Bacteroides disiens sp. nov. and *Bacteroides bivius* sp. nov. from Human Clinical Infections

LILLIAN V. HOLDEMAN AND J. L. JOHNSON

Anaerobe Laboratory, College of Agriculture and Life Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

Bacteroides disiens sp. nov. and *Bacteroides bivius* sp. nov., both isolated from human clinical specimens, are described. They are species of obligately anaerobic, nonsporeforming, nonmotile, nonpigmenting, gram-negative rods that produce succinic and acetic acids (often with traces of isobutyric, isovaleric, and lactic acids) from peptone-yeast-glucose cultures. Gelatin, milk, casein, and meat are digested. Glucose, maltose, and starch, but not sucrose, are fermented. Most strains of *B. bivius* also ferment lactose. Hemin is required for growth. Strains of these species are phenotypically similar to those of *B. melaninogenicus* subsp. *intermedius* except that they do not form pigmented colonies. However, they do not show a high level of deoxyribonucleic acid/deoxyribonucleic acid homology with *B. melaninogenicus* subsp. *intermedius* or with each other. ATCC 29426 (= VPI 8057) is designated the type strain of *B. disiens*, and ATCC 29303 (= VPI 6822) is designated the type strain of *B. bivius*.

During the past 5 years, our reference laboratory has received more than 50 similar isolates, mostly from human clinical infections, that were phenotypically similar to strains of *Bacteroides melaninogenicus* subsp. *intermedius* except that they never produced dark brown or black colonies. Phenotypically, these isolates were separated into two groups differentiated by the inability of one of the groups to ferment lactose. These groups have been previously described and referred to as *Bacteroides* Group I and *Bacteroides* Group PS, respectively, in the October 1974 and February 1976 issues of the *Anaerobe Newsletter* (VPI Anaerobe Laboratory, Blacksburg, Va.). The *Bacteroides* Group I and *Bacteroides* Group PS designations have also been used by Lambe and colleagues (10, 11) in recent reports.

Because of the close phenotypic resemblance of members of the two groups to *B. melaninogenicus* subsp. *intermedius* and the phenotypic similarity between the two groups, we did not know whether these groups represented two new species, one new species, or nonpigmenting variants of *B. melaninogenicus* subsp. *intermedius*. However, deoxyribonucleic acid (DNA)/DNA homology studies presented here have shown that the two groups are distinct.

One of the major characteristics of these two groups is that they have both saccharolytic and proteolytic properties, and this is reflected in the derivation of the proposed specific epithets. We propose the name *Bacteroides disiens* sp. nov., with ATCC 29426 (= VPI 8057) as the type strain, for the group formerly designated

Bacteroides Group I and the name *Bacteroides bivius* sp. nov., with ATCC 29303 (= VPI 6822) as the type strain, for the group formerly designated *Bacteroides* Group PS.

MATERIALS AND METHODS

Bacterial strains. The sources of the 11 strains of *B. disiens*, the 14 strains of *B. bivius*, and of five phenotypically similar strains used in this study are given in Table 1. In addition, VPI 4197 (= Finegold B422 → VPI → ATCC 25611) and VPI 8944 (← NCTC 9336) were used to determine the DNA relatedness of the study strains to *B. melaninogenicus* subsp. *intermedius*. Both VPI 4197 and 8944 had the phenotypic characteristics of *B. melaninogenicus* subsp. *intermedius* (6).

Culture media and methods. Cultures of the strains studied were characterized using media and methods described previously (6). Prereduced media (6) were gassed (with oxygen-free carbon dioxide or with nitrogen [90%]-carbon dioxide [10%] passed over hot copper to remove oxygen) while they were being inoculated. In addition to specific substrates, all media, unless otherwise stated, contained (per 100 ml) 0.5 g of peptone (Difco), 0.5 g of Trypticase (Baltimore Biological Laboratories), 1.0 g of yeast extract (Difco), and 0.5 mg of crystalline equine hemin (Sigma). Unless otherwise specified, the concentration of vitamin K₁ (Sigma V-3501) was 0.17 nl/ml (about 0.17 µg/ml) in broth and 1.0 nl/ml (about 1 µg/ml) in solid media.

