

Description of *Bacteroides loescheii* sp. nov. and Emendation of the Descriptions of *Bacteroides melaninogenicus* (Oliver and Wherry) Roy and Kelly 1939 and *Bacteroides denticola* Shah and Collins 1981

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Bacteroides loescheii, a new species from periodontal pockets and superficially cleaned tooth surfaces coronal to gingival margins and from various types of human infections, is described. Strains of *B. loescheii* are obligately anaerobic, gram-negative, usually pigmented, nonmotile, nonsporeforming rods that do not grow well in 10% bile and that ferment carbohydrates. These strains previously were identified as *Bacteroides melaninogenicus* or *Bacteroides oralis*, but they have no deoxyribonucleic acid homology with the type strain of either of these two species. The type strain of *B. loescheii* is ATCC 15930. The descriptions of *B. melaninogenicus* and *Bacteroides denticola* are emended.

In 1921 Oliver and Wherry (28) described as "*Bacterium melaninogenicum*" strains of obligately anaerobic, nonmotile, gram-negative, saccharoclastic rods that produce black growth on agar slants containing human blood. Oliver and Wherry noted that "growth of the bacteria on infusion agar is greatly facilitated by the presence of hemoglobin, and pigment production is absolutely dependent on it." The strains studied by Oliver and Wherry were isolated from throats, tonsils, an infected surgical wound of the abdomen, urine, and the "feces of a case of chronic dysentery superimposed on an original amebic (sic) infection." These authors also stated that similar pigment-producing colonies had been found by other investigators in cultures from carious teeth.

Despite its requirement of an anaerobic environment for growth, in the third edition of *Bergey's Manual of Determinative Bacteriology* the species was classified in the genus *Hemophilus* (sic), which was described as containing aerobic species (2). In the fifth edition of *Bergey's Manual*, Roy and Kelly placed the species in the genus *Bacteroides* (31). Schwabacher et al. (34) reported that the black pigment was not melanin, as originally postulated by Oliver and Wherry, suggested that the specific epithet of the species should be changed from "*melaninogenicus*" to "*nigrescens*" and, in keeping with the nomenclature used in *Topley and Wilson's Principles of Bacteriology and Immunity* (44), proposed the new combination "*Fusififormis*

nigrescens." Other synonyms are "*Ristella melaninogenica*" (Oliver and Wherry) Prévot 1938 (29) and "*Fusififormis melaninogenicus*" (44).

Until 1976, anaerobic gram-negative bacteria that produced brown or black colonies on blood agar were identified as *B. melaninogenicus* (or one of the synonyms), despite the fact that phenotypic diversity had been reported for 45 years. In 1928 Burdon (4) described strains of "*B. melaninogenicum*" that were not saccharoclastic, were proteolytic, and produced indole. These strains resembled those studied by Oliver and Wherry only in that they formed colonies with a black pigment on blood agar. Through the seventh edition of *Bergey's Manual* and through the 1957 edition of the *Manuel de Classification et de Détermination des Bactéries Anaérobies* (30), descriptions of *B. melaninogenicus* were composites of the characteristics reported by Oliver and Wherry and those reported by Burdon. Based on their own observations and the reports of Sawyer et al. (33), Holdeman and Moore (13) recognized the following three subspecies: *B. melaninogenicus* subsp. *melaninogenicus*, *B. melaninogenicus* subsp. *intermedius*, and *B. melaninogenicus* subsp. *asaccharolyticus*. In a report of the ICSB Taxonomic Subcommittee on Gram-Negative Anaerobic Rods, Finegold and Barnes proposed reclassification of *B. melaninogenicus* subsp. *asaccharolyticus* as *Bacteroides asaccharolyticus* (7). *Bacteroides gingivalis* and *Bacteroides macacae*, also anaerobic gram-negative rods that produce

colonies with black pigment, have been recognized as distinct from both *B. asaccharolyticus* and *B. melaninogenicus* (5).

Because there was considerable diversity among the strains classified as *B. melaninogenicus*, we initiated a study of deoxyribonucleic acid (DNA) homology among saccharolytic *Bacteroides* strains that form black pigment on blood agar medium. In 1980 one of us (J.L.J.) reported that there were three distinct homology groups, represented by the type strain and by strains VPI 10043 and VPI 12530, among 19 strains with the characteristics of *B. melaninogenicus* subsp. *melaninogenicus* (16). Phenotypic characteristics useful in differentiating among these homology groups also were described (16). The names "*B. socranskii*" and *B. loescheii* were proposed for the homology groups represented by VPI 10043 and VPI 12530 (L. V. Holdeman, J. L. Johnson, and W. E. C. Moore, *J. Dent. Res.* 60[Sp. Issue A]:414, abstr. no. 415, 1981). Later in 1981 Shah and Collins published the description of *B. denticola* (36) based on the study of NCDO 2352 (strain 1210) and strain 1221, presumably from E. Barnes. DNA from strain 1221 has 100% homology with DNA from NCDO 2352, the type strain of *B. denticola* (36). Strain 1221, which we received from E. Barnes, has 85% homology with strain VPI 10043, which indicates that the homology group for which we had proposed the name "*B. socranskii*" is *B. denticola*. The name *B. denticola* has priority (38).

