Description of *Porphyromonas circumdentaria* sp. nov. and Reassignment of *Bacteroides salivossus* (Love, Johnson, Jones, and Calverley 1987) as *Porphyromonas* (Shah and Collins 1988) *salivosa* comb. nov.

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A new species, *Porphyromonas circumdentaria*, is proposed for pigmented, asaccharolytic strains that were isolated from the gingival margins or mouth-associated diseases of cats. This bacterium is an obligately anaerobic, gram-negative, brown- or black-pigmented, asaccharolytic, nonmotile, nonsporing, rod-shaped organism which does not grow in bile and has a guanine-plus-cytosine content of 40 to 42 mol%. It produces major amounts of acetic, butyric, and isovaleric acids and minor amounts of propionic, isobutyric, and phenylacetic acids as end products of metabolism in cooked meat medium. Glutamate and malate dehydrogenases are present, while 6-phosphogluconate and glucose-6-phosphate dehydrogenases are absent. The major cellular fatty acid is 13-methyltetradecanoic acid (iso-C_{15:0} acid). *P. circumdentaria* strains are catalase positive and produce ammonia, and colonies fluoresce under short-wavelength UV light. These strains do not hemagglutinate erythrocytes, exhibit trypsinlike activity, or produce chymotrypsin or α -fucosidase. They are heavily piliated and produce a capsule. The type strain is strain VPB 3329 (= NCTC 12469). *Bacteroides salivossus* (D. N. Love, J. L. Johnson, R. F. Jones, and A. Calverley, Int. J. Syst. Bacteriol. 37:307–309, 1987) is an obligately anaerobic, gram-negative, pigmented, asaccharolytic, nonmotile, rod-shaped organism which does not grow in bile and has a guanine-plus-cytosine content of 42 to 44 mol%. This organism produces major amounts of acetic, butyric, and phenylacetic acids and minor amounts of isobutyric and isovaleric acids as end products of metabolism in cooked meat medium. *B. salivossus* has been shown to contain 13-methyltetradecanoic acid (iso-C_{15:0} acid) as its major cellular fatty acid and to have glutamate and malate dehydrogenases, while 6-phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase are absent. Nomenclature changes published in 1988 (H. N. Shah and M. D. Collins, Int. J. Syst. Bacteriol. 38:128–131, 1988) for the asaccharolytic, pigmented, anaerobic, rod-shaped bacteria require reassignment of this organism to the genus *Porphyromonas* as *Porphyromonas salivosa* comb. nov.

Recently, members of the genus *Bacteroides* (6) have been reassigned to a number of new genera, and a proposal to restrict the genus *Bacteroides* to the highly fermentative organism *Bacteroides fragilis* and other closely related species has been published (20). Also, the moderately saccharolytic species that do not grow well in bile have been assigned to the genus *Prevotella* (21), while the asaccharolytic pigmented *Bacteroides* species have been placed in the genus *Porphyromonas* (19). These changes in nomenclature make reassignment of the asaccharolytic pigmented organism *Bacteroides salivossus* (9) a necessity. Also, during investigations of the asaccharolytic, pigmented, anaerobic bacteria isolated from oral cavities and mouth-associated diseases of cats (9), we described a group of organisms that were similar to *Porphyromonas asaccharolytica* but were of oral origin, were catalase positive, and exhibited only 4 to 11% DNA-DNA homology with *Porphyromonas asaccharolytica* ATCC 25260^T (T = type strain). With the data available at that time, no new species was described. However, since then, more strains have been isolated and additional characteristics have been determined, and there is enough information to justify description of a new species in the genus *Porphyromonas* (19).

