATCC 14955	0	15	0	73	2
GIFU 7644	0	T	T	69	26
Peptostreptococcus micros VPI 5464^T	0	T	0	70	28
Peptostreptococcus productus ATCC 27340^T	0	7	28	29	3
Streptococcus parvulus VPI 0546^T	0	7	28	61	3
Staphylococcus saccharolyticus ATCC 14953^T	0	2	0	30	T

[a] The number to the left of the colon indicates the number of carbon atoms. Br to the right of the colon indicates a branched-chain acid, 0 indicates a saturated acid, and 1 indicates an unsaturated acid.
[b] T, Trace (<1%).

produce trace amounts of catalase. Major metabolic end products from PYG broth cultures are butyrate, lactate, and acetate; an occasional strain also may produce propionate. Most strains produce ammonia from threonine and serine, but not from glycine. Some strains produce ammonia from glutamate. Some strains weakly ferment glucose, fructose, mannose, maltose, or sucrose. Most strains do not ferment arabinose, cellobiose, lactose, mannitol, raffinose, rhamnose, trehalose, or xylose.

Other characteristics of this species are given in Tables 3 and 4.

Members of the normal floras of skin, oral cavities, guts, and vaginas. Isolated from pathological vaginal secretions and lung abscesses.

The G+C content of the DNA ranges from 29 to 33 mol% (T_m).

The type strain is strain ATCC 9321.

Peptostreptococcus prevotii is easily separated from other butyrate-producing *Peptostreptococcus* species by the characters shown in Tables 3 and 5. However, *Peptostreptococcus asaccharolyticus* strains sometimes may be misidentified as *Peptostreptococcus prevotii* because their indole production is often feeble and indole is not detected unless it is extracted with xylene before the reagent is added. Production of indole and ammonia from glutatmate by *Peptostreptococcus asaccharaolyticus* is helpful for differentiating this species from *Peptostreptococcus prevotii*.

In addition, DNA-DNA homologies between the type strain and clinical isolates of *Peptostreptococcus prevotii* and determinations of the DNA base contents of these strains revealed heterogeneity among the strains of this species (Table 2). The levels of homology between the type strain and three clinical isolates (GIFU 7678, GIFU 7695, and GIFU 7954) were 35 to 37%. Phenotypic differentiation of these strains from the type strain was not successful (Table 3). We think that these strains should be retained in *Peptostreptococcus prevotii* until useful biochemical characteristics are available to separate them from the type species.

Two genetically unrelated strains, strains GIFU 7668 and GIFU 7878, were tentatively identified as *Peptostreptococcus prevotii* according to the *Anaerobe Laboratory Manual*,

TABLE 5. Key characters to separate the species of the genus *Peptostreptococcus*

Organism	G+C content (mol%)[a]	Metabolic end product(s) from PYG[b]	Indole production	Nitrate reduction	Coagulase production	Urease production	Milk coagulation	Gelatin digestion	Carbohydrate fermentation						Ammonia from glycine	Ammonia from glutamate	Gas from PYG
									Cellobiose	Glucose	Lactose	Maltose	Sucrose	Mannose			
Peptostreptococcus anaerobius	33–34	IC, A (iv, ib, b)	–[c]	–	–	–	–	–	–	(w)	–	(w)	(w)	–	–	(–)	+
Peptostreptococcus magnus[d]	32–34	A	–	–	–	–	–	v	–	(–)	–	–	–	–	+	(–)	–
Peptostreptococcus micros	28–29	A (s)	–	–	–	–	–	–	–	–	–	–	–	–	(–)	–	–
Peptostreptococcus indolicus[e]	33–34	B (A, l, p)	+	(+)	(+)	–	–	–	–	–	–	–	–	–	–	+	+
Peptostreptococcus asaccharolyticus	30–34	B (A, l, p)	+	–	–	–	–	–	–	(–)	–	–	–	–	–	+	+
Peptostreptococcus prevotii	29–33	B (L, A, p)	–	–	–	(–)	–	–	–	(w)	–	(w)	(w)	(w)	–	(–)	+
Peptostreptococcus tetradius	30–32	L, B (a, p)	–	–	–	+	–	–	–	+	–	+	+	+	–	(–)	+
Peptostreptococcus productus	45	A, l, s	–	–	–	–	+	–	+	+	+	+	+	+	–	+	+

[a] As determined by the T_m method.

[b] a and A, Acetic acid; b and B, butyric acid; IC, isocaproic acid; iv, isovaleric acid; ib, isobutyric acid; s, succinic acid; l and L, lactic acid; p, propionic acid. The capital letters indicate major products, and the lower-case letters indicate minor products. The products in parentheses are occasional products.

[c] +, Positive; –, negative; (+), negative strains may exist; (–), most strains are negative (>90%); v, 68% of the strains tested are positive; (w), 15 to 90% of the strains are weakly positive.

[d] Cell size, soluble proteins, and alkaline phosphatase are used to differentiate *Peptostreptococcus magnus* and *Peptostreptococcus micros* (see text).

[e] Propionate production from lactate is helpful in differentiating *Peptostreptococcus indolicus* from *Peptostreptococcus asaccharolyticus* (14).

4th ed., but were finally excluded from *Peptostreptococcus prevotii* because of their low G+C values (22 mol%). These strains are listed as unclassified group P-1 in Tables 1 through 4. One strain was isolated from the lacrimal sac of a newborn baby, and the other was isolated from a uterine cavity. Both strains grew slowly in PYG medium and died within 5 to 7 days when they were kept in PYG medium or chopped meat medium at room temperature. Production of ammonia from glutamate and the lack of an alkaline phosphatase separate the two strains of group P-1 from *Peptostreptococcus prevotii*.