The effect of hemin on growth and fermentation products was tested in the peptone-yeast-glucose-hemin (PYGH) medium described above with 1 µg of vitamin K₁ per ml and in the same medium that was hemin-free (PYG-HF). In preparing PYG-HF medium, all glassware was cleaned in a Wilt glassware cleaning oven (Laboratory Glass Blowing, Inc., Lathan, N.Y.) by heating at 450°C for 4 h. All rubber

TABLE 1. *Strains Studied*

VPI no.	Isolated or referred by ^a	Sender no.	Sex of patient ^b	Type or source of specimen (comments)
<i>Bacteroides disiens</i>				
8057	DWL	1304-72B	F	Bartholin abscess (leukemia)
7617	DWL	339-72A	F	cervical swab (dilation and curettage)
7852	DWL	894-72A	M	draining stomach infusion (cancer of esophagus)
7874	DWL	860-72G	F	pelvic abscess
8573	DWL	343-73C	F	vaginal swab (cancer of cervix)
9061	DWL	1314-73A	F	fluids (2 weeks post-operative hysterectomy)
9495	DWL	709-74D	M	perineum
9627	DWL	854-74A	F	groin (post-operative adrenalectomy)
9629	DWL	873-74B	M	penile ulcer drainage
10723	DWL	1393-75D	M	scrotal cyst drainage
10798	DWL	1751-75B	M	penis wound
<i>Bacteroides bivius</i>				
6822	DB	653C	F	endometrium
5965	DWL	813-70A	F	chest fluid
5966	DWL	826-70A	M	penis drainage
6318	VPI	—	F	cervico-vaginal swab (clinically healthy)
7962	DWL	1072-72	F	breast abscess
8478	DWL	195-73B	F	transabdominal hysterectomy
8587	JMR	—	F	blood (septic abortion)
9346	DWL	374-74C	M	urethral discharge
9498	E.O'D.	6444	F	blood
9725	DWL	1005-74D	M	cyst drainage
10307	DWL	492-75B	F	infected thigh wound
10385	DWL	588-75C	F	peritoneal fluid (pelvic inflammatory disease)
10662	DMN	751510	F	vaginal discharge
10752	DMN	752195	F	post-cesarean section
Other Strains				
7620	DWL	404-72B	F	anal fistula
9447	DWL	—	F	cervical tumor
9754	DWL	1153-74A	F	breast (recurrent breast cancer)
10709	VPI	—	F	bone fragment (osteomyelitis of canine jaw)
10879	PW	74505	—	brain abscess

^aDB, Donna Blazevec, Diagnostic Microbiology, Division of Clinical Laboratories, University of Minnesota, Minneapolis, Minnesota; DMN, Dianne M. Norris, National Health Institute, Wellington, New Zealand; DWL, Dwight W. Lambe, Jr., Laboratory of Pathology, Northeast Deaconess Hospital, Boston, Massachusetts; E.O'D., Ed O'Donnell, Medical College of Ohio, Toledo; JMR, James M. Reid, Memorial Medical Center, Savannah, Georgia; PW, Palma Wideman, Wadsworth VA Hospital, Los Angeles, California; VPI, our laboratory.

^bF, female; M, male; —, sex not indicated with information received.

stoppers and rubber tubing used were soaked in a 1% solution of 7X (Linbro Scientific, Inc., Hamden, N.Y.) overnight and then rinsed, first with tap water and then with distilled water. A separate medium-dispensing system, through which hemin-containing medium never passed, was used in dispensing the hemin-free

medium. We found that these special cleaning procedures were necessary because trace amounts of residual hemin on glassware or rubber stoppers cleaned in the usual manner permitted slight growth of the organisms. To determine growth in hemin-free medium, cultures were serially transferred at least three times

in PYGH medium and in PYG-HF medium. Serial transfers were made with about 0.15 ml of inoculum into 5 ml of medium. The original inoculum was from an overnight culture in chopped meat-carbohydrate (CMC) medium (6). Serial transfers were made after overnight incubation from cultures that had 2+ growth (optical density = at least 0.15) or greater; cultures with lesser growth were incubated another 24 h and then transferred. Optical density readings were determined in a Spectronic 20 (Bausch and Lomb) spectrophotometer at a wavelength of 640 nm.