MATERIALS AND METHODS

Bacterial strains. Twelve asaccharolytic strains were examined in this study. In a previous study strains NCTC 11632^T (= Veterinary Pathology and Bacteriology Collection [VPB] 157), VPB 3313, VPB 3420, and VPB 3444 were considered *B. salivossus* strains, while strains VPB 3325, VPB 3329^T, and VPB 3497 were assigned to group 2 (9). All of the strains were isolated from subcutaneous fight wound abscesses or pyothorax of cats or from normal feline gingival margins. Additional asaccharolytic strains (strains VPB 4480, VPB 4503, VPB 4505, and VPB 4506), which were isolated from diseased or normal gingivae of cats (10, 13), and strain VPB 4097, which was isolated from gingival plaque of a jaguar (*Panthera onca*) and was originally isolated by D. Mayrand, Laval University, Quebec, Canada, were also included in this study. For the allozyme and cellular fatty acid studies, the strains listed in Table 1 were included as representatives of the genera *Porphyromonas*, *Prevotella*, and *Bacteroides*. For the dot blot hybridization studies, we used the feline strains mentioned above, the type strains of the *Porphyromonas* and *Bacteroides* species listed in Table 1, *Prevotella heparinolytica* ATCC 25285^T, other members of the genera *Prevotella*, *Fusobacterium*, and *Bacteroides*, and *Eubacterium fossor* (a total of 79 strains [7] were used).

Growth conditions and biochemical methods. The general methods which we used for growth and biochemical characterization have been described previously (3, 9, 11, 12). The

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TABLE 1. Distribution of fatty acids in *Porphyromonas*, *Prevotella*, and *Bacteroides* strains as determined by gas-liquid chromatography after extraction of methylated whole cells

Species	Strain	Relative % of ^a :										
		C _{14:0}	iso-C _{15:0}	anteiso-C _{15:0}	C _{15:0}	C _{16:0}	C _{18:0}	ECL 12:6	ECL 12:9	ECL 13:6	ECL 17:5	ECL 18:15
<i>Porphyromonas gingivalis</i>	ATCC 33277 ^T	2.5	29.3	9.2	0	9.7	1.2	2.0	1.7	11.2	3.8	12.8
<i>Porphyromonas salivosa</i>	NCTC 11632 ^T	2.3	41.9	9.8	0	8.0	1.5	3.9	2.6	17.2	1.3	2.9
	VPB 3313	2.7	40.4	8.3	0	8.6	2.1	3.4	1.6	4.7	4.4	10.5
	VPB 3444	1.8	41.3	7.5	0	6.9	2.0	4.2	1.0	6.0	2.9	11.7
<i>Porphyromonas circumdentaria</i>	NCTC 12469 ^T	1.4	55.7	1.8	0	10.9	1.5	0.4	0	3.2	0.7	9.8
	VPB 3325	1.9	45.5	2.8	0	7.6	1.3	0.5	0.5	6.6	2.0	21.4
	VPB 3497	2.4	48.2	2.3	0	6.9	1.0	0.6	0.6	6.8	2.1	21.8
<i>Porphyromonas endodontalis</i>	ATCC 35406 ^T	0	31.5	2.6	0	6.6	1.1	0.2	0.4	12.5	1.1	29.0
<i>Prevotella buccae</i>	ATCC 33574 ^T	0.7	5.9	30.2	1.1	6.9	2.2	0.7	3.2	10.6	3.2	5.8
<i>Prevotella oralis</i>	ATCC 33269 ^T	1.2	6.3	20.5	2.2	18.1	2.1	0.5	3.4	4.4	7.2	5.6
<i>Prevotella bivia</i>	ATCC 29303 ^T	1.5	4.1	19.7	6.8	4.7	2.7	2.6	2.4	33.2	0	3.6
<i>Bacteroides fragilis</i>	ATCC 25285 ^T	0	10.4	41.4	5.9	10.3	0	0	0	0	4.1	16.8
	VPB 4044	0.5	13.6	37.3	5.5	12.9	0.7	0	0.2	0	4.8	13.1
	VPB 4045	0	12.5	39.8	5.5	9.8	1.5	0	0	0	3.7	12.7
<i>Bacteroides tectum</i>	NCTC 11853 ^T	0	11.6	29.0	4.0	5.6	0	0	0	15.7	7.5	14.0
	VPB 3312	0	13.0	29.1	7.2	5.5	1.2	0	1.4	10.7	3.6	18.7

