Descriptions of *Prevotella tannerae* sp. nov. and *Prevotella enoea* sp. nov. from the Human Gingival Crevice and Emendation of the Description of *Prevotella zoogloiformans*

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***Prevotella tannerae* sp. nov. and *Prevotella enoea* sp. nov. from the human gingival crevice are described. These organisms are obligately anaerobic, non-spore-forming, nonmotile, gram-negative, rod-shaped bacteria that ferment carbohydrates and produce succinic and acetic acids. Bile inhibits growth. Some strains (38%) of *P. tannerae* produce colonies with a tan to black pigment when they are grown on rabbit blood agar. The type strains are *P. tannerae* ATCC 51259 and *P. enoea* ATCC 51261. In addition, the description of *Prevotella zoogloiformans* is emended to exclude strains now recognized as members of *Prevotella heparinolytica*.**

During quantitative and qualitative studies of the bacterial flora of samples obtained from the human gingival crevice, we isolated numerous strains of anaerobic, gram-negative, rod-shaped bacteria that represent species unlike any that have been described previously. Two of these organisms, designated “*Bacteroides* D28” and “*Bacteroides* D84” in our laboratory, were selected for further taxonomic study.

In addition, we propose that the description of *Prevotella zoogloiformans* should be emended to exclude strains now recognized as members of *Prevotella heparinolytica*. In 1938 Prévot described a new genus, *Capsularis*, with *Capsularis zoogloiformans* (Weinberg et al. 1937) as the type species (12). Because there was no strain to represent this species, it was not included on the 1980 Approved Lists of Bacterial Names (15). Following isolation from the gingival crevice of strains with the characteristics of “*C. zoogloiformans*,” Cato et al. proposed the name *Bacteroides zoogloiformans* (Weinberg et al. 1937) (corrig.) comb. nov. (2). In 1988 Bailey et al. (1) showed that some strains originally thought to be *B. zoogloiformans* strains by Cato et al. and other strains whose characteristics corresponded to the characteristics of *Bacteroides zoogloiformis* (Weinberg et al. 1937) Cato et al. exhibited only 47 to 52% DNA relatedness with the type strain of *B. zoogloiformans* and concluded that these organisms should be members of a distinct species. Bailey et al. named the new species *Bacteroides heparinolyticus* and showed that strains of *B. heparinolyticus* produce indole and strains of *B. zoogloiformans* do not. Both species were transferred to the genus *Prevotella* (13), but an emended description of *P. zoogloiformans* that includes only the indole-positive strains has not been published previously. An emended description based on five indole-positive strains is given below.

MATERIALS AND METHODS

Bacterial strains. In this study we examined *Prevotella tannerae* (“*Bacteroides* D28”) ATCC 51259^T (American Type Culture Collection, Rockville, Md.) (T = type strain), which was isolated from the gingival crevice of an adult with gingivitis, and 47 other isolates obtained from 38 samples from the gingival crevices of 33 other people (10 juveniles or adults with healthy gingiva, 4 people with gingivitis, 16 people with adult

periodontitis, 2 people with juvenile periodontitis, and 1 person with rapidly progressive periodontitis).

We also examined *Prevotella enoea* (“*Bacteroides* D85”) ATCC 51261^T, which was isolated from the gingival crevice of a person with adult periodontitis, and seven other isolates obtained from six other people (four juveniles or adults with healthy gingiva, one person with adult periodontitis, and one person with juvenile periodontitis).

P. zoogloiformans ATCC 33285^T and four other strains isolated from people with adult periodontitis were also included in this study.

Type strains of other species were also examined for comparison (Table 1).

Characterization of strains. Fermentation, enzymatic, and antimicrobial agent susceptibility tests in prereduced media and polyacrylamide gel electrophoresis of soluble proteins were performed as described previously (3, 9). The fatty acid methyl ester contents of whole-cell sediments obtained from prereduced anaerobically sterilized peptone-yeast extract-glucose broth cultures were determined as described previously (8).

DNA isolation and hybridization. Strains were grown in

TABLE 1. Levels of DNA relatedness among some *Prevotella* species

Unlabeled DNA from:	% Homology with reference DNA from:		
	<i>P. tannerae</i> ATCC 51259 ^T	<i>P. enoea</i> ATCC 51261 ^T	<i>P. oulora</i> ATCC 43324 ^T
<i>P. tannerae</i> strains			
ATCC 51259 ^T (= VPI N14B-15 ^T)	(100) ^a	44	9
VPI D212A-14	82	34	5
<i>P. enoea</i> strains			
ATCC 51261 ^T	40	(100) ^a	9
(= VPI D194A-25A ^T)			
VPI D42D-22	44	85	NT ^b
<i>P. nigrescens</i> VPI 8944 ^T	5	5	5
<i>P. melaninogenica</i> VPI 2381 ^T	13	9	12
<i>P. denticola</i> ATCC 35308 ^T	14	12	13
(= VPI 14460 ^T)			
<i>P. oris</i> VPI D1A-1A ^T	9	9	16
<i>P. oulora</i> ATCC 43324 ^T	8	14	(100) ^a

^a Value normalized to 100%.

