

Comparison of *Bacteroides zoogloformans* Strains Isolated from Soft Tissue Infections in Cats with Strains from Periodontal Disease in Humans

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A total of 11 strains of *Bacteroides zoogloformans* were isolated from 11 of 106 different cat subcutaneous "fight wound" abscesses and were among a total of 143 *Bacteroides* species isolated from these samples. They constituted 3.4% (11 of 325) of all anaerobic isolates. The cat strains and strains of *B. zoogloformans* isolated from humans with periodontal disease were similar phenotypically as determined by biochemical reactions, polyacrylamide gel electrophoresis patterns of soluble proteins, and guanine plus cytosine ratios of DNA. Eight cat strains and five human strains tested had 45 to 54% DNA homology with the type strain of *B. zoogloformans*. The eight cat strains and five human strains (excluding the type strain) were related by DNA homology at 70 to 77%. There was 85 to 90% intragroup DNA homology among the cat strains and 86 to 89% intragroup homology among the five human strains. The implications for epidemiology and human and animal ecology are discussed.

During an investigation of the bacteria found in subcutaneous abscesses in cats (6), we isolated a number of strains of *Bacteroides* which were similar phenotypically to *B. zoogloformans* described by Cato et al. (1) from human periodontal disease. Since it has been contended that the source of the bacteria in subcutaneous abscesses of cats was the mouth of the assailant (6), it was of interest taxonomically and ecologically to determine the relatedness of the cat strains to the human isolates.

MATERIALS AND METHODS

Bacterial strains. Eleven cat strains of bacteria designated VPB (veterinary pathology and bacteriology) 155, VPB 3324, VPB 3347, VPB 3357, VPB 3369, VPB 3397, VPB 3431, VPB 3459, VPB 3460, VPB 3471, and VPB 3480 were isolated over a period of 3 years as part of a mixed anaerobic and facultative flora from soft tissue "fight wound" abscesses in cats.

Human strains. Six strains designated VPI (Virginia Polytechnic Institute) D28K-1, VPI D13D-2B, VPI D84B-12, VPI D84C-7, VPI D490-21, and VPI D82M-10 were kindly supplied by W. E. C. Moore and L. V. Holdeman from their collection of dental strains. These were selected from a total of 14 isolates from three persons with moderate periodontitis and three persons with severe periodontitis. Strains VPI D84B-12, VPI D49D-21, and VPI D82M-10 were from persons with moderate periodontitis, and strains VPI D28K-1, VPI D13D-2B, and VPI D48C-7 were from persons with severe periodontitis.

Media and methods. The cat strains were isolated on the surface of blood agar plates incubated anaerobically. They were freeze-dried in skim milk after preliminary characterization and stored at 5°C. Subsequently, they were examined phenotypically along with *B. zoogloformans* ATCC 33285^T.

The methods used for the cat strains and *B. zoogloformans* ATCC 33285^T have been described previously (7, 8).

For fermentation tests, the pH was measured after 5 days of incubation at 37°C. A pH of <5.5 was considered positive fermentation whereas a pH of 5.5 to 5.7 was interpreted as weak fermentation (3). Fatty acid production was measured from cooked meat glucose (7) and from peptone-yeast extract, peptone-yeast extract-threonine, peptone-yeast extract-lactate, and peptone-yeast extract-pyruvate (3) with a Hewlett-Packard model 5830A gas chromatograph with a flame ionization detector and automatic sampling device as described previously (7). Colonies grown for 14 days on blood agar plates and on laked blood agar plates were examined for pigmentation and for fluorescence by UV light at 365 nm. Polyacrylamide gel electrophoresis patterns of soluble cellular proteins were determined as described by Moore et al. (11).

DNA isolation. The organisms were grown in a medium which was prepared as described previously (2); the 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 sufoxalate, 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 liter of medium. Flasks containing 1 liter of medium were inoculated with 20 ml of an overnight culture grown in chopped meat carbohydrate (3) and then incubated 10 to 12 h at 37°C. The harvested cells were suspended in a 0.15 M NaCl-0.01 M EDTA 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 were isolated by a hydroxylapatite procedure (5). High-molecular-weight DNA preparations for guanine plus cytosine content were isolated by the method of Marmur (9).