The results of studies of 59 strains that have characteristics originally described for *B. melaninogenicus* (28) are presented in this paper.

MATERIALS AND METHODS

Strains. The sources of the strains used in the DNA homology studies are given in Tables 1 through 3. In addition, homology was tested with DNAs from VPI D27B-24 (the parent strain of ATCC 33269, the type strain of *Bacteroides oralis*, isolated from the gingival crevice of a person with severe periodontitis) and from reference strains for the three homology groups within *B. melaninogenicus* subsp. *intermedius* (Holdeman et al., *J. Dent. Res.* 60[Sp. Issue A]:414, abstr. 415, 1981). These included VPI 4197, the parent strain of ATCC 25611 (*B. melaninogenicus* subsp. *intermedius*); VPI 8944 (NCTC 9336), the parent strain of ATCC 33563; and VPI 9343, the parent strain of ATCC 33547 (Lambe strain 58-71A, isolated from a cervical swab [20]).

Methods of characterization. The phenotypic reactions of the strains were studied with the pre-reduced media and methods described previously (11, 32). For characterization of some strains, 10% (vol/vol) sterile rabbit serum was added to differential media to obtain maximum growth. In such cases, the pH values of cultures in media containing maltose, glycogen, and starch without added serum also were tested to verify fermentation of these substrates in the absence of

serum amylase. To determine the effects of vitamin K and hemin on the growth of the organisms, peptone-yeast extract-glucose medium without vitamin K and hemin was prepared by using glassware and a dispensing apparatus that were free of vitamin K and hemin. Cultures were transferred sequentially for five transfers (or until no growth occurred) in the peptone-yeast extract-glucose medium without vitamin K and hemin and in the same medium to which vitamin K₁ and hemin were added either singly or together to give final concentrations of 1 µg/ml and 0.5 mg/ml, respectively. The susceptibilities of the organisms to chloramphenicol (12 µg/ml), clindamycin (1.6 µg/ml), penicillin G (2 U/ml), and tetracycline (6 µg/ml) were tested by using the broth disk method of Wilkins and Thiel (42). Polyacrylamide gel electrophoretic (PAGE) patterns of soluble cellular proteins were determined as described by Moore et al. (26).

DNA isolation. The organisms were grown in a medium that was prepared as described previously (6); this medium contained mineral salts, 1% pepticase, 0.5% yeast 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 10% sterile NaHCO₃ was added to each 1 liter of medium. Flasks containing 1 or 2.5 liters of medium were inoculated with 10 to 20 ml of an overnight culture grown in chopped meat carbohydrate medium (11) and then incubated for 18 to 24 h at 37°C. The harvested cells were suspended in a 0.15 M NaCl-0.01 M ethylenediaminetetraacetic acid salt solution (pH 8.0). The cells were lysed by adding sodium dodecyl sulfate to a final concentration of 1%. After a preliminary extraction with chromatography-grade phenol, high-molecular-weight DNA (for nick-translation labeling) was isolated by the method of Marmur (23). Other DNA preparations were isolated by a hydroxyapatite procedure (17).

Guanine-plus-cytosine content of DNA. Thermal melting points were used to determine the guanine-plus-cytosine contents of the DNA preparations (17, 24).

Preparation of labeled DNA. Labeled DNA was prepared in vitro either by nick translation (17) or by iodination. Fragmented denatured DNA was labeled with ¹²⁵I by using a variation of the thallium chloride method of Tereba and McCarthy (39). The reaction mixture containing 25 µl of 0.2 M sodium acetate buffer (pH 4.8), 25 µl of DNA (0.4 mg/ml), 10 µl of 6 × 10⁻⁵ M potassium iodide, 12.5 µl of distilled water, and 2.5 µl of ¹²⁵I (40 µCi/µl) was added to a 1-ml serum vial. The vial was capped, and a partial vacuum was formed by withdrawing 1 ml of air with a syringe. The reaction was initiated by injecting 25 µl of TICl₃ (1 mg/ml in 50 mM sodium acetate buffer, pH 4.8). The vial was incubated in a 70°C water bath for 20 min. The reaction was stopped by chilling the vial in an ice bath and then adding 0.2 ml of 0.5 M sodium phosphate buffer (pH 6.8) containing 10 mM mercaptoethanol. The vial was then incubated for an additional 20 min at 70°C and again cooled in an ice bath. The mixture was removed with a syringe and then passed through a Sephadex PD10 disposable column (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) by using 1 × SSC (0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0) as

the elution buffer. The front peak (2.0 ml) was collected in a tube to which 45 μg (75 μl of a 600- $\mu\text{g}/\text{ml}$ solution) of sheared, denatured salmon sperm DNA had been added. The front peak material was heated for 20 min at 70°C and then passed through a second Sephadex PD10 column equilibrated against 0.1 \times SSC-1 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 7.0). The front peak was collected in a 3.5-ml volume. The specific activities of the DNA preparations were 1×10^6 to 2×10^6 cpm/ μg .

