Three New Species of the Genus *Peptostreptococcus* Isolated from Humans: *Peptostreptococcus vaginalis* sp. nov., *Peptostreptococcus lacrimalis* sp. nov., and *Peptostreptococcus lactolyticus* sp. nov.

NA LI, YASUHIRO HASHIMOTO, SHATHA ADNAN, HIROAKI MIURA, HIROYUKI YAMAMOTO,
AND TAKAYUKI EZAKI*

Department of Microbiology, Gifu University School of Medicine, Tsukasa-machi 40,
Gifu 500, Japan

We describe three new species of the genus *Peptostreptococcus* which were isolated from human specimens and were tentatively identified as *Peptostreptococcus prevotii*. These three organisms were not homologous with previously described type strains of the genus *Peptostreptococcus*. A total of 12 strains that were identified biochemically as *P. prevotii* were divided into five independent DNA similarity groups; 10 of these strains were divided into three similarity groups which exhibited significant phenotypic differences from previously described species. Therefore, we propose the following new species: *Peptostreptococcus vaginalis* for group 1 strains, *Peptostreptococcus lacrimalis* for group 2 strains, and *Peptostreptococcus lactolyticus* for group 3 strains. The type strain of *P. vaginalis* is strain GIFU 12669 (= JCM 8138), the type strain of *P. lacrimalis* is strain GIFU 7667 (= JCM 8139), and the type strain of *P. lactolyticus* is strain GIFU 8586 (= JCM 8140).

Classification of members of the genus *Peptostreptococcus* has been evaluated by using both genetic and phenotypic methods (3, 5, 7, 8). However, in our identification work on anaerobic cocci by the microplate hybridization method (2), we found that many strains that were identified biochemically as *Peptostreptococcus prevotii* did not hybridize with the type strain of *P. prevotii*, strain ATCC 9321. Further investigation of these strains revealed that their DNAs did not hybridize with DNAs of previously described type strains of the genus *Peptostreptococcus*. Among the 12 strains whose DNAs did not hybridize with *Peptostreptococcus* DNAs, 10 strains were divided into three similarity groups which we were able to differentiate from previously described species by their phenotypic traits. In this paper we describe these strains and propose that they are members of independent species of the genus *Peptostreptococcus*.

MATERIALS AND METHODS

Strains and characterization. The strains used in this study are listed in Table 1. A total of 12 strains that were isolated from human clinical specimens were tentatively identified as *P. prevotii* by using conventional biochemical tests (5, 6). API ZYM and AN-IDENT tests (API, La Balme Les Grottes, Montalieu Vercieu, France) were also used to determine oxidase and peptidase activities. Bacterial strains were incubated on Columbia sheep blood agar plates at 37°C for 48 h, and the cells were harvested for analysis of their enzyme activities.

Determination of cell wall peptidoglycan. Cells were subcultured in Gifu anaerobic medium (GAM; Nissui, Tokyo, Japan) broth and were incubated anaerobically at 37°C for 2 days. Cell walls were purified by using a previously de-

scribed method (9, 10). Purified cell walls were hydrolyzed with 6 N hydrochloric acid for 18 h at 100°C and were analyzed by using an amino acid analyzer.

Determination of base composition of DNA and quantitative DNA-DNA hybridization. G+C contents of DNAs were determined by using the high-performance liquid chromatography method described previously (1, 4, 11). The DNA-DNA homology experiments were conducted in a microdilution plate as described previously (2). Hybridization was carried out in 2× SSC buffer (1× SSC is 0.15 M NaCl plus