^a The relative amount of each fatty acid is expressed as the area of its peak as a percentage of the total area of the peaks of all of the fatty acids detected. ECL, equivalent chain length.

cells used for cellular fatty acid and allozyme assays were grown anaerobically for 3 days on 5% defibrinated sheep blood agar plates (blood agar base no. 2 [Oxoid Ltd., Basingstoke, England]) supplemented with additional hemin-menadione, formate-fumarate (5), and 1.5% (wt/vol) proteose peptone (Difco Laboratories, Detroit, Mich.) in the presence of a streak of *Staphylococcus epidermidis*.

DNA hybridization assays. The levels of DNA-DNA homology for strains VPB 4480, VPB 4503, VPB 4505, VPB 4506, and VPB 4097 and *Porphyromonas endodontalis* ATCC 35406^T were determined by dot blot hybridization and digoxigenin labeling, using whole-chromosome probes from strain VPB 3329^T (as a representative of group 2), *Porphyromonas gingivalis* ATCC 33277^T, *B. salivosa* NCTC 11632^T, and strain VPB 3313 (7). Included as sensitivity controls were the 79 strains described above which represented members of genera *Porphyromonas*, *Prevotella*, *Bacteroides*, *Fusobacterium*, and *Eubacterium*. Each probe was tested against high-molecular-weight isolated DNA preparations obtained from the 79 strains and/or DNA from lysed cell preparations of each of the asaccharolytic pigmented strains and representatives of the other genera mentioned above (51 strains).

Allozyme electrophoresis. Cells grown for 3 days were harvested from plates by using a bent glass rod and were suspended in sterile water. Electrophoresis on Cellogel (Chemetron, Milan, Italy) was carried out by using supernatants from sonicated cell preparations (Wellcome tube 5 density) and the method of Richardson et al. (17) to determine the presence of characteristic metabolic pathway enzymes. End points of reactions were read after no more than 15 min.

Preparation and analysis of fatty acids of whole bacterial cells. Cells were harvested from plates after 3 days by using a glass rod (1) and were treated immediately essentially as described by Ghanem et al. (4). A model HP 5890 series II gas chromatograph and a model HP 7673 injector (Hewlett-Packard) were used for detection. The organic phase was chromatographed on a fused silica capillary column (type 19091J-102; Hewlett-Packard). The column running condi-

tions which we used have been described previously (4). For fatty acid identification, a bacterial acid methyl ester CP mixture (catalog no. 4-7080; Supelco, Bellefonte, Pa.) was chromatographed at the beginning of each run and after each four samples. The total area of each compound and the area percentage of each peak were determined by using a computer program. Equivalent chain lengths were calculated as described by the MIS system (Microbial ID, Inc.).

RESULTS AND DISCUSSION

Tables 1 through 3 show the results of our allozyme electrophoresis (Table 2), cellular fatty acid analyses (Table 1), and ammonia production (Table 3) for group 2 and *B. salivosa* strains along with results for representative strains belonging to the genera *Porphyromonas*, *Prevotella*, and *Bacteroides* and having the characteristics defined by Shah and Collins (19–21) for these genera.