Fatty acids produced were analyzed as previously described (6) using a Dohrmann Anaerobic Bacteriology Analyzer gas chromatograph and a Hewlett-Packard 3380A integrator recorder. The column packing was Supelco 1000, the oven temperature was 130°C, and the carrier gas was 120 cm³ of helium per min. The analytical method for hydrogen was previously described (15).

Colonies on supplemented Difco brain heart infusion (BHIA)-5% rabbit blood agar (6) and on Pfizer brucella (Albimi) agar with vitamin K₁, hemin, and 5% laked (hemolyzed) rabbit blood were examined for pigmentation and for fluorescence under long-wave ultraviolet light after plates had been incubated for 40 to 48 h in anaerobic GasPak (BBL) jars in an atmosphere of 90% hydrogen-10% carbon dioxide. After the initial examination for pigmentation, plates were reincubated anaerobically for an additional 3 to 5 days and then reexamined for pigmenting colonies. Colonies in anaerobic streak tubes on supplemented BHIA with laked rabbit blood were also examined for pigmentation. Colonies in laked blood streak tubes were held for 14 days before recording absence of pigmentation.

The broth disk test of Wilkins and Thiel (16) was used to determine susceptibility to antimicrobial agents.

DNA isolation. Methods used for the isolation of DNA have been described (3, 13). High-molecular-weight DNA for immobilizing on nitrocellulose filters was isolated from cells suspended in 0.15 M NaCl-0.01 ethylenediaminetetraacetic acid buffer, pH 8.0 (13). The cells were treated with 1% sodium lauryl sulfate and disrupted by shaking with glass beads (8). After a preliminary extraction with chromatography-grade phenol, the DNA was isolated by the method of Marum (13). Preparations of competitor DNA and labeled DNA were isolated using a hydroxyapatite procedure (J. L. Johnson, unpublished data).

Determination of G+C content and DNA/DNA homology methods. The moles percent guanine-plus-cytosine (G+C) values of the DNA preparations were determined by the thermal melting point (T_m) method (14) by use of a Gilford 2400 automatic recording spectrophotometer equipped with a model 2527 thermal programmer. DNA from *Escherichia coli* B was included in each set as a standard.

The methods used for immobilizing DNA on nitrocellulose filters and for doing the DNA competition experiments have been described (3, 9). For some competitor DNA preparations, we were unable to obtain the desired 1.5 mg/ml concentration of DNA. In competition experiments with these preparations, the

concentration of the homologous competitor DNA was lowered so that the comparisons were made at the same point on the competition curve.

RESULTS AND DISCUSSION

The Group I (*B. disiens*) and Group PS (*B. bivius*) strains were compared by DNA homology. Strains VPI 8057 (= ATCC 29426) and VPI 6822 (= ATCC 29303) were used as the reference strains. The results are shown in Table 2. Although the DNA preparations had the same G+C contents, two rather homogeneous DNA homology groups were obtained. Ten strains studied had DNA with 82 to 98% homology with DNA of strain 8057, and 13 strains had DNA with 83 to 96% homology with DNA of strain 6822. The levels of DNA homology found between DNA preparations of the two groups are in a range that is usually considered to constitute different species (7). Also included in Table 2 are the results from five strains that were similar, in many phenotypic characteristics, to the other strains. The G+C values were different for these five strains, and all had little or no DNA homology with the two reference strains used, indicating that these five strains belong to one or more other species.