DNA homology methods. DNA homology values were determined by using an S1 nuclease procedure, as described previously (17). The reassociation vials were incubated for 24 h at 61°C for reference strain VPI 2381, 62°C for reference strain VPI 9343, 64°C for reference strains VPI 12530 and VPI D1C-20, and 65°C for reference strain VPI 10043.

RESULTS AND DISCUSSION

Consistent with the original report by Oliver and Wherry (28), all strains in this study fer-

mented glucose, lactose, maltose, and sucrose, and most strains produced dark pigment on hemolyzed blood agar. Of the 61 strains studied, 11 had 62 to 78% homology with the type strain of *B. melaninogenicus* subsp. *melaninogenicus* (VPI 2381, the parent strain of ATCC 25845), 8 had 26 to 42% homology with VPI 2381 (Table 1), and 41 had no or only negligible homology with VPI 2381. Of the eight strains having moderate DNA homology with VPI 2381, five of five strains tested had 66 to 82% DNA homology with reference DNA from VPI 9343 (Table 1). Of the 41 isolates which had negligible homology with VPI 2381, 26 had 48 to 98% homology with reference DNA from VPI 10043 (Table 2), 7 had 61 to 86% homology with reference DNA from ATCC 15930 (VPI 0037) and 6 having moderate homology with ATCC 15930 had 67 to 73% homology with reference DNA from VPI D1C-20 (Table 3).

TABLE 1. Strains in the VPI 2381 homology group (*B. melaninogenicus*) and their sources

VPI no.	% DNA homology with reference DNA of:		Strain derivation and other information ^a
	VPI 2381	VPI 9343	
2381	100 ^b	44	Finegold B282, sputum; parent strain of ATCC 25845 (type strain of <i>B. melaninogenicus</i> subsp. <i>melaninogenicus</i> [Approved Lists, 1980])
5693	76	45	Cystic acnes
7169A	71		Fulghum 1502a1; intestinal contents, Whipple's disease
7216D	67	26	Mouth abscess
7465	78	46	Abdominal abscess
8227	69		Gingival crevice, periodontally healthy person
9847	76	45	Gingival crevice, periodontally healthy person
12509	66	48	Williams S2-77
13018	63		Socransky 2
13019	62		Socransky 7 (from same patient as Socransky 2)
13020	69		Socransky 8 (from same site as Socransky 7)
D26C-21B	64	44	Gingival sulcus, severe periodontitis
9343	31	100 ^b	Lambe 58-71A ^c
7673A	37	74	Anticubical space
13040	37	80	Socransky 95
13041	33	66	Socransky 96 (from same site as Socransky 95)
D13A-25	42	82	Tooth surface, severe periodontitis
D45A-30	42	78	Tooth surface, juvenile periodontitis
13022	39		Socransky 11
6093	26		Lambe 961-70C, colostomy wound

^a ATCC, American Type Culture Collection, Rockville, Md.; Finegold, S. M. Finegold, Wadsworth Veterans Administration Hospital, Los Angeles, Calif.; Fulghum, R. S. Fulghum, Department of Microbiology and Immunology, School of Medicine, East Carolina University, Greenville, N.C.; Lambe, D. W. Lambe, Jr., Department of Microbiology, East Tennessee State University, Johnson City, Tenn.; 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 margins of affected sites after superficial cleaning of the surfaces with sterile toothpicks. Juvenile periodontitis samples were from individuals 11 to 25 years old with severe destruction essentially limited to permanent first molars and incisors; severe periodontitis samples were from persons 18 to 30 years old with 5 mm or more loss of attachment on any surface of eight or more teeth not limited to first molars or incisors.

^b Percent homology with reference DNA was normalized to 100%.

^c See reference 20.

TABLE 2. Strains in the VPI 10043 (*B. denticola*) homology group and their sources

VPI no.	% DNA homology with reference DNA of		Strain derivation and other information ^a
	VPI 10043		
10043	100 ^b		Lambe 1282-74, maxillary antrium; parent strain of ATCC 33185 ^c
7570A	87		Abscess of mandible
9955	86		Lambe 1504-74A, pleural abscess
10099	91		Lambe 57-75D
11963	72		Parkland Hospital, Dallas, Tex., strain 82316, spinal fluid
12406	85		Barnes 1221, originally from Socransky
12512	82		Williams S-3-77, plaque
13023	80		Socransky 13
D2B-18	79		Gingival sulcus, moderate periodontitis
D2B-22	65		Moderate periodontitis, same site as D2B-18
D2B-34A	93		Moderate periodontitis, same site as D2B-18
D16A-3	77		Tooth surface, moderate periodontitis
D19B-6B	85		Gingival sulcus, juvenile periodontitis
D22A-18	86		Tooth surface, juvenile periodontitis
D25A-28	80		Tooth surface, juvenile periodontitis
D25C-2A	84		Tooth surface, juvenile periodontitis, same patient as D25A-28; parent strain of ATCC 33184
D27A-11	50		Tooth surface, severe periodontitis
D27B-3	48		Gingival sulcus, severe periodontitis; same patient as D27A-11
D27B-16	52		Severe periodontitis, from same sample as D27B-3
D27B-25	83		Severe periodontitis, from same sample as D27B-3
D27C-3	88		Tooth surface, severe periodontitis, same patient as D27B-25
D28C-5	91		Tooth surface, severe periodontitis
D28C-22	53		Tooth surface, severe periodontitis, from same sample as D28C-5
D28J-29A	98		Severe periodontitis, same site as D28C-5, sampled 6 weeks later
D33B-28	58		Gingival sulcus, juvenile periodontitis
D33D-10B	83		Juvenile periodontitis, from same sample as D33B-28
E1H-6	86		Gingival crevice, experimental gingivitis, no-brushing day 11