TABLE 1. Butyrate-producing *Peptostreptococcus* strains used in this study

Name as determined by biochemical tests	GIFU no.	Other designation	Source ^a
<i>P. prevotii</i>	7658 ^T	ATCC 9321 ^T	ATCC
<i>P. prevotii</i>	7667 ^T	JCM 8139 ^T	Eye discharge
<i>P. prevotii</i>	7668		Eye discharge
<i>P. prevotii</i>	7714		Vaginal discharge
<i>P. prevotii</i>	7721		Vaginal discharge
<i>P. prevotii</i>	7752	JCM 8141	Umbilicus smear
<i>P. prevotii</i>	7756		Umbilicus smear
<i>P. prevotii</i>	8124	JCM 8142	Vaginal discharge
<i>P. prevotii</i>	8376		Vaginal discharge
<i>P. prevotii</i>	8586 ^T	JCM 8140 ^T	Vaginal discharge
<i>P. prevotii</i>	8587		Vaginal discharge
<i>P. prevotii</i>	12669 ^T	JCM 8138 ^T	Ovarian abscess
<i>P. prevotii</i>	12670		Vaginal discharge
<i>P. asaccharolyticus</i>	7656 ^T	ATCC 14963 ^T	ATCC
<i>P. hydrogenalis</i>	7662 ^T		Human feces
<i>P. tetradius</i>	7672 ^T		Vaginal discharge
<i>P. indolicus</i>	7848 ^T	ATCC 29427 ^T	ATCC
<i>P. anaerobius</i>	7882 ^T	ATCC 27337 ^T	ATCC

* Corresponding author.

^a ATCC, American Type Culture Collection, Rockville, Md.

TABLE 2. Guanine-plus-cytosine contents and DNA-DNA homology values for 18 strains of *Peptostreptococcus* species

Name as determined by biochemical tests	GIFU no.	G+C content (mol%) ^a	% Homology with labeled DNA from ^b :											
			Strain GIFU 7658 ^T		Strain GIFU 12669 ^T		Strain GIFU 7667 ^T		Strain GIFU 8586 ^T		Strain GIFU 7752		Strain GIFU 8124	
			37°C	45°C	37°C	45°C	37°C	45°C	37°C	45°C	37°C	45°C	37°C	45°C
<i>P. prevotii</i>	7658 ^T	34	100	100	— ^c	—	—	—	—	—	—	—	—	—
<i>P. prevotii</i> group 1	12669 ^T	30	32	—	100	100	—	—	—	—	—	—	—	—
	7721	30	—	—	100	98	NT ^d	NT	NT	NT	NT	NT	NT	NT
	7756	29	30	—	100	95	NT	NT	NT	NT	NT	NT	NT	NT
	12670	30	—	—	94	90	NT	NT	NT	NT	NT	NT	NT	NT
	7714	29	—	—	90	89	NT	NT	NT	NT	NT	NT	NT	NT
	8376	28	—	—	86	80	NT	NT	NT	NT	NT	NT	NT	NT
<i>P. prevotii</i> group 2	7667 ^T	30	—	—	44	—	100	100	—	—	—	—	—	—
	7668	31	—	—	32	—	100	95	43	—	—	—	—	—
<i>P. prevotii</i> group 3	8586 ^T	34	—	—	—	—	—	—	100	100	—	—	—	—
	8587	34	—	—	—	—	—	—	86	79	—	—	—	—
<i>P. prevotii</i> group 4	7752	35	—	—	—	—	—	—	—	—	100	100	—	—
<i>P. prevotii</i> group 5	8124	40	—	—	—	—	—	—	—	—	—	—	100	100
<i>P. tetradius</i>	7672 ^T	32	38	—	—	—	—	—	—	—	—	—	—	—
<i>P. asaccharolyticus</i>	7656 ^T	32	—	—	—	—	—	—	—	—	—	—	—	—
<i>P. hydrogenalis</i>	7662 ^T	30	—	—	38	—	—	—	—	—	—	—	—	—
<i>P. indolicus</i>	7848 ^T	34	—	—	—	—	—	—	—	—	—	—	—	—
<i>P. anaerobius</i>	7882 ^T	34	—	—	—	—	—	—	—	—	—	—	—	—

^a As determined by the high-performance liquid chromatography method.^b DNA-DNA hybridization experiments were carried out at 37°C (optimal conditions) and 45°C (stringent conditions) in 2× SSC containing 50% formamide.^c —, homology value less than 30%.^d NT, not tested.

0.015 M sodium citrate) containing 50% formamide at 37 and 45°C for 2 h.

RESULTS

G+C contents and DNA-DNA hybridization. The DNA base compositions of 12 strains and their homology values are shown in Table 2. The G+C content of *P. prevotii* ATCC 9321^T (T = type strain) was determined to be 34 mol% by the high-performance liquid chromatography method, and the G+C contents of the 12 strains isolated from clinical specimens ranged from 28 to 40 mol%. The data obtained from DNA hybridization experiments are summarized in Table 2. DNA-DNA hybridization experiments revealed no similarity between the DNAs of these 12 strains and the DNA of *P. prevotii* ATCC 9321^T. The 12 strains isolated from clinical specimens were classified into five distinct similarity groups by using hybridization data.