Members of group 2, including strains VPB 3325, VPB 3329^T, VPB 3497, VPB 4097, VPB 4480, VPB 4503, VPB 4505, and VPB 4506, are asaccharolytic, pigmented, anaerobic, rod-shaped organisms which, as Tables 1 and 2 show (as represented by the type strain and strains VPB 3325 and VPB 3497), produced major amounts of acetic, butyric, and isovaleric acids, contained malate and glutamate dehydrogenases, and lacked 6-phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase as metabolic enzymes. These strains had iso-C_{15:0} as their major cellular fatty acid (Table 1) and a DNA guanine-plus-cytosine ratio of 40 to 42 mol%. Levels of DNA-DNA homology for strains VPB 3325, VPB 3329^T, and VPB 3497 have been published previously (9). Dot blot hybridization studies showed that strain VPB 3329^T did not react with *Porphyromonas endodontalis* ATCC 35406^T or with any strain other than strains VPB 3325, VPB 3329^T, VPB 3497, VPB 4097, VPB 4480, VPB 4503, VPB 4505, and VPB 4506. Thus, these strains form a distinct species in the genus *Porphyromonas*, the description of which is given below.

Description of *Porphyromonas circumdentaria* sp. nov. *Porphyromonas circumdentaria* (cir.cum.den.ta'ri.a. L. adj. cir-

TABLE 2. Characteristics that are useful for distinguishing members of the genus *Porphyromonas* from *Prevotella* and *Bacteroides* species^a

Organism	Metabolism ^b	End products from CMG ^c	Presence of ^d :				Major cellular fatty acid	Guanine-plus-cytosine content (mol%)
			MDH	GDH	G6PD	6PGD		
<i>Porphyromonas gingivalis</i> ATCC 33277 ^T	NF	A, B, IV, S, PA	+	+	—	—	iso-C _{15:0}	49
<i>Porphyromonas salivosa</i> NCTC 11632 ^T	NF	A, B, ib, iv, PA	+	+	—	—	iso-C _{15:0}	42–44
<i>Porphyromonas circumdentaria</i> NCTC 12469 ^T	NF	A, B, ib, iv, pa	+	+	—	—	iso-C _{15:0}	40–42
<i>Porphyromonas endodontalis</i> ATCC 35406 ^T	NF	a, b, ib, iv	+	+	—	—	iso-C _{15:0}	49–51 ^e
<i>Porphyromonas asaccharolytica</i> ATCC 25260 ^T	NF	A, P, IB, B, IV, pa	+	+	—	—	iso-C _{15:0}	48
<i>Prevotella buccae</i> ATCC 33574 ^T	MF	A, p, ib, iv, S	+	+	—	—	anteiso-C _{15:0}	42–43
<i>Bacteroides fragilis</i> ATCC 25285 ^T	F	A, p, S	+	+	+	+	anteiso-C _{15:0}	42
<i>Bacteroides tectum</i> NCTC 11853 ^T	F	A, p, S	+	+	+	+	anteiso-C _{15:0}	48

^a The table format was taken from reference 19; most of the data were derived from this study and references 8 and 9.

^b F, fermentative; NF, nonfermentative; MF, moderately fermentative.

^c CMG, cooked meat-glucose medium; A and a, acetic acid; B and b, butyric acid; IB and ib, isobutyric acid; IV and iv, isovaleric acid; P and p, propionic acid; S, succinic acid; PA and pa, phenylacetic acid. Uppercase letters indicate major amounts (≥ 10 $\mu\text{mol/ml}$), and lowercase letters indicate minor amounts (< 10 $\mu\text{mol/ml}$).

^d MDH, malate dehydrogenase; GDH, glutamate dehydrogenase; G6PD, glucose-6-phosphate dehydrogenase; 6PGD, 6-phosphogluconate dehydrogenase.

^e Data from references 19, 22, and 23.

cum, around, about; L. adj. *dentaria*, pertaining to the teeth; L. adj. *circumdentaria*, referring to the isolation of organisms from the vicinity of the teeth). Cells are anaerobic, nonsporing, nonmotile, gram-negative rods or coccoid forms. In cooked meat-carbohydrate medium (11) and on sheep blood agar plates, cells are 0.3 to 0.6 by 0.8 to 1.5 μm and occur singly and in groups; occasionally filaments up to 10 μm long are present. On sheep blood agar plates, surface colonies after 72 h are 1 to 2 mm in diameter, circular, entire, dome shaped, opaque, and greenish brown, changing to creamy brown by day 7. By day 14, colonies are brown to black. Both colony size and pigment formation are enhanced by growth in the presence of *S. epidermidis*. Colonies exhibit a brick red fluorescence (at 265 nm) after 24 h, which persists up to day 7. After incubation at 37°C for 48 h in prereduced broth, cultures exhibit a heavy uniform gelatinous to ropy turbidity. Vitamin K and hemin are required for growth. After incubation for 5 days, the pH values of media contain-

