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Characterization of Saccharolytic *Bacteroides* and *Prevotella* Isolates from Infected Dog and Cat Bite Wounds in Humans

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Saccharolytic, nonpigmented, anaerobic gram-negative rods isolated from infected dog and cat bite wounds in humans have been poorly characterized, and most are not included in the databases of kits used for anaerobic identification; thus, they are problematic for clinical laboratories to identify. Fifty strains isolated from such wounds were characterized with commercial kits for preformed-enzyme detection, carbohydrate fermentation, and other biochemical tests. PCR fingerprinting was performed on these strains to further characterize subgroups within these species. *Bacteroides tectum* is a frequent isolate in bite wounds and resembles *Prevotella bivia* in colony morphology and saccharolytic activity, except that it grows in 20% bile and hydrolyzes esculin. Profile numbers generated by various kits associate *B. tectum* with *P. bivia*, *Prevotella oralis* group, or *Prevotella melaninogenica*. PCR fingerprinting identified at least four subgroups and confirmed the heterogeneous nature of this species. *Prevotella heparinolytica* was also frequently isolated from these bite wounds. It produces indole and generates a profile number in preformed-enzyme kits that is usually associated with *Bacteroides uniformis*. However, it is bile sensitive and quite distinct from the *Bacteroides fragilis* group of anaerobes. The PCR fingerprint profiles generated by strains of *P. heparinolytica* were very similar to that of the type strain and to each other. *Prevotella zoogleoformans*, occasionally isolated from dog and cat bite wounds in humans, resembles *P. heparinolytica* except for a negative indole test. Clinical laboratories should be aware of the characteristics of these animal species when identifying isolates from animal bite wounds in humans.

Dog and cat bite wounds in humans, which account for 1% of the emergency department visits in the United States (5), can become infected with the oral flora from the biting animal. *Bacteroides* and *Prevotella* species and other anaerobic gram-negative bacilli, major components of the oral anaerobic flora of cats and dogs, are involved in bite wound infections. Animal isolates of these species are often fastidious, and many are distinct from human isolates of the same species. Their characteristics are usually not included in the databases of identification kits used by clinical laboratories and thus may be problematic to identify. In order to assist clinical laboratories in the identification of such strains, we compared the capabilities of conventional fermentation reactions in prereduced anaerobically sterilized (PRAS) biochemicals and of preformed enzymes as detected by the RapID ANA II system and the API ZYM system to identify them. In addition, PCR fingerprinting was used to compare our clinical isolates to the American Type Culture Collection (ATCC) type strains.

MATERIALS AND METHODS

Specimens. Fifty clinical isolates from infected dog and cat bite wounds were selected for this study and included *Bacteroides tectum* ($n = 30$), *Prevotella heparinolytica* ($n = 16$), *Prevotella zoogleoformans* ($n = 2$), and *Prevotella bivia* ($n = 2$) isolates. Control strains included *B. tectum* ATCC 43331 (cat abscess isolate), *P. heparinolytica* ATCC 39285 (subgingival dental plaque isolate), and *P. zoogleoformans* ATCC 33285 (human oral cavity isolate) (2, 8, 9). All of the strains studied were obligately anaerobic, nonpigmented, saccharolytic, gram-negative rods. Gram stains of pure cultures showed predominantly short rod-shaped and coccobacilliary organisms.

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Biochemical identification. The methods used to identify the isolates included carbohydrate fermentation, esculin hydrolysis, growth in 20% bile, gelatin liquefaction, nitrate reduction, and indole production in PRAS biochemicals (Carr Scarborough, Microbiologicals, Inc., Atlanta, Ga.) and detection of preformed enzymes with the RapID ANA II (Innovative Diagnostic Systems, Norcross, Ga.) and API ZYM (BioMerieux, St. Louis, Mo.) test kit systems. Pure cultures were stored in 20% skim milk at −70°C. Prior to testing, each isolate was subcultured onto brucella blood agar (BBA) plates supplemented with vitamin K and hemin (Anaerobe Systems, San Jose, Calif.) and incubated for 48 h at 35°C under anaerobic conditions. Cell paste was harvested and suspended in either (i) broth for inoculation into PRAS biochemical tubes, (ii) buffer as provided by the manufacturer of the RapID ANA II system, or (iii) sterile water for the API ZYM system. The turbidity was matched to the appropriate McFarland standard according to the manufacturers' instructions.

The PRAS biochemicals are tubes containing 5 ml of peptone yeast broth plus a carbohydrate. They are plugged with rubber stoppers which can be penetrated with a needle rather than having to be removed and inoculated while gassing through a canula. The PRAS biochemical tests were inoculated with a tuberculin syringe to penetrate the rubber stopper; thus, no carbon dioxide was introduced into the tubes (Table 1). They were incubated for 2 to 4 days at 37°C prior to measurement of pH. A pH of <5.5 was considered positive, a pH of 5.5 to 6.0 was weakly positive, and a pH of >6.0 was negative. The gelatin tube was chilled and then placed at room temperature in an inverted position for 30 min to observe for liquefaction. Indole formation from tryptophan was detected in broth with Kovács reagent.