Because strains of *B. disiens* and *B. bivius* had phenotypic characteristics similar to those described for *B. melaninogenicus* subsp. *intermedius* except that they did not form colonies with brown or black pigment on blood agar, the DNA relationships between the reference strains of *B. bivius* and *B. disiens* and two strains with characteristics as described for *B. melaninogenicus* subsp. *intermedius* (6) were investigated. The results are shown in Table 3. There was negligible DNA homology between reference DNAs of *B. bivius* and *B. disiens* and DNAs of the two strains with characteristics of *B. melaninogenicus* subsp. *intermedius*, which indicates that these strains are not merely nonpigmenting variants of *B. melaninogenicus*. The two strains with phenotypic characteristics of *B. melaninogenicus* subsp. *intermedius* had only low levels of DNA homology with each other. The DNA homologies among strains of *B. melaninogenicus* are currently being investigated. Preliminary results indicate that the group may be heterogeneous, but any taxonomic recommendations would be premature at this time.

The strains with a high level of DNA relatedness to reference DNA of VPI 8057 are genetically distinct from those which show high DNA relatedness to strain VPI 6822 as well as from similar organisms. Because the strains which show a high degree of relatedness to VPI 8057 and those which show a high degree of related-

TABLE 2. DNA homologies among strains of *Bacteroides disiens*, *B. bivius*, and some unidentified strains of *Bacteroides*

% homology to:				% homology to:			
Strain no.	Mol% G+C ^a	<i>B. di- siens</i> VPI 8057 (ATCC 29426)	<i>B. bivius</i> VPI 6822 (ATCC 29303)	Strain no.	Mol% G+C ^a	<i>B. di- siens</i> VPI 8057 (ATCC 29426)	<i>B. bivius</i> VPI 6822 (ATCC 29303)
<i>B. disiens</i>				<i>B. bivius</i> (cont.)			
8057	40	100	25	7962	40	27	89
7617	40	82	21	8478		31	96
7852		82	20	8587		20	93
7874	42	89	28	9346		29	84
8573		80	17	9498		21	91
9061		84	25	9725		26	85
9495		82	25	10307		27	94
9627		83	24	10385	40	21	94
9629		82	16	10662		18	91
10723	40	98	26	10752		23	95
10798		85	21	Unidentified			
<i>B. bivius</i>				7620		6	15
6822	40	26	100	9447		16	14
5965	40	25	89	9754	51	17	16
5966		30	95	10709	46	0	7
6318	40	22	83	10879	47	12	17

^a G + C, Guanine + cytosine.TABLE 3. Percent DNA/DNA homologies among *Bacteroides disiens*, *B. bivius*, and *B. melaninogenicus* subsp. *intermedius*

Competitor DNA			Reference DNA			
Species or group	VPI strain no.	G+C (mol%)	<i>B. disiens</i> 8057	<i>B. bivius</i> 6822	<i>B. melaninogenicus</i> subsp. <i>intermedius</i>	
					8944	4197
<i>B. disiens</i>	8057	40	100	25	7	21
<i>B. bivius</i>	6822	40	26	100	3	4
<i>B. melaninogenicus</i> subsp. <i>intermedius</i>	8944	42	15	12	100	42
<i>B. melaninogenicus</i> subsp. <i>intermedius</i>	4197	44	34	3	30	100

ness to VPI 6822 are obligately anaerobic, non-motile, gram-negative rods that produce succinic and acetic acids, usually with isovaleric and isobutyric acids, they are placed in the genus *Bacteroides* (5) as two new species, descriptions of which follow.

Bacteroides disiens sp. n. (di'siens L part. adj. *disiens* going in two different directions; intended to refer to the fact that the organism is both saccharolytic and proteolytic). This species comprises obligately anaerobic, nonmotile, nonsporeforming, nonpigmenting, gram-negative rods. Gram stains of pure cultures in broth or of single colonies from agar show predominantly short gram-negative rods in pairs or, occasionally, short chains with longer rods (Fig. 1). The longer rods occur both singly and in chains with the short rods. Since the variation

in cell length often makes one think that the culture is mixed, the observation of short and long rods in the same chain can be a guide to judging the purity of a culture.