ing a fermentable carbohydrate generally range from 6.3 to 6.5 in all of the media tested. Carbohydrate fermentation is not detected in media containing adonitol, cellobiose, fructose, glucose, glycogen, inositol, lactose, maltose, mannitol, rhamnose, salicin, starch, sucrose, trehalose, and xylose.

The major fermentation products in cooked meat-carbohydrate medium are acetic acid (average concentration, 24.5 $\mu\text{mol/ml}$), butyric acid (average concentration, 35.5 $\mu\text{mol/ml}$), and isovaleric acid (average concentration, 21.6 $\mu\text{mol/ml}$). Smaller quantities of propionic acid (average concentration, 1.0 $\mu\text{mol/ml}$), isobutyric acid (average concentration, 8.5 $\mu\text{mol/ml}$), succinic acid (average concentration, 8.4 $\mu\text{mol/ml}$), and phenylacetic acid (average concentration, 6.7 $\mu\text{g/ml}$) are produced. Neither lactate nor pyruvate is utilized, and threonine is not converted to propionate. Strains are catalase positive, produce ammonia, liquefy gelatin, and are indole positive. Strains produce acid and alkaline phosphatase and small amounts of naphthol as β -1-phosphate.

FIG. 1. Electron micrograph of a thin section of *Porphyromonas circumdentaria* NCTC 12469^T, showing numerous extracellular vesicles (V). The preparation was stained with ruthenium red (3). Bar = 100 nm.

TABLE 3. Comparison of major distinguishing characteristics of *Porphyromonas circumdentaria* sp. nov., *Porphyromonas salivosa* comb. nov., and other members of the genus *Porphyromonas*

Organism	Catalase	Ammonia	Trypsin	Chymotrypsin	Phenylacetic acid	Hemagglutination	Fucosidase	Glucosaminidase	Butyryl esterase	Capryl esterase
<i>Porphyromonas circumdentaria</i>	+	+	+	+	+	+	+	+	+	+
<i>Porphyromonas salivosa</i>	+	+	+	+	+	+	+	+	+	+
<i>Porphyromonas gingivalis</i> ATCC 33277 ^T	+	+	+	+	+	+	+	+	+	+
<i>Porphyromonas asaccharolytica</i> ATCC 25260 ^T	+	ND	+	+	+	+	+	+	+	+
<i>Porphyromonas endodontalis</i> ATCC 35406 ^T	+	+	+	+	+	+	+	+	+	+

^a +, activity; -, no activity; ND, not determined.

Cells do not hemagglutinate sheep, horse, or pig erythrocytes or produce trypsinlike activity. Nitrate is not reduced, urea is not split, and esculin is not hydrolyzed. Malate dehydrogenase and glutamate dehydrogenase are present; 6-phosphogluconate dehydrogenase and glucose 6-phosphate dehydrogenase are absent. The cellular fatty acids consist predominantly of 13-methyltetradecanoic acid (iso-C_{15:0} acid), and there are smaller amounts of the acids shown in Table 1. In transmission electron micrographs thin sections of cells reveal a typical gram-negative cell wall, and large numbers of long (up to 0.4-μm), thin, peritrichous fimbriae are observed with negative stains (3). Outer membrane vesicles are numerous at the cell surface and in the medium (Fig. 1), and a capsule or slime layer up to 0.5 μm thick is present in preparations from plates and from broth cultures (3).