^a 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. Also see Table 1, footnote a.

^b Percent homology with reference DNA was normalized to 100%.

^c See reference 21.

TABLE 3. Strains in the ATCC 15930 (VPI 0037) (*B. loeschei*) homology group and their sources

VPI no.	% DNA homology with reference DNA of:		Strain derivation and other information ^a
	VPI 0037	VPI D1C-20	
0037	100 ^b	41	ATCC 15930, Loesche 8B, gingival crevice
9621	82	41	Lambe 221-72E, Lambe serotype A ^c
12530	76		Socransky 287
12531	86		Socransky 379
D16B-5	61	42	Gingival sulcus, moderate periodontitis
D45C-10A	81		Tooth surface, periodontally healthy person
E1S-8	79		Gingival crevice, experimental gingivitis, no-brushing day 11
E1X-22A	80	45	Gingival crevice, experimental gingivitis, no-brushing day 26, same person as E1S-8
D1C-20	38	100 ^b	Gingival sulcus, moderate periodontitis
D18A-29A	39	73	Tooth surface, moderate periodontitis
D25B-5		70	Gingival sulcus, juvenile periodontitis
D26C-18	37	73	Tooth surface, severe periodontitis
D39D-9	44	71	Gingival sulcus, juvenile periodontitis
D41A-24B	38	67	Tooth surface, periodontally healthy person

^a See Table 1, footnote a.

^b Percent homology with reference DNA was normalized to 100%.

^c See reference 20.

TABLE 4. DNA homology among some saccharoclastic *Bacteroides* strains

Strain ^a	% Homology with reference DNA from strain: ^b		
	VPI 2381	VPI 10043	VPI 0037
<i>B. melaninogenicus</i> VPI 2381 (ATCC 25845)	100	7	2
<i>B. denticola</i> VPI 10043, (ATCC 33185)	8	100	3
<i>B. loescheii</i> ATCC 15930 (VPI 0037)	2	5	100
<i>B. melaninogenicus</i> subsp. <i>intermedius</i> VPI 4197 (ATCC 25611)	2	6	1
VPI 8944 (ATCC 33563)	2	4	1
VPI 9342 (ATCC 33547)	4	9	2
<i>B. oralis</i> VPI D27B-24 (ATCC 33269)	3	3	0

^a Strains ATCC 25845, ATCC 15930, ATCC 25611, and VPI D27B-24 are type or proposed type strains.

^b Percent homology with reference DNA was normalized to 100%.

The homology values among VPI 2381, VPI 10043, and VPI 0037 (ATCC 15930) reference DNA preparations and DNAs from the type and reference strains of *B. melaninogenicus* subsp. *intermedius* and the type strain of *B. oralis* are given in Table 4.

There was negligible intragroup homology among the groups represented by strains ATCC 25845 (VPI 2381), ATCC 33185 (VPI 10043), and ATCC 15930 (VPI 0037). These strains also had negligible homology either with the type strains of species that are phenotypically similar or with other strains of saccharoclastic pigmented *Bacteroides* that have been classified as *B. melaninogenicus* subsp. *intermedius*. Therefore, we believe that strains ATCC 25845, ATCC 33185, and ATCC 15930 represent separate species.

Because all strains studied are anaerobic, nonmotile, nonsporeforming, gram-negative rods that produce succinate and acetate as major fermentation products, they are members of the genus *Bacteroides* (12, 13).

ATCC 25845 is the type strain of *B. melaninogenicus*. ATCC 33185 (VPI 10043) is representative of the 41 strains of *B. denticola* that we studied. The name *B. loescheii*, with ATCC 15930 as the type strain, is proposed for the third homology group.

Strains in the VPI 9343 group show only moderate homology with *B. melaninogenicus* VPI 2381 and cannot be differentiated from strains in the VPI 2381 group by usual phenotypic reactions. The relationship between the VPI 9343 and VPI 2381 homology groups (36 to 37%) is lower than that previously observed by mem-

brane competition experiments (65 to 70%) (15) between the two homology groups within *Bacteroides fragilis* (VPI 2553 and VPI 2393). Even though values in the homology range of 60% or below usually are 10 to 15% lower with the S1 nuclease method (used in our experiments) than with the membrane competition method (unpublished data), there is justification for considering the VPI 9343 homology group a separate species. However, until new tests reveal distinct phenotypic differences between the VPI 2381 and VPI 9343 homology subgroups or until differences in clinical or ecological significance can be established, we will consider strains in the VPI 9343 group to be members of *B. melaninogenicus*.

