Bacteroides intermedius comb. nov. and Descriptions of *Bacteroides corporis* sp. nov. and *Bacteroides levii* sp. nov.

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Deoxyribonucleic acid homology studies of 66 strains formerly classified as *Bacteroides melaninogenicus* subsp. *intermedius* showed two distinct groups with negligible intergroup homology. The larger group, with homology to the type strain of *B. melaninogenicus* subsp. *intermedius*, is designated *Bacteroides intermedius*, and the description of the taxon is emended. The other deoxyribonucleic acid homology group, which includes strains that differ from *B. intermedius* in not fermenting sucrose or producing indole, is designated *Bacteroides corporis* sp. nov.; ATCC 33547^T (= VPI 9342) is the type strain. Strains of *B. intermedius* and *B. corporis* do not ferment lactose, as strains of *B. melaninogenicus*, *Bacteroides loescheii*, and *Bacteroides denticola* do. Strain ATCC 29147^T is designated the type strain of *Bacteroides levii* sp. nov. This organism is a pigmented bacteroides whose strains have been isolated from cattle and include the LEV strain (previously called *B. melaninogenicus* or "*B. melaninogenicus* subsp. *levii*"). Phenotypic characteristics useful in differentiating among nine species that formerly have been included in or identified as *B. melaninogenicus* are listed.

Holdeman and Moore (10, 11) recognized two subspecies of saccharoclastic pigmented bacteroides, *Bacteroides melaninogenicus* subsp. *melaninogenicus* and *Bacteroides melaninogenicus* subsp. *intermedius*. Because of the phenotypic heterogeneity observed among the saccharoclastic strains of *B. melaninogenicus*, we initiated a study of the deoxyribonucleic acid (DNA) homologies of strains of fermentative bacteroides classified as *B. melaninogenicus*.

In 1980 one of us (J.L.J.) reported that there were three distinct homology groups among 19 strains previously identified as *B. melaninogenicus* subsp. *melaninogenicus* and three homology groups among 56 strains previously identified as *B. melaninogenicus* subsp. *intermedius* and gave the phenotypic characteristics useful in differentiating among these homology groups (12). The three major homology groups of strains previously identified as *B. melaninogenicus* subsp. *melaninogenicus* have been designated *B. melaninogenicus*, *Bacteroides loescheii* sp. nov. (9a), and *Bacteroides denticola* (37, 40).

Studies of 66 strains previously characterized as *B. melaninogenicus* subsp. *intermedius* (8, 11) are described in this paper. The names proposed here for these homology groups have been published previously (L. V. Holdeman, J. L. Johnson, and W. E. C. Moore, J. Dent. Res. 60[Special Issue A]:414, Abstr. 415, 1981).

In 1977 "*Bacteroides melaninogenicus* subsp. *levii*" was described, and the LEV strain (17)

was designated the type strain (8). However, this taxon was omitted from the Approved Lists of Bacterial Names (39). This pigmented bacteroides is slightly saccharoclastic and proteolytic and produces acetic, butyric, isobutyric, and isovaleric acids. This subspecies is phenotypically unlike other subspecies of *B. melaninogenicus* (8). On the basis of phenotypic characteristics and the guanine-plus-cytosine (G+C) content of the DNA, reclassification of *B. melaninogenicus* subsp. *levii* has been suggested (46). The taxonomic status of this subspecies is evaluated.

MATERIALS AND METHODS

Strains. The sources of the strains used in the DNA homology studies are shown in Tables 1 and 2. In addition, homology was tested with the DNAs of strain VPI 6822 (parent strain of American Type Culture Collection [ATCC] 29303^T, type strain of *Bacteroides bivius*) and strain VPI 8057 (parent strain of ATCC 29426^T, type strain of *Bacteroides disiens*), which are similar phenotypically to strains of *B. melaninogenicus* subsp. *intermedius*.

Methods of characterization. Phenotypic reactions of the strains were studied by using prereduced media and methods described previously (8). The polyacrylamide gel electrophoretic patterns of soluble cellular proteins were determined as described by Moore et al. (32).

DNA studies. The organisms were grown in a medium prepared as described previously (2); this medium contained mineral salts, 1% peptidase, 0.5% yeast

TABLE 1. Strains having DNA homology with *B. intermedius* strains VPI 4197 and VPI 8944

