Bacteroides endodontalis sp. nov., an Asaccharolytic Black-Pigmented *Bacteroides* Species from Infected Dental Root Canals

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A new species, *Bacteroides endodontalis*, is proposed for black-pigmented asaccharolytic *Bacteroides* strains that have negligible deoxyribonucleic acid homology with either *Bacteroides gingivalis* or *Bacteroides asaccharolyticus*. Strain HG370 (= ATCC 35406) is the type strain. Unlike *B. gingivalis*, *B. endodontalis* does not agglutinate sheep erythrocytes or produce phenylacetic acid. *B. endodontalis* resembles *B. asaccharolyticus* physiologically but can be distinguished by its lower deoxyribonucleic acid guanine-plus-cytosine content (50 versus 53 to 54 mol%), by its serological reactions, by its electrophoretic patterns of proteins, and possibly by its menadione requirement.

The classification of black-pigmented *Bacteroides* strains, which were formerly classified as *Bacteroides melaninogenicus*, has been much improved recently, and many new species have been proposed, mainly on the basis of deoxyribonucleic acid (DNA) homology studies (1, 2, 5–7, 13, 19, 20). The following two asaccharolytic species have been described previously: *Bacteroides asaccharolyticus* (2) and *Bacteroides gingivalis* (1). Strains of *B. asaccharolyticus* are mostly from nonoral sources, whereas strains of *B. gingivalis* are mainly from periodontal pockets. *B. gingivalis* can be easily distinguished from *B. asaccharolyticus* by its ability to agglutinate erythrocytes (15), to produce phenylacetic acid (8), and to demonstrate a strong trypsin-like activity (14).

Previously, we reported (19) that two asaccharolytic black-pigmented *Bacteroides* strains that were isolated by Sundqvist (G. Sundqvist, Ph.D. thesis, University of Umea, Umea, Sweden, 1976) from dental root canals had negligible DNA homology with either *B. gingivalis* or *B. asaccharolyticus*. In this paper we describe DNA hybridization studies which showed that these strains and another isolate from an infected dental root canal form a distinct DNA homology group. We propose a new species for these strains, *Bacteroides endodontalis*; strain HG370 (= ATCC 35406) is the type strain of this species.

MATERIALS AND METHODS

Bacterial strains. The strains which we used are listed in Table 1. Strain HG370^T (T = type strain) was isolated along with anaerobic streptococci from an infected root canal with a very large area of periapical destruction. The strains were maintained on sheep blood agar plates (Oxoid no. 2 agar supplemented with 0.05% hemin and 0.01% menadione) in an atmosphere containing 80% N₂, 10% CO₂, and 10% H₂.

Physiological characteristics. The fermentation and biochemical tests, as well as the procedures for extraction and methylation of volatile and nonvolatile fatty acids, were done according to the methods outlined in the *Anaerobe Laboratory Manual* (4) and by the Minitex anaerobic system (BBL Microbiology Systems, Cockeysville, Md.). The compounds used in the fermentation assays were glucose, sucrose, lactose, cellobiose, mannose, arabinose, maltose, rhamnose, trehalose, xylose, raffinose, sorbitol, salicin, mannitol, and glycerol. Menadione requirement was deter-

mined by using BM agar plates (18) without menadione for three passages.

The enzymatic activities of the strains were studied with the API ZYM system (API Produits de Laboratoire Ltee., St.-Laurent, Canada) by using growth conditions and test procedures which have been described previously (14). Hemagglutination assays using sheep erythrocytes were performed in titer plates as described by Mayrand et al. (10), except that no reducing agent was added, and by the slide test described by Slots and Genco (15). Protease activity (Azocoll) was measured as previously described (9). Collagenase activity was determined by two methods; the first involved growth in broth in the presence of collagen (16), whereas the second measured the collagenolytic activity of washed cells as described by Gisslow and McBride (3). Polyacrylamide gel electrophoresis of soluble proteins was done according to the methods and procedures described by Moore et al. (11).

Isolation of DNA. Unlabeled DNA was isolated from cells lysed with sodium dodecyl sulfate or from cells lysed mechanically with a Vibrogen cell mill (Bühler, Tübingen, Federal Republic of Germany), as described previously (17). Tritiated DNA was isolated from Sarcosyl-lysed cells by means of CsCl density gradient centrifugation (19).

