*Bacteroides microfus*us, a New Species from the Intestines of Calves, Chickens, and Japanese Quails

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*Bacteroides microfus*us sp. nov. is described on the basis of 16 strains isolated from a number of fecal or cecal specimens from calves, chickens, and Japanese quails. The isolates are obligately anaerobic, gram-negative, nonsporeforming, nonmotile, relatively small rods with pointed ends; the cells occur singly, in pairs, and sometimes in short chains. *B. microfus*us differs from other species in the genus *Bacteroides* principally in morphology; the small spindle-shaped cells, particularly on agar media, are rather distinctive. The guanine-plus-cytosine contents of the deoxyribonucleic acids of the new strains range from 59 to 61 mol%, the highest value so far detected in the genus *Bacteroides*. The new strains are similar to those of *B. putredinis*, *B. furcosus*, *B. amylophilus*, and *B. succinogenes* in fermenting a relatively small number of carbohydrates; however, *B. microfus*us can clearly be differentiated from these organisms by numerous biochemical properties and/or fermentation products. The type strain of *B. microfus*us is Q-1 (= ATCC 29728 = NCTC 11190).

During studies on the intestinal flora of various animals, a number of obligately anaerobic, nonsporeforming, gram-negative, small rods with pointed ends were encountered. The strains differed sufficiently from other species in the genus *Bacteroides* in their morphology and in the guanine-plus-cytosine (G+C) contents of their deoxyribonucleic acids (DNAs) to suggest that they might belong to new species. A study was undertaken, accordingly, to determine the systematic position of these strains.

MATERIALS AND METHODS

Bacterial strains. Sixteen strains were studied. Of these, seven were isolated from the feces of healthy 2-month-old calves sustained on an artificial diet, six were from the feces of adult Japanese quails, and three were from the cecal contents of chickens. *Bacteroides multiacidus* (11) ATCC 27723 was obtained from the American Type Culture Collection, Rockville, Md., and was included in this study because it had a high G+C content relative to that of the genus *Bacteroides*, as did the new isolates. Twelve strains isolated from the ceca of chickens were obtained from Ella M. Barnes, Food Research Institute, Norwich, England, as *Bacteroides* sp. They were also included in this study because of their morphological similarity to the present organism in having cells with pointed ends.

Isolation and maintenance of cultures. Fecal or cecal materials emulsified in an anaerobic buffer solution were plated out on modified Eggerth-Gagnon agar and glucose-blood-liver agar (10) plates. Eggerth-Gagnon and glucose-blood-liver agar plates were incubated at 37°C in an anaerobic steel-wool jar (12) filled with an atmosphere of 100% CO₂. After incubation for 2 to 3 days, grayish to slightly reddish mottled

colonies were selected. Strains were maintained on prereduced Eggerth-Gagnon-liver slants with H₂CO₃-CO₂ buffer (10) and stored at 4°C. New transfers were usually made at 3-month intervals.

Cultural and biochemical tests. The methods for the cultural and biochemical characterization of the strains used in this study were described previously (6). Growth in the presence of antimicrobial agents was tested in peptone-yeast extract-Fildes glucose (PYFG) broth (6). β -Glucuronidase activity was tested for by the method of Kent et al. (7) with partial modification.

Fermentation products and DNA base composition. Alcohols, volatile acids, and nonvolatile acids were detected by the method of Holdeman and Moore (4). The G+C content of DNA was determined by the methods of Marmur (8) and Marmur and Doty (9) with partial modification. DNA from calf thymus (Worthington Biochemicals Corp., Freehold, N.J.) was included in each set of analyses as a standard; the G+C content of the calf thymus was regarded as 42 mol%.

RESULTS AND DISCUSSION

The characteristics of the 16 new isolates are as follows.

Morphology. Obligately anaerobic, nonmotile, nonsporeforming, gram-negative, relatively small rods with pointed ends. Cells occurred singly, in pairs, and sometimes in short chains (Fig. 1). After incubation for 2 days, cells grown on Eggerth-Gagnon agar were 0.15 to 0.3 by 0.3 to 1.3 μ m. Short, filamentous or swollen forms were often seen on glucose-blood-liver agar or in PYFG broth containing a relatively large amount of fermentable carbohydrate.

After incubation for 2 days, surface colonies on Eggerth-Gagnon agar were 0.5 to 1.0 mm in diameter, circular, convex, entire, smooth, translucent, grayish, and beta-hemolytic. Some strains formed larger (1.0- to 1.5-mm), raised, opaque, gray colonies which contained stouter (swollen), more filamentous cells.

Cultural and biochemical characteristics (Tables 1 to 3). There was slight to moderate growth in PYF broth. PYFG cultures had abundant growth, a smooth sediment, and a terminal pH of 5.5 to 5.8. Growth in PYG broth was enhanced by the addition of 20% bile or both

FIG. 1. *B. microfusum* ATCC 29728, the type strain. Cells from 24-h-old PYG broth culture. Bar represents 10 μ m.