VPI no.	% DNA homology with reference DNA of:		Strain derivation and other information ^a
	VPI 4197	VPI 8944	
Group I			
VPI 4197	100 ^b	36	Finegold B422; empyema; parent strain of ATCC 25611 ^T , the type strain of <i>B. intermedius</i> (39) ^c
VPI 13043	87	55	Socransky S-98
VPI 9849	84		Lambe 275-70A, serogroup C immunizing strain (15)
VPI 13042	81	46	Socransky S-97
VPI 13048	80	45	Socransky S-108
VPI D10A-24	78	38	Tooth surface, severe periodontitis
VPI 13044	77	42	Socransky S-101
VPI D16B-12A	77	42	Gingival sulcus, moderate periodontitis; same person as D16A-32; same sample as VPI D16B-28A
VPI 12510	76	41	Williams 3b/L-75
VPI 12528	76	39	Socransky 581
VPI 13024	76	47	Socransky S-14
VPI 13029	76	50	Socransky S-22
VPI D11B-5	76	48	Gingival sulcus, moderate periodontitis
VPI D16A-32	76	39	Tooth surface, moderate periodontitis; same person as VPI D16B-12A
VPI D1C-2	74	46	Gingival sulcus, moderate periodontitis
VPI D10D-1	74	40	Gingival sulcus, severe periodontitis; same person as VPI D10A-24
VPI D10B-7	73	40	Gingival sulcus, severe periodontitis; same person as VPI D10D-1
VPI D10D-18	73	37	Gingival sulcus, severe periodontitis; same site as VPI D10D-1; same person as VPI D10A-24
VPI 6092	72		Lambe 935F; abdominal wound
VPI 13049	72		Socransky S-109
VPI D2B-42	72	40	Gingival sulcus, moderate periodontitis
VPI D13D-11	72	39	Gingival sulcus, moderate periodontitis
VPI 9123	71	39	Wisconsin Department of Hygiene B-12; VPI 4200; Finegold B485; maxillary sinus drainage
VPI 9182	71	37	Throat
VPI 13028	71	41	Socransky S-20
VPI D1C-4	71	45	Gingival sulcus, moderate periodontitis; same sample as VPI D1C-2
VPI D16B-28A	71	38	Gingival sulcus, moderate periodontitis; same sample as VPI D16B-12A
VPI 13039	69	41	Socransky S-90
VPI D19B-24	69	40	Gingival sulcus, juvenile periodontitis
VPI 12507	68		Williams B9-74
VPI 12527	68	48	Socransky 576
VPI 13032	68	42	Socransky S-47
VPI D25D-29	68	46	Gingival sulcus, juvenile periodontitis
VPI D28M-18	68	46	Gingival sulcus, severe periodontitis; same person as VPI D28C-1
VPI D30A-17	68	42	Gingival sulcus, advanced periodontitis
VPI 13038	66	46	Socransky S-89
VPI D12B-20	66	41	Gingival sulcus, moderate periodontitis
VPI 13037	65	41	Socransky S-88
VPI 9042	63	50	Hill (CDC 1976); Lambe 730-74, serogroup C (16)
VPI 12987	62	32	Slots 15-7K-2
VPI D28C-1	62	33	Tooth surface, severe periodontitis
VPI 13033	61		Socransky S-51
VPI D33B-8	59	40	Gingival crevice, healthy gingiva
Group II			
VPI 8944	34	100 ^b	NCTC 9336; Vincent's angina; parent strain of ATCC 33563; Lambe 729-74, serogroup C (15)
VPI 12526	42	99	Socransky 377; oral
VPI 13025	39	93	Socransky S-15
VPI 12516	41	90	Williams JPI-77
VPI 12515	41	88	Williams LH-107; dental

TABLE 1—Continued

VPI no.	% DNA homology with reference DNA of:		Strain derivation and other information ^a
	VPI 4197	VPI 8944	
VPI D25B-1	37	87	Gingival sulcus, juvenile periodontitis
VPI D16B-7	38	86	Gingival sulcus, moderate periodontitis
VPI 4203	37	85	Finegold B515; laryngotomy; Lambe 724-74, serogroup C (16); parent strain of ATCC 25261
VPI 8946	35	84	NCTC 9338; empyema; Lambe 206-74, serogroup C (15)
VPI 11328	31	83	Williams B-18; gingiva
VPI D27C-15	31	81	Tooth surface, severe periodontitis
VPI D28D-12	29	83	Gingival sulcus, periodontitis
VPI 11335	34	80	Carlsson G11a-d2; root canal
VPI 9854	36	79	Lambe 297-74G, Lambe serogroup C (16)
VPI D22B-23	33	73	Gingival sulcus, juvenile periodontitis
VPI 9145		62	Williams T 584/72; plaque; Lambe 731-74, serogroup C (16)