Determination of DNA base composition and DNA homology. DNA base composition was determined by the buoyant density method, and the amount of DNA-DNA hybridization was determined by the S1 nuclease method essentially as described previously (19).

Indirect fluorescent antibody staining. The procedures used for antiserum production in rabbits, conjugate preparation (goat immunoglobulin monospecific for rabbit immunoglobulin labeled with fluorescein isothiocyanate isomer I [BBL]), and actual assay were those described by Mouton et al. (12). Fluorescence of single cells was graded as follows: 4+, brilliant fluorescence with good cell envelope definition, center of cell dark; 3+, moderate fluorescence with good cell envelope definition, center of cell dark; 2+, faint fluorescence, single cells visible with no definition of cell envelope; 1+, bare fluorescence, single cells not distinguishable; 0, no visible staining of bacteria.

RESULTS

Three asaccharolytic, black-pigmented *Bacteroides* strains from infected dental root canals with periapical

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TABLE 1. Strains used in this study

Strain	Source ^a	Site of isolation
<i>B. endodontalis</i> sp. nov.		
HG181 (= H11 a-e)	1	Infected root canal
HG182 (= BN11 a-f)	1	Infected root canal
HG370 ^T (= ATCC 35406)	2	Infected root canal
<i>B. asaccharolyticus</i>		
HG78 (= B536)	3	Human feces
HG107 ^T (= ATCC 25206 ^T)	3	Empyema
HG364 (= BM4)	4	Dental plaque
HG111 (= ATCC 27067)	5	Human leg wound
<i>B. gingivalis</i>		
HG66 (= W83)	3	Clinical specimen
HG372 ^T (= ATCC 33277 ^T)	6	Human gingival sulcus

^a Sources: 1, G. Sundqvist, University of Umea, Umea, Sweden; 2, our isolate; 3, H. N. Shah, London Hospital Medical College, London, United Kingdom; 4, G. H. Bowden, University of Winnipeg, Winnipeg, Canada; 5, E. C. S. Chan, McGill University, Montreal, Canada; 6, J. Slots, State University of New York, Buffalo.

destruction were studied. A DNA analysis of these strains showed a guanine-plus-cytosine content of 49.6 to 50.2 mol% (Table 2). DNA-DNA hybridization experiments showed little or no homology of the DNAs of these three strains with reference DNAs of *B. asaccharolyticus* and *B. gingivalis*. The three strains formed a distinct DNA homology group with mutual DNA hybridization values of 87% or more (Table 2). From these results we concluded that these strains should be placed in a separate species, for which we propose the name *Bacteroides endodontalis* (en.do.don'ta.lis. Gr.prep.endon within; Gr.n.odon tooth; *endodontalis* within teeth). The cells of *B. endodontalis* strains are obligately anaerobic, nonsporeforming, nonmotile, gram negative, and rod shaped. Cells grown in broth are 0.4 to 0.6 by 1.0 to 1.5 μ m. The organisms require hemin and vitamin K1 or menadione for growth. Colonies grow slowly on blood agar plates. After 48 h of incubation colonies are 1 to 2 mm in diameter, low convex, circular, smooth, and entire. Dark brown or black pigmentation develops only after incubation for more than 7 days at 37°C. When hemolyzed sheep blood is used, pigmentation develops within 3 days. Hemolysis is visible on

TABLE 2. Guanine-plus-cytosine contents and relative levels of hybridization of DNAs from asaccharolytic black-pigmented *Bacteroides* strains with reference DNAs from strains of *B. endodontalis*, *B. asaccharolyticus*, and *B. gingivalis*

Source of unlabeled DNA	Guanine-plus-cytosine content (mol%)	Relative hybridization (%) with labeled DNA from strain:		
		HG181	HG107 ^T	HG66
<i>B. endodontalis</i>				
HG181	50.0	100	12	0 ^a
HG182	50.2	87	20	0 ^a
HG370 ^T	49.6	97	19	0
<i>B. asaccharolyticus</i>				
HG78	54.2	38	83	0 ^a
HG107 ^T	53.0	15	100	22 ^a
HG364	54.3	31	87	0
<i>B. gingivalis</i>				
HG66	48.2	25	9	100 ^a
HG372 ^T	49.2	3	0	68