TABLE 1. Biochemical characteristics of *B. microfusum*: tests in which all strains are positive

Acid from:
Glucose
Lactose
Mannose
Melibiose
Gas from PYFG ^a
Gelatin liquefaction ^b
Growth at 45°C ^c
Growth enhanced by:
Bile (20%)
Hemin (0.0005%) and menadione (0.00005%)
Growth in presence of: ^d
Bacitracin (3 U/ml)
Cefalotin (300 μ g/ml)
Colistin (10 μ g/ml)
Erythromycin (60 μ g/ml)
Kanamycin (1,000 μ g/ml) ^e
Penicillin (15 μ g/ml) ^c
Polymyxin B (10 μ g/ml)
Vancomycin (10 μ g/ml)
Hemolysis
Litmus milk:
Acid
Curd
Production of:
β -Glucuronidase
Glutamic acid decarboxylase
Products from PYFG:
Alcohols ^f
Acetic acid ^c
Propionic acid ^f
Succinic acid ^f

^a Trace amounts.

^b Weak reaction.

^c Slight to moderate growth.

^d Basal medium, PYFG broth.

^e Moderate product.

^f Major product.

TABLE 2. Biochemical characteristics of *B. microfusum*: tests in which all strains are negative

Acid from:	Digestion of litmus milk
Amygdalin	Esculin hydrolysis
Arabinose	Growth at 15°C
Cellobiose	Growth in presence of:
Dextrin	Brilliant green (0.001%)
Dulcitol	Crystal violet (0.005%)
Erythritol	Neomycin (1,600 μ g/ml) ^a
Esculin	Rifampin (15 μ g/ml) ^a
Fructose	Heat resistance at 70°C for 10 min
Glycerol	Lactate utilization to propionic acid
Glycogen	Motility
Inositol	Neutral red reduction
Inulin	Nitrate reduction
Maltose	Orotic acid hydrolysis
Mannitol	Production of:
α -Methylglucoside	Acetyl-methylcarbinol
α -Methylmannoside	Catalase
Melezitose	Indole
Raffinose	Hydrogen-sulfide ^b
Rhamnose	Lecithinase ^c
Ribose	Lipase (tributyrin)
Salicin	Urease
Sorbose	Products from PYFG:
Sorbitol	Butyric acid
Starch	iso-Butyric acid
Sucrose	Lactic acid
Trehalose	Valeric acid
Xylose	iso-Valeric acid
Casein digestion	Starch hydrolysis
	Threonine conversion

^a Slight growth occurred sometimes with a few stains.

^b SIM, Sulfide-indole-motility medium (Difco).

^c EYA, McClung-Toabe egg yolk agar (modified) (4).

TABLE 3. Biochemical characteristics of *B. microfusum*: tests in which there are strain differences

Characteristic	No. of strains positive	Result of type strain (Q-1)
Ammonium production ^a	4/16	—
Acid from galactose ^b	12/16	+

^a The positive strains had weak reactions.

^b The positive strains had weak reactions; the pH values ranged from 5.6 to 6.2 against control pH values of about 6.7.

0.0005% hemin and 0.00005% menadione but was not affected by the addition of 10% rumen fluid or 0.1% Tween 80. When grown in PYFG broth with added antimicrobial agents, the strains were usually susceptible to neomycin (1,600 μ g/ml), rifampin (15 μ g/ml), brilliant green (0.001%), and crystal violet (0.005%); slightly susceptible to erythromycin (60 μ g/ml) and penicillin (15 μ g/ml); and tolerant to bacitracin (3 U/ml), cefalotin (300 μ g/ml), colistin (10 μ g/ml), kanamycin (1,000 μ g/ml), polymyxin B (10 μ g/ml), and vancomycin (10 μ g/ml). The temperature for optimal growth was 37°C. The strains grew weakly at 45°C but usually not at

15 or 25°C. No strains resisted heating at 70°C for 10 min after incubation for 7 days at 30°C on CM agar (4) slants or medium 10 agar (3).

Acid was produced from glucose, mannose, lactose, melibiose, and galactose. Some strains did not ferment galactose. Arabinose, Xylose, rhamnose, sorbose, ribose, fructose, sucrose, maltose, cellobiose, trehalose, raffinose, melezitose, dextrin, starch, glycogen, inulin, glycerol, dulcitol, mannitol, sorbitol, erythritol, esculin, salicin, amygdalin, α -methylglucoside, and α -methylmannoside were not fermented. Starch, esculin, and orotic acid were not hydrolyzed.