^a ATCC, American Type Culture Collection, Rockville, Md.; Carlsson, J. Carlsson, University of Umea, Umea, Sweden; CDC, Centers for Disease Control, Atlanta, Ga.; Finegold, S. M. Finegold, Wadsworth Veterans Administration Hospital, Los Angeles, Calif.; Hill, G. Hill, Duke University, Durham, N.C.; Lambe, Dwight W. Lambe, Jr., Department of Microbiology, East Tennessee State University, Johnson City; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, England; Slots, J. Slots, State University of New York, Buffalo; Socransky, S. S. Socransky, Forsyth Dental Center, Boston, Mass.; Williams, R. A. D. Williams, The London Hospital Medical College, London, England. Unless otherwise designated, strains were isolated at the Anaerobe Laboratory, Virginia Polytechnic Institute and State University. All isolates were from humans. Tooth surface samples were taken coronal to the gingival margin of affected sites after superficial cleaning of the surface with sterile toothpicks. The advanced periodontitis sample was from an individual (ca. 50 years old) with advanced generalized periodontitis. Juvenile periodontitis samples were from individuals 11 to 25 years old with severe destruction essentially limited to permanent first molars and incisors. Moderate periodontitis samples were from individuals 35 to 55 years old with chronic periodontitis generalized to all four quadrants, pocket depths of 5 to 7 mm, and no history of rapid loss of attachment. Severe periodontitis samples were from persons 18 to 30 years old with a loss of attachment of 5 mm or more on any surface of eight or more teeth not limited to first molars or incisors.

^b Homology with reference DNA was normalized to 100%.

^c The numbers in parentheses are reference numbers.

extract, 1% dehydrated brain heart infusion broth (Difco Laboratories, Detroit, Mich.), 1% glucose, 0.03% cysteine, 0.03% sodium formaldehyde sulfoxalate, 0.01% heme, and 0.05 M potassium phosphate buffer (pH 7.0). At the time of inoculation, 10 ml of sterile 10% NaHCO₃ was added to each 1 liter of medium. Cultures were grown and DNA was harvested as described previously (9a). High-molecular-weight DNA (for nick-translation labeling) was isolated by the method of Marmur (28), and other DNA preparations were isolated by an hydroxyapatite procedure (13). Thermal melting points were used to determine the G+C contents of the DNA preparations (13). Labeled DNA was prepared in vitro either by nick translation (13) or by iodination. Fragmented denatured DNA was labeled with ¹²⁵I by using a variation of the thallium chloride method of Tereba and McCarthy (44). DNA homology values were determined by using an S1 nuclease procedure (13). The reassociation vials were incubated for 24 h at 62°C. We have described other details of the methods used elsewhere (9a).

RESULTS AND DISCUSSION

Of the 66 isolates studied, 42 had relatively high homology with reference DNA from *B. melaninogenicus* subsp. *intermedius* strain VPI 4197, and 15 had high homology with reference

DNA from strain VPI 8944 (Table 1). Homologous and heterologous values for individual strains are given in Table 1, and average intra-group homology values are shown in Table 3. Although the reciprocal homology values for the two reference strains were nearly equal (34 and 36% [Table 1]), there was a 6% difference in the average heterologous values (36 and 42% [Table 3]). In general, lower values were obtained with reference DNA from strain VPI 4197 than with reference DNA from strain VPI 8944. Strains in the VPI 4197 and VPI 8944 homology groups produced different polyacrylamide gel electrophoresis patterns (Fig. 1), but none of the cultural phenotypic traits which we tested clearly differentiated between the two homology groups.

The intergroup homology observed between strains in the VPI 4197 and VPI 8944 homology groups was similar to that previously reported (9a) between *B. melaninogenicus* subgroups (represented by VPI 2381 [= ATCC 25845^T] and VPI 9343) or between *B. loescheii* subgroups (represented by ATCC 15930^T and VPI D1C-20).

We believe that the VPI 4197 and VPI 8944 homology groups are sufficiently distinct to be

TABLE 2. Strains in the *B. corporis* VPI 9342 homology group and their sources

VPI no.	% DNA homology with reference DNA from VPI 9342	Strain derivation and other information ^a
VPI 9342	100 ^b	Lambe 532-70A; cervical swab; Lambe serogroup C-1 (16); parent strain of ATCC 33547 ^T , the proposed type strain ^c
VPI 6018	94	Lambe 867-70D; perineal incision
VPI 8667	88	University of Virginia School of Medicine; nasal septum; Lambe 728-24, serogroup C-1 (16)
VPI 10443	85	Lambe 1701-74E; wound
VPI 8558	81	Lambe 237-73C; rectal abscess
VPI 11329	76	Williams 2B (JK2B); urethra
VPI 12517	64	Williams 532-70A

^a See Table 1, footnote a.^b Homology with reference DNA was normalized to 100%.^c The numbers in parentheses are reference numbers.

separate species, but at this time we are not proposing that they be classified as distinct species, principally because we know of no cultural phenotypic characteristic that is useful for differentiating between them.