All of the strains were isolated from soft-tissue infections (abscesses and empyemas), gingival margins, and gingival plaques of felines. The guanine-plus-cytosine content of the DNA is 40 to 42 mol% as determined by the thermal denaturation method (14). Members of the species exhibit an average intraspecies DNA-DNA homology level of 98%, and strain VPB 3325 exhibited average DNA-DNA homology levels of 9, 16, 11, and 4% with *Porphyromonas gingivalis* ATCC 33277^T, *Porphyromonas salivosa* comb. nov., *Porphyromonas gingivalis* subsp. *felis*, and *Porphyromonas asaccharolytica* ATCC 25269^T, respectively, and did not react in dot blot hybridization assays with *Porphyromonas endodontalis* ATCC 35406^T or the representatives of the genera *Bacteroides*, *Prevotella*, *Fusobacterium*, and *Eubacterium* included in the assays.

The type strain is strain NCTC 12469 (= VPB 3329).

Characteristics which distinguish *Porphyromonas circumdentaria* from other members of the genus *Porphyromonas* are shown in Table 3. These characteristics include the production of catalase (which distinguishes it from *Porphyromonas gingivalis*, *Porphyromonas asaccharolytica*, and *Porphyromonas endodontalis*), the production of ammonia and phenylacetic acid (which distinguishes it from *Porphyromonas endodontalis*), the lack of trypsinlike activity (which distinguishes it from *Porphyromonas gingivalis* and *Porphyromonas salivosa*), the lack of chymotrypsin activity (which distinguishes it from *Porphyromonas salivosa*), and the lack of β-glucosaminidase and butyryl and capryl esterase activities (which distinguishes it from all other members of the genus *Porphyromonas* except *Porphyromonas asaccharolytica*). Although strains elaborate numerous fimbriae, they do not hemagglutinate sheep erythrocytes, which is a characteristic of *Porphyromonas gingivalis*. The new species differs from *Porphyromonas asaccharolytica* in not producing α-fucosidase. The major features which distinguish *Porphyromonas circumdentaria* and other members of the genus *Porphyromonas* from the genera *Prevotella* and *Bacteroides* are shown in Table 2 and have been described previously (2, 15, 16, 18-21).

In addition to previously published characteristics, our studies on the asaccharolytic, pigmented organism *B. salivosa* (3, 9) have shown that this species contains malate and glutamate dehydrogenases but lacks 6-phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase (Table 2) and that the major cellular fatty acid of this organism is iso-C_{15:0} acid (Table 1). Thus, the combination of the end products resulting from metabolism of cooked meat-glucose or cooked meat-carbohydrate medium (major amounts of acetic, butyric, and phenylacetic acids and smaller quantities of isobutyric and isovaleric acids), a DNA

guanine-plus-cytosine content of 42 to 44 mol%, the set of four metabolic enzymes, and the cellular fatty acid composition, along with nonfermentation of sugars and pigment production, places *B. salivosus* in the genus *Porphyromonas*; an amended description of this taxon is given below.

Description of *Porphyromonas salivosa* (Love, Jones and Calverley 1987) comb. nov. A description of *Porphyromonas salivosa* is given in references 3 and 9, and the type strain is strain NCTC 11632 (= VPB 157). The members of this species exhibit intraspecies DNA-DNA homology levels of 96 to 100%, and *B. salivosus* NCTC 11632^T exhibits average DNA-DNA homology levels of 20, 9, 12, and 6% with *Porphyromonas circumdentaria* sp. nov., *Porphyromonas gingivalis* subsp. *felis*, strain ATCC 33277^T, and *Porphyromonas asaccharolytica* ATCC 25260^T, respectively (9). In addition, strains NCTC 11632^T, VPB 3313, VPB 3420, and VPB 3444 contain malate and glutamate dehydrogenases but lack 6-phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase, and they contain iso-C_{15:0} acid as the major cellular fatty acid and the other acids shown in Table 1.

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