Cells of strain VPI 8057 were 0.8 μ m wide and 2.0 to 8.2 μ m long. The average minimum and maximum cell dimensions of five strains measured were 0.6 to 0.9 μ m in width and 2.0 to 7.4 μ m in length. No flagella were detected with Leifson flagella stain.

Cultures did not survive heating at 80°C for 10 min.

Colonies. After incubation for 1 to 2 days on BHIA or BHIA-laked blood streak tubes or on freshly prepared brucella-laked blood agar plates incubated for 2 days, colonies were minute to 2.0 mm in diameter, circular, entire, convex, translucent to opaque, smooth, shiny, and white.

There usually was no hemolysis on blood agar plates; occasionally a slight greening or indefinite hemolysis was observed, usually in the area of confluent growth.

No brown or black pigmentation was observed in colonies on BHIA-laked blood streak tubes or on laked blood agar plates. Colonies of VPI 9627 and 10723, but not of ATCC 29426, showed light orange to pink fluorescence on blood agar plates.

There was no growth on blood agar plates incubated in a candle-extinction jar or in an aerobic atmosphere.

Cultural characteristics. Cultures in pre-reduced peptone-yeast broth were moderately turbid, sometimes with a slight, smooth sediment. Growth was somewhat enhanced in broth with a fermentable carbohydrate, and these cultures were turbid with a smooth (sometimes granular or flocculent) sediment. Cultures grew well in CMC broth (6).

Best growth was at 37°C, although some strains grew to some extent at 25°C and 45°C.

After good growth was observed in PYG cultures, usually after incubation for 1 day, the pH of the culture was 4.9 to 5.2. The pH of cultures in broth media without fermentable carbohydrate was often 5.9 to 6.1; therefore, a pH of 5.7 or above was interpreted as no fermentation, a pH between 5.5 and 5.7 was interpreted as weak fermentation, and a pH below 5.5 was interpreted as strong fermentation. In general, the terminal pH of cultures in broth media containing fermentable carbohydrates was the same in an atmosphere of 90% nitrogen-10% carbon dioxide as it was in 100% carbon dioxide.

Biochemical reactions. Some biochemical reactions of strain ATCC 29426 and of other

strains of this species included in this study are given in Table 4. Cultures that had been transferred several times in the laboratory digested gelatin, milk, and meat in 1 to 3 days, but newly isolated strains often did not show evidence of digesting milk and meat until after incubation for 10 to 14 days. Casein digestion, however, was apparent in 48 h, even with freshly isolated strains.

Strains grew very poorly, if at all, in PYG-20% bile broth or in PYG-6.5% NaCl broth.

Fermentation products. The fermentation acids (in average milliequivalents per 100 ml) from PYG culture were succinic (3.5) and acetic (1.3), usually with traces of isovaleric and isobutyric and sometimes with small amounts of formic, propionic, or lactic acids. Products from CMC cultures were succinic (12.7 meq/100 ml), and acetic (2.3 meq/100 ml) acids, usually with traces of isovaleric and isobutyric acids and sometimes with small amounts of propionic, butyric, and lactic acids.

Lactate and pyruvate were not utilized.

Hydrogen was not produced and there were no, or only a few, gas bubbles in PYG agar deep cultures.

Susceptibility to antimicrobial agents. All 11 strains were susceptible to chloramphenicol (12 µg/ml), clindamycin (1.6 and 3.2 µg/ml), erythromycin (3 µg/ml), and tetracycline (6 µg/ml). Strain VPI 8057 and six other strains were susceptible and four strains were resistant to penicillin (2 U/ml).

G+C content. The G+C content of the DNA was 40 mol% for strain ATCC 29426 (= VPI 8057) and 40 or 42 mol% for the other three strains tested (Table 2).

Type strain. ATCC 29426 (= VPI 8057 [D. W. Lambe, Jr., EUH no. N-1304-72B, isolated from Bartholin abscess]).

FIG. 1. *Bacteroides disiens* ATCC 29426. Left, Cells from 24-h-old peptone-yeast broth culture. Right, Cells from 24-h-old peptone-yeast-glucose broth culture. Bar represents 10 µm.