^a Data from reference 19.

blood agar plates after incubation for 24 h in air. The organisms do not ferment any of the sugars listed above. The final pH in broth containing glucose is 6.8 to 7.2. Acetic, propionic, butyric, isobutyric, and isovaleric acids are produced. Phenylacetic acid is not produced. No collagenolytic or proteolytic activity is detected, except for a positive gelatinase reaction. API ZYM tests show no trypsin-like activity and strong acid and alkaline phosphatase and phosphamidase activities. The organisms do not produce hemagglutinin or catalase; indole and H₂S are produced. Arginine is hydrolyzed; starch and esculin are not hydrolyzed. Nitrate is not reduced.

The protein patterns of the three strains which we studied were not identical, although similar bands were present throughout the gel. However, the general pattern of these three strains was very different from the patterns of *B. asaccharolyticus* and *B. gingivalis* isolates (Fig. 1). A serological analysis using an indirect fluorescence antibody staining method showed that all three isolates react poorly or not at all (reactions of 0 or 1+ with an antiserum diluted 1 in 20) with antisera directed against *B. gingivalis* or *B. asaccharolyticus*. The homologous strains and antisera gave results of 3+ or 4+ (with an antiserum diluted 1 in 640). These data indicate that the three strains which we studied do not share common antigens with *B. gingivalis* or *B. asaccharolyticus*.

The type strain of *B. endodontalis* is strain HG370 (= ATCC 35406).

FIG. 1. Soluble protein patterns. Lane 1, Standard mixture of bovine serum albumin, carbonic anhydrase, chicken egg albumin, and lactalbumin; lane 2, *B. asaccharolyticus* strain HG107^T; lane 3, *B. endodontalis* strain HG370^T; lane 4, *B. endodontalis* strain HG182; lane 5, *B. endodontalis* strain HG181; lane 6, *B. gingivalis* strain HG372^T.

DISCUSSION

It has been shown that most oral asaccharolytic black-pigmented *Bacteroides* strains belong to *B. gingivalis* and that most of the nonoral or "clinical" isolates belong to *B. asaccharolyticus* (1, 19). However, there are exceptions; strain HG364, which was isolated from dental plaque, was identified as *B. asaccharolyticus*. This strengthens the conclusion that an identification based only on isolation site is inappropriate.

Asaccharolytic black-pigmented *Bacteroides* strains HG181 and HG182 were originally isolated by Sundqvist (Ph. D. thesis) from dental root canals; his description is fully consistent with the description given in this paper.

In a previous paper (19), we reported that strains HG181 and HG182, which were isolated from dental root canals, were different from both *B. gingivalis* and *B. asaccharolyticus* on the basis of DNA-DNA hybridization. It may be significant that the three strains of *B. endodontalis* were all isolated from dental root canals with periapical destruction. Further research will be necessary to determine the prevalence of *B. endodontalis* in dental root canals, in other samples from oral cavities, and in nonoral infections. In a mouse virulence test the two strains of *B. endodontalis* studied produced an inflammation pattern which was different from the inflammation patterns found with strains of *B. gingivalis* and *B. asaccharolyticus* (18), suggesting a pathogenic potential different from that demonstrated by *B. gingivalis* and *B. asaccharolyticus* strains.

B. endodontalis can be easily distinguished from *B. gingivalis*; *B. gingivalis* hemagglutinates sheep erythrocytes (15), produces phenylacetic acid (8), and shows a strong trypsin-like activity, whereas *B. endodontalis* does not. It is more difficult to distinguish *B. endodontalis* from *B. asaccharolyticus*. Other than by DNA hybridization, *B. endodontalis* can be distinguished from *B. asaccharolyticus* by the lower guanine-plus-cytosine content of its DNA, by serological methods, and by electrophoretic patterns of proteins. The three strains of *B. endodontalis* described in this paper grew very slowly both in broth and on enriched blood agar plates. Although growth was slow compared with almost all strains of *B. gingivalis* and *B. asaccharolyticus*, it is probably inappropriate to use this as a differential character. The three strains of *B. endodontalis* described here required menadione for growth, whereas six tested strains of *B. asaccharolyticus*, including the type strain, did not. Although more strains should be examined, menadione requirement might be a valuable test for identification of *B. endodontalis*.

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