(iv) *Peptostreptococcus magnus* (Prévot 1933) Smith 1957 comb. rev. Cells are 0.8 to 1.6 μm in diameter and occur predominantly in masses but occasionally in pairs or short chains. Colonies on sheep blood agar range from pinpoint to 1.2 mm in diameter. No strain produces coagulase or indole. No strain reduces nitrate. Many strains show feeble catalase activity. All strains produce acetic acid but not butyric or caproic acid from PYG broth. All strains produce ammonia from glycine. Most strains produce ammonia from threonine and serine.

Other characteristics are given in Tables 3 and 4.

May be pathogenic for humans.

Often isolated from human infections, including lung, brain, ovarian, and skin abscesses, endometritis, sepsis, and peritonitis, and from normal feces and vaginas.

The G+C content of the DNA is 32 to 34 mol% (T_m).

The type strain is strain ATCC 15794.

Peptostreptococcus magnus can be differentiated from anaerobic strains of *Staphylococcus saccharolyticus* by nitrate reduction, urease, and mannose fermentation tests (Table 5). Differentiation of *Peptostreptococcus magnus* from *Peptostreptococcus micros* is sometimes difficult. *Peptostreptococcus micros* is smaller than 0.6 μm (14); the small cell size of this species has been used to differentiate it from *Peptostreptococcus magnus* (14). It has been reported that the inability of *Peptostreptococcus magnus* to produce alkaline phosphatase is helpful in differentiating these two species (4, 30). In our study, *Peptostreptococcus magnus* strains had generally weak alkaline phosphatase activities (Table 3), whereas strains of *Peptostreptococcus micros* had strong activities. Gel electrophoretic patterns of soluble proteins also may be helpful in differentiating these two species (4). Occasionally, gelatin liquefaction and catalase activity of *Peptostreptococcus magnus* are helpful in separating the two species.

In addition, DNA-DNA homology studies among the type strain *Peptostreptococcus magnus* and clinical isolates revealed that there is little genetic heterogeneity among the strains generally recognized as *Peptostreptococcus magnus*. There was no difference between gelatin-negative strains and gelatin-positive strains (8, 19).

History of *"Gaffkya anaerobia"* and proposal of *Peptostreptococcus tetradius* sp. nov. *"Gaffkya anaerobia"* was first described by Choukevitch as *"Tetracoccus anaerobius"* (5). This species also appeared in the 6th edition of *Bergey's Manual* under the name *"Gaffkya anaerobia"* (Choukevitch) Prévot (16). However, the name *"Gaffkya anaerobia"* (Choukevitch) Prévot lost standing in nomenclature when the generic name *"Gaffkya"* was placed on the list of rejected names by the Judicial Commission (18). *"G. anaerobia"* was a genetically and biochemically distinct species both in a previous study (9) and in this study. Our isolates did not survive heat treatment at 70°C for 15 min. The cells were generally less than 1.8 μm in diameter and did not occur in clusters of eight or more. Carbohydrates did not stimulate the growth of these organisms, so they are not members of the genus *Sarcina*. They produced butyric acid from PYG medium but did not require fermentable carbohydrates, so they are not members of the genus *Coprococcus* (15). Genetic and biochemical evidence indicates that the species should be placed in the genus *Peptostreptococcus*. *Peptostreptococcus tetradius* sp. nov. (te.tra'di.us. Gr. adj. *tetradios* by fours; N. L. masc. adj. *tetradius* occurring in groups of four) is proposed for this group of strains.

Description of *Peptostreptococcus tetradius* sp. nov. Cells are 0.8 to 1.8 μm in diameter and occur mostly in pairs or tetrads, occasionally in short chains or masses (Fig. 1). Colonies on sheep blood agar range from pinpoint to 1.2 mm in diameter. Obligately anaerobic. Strains are nonhemolytic and make no black colonies on either rabbit or sheep blood agar after 7 days of anaerobic incubation. The major metabolic end products in PYG broth cultures are butyrate and lactate. No strain produces coagulase or indole or reduces nitrate. Catalase production is variable among strains. All strains produce urease and ferment fructose, glucose, maltose, mannose, and sucrose. They do not make acid from arabinose, cellobiose, esculin, lactose, mannitol, raffinose, rhamnose, or sorbitol. They produce ammonia from threonine and serine, but not from glycine.

Other characteristics of this species are given in Tables 3 and 4.

The pathogenicity is unknown.

Isolated from human vaginal discharge and various purulent secretions.

The G+C content of the DNA is 30 to 32 mol% (T_m).

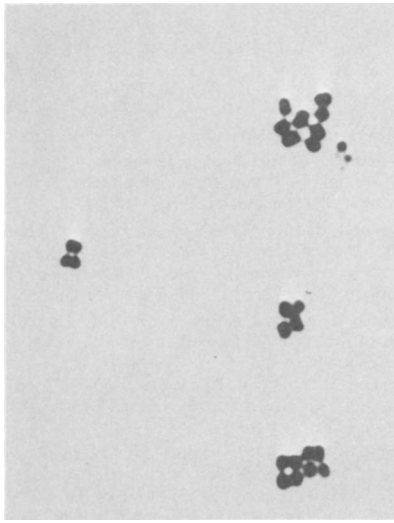

FIG. 1. *Peptostreptococcus tetradius*. ×2,000.

The type strain is strain GIFU 7672 (= ATCC 35098 = JCM 1964 = CCM 3634).

Tetrad formation is not always observed and cannot be used as a distinctive trait to separate this species from other anaerobic cocci. Urease and β-glucuronidase activities and fermentation of fructose, glucose, maltose, mannose, and sucrose are useful characters for separating *Peptostreptococcus tetradius* from *Peptostreptococcus prevotii*.

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