Bacteroides bivius sp. n. (bi'vi.us L. adj. *bivius* having two ways, pertaining to the saccharolytic and proteolytic activities of the species). This species comprises obligately anaerobic, nonmotile, nonsporeforming, gram-negative rods. Gram stains of pure cultures in broth or of single colonies from agar show predominantly short, gram-negative rods in pairs or short chains with longer rods only occasionally seen (Fig. 2). In fact, in some cultures the cells may be so short and the short cells so numerous that one is inclined to think that one has a culture of gram-negative cocci. Cells of strain VPI 6822 ranged from 0.6 to 1.3 µm in width and from 1.3 to 2.8 µm in length. The average minimum and maximum cell dimensions of all strains measured were 0.7 to 1.2 µm in width and 1.3 to 4.6 µm in length. No flagella were detected with Leifson

flagella stain. Cultures did not survive heating at 80°C for 10 min.

Colonies. After incubation for 1 to 2 days on BHIA or BHIA-laked blood streak tubes or for 2 days on freshly prepared brucella-laked blood agar, colonies were 0.5 to 2.0 mm in diameter, circular, entire to slightly erose, convex, translucent to semiopaque, smooth, and glistening. Colonies of VPI 7962, 8587, 9498, and 9725, but not of ATCC 29303, showed light orange to pink fluorescence under long-wave ultraviolet light. Slight greening or an indefinite hemolysis often was apparent on blood agar plates. No black or brown pigmentation was observed in colonies on BHIA-laked blood streak tubes incubated for 14 days or on laked blood agar plates incubated for 5 days in an anaerobe jar.

There was no growth on blood agar plates incubated in a candle-extinction jar or in an aerobic atmosphere.

Cultural characteristics. Cultures in pre-reduced peptone-yeast extract broth were turbid with moderate sediment. Growth was somewhat enhanced in broth with a fermentable carbohydrate, and these cultures were turbid with a

smooth (sometimes slightly stringy) sediment. Best growth was reliably obtained at 37°C. Some strains also grew maximally at 30°C. Some strains grew moderately well at 45°C; no strain grew at 25°C.

After good growth was observed in PYG cultures (1 to 2 days), the pH was 4.7 to 5.0. Fructose was often fermented less well (more slowly and to only pH 5.0 to 5.7) than the other fermentable carbohydrates. The pH of cultures in broth media without fermentable carbohydrate was often 5.85 to 6.0; therefore, pH 5.7 or above was interpreted as no fermentation, pH between 5.5 and 5.7 was interpreted as weak fermentation, and pH below 5.5 was interpreted as strong fermentation. In general, the terminal pH of cultures in broth media containing fermentable sugars was the same in an atmosphere of 90% nitrogen-10% carbon dioxide as it was in 100% carbon dioxide.

Biochemical reactions. Some biochemical reactions of strain ATCC 29303 and of 13 other similar strains are given in Table 4. The strains grew very poorly, if at all, in PYG-20% bile broth or in PYG-6.5% NaCl broth.

TABLE 4. *Reactions of B. disiens, B. bivius, and 5 other strains of bacteroides studied.**

Reaction	<i>B. disiens</i>		<i>B. bivius</i>		Unidentified strains of bacteroides				
	ATCC 29426	% pos. 11 strains	ATCC 29303	% pos. 14 strains	7620	9447	9754	10709	10879
Acid from:									
Dextrin	A	100	A	100	A	nt	nt	nt	nt
Fructose	A	91	A	93	—	A	A	A	A
Galactose	—	0	A	71	—	nt	nt	nt	nt
Glucose	A	100	A	100	A	A	A	A	A
Glycogen	A	100	A	100	A	A	A	A	—
Lactose	—	0	A	93	w	—	A	A	A
Maltose	A	100	A	100	—	A	A	A	A
Mannitol	—	0	—	0	—	—	—	—	—
Mannose	—	18	A	93	A	A	A	—	A
Starch	A	100	A	100	—	A	A	A	—
Sucrose	—	0	—	0	—	A	—	—	—
Digestion of									
Gelatin	+	100	+	100	+	+	—	—	+
Milk	+	100	+	100	+	+	—	—	—
Casein	+	100	+	100	—	+	—	—	—
Meat	+	100	+	100	+	+	—	—	—
Hydrolysis of									
Esculin	—	0	—	0	—	—	+	+	—
Starch	+	100	+	100	+	+	+	+	—