Similarly, strains in the VPI D1C-20 homology group show moderate (average, 39%) intragroup homology with *B. loescheii* and are not distinguishable from *B. loescheii* by the usual phenotypic characteristics. We will consider strains with high homology with VPI D1C-20 to be members of *B. loescheii* until distinct differences in phenotypic characteristics or significance can be established.

The VPI 2381 and VPI 9343 homology subgroups are distinguishable by PAGE patterns of soluble proteins (Fig. 1), but the patterns of the strains in the ATCC 15930 and VPI D1C-20 subgroups are very similar (Fig. 1).

The phenotypic characteristics of the type or reference strains of the three species are given in Table 5 and in the descriptions below. The PAGE patterns of representative strains within the groups are given in Fig. 1 and 2.

Because there are several distinct taxa among the strains that have been classified as *B. melaninogenicus* subsp. *melaninogenicus*, previous research concerning the characteristics of "*B. melaninogenicus*" is difficult to interpret unless specific strains can be identified. The relationships of some reference strains to the homology groups described here and elsewhere are given in Table 6. Information from many literature citations has been included in the species descriptions given below.

Emended description of *Bacteroides melaninogenicus* (Oliver and Wherry 1921) Roy and Kelly 1939, 569. (*Bacteroides melaninogenicus* subsp. *melaninogenicus* (Oliver and Wherry 1921) Holdeman and Moore 1970, 33 (Approved Lists, 1980)). This species is composed of obligately anaerobic, nonsporeforming, nonmotile, gram-negative rods. Cells from glucose broth cultures were 0.5 to 0.8 by 0.9 to 2.5 μm , with occasional cells 10 μm long or longer (Fig. 3).

Surface colonies on blood agar were 0.5 to 2.0 mm in diameter, circular, entire, convex, and shiny; they usually were darker in the center of the colony, and the edges were gray to light

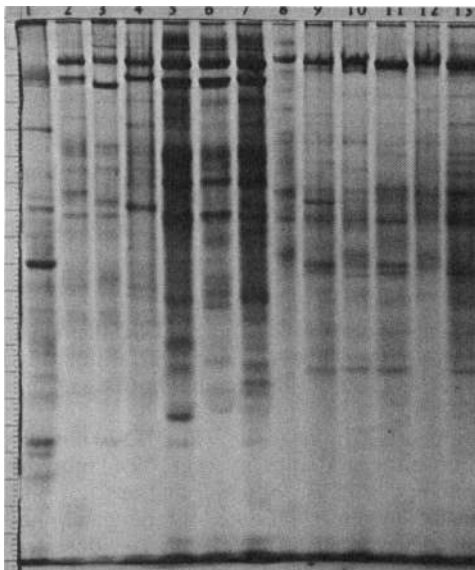


FIG. 1. PAGE patterns of strains that have high homology with *B. melaninogenicus* VPI 2381 (lane 2, VPI 5693; lane 3, VPI 7169A; lane 4, VPI 2381) and VPI 9343 (lane 5, VPI 9343; lane 6, VPI D13A-25; lane 7, VPI 13040) and with *B. loescheii* ATCC 15930 (lane 8, VPI 12530; lane 9, VPI D45C-10A; lane 10, ATCC 15930) and VPI D1C-20 (lane 11, VPI D1C-20; lane 12, VPI D18A-29A; lane 13, VPI D26C-18). Lane 1 contained a *Streptococcus faecalis* VPI U4-20 reference culture.

brown. Colonies became darker upon continued incubation (5 to 14 days). Dark pigment usually developed more rapidly when hemolyzed (laked) blood rather than blood containing whole erythrocytes was used. Some strains did not produce colonies with black pigment on horse blood agar but pigmented well on agar containing rabbit blood. The type strain and two other strains were beta-hemolytic on rabbit blood agar.

Glucose broth cultures usually were turbid, with smooth or stringy sediment and a pH of 4.6 to 5.0.

The major fermentation acids (from 1% peptone-1% yeast extract-1% glucose broth cultures containing vitamin K₁ and hemin [11]) of the type strain were succinate (2 meq/100 ml of culture) and acetate (2 meq/100 ml); sometimes formate (0.5 to 1.0 meq/100 ml) was also present. Small amounts (less than 0.3 meq/100 ml) of isobutyrate and isovalerate usually were detected in medium containing 1 to 2% peptone. No hydrogen was detected in headspace gas. Similar products were obtained with other strains. Neither lactate nor pyruvate was utilized. Threonine was not converted to propionate.

Hemin (1 µg/ml) was required for growth of the type strain. Hemin or vitamin K₁ (0.1 µg/ml)

or both were required for or enhanced growth of most strains. Most strains grew at pH 8.5 and 25°C; some grew at 45°C.