Peptidoglycan type. From the molecular ratios of the amino acids we estimated the peptidoglycan types of the anaerobic cocci (Table 3). All 12 strains had D-glutamic acid as the interpeptide when cells were cultured in GAM broth, although both D-glutamic acid and D-aspartic acid were detected in members of similarity group 5. The hydrolysate of strain GIFU 12669^T contained both ornithine and lysine (molecular ratio, 1:0.83). The amino acids in positions 1 and 3 are shown in Table 3.

Biochemical characteristics. All 12 strains were stored in our culture collection as *P. prevotii*, since they produced butyric acid as a major metabolic product from peptone-yeast extract-glucose (PYG) medium and did not produce indole. Group 3 strains produced urease and fermented sugars. Thus, these strains are phenotypically related to *Peptostreptococcus tetradius*. *P. prevotii* ATCC 9321^T and the 12 clinical strains were all negative in the indole test. In

the urease test, *P. prevotii* ATCC 9321^T and the group 3 strains were all positive. In carbohydrate fermentation tests, only group 3 strains fermented lactose and only group 4 strains fermented sucrose, whereas *P. prevotii* ATCC 9321^T fermented neither lactose nor sucrose. Other characteristics are shown in Table 4. Differential characteristics for similarity groups 1 to 5 and previously described species are shown in Table 5.

DISCUSSION

Twelve strains that were isolated from clinical specimens and were previously identified as *P. prevotii* were genetically

TABLE 3. Peptidoglycan types of representative strains of the homology groups

Taxon	GIFU no.	Cell wall peptidoglycan type ^a		
		Position 1	Position 3	Interpeptide
<i>P. prevotii</i>	7658 ^T	Gly	Lys	D-Glu
Group 1	12669 ^T	Ala	Lys (Orn) ^b	D-Glu
Group 2	7667 ^T	Ala	Orn	D-Glu
Group 3	8586 ^T	Gly	Lys	D-Glu
Group 4	7752	Ala	Orn	D-Glu
Group 5	8124	Ala	Orn	D-Glu (D-Asp) ^c
<i>P. tetradius</i>	7672 ^T	Gly	Lys	D-Glu
<i>P. asaccharolyticus</i>	7656 ^T	Ala	Orn	D-Glu
<i>P. hydrogenalis</i>	7662 ^T	Ala	Lys	D-Glu
<i>P. indolicus</i>	7848 ^T	Ala	Orn	D-Glu

^a Ala, alanine; Gly, glycine; Orn, ornithine; Lys, lysine; D-Glu, D-glutamic acid; D-Asp, D-aspartic acid.^b The molecular ratio of Lys and Orn was 1:0.83.^c The molecular ratio of D-Glu and D-Asp was 1:1.

TABLE 4. Biochemical characteristics of members of the five genetic groups and reference strains^a

Name as determined by biochemical tests	GIFU no.	Production of:			Carbohydrate ferment- ation				Saccharolytic enzymes				Proteolytic enzymes						
		Indole	Urease	ALP-ase	Glucose	Lactose	Maltose	Sucrose	NPG	ADG	GAL	β-GLU	LEU	ALA	HIS	GLY	PHA	INA	PRO
<i>P. prevotii</i>	7658 ^T	— ^b	+	+	w	—	w	—	—	+	+	w	+	+	+	+	+	—	+
<i>P. prevotii</i> group 1	12669 ^T	—	—	+	w	—	w	—	—	—	—	—	+	+	+	+	—	—	—
	7714	—	—	+	w	—	w	—	—	—	—	—	+	+	+	+	—	—	—
	7721	—	—	+	w	—	w	—	—	—	—	—	+	+	+	+	—	—	—
	7756	—	—	+	w	—	w	—	—	—	—	—	+	+	+	+	—	—	—
	8376	—	—	+	w	—	w	—	—	—	—	—	+	+	+	+	—	—	—
	12670	—	—	+	w	—	w	—	—	—	—	—	+	+	+	+	—	—	—
<i>P. prevotii</i> group 2	7667 ^T	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	—	—
	7668	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	—	—
<i>P. prevotii</i> group 3	8586 ^T	—	+	+	+	+	+	—	+	—	—	—	+	+	+	+	—	—	+
	8587	—	+	+	+	+	+	—	+	—	—	—	+	+	+	+	—	—	+
<i>P. prevotii</i> group 4	7752	—	—	—	—	—	—	—	—	+	+	—	—	+	+	+	+	w	—
<i>P. prevotii</i> group 5	8124	—	—	—	—	—	—	—	—	—	w	—	—	+	+	+	—	—	—
<i>P. tetradius</i>	7672 ^T	—	+	—	+	—	+	+	—	+	ND	+	+	+	+	+	+	—	+
<i>P. asaccharolyticus</i>	7656 ^T	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	w	—
<i>P. hydrogenalis</i>	7662 ^T	+	—	—	+	—	+	+	—	+	—	—	—	—	—	—	—	w	—
<i>P. indolicus</i>	7848 ^T	+	—	—	—	—	—	—	—	—	—	—	+	+	+	+	w	—	—
<i>P. anaerobius</i>	7882 ^T	—	—	—	+	—	+	—	—	+	—	—	—	—	—	—	—	—	+