*Symbols: —, Negative reaction; +, positive reaction; A, acid; w, weak acid (pH 5.5 — 5.7); nt, not tested. All strains were negative for fermentation of amygdalin, arabinose, cellobiose, erythritol, esculin, inositol, melezitose, melibiose, raffinose, rhamnose, salicin, sorbitol, trehalose, and xylose. No strain reduced nitrate or produced lecithinase or lipase (on egg yolk agar), indole, or catalase.

Gelatin was usually completely digested after incubation for 2 days. Milk cultures usually developed a soft curd, which was subsequently digested. Obvious digestion of milk was often apparent within 3 days, although about 20% of the cultures required incubation for 2 weeks before digestion was certain. Meat was digested within 1 week, often within 1 to 3 days. Many freshly isolated strains did not digest milk and meat for 10 to 14 days, but casein digestion was usually apparent in 48 h, even with freshly isolated strains.

Fermentation products. The fermentation acids (in average milliequivalents per 100 ml) from PYG cultures were succinic (5.2) and acetic (1.5) acids, usually with a trace of isovaleric acid, often with a trace of isobutyric acid, and occasionally with a small amount of lactic acid.

The products from CMC broth cultures were succinic (11.2 meq/100 ml), acetic (3.9 meq/100 ml), and isovaleric (0.64 meq/100 ml) acids, usually with a small amount of isobutyric acid.

Lactate and pyruvate were not utilized.

Hydrogen was not produced; there were no, or only a few, gas bubbles in PYG agar deep cultures.

Resistance to antimicrobial agents. All strains were susceptible to chloramphenicol (12 µg/ml), clindamycin (1.6 and 3.2 µg/ml), and erythromycin (3 µg/ml). Ten of 14 strains (71%) were susceptible to tetracycline (6 µg/ml); only 4 of 14 were susceptible to penicillin (2 U/ml).

G+C content. The G+C content of the DNA was 40 mol% for strain ATCC 29303 (= VPI 6822) and for four other strains tested.

Type strain. ATCC 29303 (= VPI 6822 [Donna Blazevic, University of Minnesota, no. 653C, isolated from endometrium]).

Strains of *B. disiens* and *B. bivius* do not

grow well in PYG-20% bile broth or ferment sucrose, as do *B. fragilis*, *B. thetaiotaomicron*, *B. vulgatus*, *B. distasonis*, and *B. ovatus* (2). They are similar to *B. oralis* in many morphological and cultural characteristics; however, they differ by not fermenting sucrose or raffinose and by digesting milk and meat. *B. disiens* and *B. bivius* do not form black or brown colonies on blood agar, as do strains of *B. melaninogenicus*. They may, however, show some light orange to pink fluorescence on blood agar plates incubated for 48 h, as do some of the saccharolytic strains of *B. melaninogenicus*. None, however, has been observed to show a brick red fluorescence. Characteristics useful in differentiating *B. disiens* and *B. bivius* from other saccharolytic bacteroides that do not grow well in bile are given in Table 5.

Characteristically, strains of *B. bivius* ferment lactose and strains of *B. disiens* do not. However, one strain of *B. bivius* (VPI 7962) that showed 89% homology with DNA of *B. bivius* ATCC 29303 was phenotypically indistinguishable from strains of *B. disiens*. VPI 7962 consistently did not ferment galactose, lactose, or mannose and, in all respects tested, had phenotypic characteristics like strains of *B. disiens*. Two separate DNA preparations of VPI 7962 showed a high degree of homology with reference DNA of *B. bivius* and little homology with reference DNA of *B. disiens*. Phenotypic tests of 7962 were repeated several times, using a subculture of the culture used in the DNA preparations, and all results were as described.