Cell wall peptidoglycan may contain either lysine or diaminopimelic acid. No heptose or 2-keto-3-deoxyoctulosonic acid is detected (9). Cells contain sphingolipids (25) and have superoxide dismutase activity (8).

The type strain reacts with Lambe group A fluorescent antibody conjugate (20).

Other characteristics of the species are given in Table 5.

Strains have been isolated from human gingival crevices and from human clinical specimens.

The guanine-plus-cytosine content of the DNA is 36 to 40 mol% (37, 41; this study).

Type strain: ATCC 25845 (VPI 2381, Fingold B282).

Emended description of *Bacteroides denticola* Shah and Collins 1981, 237. This species is composed of obligately anaerobic, nonmotile, nonsporeforming, gram-negative rods. Cells from glucose broth cultures were 0.5 to 0.7 by 0.7 to 6.0 µm and usually occurred in pairs and short chains (Fig. 4).

Surface colonies on blood agar were 1 to 2 mm in diameter, circular, entire, low convex, semi-opaque, shiny, and smooth, often appearing to have white or buff concentric rings. Pigmented colonies developed more rapidly and to a darker color on rabbit blood agar than on sheep blood agar. Most strains produced dark brown or black colonies within 7 days on medium containing hemolyzed rabbit blood; 2 of 20 strains never developed colonies with definite pigment.

Glucose broth cultures were turbid, with a smooth sediment and a pH of 4.2 to 4.8.

The major fermentation acids (from 1% peptone-1% yeast extract-1% glucose broth cultures containing vitamin K₁ and hemin [11]) of strain VPI 10043 (ATCC 33185) were succinate (2 meq/100 ml of culture) and acetate (2 meq/100 ml); sometimes lactate (0.2 to 0.3 meq/100 ml) was also present. Small amounts (less than 0.3 meq/100 ml of culture) of isobutyrate and isovalerate often were detected in medium containing 1 to 2% peptone. No hydrogen was detected in headspace gas. Similar products were obtained with other strains.

Hemin was required or highly stimulatory for growth of ATCC 33185 and the other strains; vitamin K₁ generally was not required.

Of 19 strains tested, 15 were susceptible to chloramphenicol (12 µg/ml), clindamycin (1.6 µg/ml), penicillin G (2 U/ml), and tetracycline (6 µg/ml). ATCC 33185 and four other strains were resistant to 2 U of penicillin G per ml, and one of these strains also was resistant to 6 µg of tetracycline per ml.

No heptose or 2-keto-3-deoxyoctulosonic acid

TABLE 5. Phenotypic characteristics of *B. melaninogenicus*, *B. denticola*, and *B. loescheii*^a

Species	Strain(s)	Acid from:										Lipase on egg yolk agar	Esculin hydrolysis	Starch hydrolysis	
		Cellobiose	Esculin	Gum arabic	Inulin	Larch arabinogalactan	Meltiliose	Ribose	Xylan						
<i>B. melaninogenicus</i>	ATCC 25845	-	-	-	A	-	-	-	-	-	-	-	-	+L	+
	11 others	(9) ^b	-	(27)	A (91)	(36)	(9)	-	-	-	-	-	(36)	+	
	VPI 9343	-	-	-	A	-	-	-	-	-	-	-	-	+	
	5 others	-	-	-	A	-	v (40)	-	-	-	-	-	v (60)	+	
<i>B. denticola</i>	ATCC 33185	-	A	-	A	-	-	A	-	-	-	-	+	+	
	17 others	-	A (89)	(21)	A	-	-	v (58)	(21)	-	-	-	+	(89)	
<i>B. loescheii</i>	ATCC 15930	A	-	A	-	-	w	-	-	-	-	-	+	+	
	8 others	A	-	(38)	v (50)	-	v (50)	-	v (43)	-	(25)	+	+	+	
	VPI DIC-20	A	w	-	A	-	A	-	A	+	+	+	+		
	4 others	A	v (50)	-	A	-	A (75)	-	v (50)	+	+	+	+		

^a All strains produced acid (pH below 5.5) from dextrin, fructose, glucose, glycogen, lactose, maltose, mannose, raffinose, sucrose, and starch; all strains digested gelatin and produced an acid curd in milk. No strain produced acid from amygdalin, arabinose, erythritol, inositol, mannitol, melzitose, rhamnose, salicin, sorbitol, trehalose, or xylose. No strain digested milk or meat. No strain grew in peptone-yeast extract-glucose medium containing 20% bile. No strain reduced nitrate or produced indole, catalase, or lecitinase. No strain produced gas in peptone-yeast extract-glucose agar deep cultures. Abbreviations: 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 medium culture; -, pH above 5.7 and within 0.3-pH unit of the pH of a peptone-yeast extract medium culture; +, positive reaction; L, late; v, variable among strains (40 to 60% of strains produced acid).

^b All strains tested produced the reaction given unless the reaction is followed by a number in parentheses, which represents the percentage of strains producing acid or a positive reaction.