Strain VPI 4197 is the parent strain of ATCC 25611^T, the type strain of *B. melaninogenicus* subsp. *intermedius*. Because the related subgroups (i.e., VPI 4197 and VPI 8944 subgroups) have negligible DNA homology with *B. melaninogenicus*, *B. loescheii* (9a), or *B. denticola* (37, 40) or with phenotypically similar species that do not form colonies with dark pigment (Table 4), we propose that this taxon, *B. melaninogenicus* subsp. *intermedius*, be elevated to species rank as *Bacteroides intermedius* comb. nov., with ATCC 25611^T as the type strain.

The phenotypic characteristics of *B. intermedius* are given in Tables 5 and 6 and in the species description below. Polyacrylamide gel electrophoresis patterns are shown in Fig. 1.

Because there are several distinct taxa among the strains that previously have been classified as *B. melaninogenicus* subsp. *intermedius*, previous research concerning the characteristics of this taxon is difficult to interpret unless specific strains were identified and can be related to proposed taxa. The relationships of some strains

to the homology groups described here are shown in Table 7. Information from many previously published reports has been included in the species descriptions given below.

***Bacteroides intermedius*: emended description.** *Bacteroides intermedius* (Holdeman and Moore) comb. nov. (in.ter.me'di.us. L. adj. *intermedius* intermediate; basionym, *Bacteroides melaninogenicus* subsp. *intermedius* Holdeman and Moore 1970, 33).

FIG. 1. Polyacrylamide gel electrophoresis patterns of strains of *B. intermedius* and *B. corporis*. Lanes 11 to 13, *B. intermedius* VPI 4197 group (lane 11, VPI 4197 [= ATCC 25611^T]; lane 12, VPI D16A-32; lane 13, VPI 9182); lanes 14 and 15, *B. intermedius* VPI 8944 group (lane 14, VPI 8944 [= NCTC 9336]; lane 15, VPI 11328); lanes 16 to 18, *B. corporis* (lane 16, VPI 9342 [= ATCC 33547^T]; lane 17, VPI 10443; lane 18, VPI 8558); lane 19, *Streptococcus faecalis* U4-20 reference culture.

TABLE 3. Reciprocal homology values for strains in the *B. intermedius* VPI 4197 and VPI 8944 groups

DNA homology group	Avg homology (%) with reference DNAs from: ^a	
	VPI 4197 group strains	VPI 8944 group strains
VPI 4197	72 ± 6 ^b	42 ± 5
VPI 8944	36 ± 4	84 ± 8

^a All values in Table 1 except the homologous values were used for these calculations.^b Average ± standard deviation.

TABLE 4. DNA homology among some saccharoclastic bacteroides

Taxon	% Homology with reference DNA from strains:		
	VPI 4197	VPI 8944	VPI 9342
<i>B. intermedius</i> VPI 4197 (= ATCC 25611 ^T) ^a	100 ^b	36	5
<i>B. intermedius</i> VPI 8944 (= ATCC 33563)	34	100 ^b	5
<i>B. corporis</i> VPI 9342 (= ATCC 33547 ^T)	9	13	100 ^b
<i>B. bivius</i> VPI 6822 (= ATCC 29303 ^T)	8	22	9
<i>B. disiens</i> VPI 8057 (= ATCC 29426 ^T)	25	28	20

^a A superscript T indicates a type or proposed type strain.

^b Homology with reference DNA was normalized to 100%.

Most cells are 0.4 to 0.7 μm wide by 1.5 to 2 μm long (Fig. 2). Some cells are up to 12 μm long.

Surface colonies on rabbit blood agar are circular, entire, low convex, 0.5 to 2 mm in diameter, translucent, smooth, and beta-hemolytic. Older or larger colonies sometimes are opaque. After anaerobic incubation for 48 h, colonies are tan, gray, reddish brown, or black. Pigmentation of colonies occurs more rapidly on agar containing hemolyzed blood than on agar supplemented with whole blood. On hemolyzed rabbit blood agar, about one-third of the strains produce dark brown to black colonies within 2 days, another third produce such colonies within 7 days, and most of the rest produce such colonies within 14 days. An occasional strain require 18 to 21 days to produce definite pigment. The pigment of the colonies is due principally to protoheme (36).

Glucose broth cultures are turbid with a smooth (sometimes ropy or slightly mucoid) sediment and have a final pH of 4.9 to 5.4.

The major fermentation acids detected in cultures of the type strain grown in broth medium containing 1% peptone, 1% yeast extract, 1% glucose, vitamin K, and hemin (8) are as follows (in milliequivalents per 100 ml of culture): succinic, 4; acetic, 2. Isobutyric acid (0.1 to 0.4 meq/100 ml) and isovaleric acid (0.1 to 0.4 meq/100 ml) also are detected. No hydrogen is detected in the headspace gas. Similar products are obtained with other strains of this species. Neither lactate nor pyruvate is used; threonine is not converted to propionate.