Somewhat similar strains of *Bacteroides* (7620, 9447, 9754, 10709, and 10879), listed as "unidentified" in Tables 1 and 2, showed negligible homology with the *B. bivius* and *B. disiens* reference DNAs. These strains produced succinic and acetic acids as major fermentation products, did not produce hydrogen, and did not grow in PYG-20% bile broth. Major phenotypic differences between these strains and *B. bivius* and *B. disiens* were the lack of proteolytic activity (9754, 10709, and 10879), absence of fermentation of starch (7620 and 10879), and fermentation of sucrose (9447).

There was no growth of any strain of *B. disiens* tested (ATCC 29426, VPI 9061, 9495, 9629) or of any strain of *B. bivius* tested (ATCC 29303, VPI 7962, 8587, 9498, 9725, 10207) in the fifth serial transfer in hemin-free PYG. Only three strains of *B. disiens* (VPI 9061, 9495, and 9629) grew in the third serial transfer of PYG-HF; VPI 9629 grew in the fourth serial transfer, but not in the fifth. *B. disiens* ATCC 29426 and *B. bivius* ATCC 29303 did not grow in the third serial transfer in the two different lots of PYG-HF media tested at two different times. All

FIG. 2. *Bacteroides bivius* ATCC 29303. Left, Cells from 24-h-old peptone-yeast broth culture. Right, Cells from 24-h-old peptone-yeast-glucose broth culture. See Fig. 1 for scale.

TABLE 5. Some differential characteristics of *B. disiens*, *B. bivius*, and some similar species of *Bacteroides*^a

Species	Indole production	Esculin hydrolysis	Lactose	Starch	Sucrose	Black pigment	Growth in 20% bile
<i>B. disiens</i>	—	—	—	+	—	—	I
<i>B. bivius</i>	—	—	+-	+	—	—	I
<i>B. melaninogenicus</i> subsp. <i>intermedius</i>	v	—	v	+-	v	+	I
<i>B. melaninogenicus</i> subsp. <i>melaninogenicus</i>	—	+-	+	+	+	+	I
<i>B. oralis</i>	—	+	+	+	+	—	I
<i>B. eggerthii</i>	+	+	+	+	—	—	+

^a—, Negative reaction or no fermentation; +, positive reaction or fermentation; v, variable reaction; I, growth inhibited. Where two reactions are given, the first is the more usual and the second is observed less frequently.

strains grew well in the first transfer in PYG-HF, probably due to the hemin that was carried over in the CMC inoculum. All strains tested grew well in the third and fourth serial transfers in PYGH medium.

As has been reported for some other species of *Bacteroides*, *B. disiens* and *B. bivius* require hemin for growth. Gibbons and Macdonald (4) found that many strains of *B. melaninogenicus*, particularly the saccharolytic strains, require hemin for growth, and Caldwell et al. (1) reported that hemin was required by *B. ruminicola*. More recently, Macy et al. (12) reported that hemin stimulated growth of and succinate production by *B. fragilis* ATCC 23745. They detected a *b*-type (and possibly a *c*-type) cytochrome and a fumarate reductase in cells grown with hemin, but not in cells grown without hemin, and postulated that adenosine 5'-triphosphate generated during reduction of fumarate to succinate could be responsible for the increased growth rate and cell yield in hemin-containing media. Although Macy et al. did not report an absolute hemin requirement for growth of *B. fragilis*, an absolute requirement has been found for strains of *B. fragilis* (M. D. Appleman, J. L. Johnson, and T. D. Wilkins, *Bacteriol. Proc.*, p. 132, 1976).

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of Luba Fabrycky, Ann Mitchell, Habiba Najafi, Carol Phelps, and Sue Smith. This research was supported by Public Health Service Grant GM-14604 from the National Institute of General Medical Sciences.

REPRINT REQUESTS

Address reprint requests to: Dr. Lillian V. Holdeman, Anaerobe Laboratory, College of Agriculture and Life Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

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