^a ALP-ase, alkaline phosphatase; NPG, β-galactosidase; ADG, α-glucosidase; GAL, D-galactosidase; β-GLU, β-glucuronidase; LEU, leucine AMD; ALA, alanine AMD; HIS, histidine AMD; GLY, glycine AMD; PHA, phenylalanine AMD; INA, indoxylacetate AMD; PRO, proline AMD; PYR, pyrrolidone AMD.

^b +, positive (pH < 5.5); —, negative (pH > 6.0); w, weakly positive (5.5 > pH > 6.0); ND, not determined.

different from the type strain of *P. prevotii*, strain ATCC 9321. These 12 strains were classified into five distinct similarity groups, three of which were phenotypically different from previously described species. The results of a cell wall analysis also supported this observation. Only group 3 strains had a peptidoglycan type similar to that of *P. prevotii* and *P. tetradius*. Butyrate-positive peptostreptococci had D-glutamic acid as an interpeptide bridge of peptidoglycan (12). D-Aspartic acid and D-glutamic acid were detected in strain GIFU 8124, a member of similarity group 5. The G+C contents of the 12 strains ranged from 28 to 40 mol%, although the G+C content of *P. prevotii* ATCC 9321^T was 33 to 34 mol%. Thus, these data also support the hypothesis that these 12 strains are heterogeneous.

Proposal of three new *Peptostreptococcus* species. Because strains belonging to groups 1 to 3 are phenotypically and genetically different from previously described species, we propose them as new species, as follows: *Peptostreptococcus vaginalis* for group 1 strains, *Peptostreptococcus lacrimalis* for group 2 strains, and *Peptostreptococcus lactolyticus* for group 3 strains. Although the strains belonging to groups 4 and 5 were differentiated by the DNA homology test, we had only one strain of each group, and the phenotypic characteristics of these organisms were not sufficient to differentiate them from previously described species. Thus, we will not propose them as new species until we acquire more strains belonging to these taxa and identify reasonable differentiating characteristics.

Description of *Peptostreptococcus vaginalis* sp. nov. Cells are strictly anaerobic, gram-positive cocci that occur in short chains or in masses. The strains produce alkaline phosphatase, but not urease, indole, or coagulase. Weak acid is produced from glucose and maltose, but not from lactose, cellobiose, sucrose, arabinose, ribose, mannitol, trehalose, sorbitol, and raffinose. The strains use peptone and oligopeptides as their major energy sources. Leucine arylamidase (AMD), alanine AMD, histidine AMD, and glycine AMD are present. The major metabolic products from GAM medium

and PYG broth are butyrate and acetate. *P. vaginalis* was isolated from human vaginas. The G+C contents of the DNAs of *P. vaginalis* strains are 28 to 30 mol%. The type strain is strain GIFU 12669 (= JCM 8138).