Hemin is required for growth of most strains. Vitamin K often is highly stimulatory or required for fermentation. Growth is inhibited by 6.5% NaCl. The type strain grows well at tem-

peratures between 25 and 45°C. In poorly buffered media (e.g., prereduced peptone-yeast extract-sugar media [8]), some strains grow better in an anaerobic atmosphere containing 10% (rather than 100%) carbon dioxide, probably due to the higher pH when the lower concentration of carbon dioxide was used.

Strains produce superoxide dismutase (4) and do not hydrolyze dextran (7).

The type strain and 11 other strains (6 strains from the VPI 4197 homology subgroup and 6 strains from the VPI 8944 homology subgroup) are strongly positive for acid and alkaline phosphatase, phosphoamidase, and alpha-glucosidase. Very weak reactions occur for C4 esterase and C8 esterase lipase (API ZYM system; Analytab Products, Plainview, N. Y.) (Roger Kelley, unpublished data).

Aspartate, asparagine, cysteine, and serine stimulate growth and are metabolized by strain ATCC 25261 (VPI 8944 subgroup [Table 7]) (31, 48).

Diaminopimelic acid is the only dibasic amino acid in the peptidoglycan. No heptose or 2-keto-3-deoxyoctulosonic acid has been detected (5).

Other characteristics of this species are given in Table 5.

This species is a common inhabitant of human gingival crevices and has been isolated from human clinical specimens.

The G+C content of the DNA is 41 to 44 mol% (45–47; this study).

The type strain is ATCC 25611^T (= VPI 4197 = Finegold B422).

Seven strains previously classified as *B. melaninogenicus* subsp. *intermedius* had negligible homology with reference DNA from either VPI 4197 or VPI 8944. These strains had from 64 to 94% homology (Table 2) with reference DNA from VPI 9342 (parent strain of ATCC 33547). For these strains we propose the name *Bacteroides corporis*, with ATCC 33547^T as the type strain.

Bacteroides corporis. *Bacteroides corporis* sp. nov. (cor'po.ris. L. gen.n. corporis of the body; pertaining to the isolation of this organism from human clinical specimens).

Cells in glucose broth cultures are 0.9 to 1.6 by 1.6 to 4 μm and occur singly, in pairs, and in short chains (Fig. 3). In many cultures the cells are coccoid. Occasionally, longer cells (up to 11 μm) are observed.

Surface colonies on anaerobic blood agar plates are minute to 1 mm in diameter, circular, entire, convex, and (after 48 to 72 h) buff with brown edges. Dark brown colonies developed after 4 to 7 days.

Glucose broth cultures are turbid, often with a smooth or ropy sediment that tend to adhere to the bottom of the tube, and have a final pH of 4.8

TABLE 5. Phenotypic characteristics of *B. intermedius* and *B. corporis*^a

Characteristic	<i>B. intermedius</i>				<i>B. corporis</i>	
	VPI 4197 ATCC 25611 ^T	38 other strains	VPI 8944 ATCC 33563	15 other strains	VPI 9343 ATCC 33574 ^T	5 other strains
Acid from:						
Fructose	A	A (74) ^b	A	A	—	A (80)
Glycogen	A	A	A	A (87)	A	A
Gum arabic	—	— (13)	—	— (7)	—	—
Inulin	w	A (78)	A	v (60)	—	—
Maltose	A	A (92)	A	A	A	A
Mannose	w	v (47)	—	—	A	A (80)
Raffinose	A	v (42)	A	A (93)	—	—
Starch	A	A (89)	A	A	A	A
Sucrose	A	A (95)	A	A	—	—
Trehalose	—	— (3)	—	—	—	—
Xylan	—	— (3)	—	— (33)	—	—
Milk digestion	+	+	+	+	+	+
Meat digestion	—	v (53)	—	v (40)	—	v (40)
Indole	+	+	+	+	—	—
Lipase on egg yolk agar	+	+	—	+	—	—
H ₂ S (SIM medium)	—	— (22)	—	—	—	—
Starch hydrolysis	+	+	+	+	+	+

^aAll strains produced acid (at pH values below 5.5) from dextrin and glucose; all strains digested gelatin. No strain produced acid from amygdalin, arabinose, cellobiose, erythritol, esculin, inositol, lactose, larch arabinogalactan, mannitol, melezitose, melibiose, rhamnose, ribose, salicin, sorbitol, or xylose; no strain grew in peptone yeast extract glucose medium containing 20% bile. No strain reduced nitrate or produced catalase or lecithinase; no strain hydrolyzed esculin. Negligible gas was produced in peptone yeast extract glucose agar deep cultures. A, pH below 5.5; w, pH 5.5 to 5.7 and at least 0.3 pH unit below the pH of a peptone yeast extract culture; —, pH above 5.7 and within 0.3 pH unit of the pH of a peptone yeast extract culture; +, positive reaction; v, variable among strains (40 to 60% of the strains acid [sugars] or were positive).