^b NT, not tested.

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TABLE 2. Characteristics of *P. tannerae*, *P. enoea*, and *P. zoogloformans*^a

Characteristic	<i>P. tannerae</i>		<i>P. enoea</i>		<i>P. zoogloformans</i>	
	Reaction of ATCC 51259 ^T	% of 47 other strains that are positive	Reaction of ATCC 51272 ^T	% of 7 other strains that are positive	Reaction of ATCC 33285 ^T	% of 4 other strains that are positive
Acid produced from:						
Amygdalin	—	0	—	0	—	50
Cellobiose	—	0	—	0	A	75
Dextrin	A	70	A	100	A	100
Fructose	A	79	A	88	A	100
Gum arabic	—	11	—	0	—	0
Glycogen	A ^b	81	A	100	A	0
Inulin	—	11	—	0	A	0
Lactose	A	81	A	100	A	0
Larcharabino-galactan	—	5	—	0	—	0
Maltose	A ^b	81 ^b	A	100	A	100
Mannose	A	46	A	71	A	75
Melibiose	—	4	—	0	A	50
Pectin	—	17	—	42	—	0
Raffinose	A	21	—	0	A	25
Salicin	—	0	—	0	A	100
Starch	A ^b	83 ^b	A	86	A	100
Sucrose	A	30	—	0	A	75
Trehalose	A ^b	26 ^b	—	0	—	100
Xylose	—	0	—	14	A	0
Hydrolysis of:						
Esculin	—	15	—	43	+	100
Starch	+ ^b	83 ^b	+	57	—	0
Digestion of:						
Gelatin	+	100	+	100	+	25
Milk	+	76	—	0	—	0
Meat	—	36	—	0	—	0
Milk curd	—	27	+	100	+	75
Nitrate reduction	—	4	—	0	—	0
H ₂ S (Simmons indole motility medium)	+	21	—	43	+	50
Peptone-yeast extract-glucose agar						
deep						
Gas	—	0	—	44	—	0
Reducing	—	0	—	0	+	0
Resistance to:						
Chloramphenicol (12 µg/ml)	—	12	—	0	—	0
Clindamycin (1.6 µg/ml)	—	4	—	0	—	0
Erythromycin (3 µg/ml)	—	4	—	0	—	0
Penicillin G (2 U/ml)	—	17	—	28	—	0
Tetracycline (6 µg/ml)	+	30	—	0	—	0

^a A, acid (pH < 5.5); —, pH > 5.7 or negative reaction; +, positive reaction. All strains produced acid from glucose. No strain produced acid from arabinose, erythritol, esculin, mannitol, melezitose, rhamnose, ribose, or sorbitol; no strain produced lecithinase or lipase on egg yolk agar (3), indole, or catalase.

^b This reaction may be positive or acid when serum (10%, vol/vol) is added to the medium but usually is not positive in the absence of serum.

peptone-yeast extract-glucose broth (3) supplemented with 1% (wt/vol) dehydrated brain heart infusion (Difco Laboratories, Detroit, Mich.). The DNA was isolated by using a variation of the Marmur procedure (7). DNA hybridization values were determined by using ¹²⁵I-labeled reference DNAs and the S1 nuclease assay procedure (7). Each reassociation vial contained 3 to 5 ng (about 30,000 cpm) of labeled DNA, 20 µg of unlabeled DNA, and 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) containing 1 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-ethanesulfonic acid) (pH 7.0). Each reassociation experiment was performed for 24 h at 65°C.

RESULTS AND DISCUSSION

"*Bacteroides* D28" and "*Bacteroides* D84" are anaerobic, fermentative, gram-negative bacilli that do not grow well in the presence of 20% bile and have phenotypic characteristics similar to those of *Prevotella* species (Tables 2 and 3). The

major cellular fatty acids of these organisms also are similar to those found in members of the genus *Prevotella* (Table 4), with anteiso-C_{15:0} fatty acid making up 30 to 36% of the total fatty acids detected. The G+C contents of the DNAs (45 and 47 mol%) are within the range found for other species in the genus *Prevotella* (5). These two closely related species exhibited negligible levels of DNA-DNA reassociation with *Prevotella nigrescens*, *Prevotella melaninogenica*, *Prevotella denticola*, and *Prevotella oris* (Table 1), species which are similar to them in cellular fatty acid content.

