**Bacteroides salivosus** sp. nov., an Asaccharolytic, Black-Pigmented Species from Cats

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A new species, *Bacteroides salivosus* sp. nov., is proposed for black-pigmented, asaccharolytic *Bacteroides* strains from cats, isolated from subcutaneous abscesses and empyemas, as well as from gingival margins of normal mouths. The bacterium is an obligately anaerobic, gram-negative, brown- or black-pigmented, asaccharolytic, nonmotile, nonsporeforming rod that does not grow in 20% bile and has a guanine-plus-cytosine content of 42 to 44 mol%. It has 12% deoxyribonucleic acid homology with the human type strain of *Bacteroides gingivalis* (ATCC 33277) and 1% deoxyribonucleic acid homology with the human type strain of *Bacteroides asaccharolyticus* (ATCC 25260). Strain VPB 157 (NCTC 11632) is the type strain. Unlike *B. gingivalis*, *B. salivosus* produces catalase, and the colonies of the type strain fluoresce at 24 and 48 h, although some other strains do not fluoresce. It does not agglutinate sheep erythrocytes. Unlike either *B. asaccharolyticus* or *Bacteroides endodontalis*, it has trypsinlike activity and produces large quantities of phenylacetic acid.

**MATERIALS AND METHODS**

**Bacterial strains.** All 18 strains studied were isolated from cats with either a solitary closed subcutaneous “fight” wound abscess or a pyothorax, or from the gingival margins in normal cats. These organisms were compared in all reactions with the type strains (obtained from the American Type Culture Collection [ATCC]) *Bacteroides*. ATCC 33277 and *Bacteroides asaccharolyticus* ATCC 25260.

**Methods and characterization.** The general methods of growth and biochemical characterization have been described previously (4).

**DNA isolation.** The organisms were grown in 800-ml amounts of preeroded brain heart infusion broth (Oxoid Ltd.) supplemented with yeast extract and vitamin K and heme (PRBHBIB) (1). Bottles containing 800 ml of medium were inoculated with 18 ml of an overnight culture grown in PRBHBIB and then incubated for 24 h at 37°C. The harvested cells were suspended in a 0.15 M NaCl-0.01 M ethylene-diaminetetraacetic acid-salt solution (pH 8.0). The cells were lysed by adding sodium dodecyl sulfate to a final concentration of 1%. DNA preparations for hybridization experiments and for DNA guanine-plus-cytosine (G+C) determinations were isolated by a hydroxyapatite procedure (2). The DNA preparations were stored in 0.1× SSC (SSC is 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0) at −20°C.

**G+C content of DNA.** Thermal melting points were used to determine the G+C contents of the DNA preparations. A Gilford spectrophotometer (model 260) fitted with a Gilford 2527 thermoprogrammer was used for melting point determinations (5). *Escherichia coli* b DNA isolated by the hydroxyapatite procedure was included in each run. Two preparations were made from each organism, and each preparation was run on at least two occasions.

**Preparation of labeled nucleic acids.** Fragmented, denatured DNA was labeled with 123I by using a variation of the thallium chloride method (7, 8).

**DNA homology methods.** Preparations of fragmented, denatured DNA were prepared by three passages through a French pressure cell at 16,000 lb/in² followed by heating in a boiling water bath for 5 min and adjusting to 0.5 mg/ml. DNA homology values were determined by using an SI nuclease procedure as described previously (2). The reassociation reaction mixtures contained 10 μl of denatured labeled DNA (0.01 to 0.03 μg), 50 μl of denatured unlabeled DNA (25 μg), 25 μl of 5.28 M NaCl plus 2 mM N,N'-2-ethanesulfonic acid at pH 7.0, and 25 μl of 0.01 M NaCl plus 0.01 M ethylenediaminetetraacetic acid, pH 8.0. The reactions were incubated for 20 h at 60°C for group 1, 56°C for group 2, 53°C for group 3, and 56°C for *B. gingivalis* ATCC 33277. For measuring background reassociation and the nuclease SI-resistant material in the labeled preparations, the denatured unlabeled DNA was replaced with 50 μl (20 μg) of sheared native salmon sperm DNA.