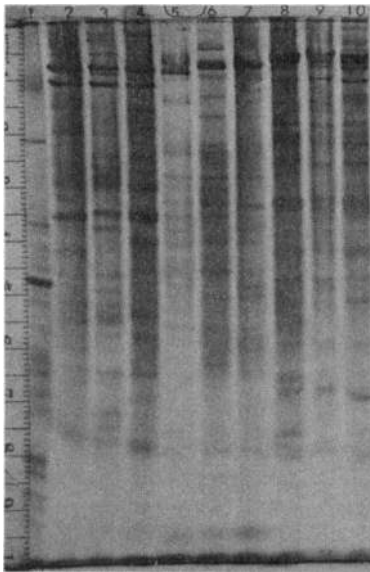


FIG. 2. PAGE patterns of strains of *B. melaninogenicus*, *B. loescheii*, and *B. denticola*. Lane 1, *S. faecalis* VPI U4-20 reference culture; lanes 2 to 4, *B. melaninogenicus* (lane 2, VPI 2381 [ATCC 25845, type strain]; lane 3, VPI 9847; lane 4, VPI 13020); lanes 5 to 7, *B. loescheii* (lane 5, ATCC 15930; lane 6, VPI 12530; lane 7, VPI 9621); lanes 8 to 10 *B. denticola* (lane 8, VPI 10043 [ATCC 33185]; lane 9, VPI 9955; lane 10, VPI 13023).

is detected (in the strain referred to as "*B. oralis* VPI 7570A") (9).

Other characteristics of the species are given in Table 5 and by Shah and Collins (36).

Tests for hydrolysis of esculin and fermentation of cellobiose are helpful in differentiating *B. denticola* from *B. melaninogenicus* and *B. loescheii*.

Strains have been isolated from human gingival crevices and human clinical specimens.

The guanine-plus-cytosine content of the DNA is 49 to 51 mol% in this study. Shah and Collins reported 51 mol% for the two strains that they studied (36).

Type strain: NCDO 2352 (Shah and Collins 1210).

Bacteroides loescheii Holdeman and Johnson sp. nov. (loesche'i.i. L. gen. noun *loescheii* of Loesche, named after Walter J. Loesche, an American dental microbiologist). This species is composed of obligately anaerobic, nonspore-forming, nonmotile, gram-negative rods. Cells in glucose broth were 0.4 to 0.6 by 0.8 to 15.0 μm and occurred singly, in pairs, and in short chains (Fig. 5).

Surface colonies on anaerobic blood agar plates were 1 to 2 mm in diameter, circular, entire, low convex, translucent, shiny, and smooth. After incubation for 48 h on plates containing whole blood, colonies were white or buff. Upon continued anaerobic incubation (up to 14 days), colonies usually became brown (often only light brown). Although pigment developed more rapidly on media containing hemolyzed blood, definite dark brown or black colonies did not develop with some strains, even if cultures were incubated for 21 days. The pigment is formed principally from protoporphyrin (35). Most strains produced slight clearing or beta-hemolysis on rabbit blood agar.

TABLE 6. Proposed classification of some commonly used reference strains of pigmenting *Bacteroides* previously classified as *B. melaninogenicus* subsp. *melaninogenicus* or *B. oralis*

Published strain no. ^a	Identification or classification		Reference(s)
	Present	Previous	
ATCC 25845 (VPI 2381, WAL B282)	<i>B. melaninogenicus</i>	<i>B. melaninogenicus</i> subsp. <i>melaninogenicus</i>	7, 16, 22, 27, 40, 41
Lambe 138-71, serogroup A (VPI 9847)	<i>B. melaninogenicus</i>	<i>B. melaninogenicus</i> subsp. <i>melaninogenicus</i>	20, 37
VPI 9343 (Lambe 58-71A)	<i>B. melaninogenicus</i>	<i>B. melaninogenicus</i> subsp. <i>melaninogenicus</i>	40, 41
ATCC 33185 (VPI 10043, DWL 1282-74B)	<i>B. denticola</i>	<i>B. melaninogenicus</i> subsp. <i>melaninogenicus</i>	16
VPI 7570A	<i>B. denticola</i>	<i>B. melaninogenicus</i> subsp. <i>melaninogenicus</i> , <i>B. oralis</i>	9, 10
Socransky 287 (VPI 12530)	<i>B. denticola</i>	<i>B. melaninogenicus</i>	16
ATCC 15930 (VPI 9085, Loesche 8B)	<i>B. loescheii</i>	<i>B. melaninogenicus</i> subsp. <i>melaninogenicus</i> , <i>B. oralis</i>	9, 22, 27, 35, 37, 40, 43, 45
Socransky 379	<i>B. loescheii</i>	<i>B. melaninogenicus</i> subsp. <i>melaninogenicus</i>	22

^a See Table 1, footnote a. Strains ATCC 25845 and ATCC 15930 are type strains.

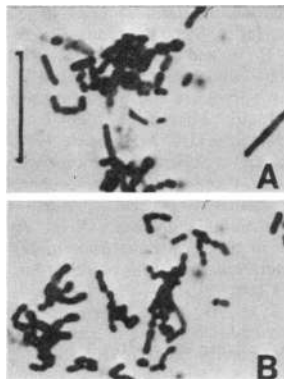


FIG. 3. Gram stains of 24-h broth cultures of *B. melaninogenicus* VPI 2381 (ATCC 25845) in peptone-yeast extract medium (A) and peptone-yeast extract-glucose medium (11) (B). Bar = 1 μ m.