^bExcept where noted, all strains tested produced the reaction shown. The numbers in parenthesis indicate the percentages of strains that produced acid or gave a positive reaction.

to 5.1. Addition of 10% (vol/vol) serum enhances growth and fermentation of some strains.

The major fermentation acids detected in cultures of the type strain grown in broth medium containing 1% peptone, 1% yeast extract, 1% glucose, vitamin K, and hemin (8) are (in milliequivalents per 100 ml of culture): succinic, 4; acetic, 2. Isobutyric acid (0.1 to 0.4 meq/100 ml) and isovaleric acid (0.1 to 0.4 meq/100 ml) also are detected. No hydrogen is detected in the headspace gas. Similar products are obtained with other strains. Neither lactate nor pyruvate is used; threonine is not converted to propionate.

Hemin and vitamin K are required for growth.

Of six strains tested, two are resistant to penicillin G (2 U/ml), one is resistant to tetracycline (6 µg/ml), and two is resistant to both penicillin G and tetracycline. All six strains are susceptible to chloramphenicol (12 µg/ml) and clindamycin (1.6 µg/ml).

Other characteristics of the species are given in Table 5.

Strains of this species have been isolated from various types of human clinical specimens.

The G+C content of the DNA is 43 to 46 mol%.

The type strain is ATCC 33547^T (= VPI 9342 = Lambe 532-70A).

In *Bergey's Manual of Determinative Bacteriology*, 8th ed. (10), *B. melaninogenicus* included the following three subspecies: *B. melaninogenicus* subsp. *melaninogenicus*, *B. melaninogenicus* subsp. *intermedius*, and *B. melaninogenicus* subsp. *asaccharolyticus*. *B. melaninogenicus* subsp. *asaccharolyticus* has been elevated to species rank (3), and we propose in this paper that *B. melaninogenicus* subsp. *intermedius* be elevated to species rank. Van Steenberg (45) has reported that the LEV strain of "*B. melaninogenicus* subsp. *levii*" has 0 to 10% homology with the type strain of either *Bacteroides asac-*

TABLE 6. Some differential characteristics for species formerly classified as *B. melaninogenicus* (Oliver and Wherry 1921) Roy and Kelly^a

Species	Acid produced from:						Indole	G+C content (mol %)	Products from PYG ^b		Other characteristics
	Glucose	Lactose	Sucrose	Esculin	Cellobiose	Ribose			Major	Other ^c	
<i>B. melaninogenicus</i>	+	+	+	—	—	—	—	40–43 ^d	S, A	IB, IV, (F)	Lipase negative
<i>B. denticola</i>	+	+	+	+	—	v	—	49–51 ^d	S, A	(IB), (IV), (L)	Lipase negative
<i>B. loeschii</i>	+	—	+	—	+	—	+	40–45 ^d	S, A	(L), (F)	Lipase negative
<i>B. intermedius</i>	+	—	+	—	—	—	+	40–45 ^e	S, A	(IB), (IV)	Lipase usually produced
<i>B. corporis</i>	+	—	—	—	—	—	—	43–46 ^e	S, A	(IB), (IV)	Lipase negative
<i>B. levii</i>	+	+	—	—	—	—	—	45–46 ^f	A, P, B	IV, IB, S	Isolated from cattle
<i>B. macacae</i>	+	+	—	—	—	—	+	43–45 ^f	P, S	IV, A, B, (PA)	Catalase positive
<i>B. asaccharolyticus</i>	—	—	—	—	—	—	+	52–54	B, A	P, IB, IV, (L), (S)	Does not produce phenylacetate (14, 29, 30) ^g
<i>B. gingivalis</i>	—	—	—	—	—	—	+	48–50 ^f	B	IB, A, P, IV, (S)	Produces phenylacetate (14, 30) and sheep erythrocyte agglutinin (41)

^a +, Positive reaction for 90 to 100% of the strains; —, negative reaction for 90 to 100% of the strains; v, 40 to 60% of the strains were positive.

^b PYG, Broth containing 1% peptone, 1% yeast extract, 1% glucose, vitamin K, and hemin (8); A, acetic acid; B, butyric acid; F, formic acid; IB, isobutyric acid; IV, isovaleric acid; L, lactic acid; P, propionic acid; PA, phenylacetic acid; S, succinic acid. The products in parentheses may or may not be produced.

^c Products produced in moderate or trace amounts.

^d Values for G+C content of DNA obtained from the data of Holdeman and Johnson (9a) and Van Steenberg (45).

^e Values for G+C content of DNA obtained from the present study and from the data of Van Steenberg (45).

^f Values for G+C content of DNA obtained from the data of Van Steenberg (45) and Coykendall et al. (1).