Description of *Peptostreptococcus lacrimalis* sp. nov. Cells are strictly anaerobic, gram-positive cocci that occur in short chains or in masses. Indole, coagulase, alkaline phosphatase, and urease tests are negative. No acid is produced from glucose, maltose, lactose, sucrose, mannitol, mannose, cellobiose, trehalose, raffinose, arabinose, ribose, and sorbitol. β-Glucuronidase and α- and β-galactosidases are not present. The strains use peptones and oligopeptides as their major energy sources. Leucine AMD, alanine AMD, histidine AMD, glycine AMD, and phenylalanine AMD are present. The major metabolic products from GAM medium and PYG broth are butyrate and acetate. *P. lacrimalis* was isolated from human eyes. The G+C contents of the DNAs of the type strain and one other strain are 30 to 31 mol%. The type strain is strain GIFU 7667 (= JCM 8139).

Description of *Peptostreptococcus lactolyticus* sp. nov. Cells are strictly anaerobic, gram-positive cocci that occur in short chains or in masses. The strains produce urease, alkaline phosphatase, and β-galactosidase but not indole or coagulase. α-Galactosidase, α-glucosidase, and β-glucuronidase are not present. Strong acid (pH less than 5.5) is produced from glucose, lactose, and maltose, but not from sucrose, mannitol, trehalose, sorbitol, and arabinose. The strains also have strong peptidase activity. Leucine AMD, alanine AMD, histidine AMD, glycine AMD, and pyrrolidone AMD are present. The major metabolic products from GAM broth and PYG medium are butyrate, lactate, and acetate. The pathogenicity of this organism is unknown. The G+C content is 34 mol%. The type strain is strain GIFU 8586 (= JCM 8140).

Differential characteristics of *P. vaginalis*, *P. lacrimalis*, *P. lactolyticus*, and other *Peptostreptococcus* species are shown in Table 5.

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TABLE 5. Differential characteristics of butyrate-producing *Peptostreptococcus* species

Species	Peptidoglycan ^a			Metabolic products ^b	Coagulase	Nitrate reduced	Indole	Urease	ALP-ase	Carbohydrate fermentation				Saccharolytic enzymes ^c		Proteolytic enzymes ^d	
	Position 1	Position 3	Peptide bridge							Glucose	Lactose	Maltose	Sucrose	ADG	β-GLU	LEU	PHA
<i>P. prevotii</i>	Gly	Lys	D-Glu	B ₁ ,a,p	- ^{e,f}	-	-	+	+	w	-	w	-	+	w	+	d
<i>P. tetradius</i>	Gly	Lys	D-Glu	B ₁ ,a,p	-	-	-	+	+	+	+	+	+	+	+	+	d
<i>P. lactobutylicus</i> sp. nov.	Gly	Lys	D-Glu (D-Asp)	B ₁ ,a	-	-	-	+	+	+	-	+	-	+	+	+	+
<i>P. vaginalis</i> sp. nov.	Ala	Lys (Orn)	D-Glu	B ₁ ,l	-	-	-	-	+	w	w	-	-	+	-	+	-
<i>P. lactinialis</i> sp. nov.	Ala	Orn	D-Glu	B ₁ ,a,p,l	-	-	-	-	-	-	-	-	-	-	-	+	-
<i>P. asaccharolyticus</i>	Ala	Orn	D-Glu	B ₁ ,a	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>P. indolicus</i>	Ala	Orn	D-Glu	B ₁ ,a	+	+	+	+	+	+	-	-	-	-	-	+	w
<i>P. hydrogenalis</i>	Ala	Lys	D-Glu	B ₁ ,a,p,l	-	-	+	-	+	+	+	+	+	+	-	-	-
<i>P. anaerobius</i>	Ala	Lys	D-Asp ^g	C ₁ ,A,b,ib,iv	-	-	-	-	+	+	+	+	+	+	+	+	+

^a Ala, alanine; Gly, glycine; Orn, ornithine; Lys, lysine; D-Glu, D-glutamic acid; D-Asp, D-aspartic acid.

^b B and b, butyrate; A and a, acetate; p, propionate; L and l, lactate; C, isocaproate; ib, isobutyrate; iv, isovalerate. Uppercase letters indicate major products, and lowercase letters indicate minor products.

^c ADG, α-glucosidase; β-GLU, β-glucuronidase.

^d LEU, leucine AMD; PHA, phenylalanine AMD; PYR, pyrrolidone AMD.

^e +, positive; -, negative; w, weakly positive; d, different reactions.

^f Some strains are positive.

^g Data from reference 12.