Glucose broth cultures were turbid, with a smooth sediment and a final pH of 4.9 to 5.4.

The major fermentation acids (from 1% peptone-1% yeast extract-1% glucose broth cultures containing vitamin K₁ and hemin [11]) of the type strain were succinate (2 to 3 meq/100 ml of culture) and acetate (1 meq/100 ml); sometimes traces of lactate (0.2 to 0.3 meq/100 ml) and formate (0.2 to 0.3 meq/100 ml) were also present. No hydrogen was detected in head-space gas. Similar products were obtained with other strains. Neither pyruvate nor lactate was used, and threonine was not converted to propionate.

Hemin was required or highly stimulatory for growth. Even in the presence of hemin, serum (10%, vol/vol) often enhanced growth and fermentation.

The six strains tested were susceptible to

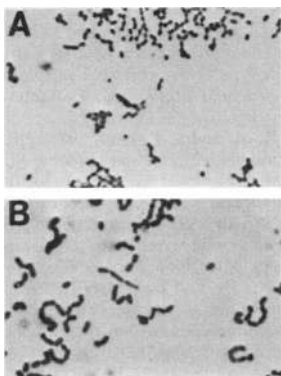


FIG. 4. Gram stains of 24-h broth cultures of *B. denticola* VPI 10043 (ATCC 33185) in peptone-yeast extract medium (A) and peptone-yeast extract-glucose medium (11) (B). Same magnification as in Fig. 3.

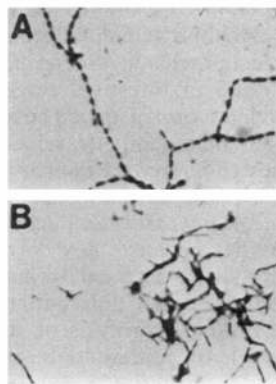


FIG. 5. Gram stains of 24-h broth cultures of *B. loescheii* ATCC 15930 in peptone-yeast extract medium (A) and peptone-yeast extract-glucose medium (11) (B). Same magnification as in Fig. 3.

chloramphenicol (12 μ g/ml), clindamycin (1.6 μ g/ml), penicillin G (2 U/ml), and tetracycline (6 μ g/ml).

The type strain (referred to as "*B. oralis* ATCC 15930") produces sphingolipids (25). Extracellular phospholipase A is produced (3).

Other characteristics of the species are given in Table 5.

Hydrolysis of esculin and fermentation of cellobiose are helpful in differentiating *B. loescheii* from *B. melaninogenicus* and *B. denticola*.

Isolated from gingival crevices of humans.

The guanine-plus-cytosine content of the DNA is 46 mol% (37) or 47 to 48 mol% (41; this study).

Type strain: ATCC 15930 (Loesche strain 8B, NCTC 11321).

Additional comments. Loesche strain 8B originally was deposited with the American Type Culture Collection as a representative of *B. oralis*. Concurrent with the interest in *Bacteroides* in the last two decades and with improved methods for culture of the organisms, many investigators independently observed that ATCC 15930 produced colonies with brown or black pigment on blood agar, particularly if the blood was hemolyzed (10, 37). Because pigment was observed in this saccharoclastic organism, ATCC 15930 was reidentified by W. E. C. Moore and one of us (L.V.H.) as a strain of *B. melaninogenicus* subsp. *melaninogenicus*, and it was so listed in the 14th edition of the catalog of the American Type Culture Collection (1). This strain has been referred to as both *B. oralis* and *B. melaninogenicus* in the literature, but it is distinctly different from the type strain of *B. oralis* (Table 4).

Phenotypic characteristics helpful in differentiating *B. melaninogenicus*, *B. denticola*, and *B. loescheii* from other species. Strains of *B. melani-*

nogenicus, *B. denticola*, and *B. loescheii* do not grow well in 20% bile, which differentiates them from the saccharoclastic *Bacteroides* strains commonly found in intestinal tracts (e.g., *B. fragilis* and related organisms). These three species differ from the recently described saccharoclastic species (*Bacteroides buccae* and *Bacteroides oris* [14], *Bacteroides capillus* [19, 38], and *Bacteroides pentosaceus* [36, 38]) isolated from oral cavities because they do not ferment arabinose or xylose. Pigment formation is the major characteristic that differentiates *B. loescheii* and *B. oralis*; hydrolysis of starch by *B. loescheii* also helps differentiate this species from *Bacteroides buccalis* (36, 38). *B. melaninogenicus*, *B. loescheii*, and *B. denticola* ferment lactose, unlike *B. melaninogenicus* subsp. *intermedius*; in addition, these three species ferment maltose, unlike strains of *B. macacae* and "*B. melaninogenicus* subsp. *levii*," and they do not produce butyric acid, as strains of *B. asaccharolyticus* and *B. gingivalis* do.

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