^g The numbers in parentheses are reference numbers.

charolyticus or *B. intermedius* or with reference strains of *Bacteroides gingivalis* and *B. melaninogenicus*. The LEV strain is most like *Bacteroides macacae* phenotypically but does not produce indole or catalase, as do strains of *B. macacae*, and is serologically distinct (as determined by immunodiffusion) from *B. macacae* (42). Therefore, we propose the name *Bacteroides levii* sp. nov., with ATCC 29147^T (= LEV strain = VPI 3300) as the type strain. The description of this species is given below.

***Bacteroides levii*.** *Bacteroides levii* sp. nov. (lev'i.i. L. gen.n. *levii* of Lev, named for Meir Lev, the American-English microbiologist who first isolated this organism; "*Bacteroides melaninogenicus* subsp. *levii*" Holdeman, Cato, and Moore 1977 [32]).

Except where otherwise noted, this description is based on our study of the LEV strain (ATCC 29147^T) and two phenotypically similar strains (including strain JP-2 [33, 43]) isolated from cattle.

In glucose broth cultures, cells are 0.6 to 1.2 by 2 to 7 μ m and occur in pairs and short chains (Fig. 4).

Surface colonies on anaerobic blood agar plates are minute, circular, entire, and low convex. After incubation for 2 to 3 days, colonies are buff to light brown; dark brown colonies develop after incubation for 5 to 7 days. No lipase or lecithinase activity is observed on egg yolk agar (8).

Glucose broth cultures are turbid with a smooth sediment and had a final pH of 5.5.

In addition to the characteristics given in Table 6, gelatin, casein, milk, and meat are digested. There is no fermentation of adonitol, amygdalin, arabinose, dextrin, dulcitol, erythritol, fructose, glycerol, glycogen, inositol, inulin, maltose, mannitol, salicin, sorbitol, sorbose, starch, trehalose, or xylose. Nitrate is not reduced, catalase is not produced, and hippurate is not hydrolyzed. No growth occurred in peptone-yeast extract-glucose broth supplemented with

TABLE 7. Proposed classification of some commonly used reference strains of pigmented bacteroides previously classified as *B. melaninogenicus* subsp. *intermedius* and of the LEV strain

Strain ^a	Identification or classification		Reference(s)
	Present	Previous	
ATCC 25611 ^T (= VPI 4197 = WAL B422)	<i>B. intermedius</i>	<i>B. melaninogenicus</i> subsp. <i>intermedius</i>	3, 5, 12, 27, 45
VPI 4200 (= Finegold B485)	<i>B. intermedius</i>	<i>B. melaninogenicus</i> subsp. <i>intermedius</i>	3
NCTC 9336 (= VPI 8944 = Lambe 729-74)	<i>B. intermedius</i> 8944 subgroup	<i>B. melaninogenicus</i>	3, 6, 12, 35, 38, 43, 45-47
ATCC 25261 (= VPI 4203 = WAL B515 = Lambe 724-74)	<i>B. intermedius</i> 8944 subgroup	<i>B. melaninogenicus</i> subsp. <i>intermedius</i>	16, 33, 38, 45
NCTC 9338 (= Lambe 206-74)	<i>B. intermedius</i> VPI 8944 subgroup	<i>B. melaninogenicus</i> subsp. <i>intermedius</i>	3, 6, 15, 38, 47
Williams T584 (= Lambe 731-74 = VPI 9145)	<i>B. intermedius</i> VPI 8944 subgroup	<i>B. melaninogenicus</i> subsp. <i>intermedius</i>	16, 35, 38
T588 (= Lambe 733-74)	<i>B. intermedius</i> VPI 8944 subgroup	<i>B. intermedius</i> subsp. <i>melaninogenicus</i>	36, 43, 45-47
C11a-d2 (Carlsson)	<i>B. intermedius</i> VPI 8944 subgroup	<i>B. melaninogenicus</i> subsp. <i>intermedius</i>	33, 38
LH-107	<i>B. intermedius</i> VPI 8944 subgroup	<i>B. melaninogenicus</i> subsp. <i>intermedius</i>	38
JPI	<i>B. intermedius</i> VPI 8944 subgroup	<i>B. melaninogenicus</i> subsp. <i>intermedius</i>	38
ATCC 33547 ^T (= VPI 9342 = Lambe 532-70A)	<i>B. corporis</i>	<i>B. melaninogenicus</i> subsp. <i>intermedius</i>	12, 16, 33, 43, 45
Lambe 532-70A (= VPI 9342)	<i>B. corporis</i>	<i>B. melaninogenicus</i> subsp. <i>intermedius</i>	16, 45
ATCC 29147 ^T (= LEV strain = VPI 10450 = VPI 3300) ^b	<i>B. levii</i>	<i>B. melaninogenicus</i> , "B. melaninogenicus subsp. <i>levii</i> "	8, 33, 43, 45, 46

^a ATCC, American Type Culture Collection, Rockville, Md.; Carlsson, J. Carlsson, University of Umea, Umea, Sweden; Lambe, Dwight W. Lambe, Jr., East Tennessee State University, Johnson City; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, England; VPI, Virginia Polytechnic Institute and State University Anaerobe Laboratory, Blacksburg; WAL, Wadsworth Veterans Administration Hospital Anaerobe Laboratory, Los Angeles, Calif. (usually received from S. M. Finegold or Vera Sutter); Williams, R. A. D. Williams, London Hospital Medical College, London, England.