**RESULTS AND DISCUSSION**

As shown in a previous publication (4) and summarized here (Table 1), on biochemical parameters the strains could be divided into three groups which differed from the human type strains of *B. gingivalis* (ATCC 33277) and *B. asaccharolyticus* (ATCC 25260). The more recently described *Bacteroides endodontalis* (9) also differs biochemically from the cat strains studied here (Table 1).

Shown in Table 2 are DNA homology results for representative strains from each group of strains from cats and for *B. gingivalis* ATCC 33277 and *B. asaccharolyticus* ATCC 25260 from humans. These data indicate three distinct homology groups. A comparison of these results with those in an earlier publication (4) shows that group 1 is a combination of groups A and E, group 2 includes C and E, and group 2 corresponds to group B. There was some variation in the G+C contents obtained in the two studies. Although the
values for group 1 were very similar for both studies, the values of 40 to 42% for group 2 and 42 to 44% for group 3 obtained in this study are 2 to 5 mol% G+C lower than those obtained previously (4). We do not have an explanation for these differences, but since we rechecked some of the data obtained previously (4). We do not have an explanation for these differences, but since we rechecked some of the data obtained previously (4). We do not have an explanation for these differences, but since we rechecked some of the values shown in Table 2.

Group 1 strains show an average of 72% DNA homology with B. gingivialis ATCC 33277T and an average of 93% homology with the two reference strains from group 1 (Table 2). These results are consistent with the protein patterns of the group A organisms which resembled closely that of B. gingivialis ATCC 33277T (4). It is considered that this group is B. gingivialis, although the strains from cats have some biochemical differences from the strains from humans (Table 1). The results confirm the "serological variant" proposal for strains from dogs made by Parent et al. (6).

The group 2 organisms had protein band patterns similar to B. asaccharolyticus, but differed in being catalase positive and having a lower G+C ratio (4). Although our homology results substantiated that the strains in this group were distinct, having an average of only about 11% DNA homology with B. asaccharolyticus ATCC 25260T (Table 2), we have chosen not to describe this group as a new species at the present time. Since organisms in this group have been rather uncommon isolates and by readily available tests differ from B. asaccharolyticus only in catalase production, species designation will await the isolation of additional strains and the determination of additional differentiation tests.

Group 3 organisms from which we selected VPB 157 as the type strain of B. salivosus sp. nov. form a cluster distinct from all the other groups of cat strains examined (Tables 1 and 2). These constitute the group C and E organisms described by Love et al. (4). They have an average of 10% DNA homology with group 1, an average of 20% with group 2, 12% with B. gingivialis ATCC 33277T, and 1% with B. asaccharolyticus ATCC 25260T. On this basis, and because they represent a distinct group of strains from the oral cavity of cats, we propose that they are of sufficient importance to deserve species identity.

**Description of Bacteroides salivosus.** Bacteroides salivosus

<table>
<thead>
<tr>
<th>Group or strain</th>
<th>No. of strains</th>
<th>Site</th>
<th>Fluorescence</th>
<th>Catalase</th>
<th>Hemagglutination</th>
<th>Trypsinlike activity</th>
<th>Production of phenylacetic acid (µg/ml)</th>
<th>G+C content (mol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (A and D)²</td>
<td>10</td>
<td>Oral</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>738</td>
<td>48–51</td>
</tr>
<tr>
<td>2 (B)</td>
<td>3</td>
<td>Oral</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>11</td>
<td>45–47</td>
</tr>
<tr>
<td>3 (C and E)</td>
<td>5</td>
<td>Oral</td>
<td>±</td>
<td>–</td>
<td>–</td>
<td>0.17</td>
<td>358</td>
<td>44–48</td>
</tr>
<tr>
<td>B. asaccharolyticus ATCC 33277T</td>
<td>10</td>
<td>Oral</td>
<td>±</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>1.6</td>
<td>49–50</td>
</tr>
</tbody>
</table>

² Phenotypic group designations used in reference 4.
³ Summary of data from reference 4.
⁴ All B. endodontalis data taken from reference 9. ND. Not described.