Because the characteristics of "*Bacteroides* D28" and "*Bacteroides* D84" are unlike the characteristics of previously described *Prevotella* species, we propose the following new species for these taxa: *Prevotella tannerae* and *Prevotella enoea*, respectively.

Description of *Prevotella tannerae* sp. nov. *Prevotella tannerae* (tan'ne.rae. N. L. gen. n. *tannerae*, of Tanner, in honor of Anne

TABLE 3. Differentiation of *P. tannerae*, *P. enoecca*, and *P. zoogloeoformans* from indole-negative *Prevotella* species^a

Species	Cellobiose acid	Esculin hydrolysis	Glycogen acid	Lactose acid	Sucrose acid	Meat digestion	Reference(s)
<i>P. disiens</i>	—	—	A	—	—	+	4
<i>P. bivia</i>	—	—	A	93	—	+	4
<i>P. enoecca</i>	—	38	A	A	—	—	
<i>P. tannerae</i>	—	14	81	81	31	35	
<i>P. oulora</i>	—	+	— ^b	A	A	—	16
<i>P. zoogloeoformans</i>	83	+	— ^c	17	67	—	
<i>P. melaninogenica</i>	15	84	A	A	A	—	17
<i>P. denticola</i>	22	+	A	A	A	15	17
<i>P. veroralis</i>	80	+	A	A	A	—	16, 17
<i>P. oralis</i>	A	+	A	A	A	—	5
<i>P. buccalis</i>	A	+	A	A	A	—	16
<i>P. oris</i>	A	+	A	A	A	—	6
<i>P. buccae</i>	A	+	A	A	A	—	6

^a —, 90 to 100% of the strains are negative; +, 90 to 100% of the strains are positive; A, 90 to 100% of the strains produce pH <5.7. The values are the percentages of strains that are positive.

^b Contrary to the original description (14), we have not been able to demonstrate that the type strain of *Prevotella oulora* lowers the pH of media containing glycogen unless serum (which contains amylase) is added to the medium. We realize that there has been confusion concerning the type strain of this species (11), but the tests for fermentation of glycogen were performed with the correct type strain obtained from the American Type Culture Collection in 1993 rather than with the strain that has been shown to be *Rikenella microfus* (11).

^c This reaction may be acid when serum is added to the medium.

C. R. Tanner, a United States microbiologist). The description below is based on studies of the type strain and 47 other isolates obtained from 37 other people. Cells of the type strain from peptone-yeast extract-glucose broth cultures are 0.3 µm wide by 0.7 to 8.0 µm long; filaments up to 14 µm long are observed. Cells occur in short chains and are not motile. Surface colonies on blood agar plates incubated for 2 days are 1 mm in diameter, circular, low convex, and translucent to transparent. On rabbit blood agar medium, 19% of the strains are black, 19% are tan to brown to red, and the rest are colorless after incubation for 5 days. Colonies in pure cultures of 2 of 41 strains are surrounded by small clear zones of hemolysis.

Broth cultures are cloudy with a smooth sediment. The terminal pH values of glucose broth cultures after incubation for 5 days are 4.8 to 5.3; 53% of the strains require the addition of 10% sterile serum to broth media for optimum growth and acid production.

The fermentation acids are succinic acid (2.6 ± 0.22 meq/100 ml of culture [mean ± standard error of the mean]), acetic acid (1.5 ± 0.11 meq/100 ml), formic acid (0.17 ± 0.03 meq/100 ml), isovaleric acid (0.15 ± 0.03 meq/100 ml), and isobutyric acid (0.03 ± 0.006 meq/100 ml). No hydrogen is produced.

Additional characteristics of the species are shown in Tables 2 and 4.

Isolated from the human gingival crevice (Table 5) (10).

The type strain is ATCC 51259 (= VPI N14B-15).

The G+C content of the DNA of the type strain is 45 mol%.

Phenotypically, *P. tannerae* can be differentiated from many saccharolytic *Prevotella* species by the inability of most strains to hydrolyze esculin (86% of the strains are negative) and by the inability of most strains to ferment sucrose (69% of the strains are negative) (Table 3). It differs from *Prevotella bivia* by not digesting meat and from the new species *P. enoecca* by its cellular fatty acid profile (iso-C_{15:0} fatty acid content) (Table 4).