Trace amounts of gas was formed from glucose. Gelatin was liquefied weakly. Indole, hydrogen sulfide, and acetylmethylcarbinol were not produced. Nitrate was not reduced to nitrite. Acid and curd were produced in litmus milk without digestion. Lecithinase was not produced on egg yolk agar. Trybutyrin was not hydrolyzed. Catalase, urease, and threonine deaminase were not produced. Glutamic acid decarboxylase was produced. Lactate was not converted to propionic acid.

Propionic and succinic acids were produced as major products in PYFG broth with moderate amounts of acetic acid and trace amounts of alcohols. Isobutyric, butyric, isovaleric, valeric, and lactic acids were not produced. Formic acid could not be detected by the method used.

The G+C contents of the DNAs were 60.3, 59.5, and 60.7 mol% for strains Q-1, CQ-5, and CHQ-3, respectively.

The strains studied were rather uniform in their characteristics, enough so as to warrant their placement in the same taxon. A comparison of their overall characteristics with those of the presently known species of the genus *Bacteroides* indicates that these strains represent a new species, for which we propose the name *Bacteroides microfus* (mic.ro.fus.us. Gr. adj. *micro* small; L.n. *fuscus* a spindle; M. L. adj.

microfus pertaining to a small spindle). The type strain of *B. microfus* is Q-1 (= ATCC 29728 = NCTC 11190). This strain was isolated from the feces (10^6 to 10^9 cells per gram [wet weight]) of a Japanese quail.

Characteristics which qualify *B. microfus* as a member of the genus *Bacteroides* and which differentiate it from other qualitatively less saccharolytic species. *B. microfus* is a member of the genus *Bacteroides* because it is an obligately anaerobic, nonmotile, nonsporeforming, gram-negative, rod-shaped organism with pointed ends. Unlike *Fusobacterium* and *Leptotrichia* (5), it produces neither butyric nor lactic acid as a major product of glucose fermentation, and the G+C content of its DNA is much more than 40 mol%. The G+C content of 60 mol% of *B. microfus* is the highest of the presently known and described *Bacteroides* species; this value is close to that of *B. multiacidus* (56 to 58 mol%) (11), which, however, differs from *B. microfus* in cellular morphology and in numerous biochemical properties. *B. microfus* is rather similar to *B. clostridiiformis*, *B. biacutus*, and *F. symbiosum* in having cells with pointed ends. These three organisms, however, have recently been transferred to the genus *Clostridium* because of their sporeforming ability (6). The presently described organism also differs as follows from morphologically similar strains isolated from the ceca of chickens by Barnes and Impey (1, 2) and identified by them as *bacteroides*: the latter organisms have a larger cell size (0.5 to 0.8 by 2.0 to 10.0 μ m), a number of biochemical reactions different from those of *B. microfus*, and G+C contents of 48 to 53 mol%. Some characters of *B. microfus*, such as cellular morphology and G+C content, are to a certain extent different from those of typical *Bacteroides* strains. However, it is felt that at present there is insufficient knowledge about the members of the

TABLE 4. Characteristics that differentiate *B. microfus* from other species with qualitatively less saccharolytic reactions^a

<i>Bacteroides</i> species	Product ^b	Cellobiose	Fructose	Lactose	Melibiose	Indole
<i>B. microfus</i>	SPa	—	—	+	+	—
<i>B. putredinis</i>	apsivbib (lf)	—	—	—	—	+
<i>B. furcosus</i>	Lsa2 (f)	—	w	—	—	—
<i>B. amylophilus</i>	Sfa (pl)	—	—w	+	—	—
<i>B. succinogenes</i>	SApiv (f)	w	—	v	—	—

^a The characteristics of the species other than *B. microfus* are from Holdeman and Moore (4, 5). Symbols: +, Reaction positive for 90 to 100% of strains or final pH of 5.5 or lower; —, reaction negative for 90 to 100% of strains or final pH of >6.0; w, reaction weak for 90 to 100% of strains or final pH of 5.6 to 6.0; v, reaction variable within the strains.

^b Products: A, acetic acid; B, butyric acid; F, formic acid; L, lactic acid; P, propionic acid; S, succinic acid; iV, isovaleric acid; iB, isobutyric acid; 2, ethanol. Capital letters, major products; small letters, minor products. Products in parentheses were not uniformly detected.

family *Bacteroidaceae* to permit the placement of *B. microfusus* in a new genus. It seems appropriate, therefore, that this organism be retained in the genus *Bacteroides*.

Table 4 shows the biochemical characteristics most useful in distinguishing *B. microfusus* from some other members of the genus *Bacteroides* with qualitatively less saccharolytic reactions. The major products of glucose fermentation by *B. microfusus* are succinic and propionic acids, usually with small amounts of acetic acid. This species produces acid from lactose and melibiose but not from cellobiose and fructose; it does not produce indole.

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REPRINT REQUESTS

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