20% bile or 6.5% NaCl. The optimum temperature for growth is 35 to 37°C; growth at 30 or 45°C is delayed and partially inhibited.

The major fermentation acids detected in cultures of the type strain grown in broth medium containing 1% peptone, 1% yeast extract, 1% glucose, vitamin K, and heme (8) are (in milliequivalents per 100 ml of culture): acetic, 2; propionic, 1.5 to 2; butyric, 1 to 1.5; and isovaleric, 1 to 1.5. Isobutyric acid (0.5 meq/100 ml) and a small amount of succinic acid (0.3 meq/100 ml) also are detected. Pyruvate is converted to acetate and propionate. Lactate is not utilized, and threonine was not converted to propionate.

Vitamin K and heme are required for growth (17-19). Succinate stimulates growth and can replace the requirement for heme. Cytochrome *b* is synthesized from heme and is not detected in the absence of heme (22). Glutamine stimulates growth and results in increased uptake of many amino acids and dipeptides (21). The cells possess superoxide dismutase activity (4).

Further information on the biochemical properties of *B. levii* has been described by Lev (18, 20) and Lev and Milford (23-26).

This species has been isolated from the bovine rumen, a cattle horn abscess, and a case of bovine summer mastitis.

The G+C content of the DNA has been reported to be 45 to 46 mol% by *T_m* (45) and 48 mol% by buoyant density (34).

The type strain is ATCC 29147^T (= LEV strain).

Additional comments. Strains with similar phenotypic characteristics have been isolated from human clinical specimens. The genetic relatedness between the human and bovine isolates has not been investigated by DNA homology studies.

The major phenotypic characteristics by which *B. intermedius*, *B. corporis*, and *B. levii* differ from each other and from other species that previously have been considered "*B. melaninogenicus*" are given in Table 6.

FIG. 2. Gram stains of 24-h broth cultures of *B. intermedius* VPI 4197 (= ATCC 25611^T) in peptone-yeast extract broth (A) and peptone-yeast extract-glucose broth (B). Bar = 10 μ m.

FIG. 4. Gram stains of 24-h broth cultures of *B. levii* VPI 3300 (= LEV strain) in peptone-yeast extract broth (A) and peptone-yeast extract-glucose broth (B). See Fig. 2 for magnification.

As we have observed and has been noted by other workers (6, 16, 45), pigment formation is not an exceedingly reliable characteristic for differentiating between "*B. melaninogenicus*" and phenotypically similar organisms that may produce buff or creamy colonies upon continued incubation. Therefore, the ability to recognize the different species by characteristics other than pigment production is highly desirable. Except for production of pigmented colonies on blood agar, *B. intermedius* and *B. corporis* are most like *B. disiens* (9), which also does not ferment lactose and does not grow well, if at all, in peptone-yeast extract-glucose medium supplemented with 20% bile (2 g of oxgall per 100 ml). Fermentation of sucrose and production of indole by *B. intermedius* but not by *B. disiens* differentiate these species. There is no one substrate reaction that reliably differentiates between *B. corporis* and *B. disiens*. Fortunately,

all strains of *B. corporis* that we have isolated have pigmented reliably on blood agar within 4 to 7 days, and distinct pigmentation has not been observed with strains of *B. disiens*. The patterns of soluble proteins in polyacrylamide gel electrophoresis gels also clearly differentiate these two species (data not shown). All strains of *B. disiens* digest milk and meat, and some strains of *B. corporis* may not digest one or the other of these complex proteins. Tests for digestion are not considered negative until cultures have been incubated for 3 weeks. Freshly isolated strains of *B. disiens* do not digest milk and meat as rapidly as laboratory stock cultures do. Fermentation of glycogen, fermentation of maltose, and fermentation of starch by *B. bivius* and *B. disiens* are helpful in differentiating between these species and *B. levii*.

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FIG. 3. Gram stains of 24-h broth cultures of *B. corporis* VPI 9342 (= ATCC 33547^T) in peptone yeast extract broth (A) and peptone-yeast extract-glucose broth (B). See Fig. 2 for magnification.

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