Description of *Prevotella enoecca* sp. nov. *Prevotella enoecca* (e.noe'ca. Gr. adj. *enoikos*, inhabiting; N.L. fem. adj. *enoeca*, inhabiting, because the organism is an inhabitant of the gingival crevice). The description below is based on studies of the type strain and seven other isolates obtained from six other people. Cells of the type strain are 0.5 µm wide by 2.2 to 4.6 µm long; filaments up to 7.7 µm long are observed. Cells are not motile and occur in pairs and short chains. Surface colonies on rabbit blood agar plates incubated for 2 days are 1 to 2 mm in diameter, circular, entire, convex, transparent to translucent, and not hemolytic. No pigment develops on either hemolyzed or whole rabbit blood agar plates or streak tubes incubated for 10 days, and there are no hemolytic zones around colonies on blood agar plates. Broth cultures are turbid with a smooth sediment. The terminal pH values of glucose broth cultures incubated for 5 days are 4.8 to 5.4.

The fermentation acids are succinic acid (3.2 ± 0.72 meq/100 ml of culture [mean ± standard error of the mean]) and acetic acid (1.4 ± 0.19 meq/100 ml); sometimes a trace of

TABLE 4. Cellular fatty acid contents at greater than 5% of the total acids

Taxon	Fatty acid content (%)						
	iso-C _{14:0}	C _{14:0}	iso-C _{15:0}	anteiso-C _{15:0}	C _{16:0}	3OH C _{16:0}	3 OH iso-C _{17:0} or C _{18:2} dimethyl acetyl
<i>Prevotella</i> spp. ^a	5 (0–13)	2 (0–9)	10 (4–18)	35 (22–45)	9 (2–24)	5 (1–13)	3 (3–9)
<i>P. tannerae</i>	3	5	20	30	6	3	2
<i>P. enoecca</i>	3	4	8	36	17	10	2
<i>P. zoogloeoformans</i>	0	1	16	25	12	5	5

^a The values for *Prevotella* spp. are the means (and ranges) obtained for the following species and subspecies: *P. bivia*, *P. buccae*, *P. buccalis*, *P. corporis*, *P. denticola*, *P. disiens*, *P. heparinolytica*, *P. intermedia*, *P. loeschii*, *P. melaninogenica*, *P. nigrescens*, *P. oralis*, *P. oris*, *P. oulora*, *P. ruminicola* subsp. *ruminicola*, *P. veroralis*, and *P. zoogloeoformans*. Data were obtained from reference 8.

TABLE 5. Incidence of *P. tannerae*, *P. enoeca*, and *P. zoogloeiformans* in gingival crevice samples

Population	No. of people	Occurrence				Isolates			
		No. of samples ^a	% of samples positive for:			No. of isolates	% of isolates positive for:		
			<i>P. tannerae</i>	<i>P. enoeca</i>	<i>P. zoogloeiformans</i>		<i>P. tannerae</i>	<i>P. enoeca</i>	<i>P. zoogloeiformans</i>
Healthy	84	275	3.6	1.1	0	8,588	0.2	0.1	0
Experimental gingivitis									
Adults	4	96	1.0	0	0	3,038	0.03	0	0
Children	4	102	2.9	0	0	3,215	0.1	0	0
Naturally occurring gingivitis	11	22	0	0	0	680	0	0	0
Adult periodontitis	93	399	11.0	0.5	0.5	12,571	0.7	0.02	0.02
Rapidly progressive periodontitis	21	84	8.3	0	0	2,880	0.5	0	0
Juvenile periodontitis	21	73	6.9	1.4	0	2,335	0.4	0.04	0

^a Each sample contained about 30 isolates.

formic acid is present. No hydrogen is detected in the head-space gas of glucose broth cultures.

Additional characteristics of the species are shown in Tables 2 and 4.

Isolated from the gingival crevices of humans with healthy gingiva and periodontitis (Table 5).

The type strain is ATCC 51261^T (= VPI D194A-25A).

The G+C content of the DNA of the type strain is 47 mol%.

The lack of sucrose fermentation and the inability of *P. enoeca* to digest gelatin differentiate this species from many *Prevotella* species (Table 3). It can be differentiated from *P. tannerae* by its cellular fatty acid profile (iso-C_{15:0} fatty acid content) (Table 4).

Description of *Prevotella zoogloeiformans* (Weinberg et al. 1937) Shah and Collins 1990. The phenotypic characteristics of the type strain and four other strains of *P. zoogloeiformans* are shown in Table 2, and the characteristic cellular fatty acids of the species are shown in Table 4. Characteristics that differentiate *P. zoogloeiformans* from some other *Prevotella* species are shown in Table 3. Production of acid from cellobiose and usually from lactose and an inability to produce colonies with dark pigment on blood agar differentiate *P. zoogloeiformans* from *Prevotella intermedia* and *P. nigrescens*, the other indole-producing strains in the genus.

The G+C content of the DNA of the type strain, ATCC 33285, is 47 mol%.

The incidence of the species in periodontally healthy and diseased individuals is shown in Table 5.

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