Guanine plus cytosine content of DNA. Thermal melting points were used to determine the guanine plus cytosine contents of the DNA preparations (10).

Preparations of labeled DNA. Fragmented denatured DNA was labeled with ¹²⁵I by using a variation of the thallium chloride method of Tereba and McCarthy (16) as described by Holdeman and Johnson (4).

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DNA homology methods. DNA homology values were determined by an S1 nuclease procedure as described previously (5). The reassociation vials were incubated for 20 h at 56°C.

RESULTS AND DISCUSSION

All cat strains were gram-negative, strictly anaerobic, non-spore-forming, nonmotile rods which produced acetic, propionic, and succinic acids as major products of fermentation and thus are members of the genus *Bacteroides*. All strains failed to fluoresce or to produce pigmented colonies on blood agar or laked blood agar. Several sugars were fermented (Table 1). All cat strains produced indole, failed to grow in bile, and were catalase negative. These characteristics were similar to those of *B. zoogeleoformans* which is of human origin. Although the type strain of *B. zoogeleoformans* ATCC 33285^T does not produce indole, other strains of this species, including those dental strains included in this study, are known to produce indole and do not grow in bile (1). The range of sugars fermented by *B. zoogeleoformans* also is similar to those fermented by the cat strains. The DNA guanine plus cytosine ratio of the cat strains is 47 mol%, which is the same as that reported for *B. zoogeleoformans* ATCC 33285^T (1).

The DNA homology data for the cat and human dental strains is given in Table 2. The cat strains show homologies of 85% or greater with each other, and the human dental strains (other than the type strain) show a similar clustering within their group. The human dental strains (other than the type strain) also show a relationship of greater than 70% with the cat strains. A similar reciprocal relationship exists between the cat strains and the human dental isolates (other

TABLE 2. DNA homology among cat and human strains of *B. zoogeleoformans*

| Strain | Homology (%) with reference DNA from: | | |
|-------------|---------------------------------------|--------------------------|-------------|
| | VPB 3324 | VPI D28K-1 ^{Tb} | VPI D13D-2B |
| VPB 3324 | 100 ^a | 49 | 74 |
| VPB 3347 | 86 | 48 | 72 |
| VPB 3480 | 93 | 48 | 75 |
| VPB 3357 | 92 | 50 | 75 |
| VPB 3459 | 85 | 47 | 73 |
| VPB 3397 | 86 | 50 | 77 |
| VPB 3471 | 90 | 48 | 73 |
| VPB 3460 | 94 | 45 | 72 |
| VPI D28K-1 | 45 | 100 | 49 |
| VPI D13D-2B | 75 | 54 | 100 |
| VPI D84B-12 | 77 | 52 | 88 |
| VPI D48C-7 | 70 | 49 | 86 |
| VPI D82M-10 | 71 | 52 | 86 |
| VPI D49D-21 | 71 | 49 | 89 |

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

^b VPI D28K-1 is the parent strain of ATCC 33285, the type strain of *B. zoogeleoformans*.

^T Type strain.

than the type strain). This similarity of cat strains with each other and with the human dental isolates is also reflected in their polyacrylamide gel electrophoresis patterns (Fig. 1).

The 11 cat strains described here were among 325 different anaerobic species isolated from 106 fight wound abscesses. They thus constituted some 3.4% (11 of 325) of all anaerobic isolates and 7.7% (11 of 143) of the *Bacteroides* species characterized from these infections. In contrast, the six strains of *B. zoogeleoformans* of human origin were from five separate patients with moderate or severe periodontitis (12, 15). The 14 isolates from which these six strains were chosen consisted of 7 isolates from 3 of 26 patients with moderate periodontitis and 7 isolates from 3 of 21 patients with severe