**TABLE 2.** DNA homologies of type strains and strains from cats

<table>
<thead>
<tr>
<th>Group</th>
<th>Unlabeled DNA from strain:</th>
<th>G+C content (mol%)</th>
<th>% Homology with labeled DNA from strain:</th>
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<tr>
<td></td>
<td></td>
<td>3492</td>
<td>3362</td>
</tr>
<tr>
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<tr>
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<td>51</td>
<td>89</td>
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<td>96</td>
</tr>
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<td>51</td>
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² With reference DNA normalized to 100%.
³ DNA extracted by the hydroxyapatite procedure; thermal melting point determination performed in Gilford spectrophotometer plus thermoprogrammer.

**TABLE 1.** Characteristics of groups of pigmented asaccharolytic Bacteroides strains isolated from soft-tissue infections or the gingival margins in normal cats and human asaccharolytic type strains.

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³ Summary of data from reference 4.
⁴ All B. endodontalis data taken from reference 9. ND. Not described.
Bacteroides salivosus

(L. adj. salivosus, of saliva, slimy). Cells are obligately anaerobic, nonsporeforming, nonmotile, gram-negative rods or cocccoid forms. In cooked-meat carbohydrate (3) and on sheep blood agar plates, cells are 0.3 to 0.6 μm by 0.8 to 1.5 μm and arranged singly and in groups; occasional filaments up to 10 μm are seen. On sheep blood agar plates, surface colonies at 72 h are 0.5 to 1.5 mm in diameter, circular, entire, dome shaped, opaque, and brown-black. Colonies may show an orange fluorescence (265 nm) at 24 h, but fluorescence is absent by 3 days. Colonies from some strains do not fluoresce. After incubation at 37°C for 24 h in prereduced broth media, cultures show a uniform turbidity with a small ropy deposit. Vitamin K and hemin are required for growth. After incubation for 5 days, the pH in media containing a fermentable carbohydrate generally ranges from 6.5 to 6.8 in all media tested. Carbohydrate fermentation is not detected in adonitol, cellobiose, fructose, glucose, glycogen, inositol, lactose, maltose, mannitol, rhamnose, salicin, starch, sucrose, trehalose, and xylose.

The major fermentation products from cooked-meat carbohydrate are acetic acid (average 21.9 μmol/ml) and butyric acid (average, 10.4 μmol/ml). Smaller quantities of isobutyric acid (average, 0.4 μmol/ml) and isovaleric acid (average, 3.6 μmol/ml) are produced. Large amounts of phenylacetic acid (average, 350 pg/ml) are produced. Neufy gelatin, and have trypsinlike activity. However, cells neither agglutinate sheep erythrocytes nor exhibit α-glucosidase activity, reduce nitrate, split urea, or hydrolyze esculin.

Strains are susceptible to penicillin (2 U/ml), amoxyccillin (2.5 μg/ml), carbenicillin (100 μg/ml), and erythromycin (3 μg/ml).

This species was isolated from soft-tissue infections (absceses and empyemas) and normal gingival margins from cats. The G+C content of the DNA was 42 to 44 mol% by $T_m$ (5).

The type strain is NCTC 11632T (VPB 157).

Phenotypic characteristics helpful in differentiating B. salivosus sp. nov. from other species are as follows. B. salivosus produces catalase which distinguishes it from the asaccharolytic pigmented Bacteroides spp. from humans. Its combined characteristics of fluorescent colonies, trypsinlike activity, production of large amounts of phenylacetic acid, and lack of hemagglutinating activity for sheep erythrocytes easily distinguished it from B. gingivalis ATCC 33277T, B. asaccharolyticus ATCC 25260T, and B. endodontalis ATCC 35405T (although the description of the latter species does not mention fluorescence of colonies at 265 nm).

ACKNOWLEDGMENTS

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Prereduced media were prepared by C. Wilcox and G. Tsoukalas.

LITERATURE CITED