TABLE 1. Biochemical reactions of *B. zoogeleoformans*

| Reaction | Strain characteristics | | |
|--------------------------|---|---|------|
| | Human ATCC 33285 ^T pH ^a | Acid production (%) 11 cat strains ^b | |
| | | Strong | Weak |
| Acid from: | | | |
| Arabinose | - | 0 | 0 |
| Cellobiose | + | 30 | 70 |
| Fructose | - | 50 | 50 |
| Glucose | w | 20 | 80 |
| Glycogen | w | 0 | 80 |
| Inositol | - | 0 | 0 |
| Lactose | w | 20 | 80 |
| Maltose | + | 20 | 80 |
| Mannitol | - | 0 | 0 |
| Melezitose | - | 0 | 0 |
| Melibiose | w | 0 | 80 |
| Raffinose | w | 0 | 0 |
| Ribose | w | 0 | 0 |
| Salicin | + | 30 | 70 |
| Sucrose | + | 90 | 10 |
| Trehalose | - | 0 | 0 |
| Xylose | + | 30 | 40 |
| Digestion of gelatin | - | 80 | 0 |
| Growth in bile | 0 | 0 | |
| Indole production | - | 100 | |
| Phenylacetate production | + | 60 | |
| Esculin hydrolysis | + | 100 | |
| Starch hydrolysis | + | 80 | |

^a Symbols: +, pH of less than 5.5; -, pH of greater than 5.7; w, pH 5.5 to 5.7.

^b Percentage of strains producing weak (pH of 5.5 to 5.7) or strong (pH of less than 5.5) acid.

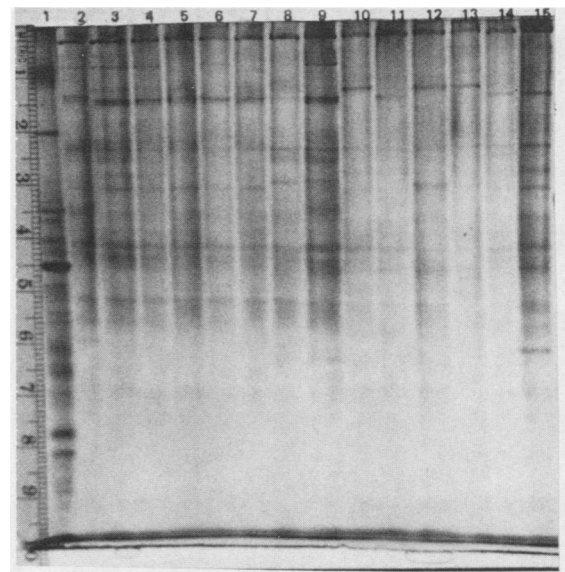


FIG. 1. Electrophoretic patterns of strains of *B. zoogeleoformans*. Lane 1, *Streptococcus faecalis* reference strain U4-20; lanes 2 to 9, VPB 3460, VPB 3459, VPB 3471, VPB 3357, VPB 3397, VPB 3480, VPB 3347, and VPB 3324, respectively, from cat abscesses; lanes 10 to 15, VPI D84B-12, VPI D82M-10, VPI D13D-2B, VPI D28K-1 (= ATCC 33285^T), VPI D49D-21, and VPI D48C-7, respectively, from periodontal disease in humans.

periodontitis. These strains were from a total of 3,969 isolates from severe periodontitis and 1,711 isolates from moderate periodontitis. *B. zooglyphiformans* was not found in supragingival samples of affected periodontitis sites nor among comparable numbers of isolates from patients with healthy gingiva, experimental gingivitis, or classical juvenile periodontitis (12, 13, 14, 15).

It is of interest to find *B. zooglyphiformans* strains from two different animal species (namely, humans and domestic cats) to be related by DNA homology at greater than 70%; their intergroup relationship is greater than 80% for most strains. This would indicate that even though several of the humans may have been cat owners (one patient in this study owned a cat, one owned a dog, and one owned neither a cat nor a dog; W. E. C. Moore, personal communication), they did not carry cat strains of *B. zooglyphiformans*. The implication is, therefore, that although different animal species may harbor bacterial species which are phenotypically very similar, each appears to have its own cluster of organisms when analyzed by DNA homologies. The evidence presented here also strengthens the supposition that cat subcutaneous abscesses are the result of implantation of oral organisms from the assailant cat.

ACKNOWLEDGMENTS

We thank W. E. C. Moore and L. V. Holdeman for supplying human dental strains and for information about their strains and the patients from whom they were isolated. We also greatly appreciate the suggestion of the implications of this work and helpful advice and discussions. We also gratefully acknowledge the help of E. P. Cato, who performed and interpreted the polyacrylamide gel electrophoresis analysis.

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