The RapID ANA II system tested for preformed enzymes (Table 2). The system was inoculated according to the manufacturer's instructions and incubated for 4 h at 37°C in an ambient atmosphere. Reactions involving urease production and the hydrolysis of the colorless aryl-substituted glycosides or phosphoester-releasing yellow *o*- or *p*-nitrophenol were interpreted. After addition of the RapID ANA II reagent, production of β-naphthylamide was recorded. Indole was detected after addition of the *p*-dimethylaminocinnamaldehyde reagent.

The API ZYM system consists of a series of microcupules containing dehydrated chromogenic enzyme substrates that test for 19 preformed enzymes (Table 2). Each microcupule of the API ZYM tray was inoculated with 0.05 ml of the standardized bacterial suspension. After aerobic incubation for 4 h at 37°C, 25 μl of reagents A and B was added to each microcupule, and the color reactions which developed within 5 min were graded from 0 to 5 according to the API ZYM color reaction chart. Tests that were given a grade of 0 to 1 were regarded as negative, whereas tests given a grade of 2 to 5 were regarded as

TABLE 1. PRAS biochemical profiles for *B. tectum*, *P. heparinolytica*, and *P. zoogleoformans* type strains and clinical isolates from dog and cat bite infections in humans

PRAS biochemical test	Result for:					
	B. tectum (n = 30)		*P. heparinolytica* (n = 16)		*P. zoogleoformans* (n = 2)	
	ATCC 43331[a]	Clinical isolates (%)[b]	ATCC 39285	Clinical isolates (%)	ATCC 33285	Clinical isolates (%)
Fermentations						
Arabinose	−	7	+	100	+	0
Cellobiose	W	56	W	100	+	100
Glucose	W	83	W	100	+	100
Lactose	−	93	W	100	+	100
Maltose	−	93	W	100	+	100
Mannose	W	100	W	100	+	50
Melibiose	−	0	W	75	W	0
Raffinose	−	13	W	100	W	50
Rhamnose	−	0	−	0	−	0
Salicin	−	7	−	81	W	50
Sucrose	−	10	W	100	W	100
Trehalose	−	7	−	0	−	0
Xylose	−	0	W	75	−	0
Esculin hydrolysis	+	100	+	100	+	100
Esculin acidification	−	53	−	63	−	50
Growth in bile	+	100	−	0	−	0
Gelatin hydrolysis	−	0	−	19	−	50
Indole production	−	0	+	94[c]	−	0
Nitrate reduction	−	0	−	0	−	0

[a] −, negative reaction (pH >6.0); +, positive reaction (acid pH <5.5); W, weak reaction (pH 5.5 to 6.0).
[b] Percentage of strains positive or weakly positive for reaction.
[c] Indole production for one isolate was detected by Kovács reagent after incubation in chopped-meat broth.

positive. A profile number based on positive reactions for each isolate was generated for comparison purposes.

PCR fingerprinting. PCR fingerprinting was performed as previously described (3, 4) using the following as single primers: the core sequence of phage M13 (M13 core; 5′-GAGGGTGGCGGTTCT-3′) (10), which anneals with mini- and microsatellite DNA sequences, and a nonspecific primer derived from t-DNA intergenic spacer (T3B; 5′-AGGTCGCGGGTTCGA ATCC-3′) (11) (National Biosciences, Inc., Plymouth, Min.). Briefly, isolates were grown anaerobically for 48 h on supplemented BBA (Anaerobe Systems). Several bacterial colonies of each isolate were extracted in 100 μl of 50 μM NaOH, and cell lysates were centrifuged (μSpeedFuge; Savant, Holbrook, N.Y.) to remove cellular debris. Each cell lysate supernatant was subjected to PCR amplification in volumes of 50 μl containing 2.5 μl of cell lysate supernatant (~25 ng of template DNA); 10× PCR buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl; 1.5 mM $MgCl_2$; and 0.001% gelatin [final concentrations]); 200 μM (each) dATP, dCTP, dGTP, and dTTP (Pharmacia LKB Biotechnology Inc., Piscataway, N.J.); 2.5 U of *Taq* DNA polymerase (Perkin-Elmer Cetus, Norwalk, Conn.); and either 25 pmol of M13 core primer or 50 pmol of the T3B primer. Amplification (DNA Thermal Cycler 480; Perkin-Elmer Cetus) for 35 or 40 cycles was performed as follows: with M13 core primer, 20 s at 93°C, 1 min at 50°C, and 20 s at 72°C, followed by one final extension cycle of 6 min at 72°C; with T3B primer, 30 s at 94°C, 30 s at 50°C, and 2 min at 72°C. Reaction tubes were held at 4°C prior to analysis. Samples were concentrated to a volume of approximately 20 to 25 μl each (Speed Vac; Savant) prior to electrophoretic separation in 1.2% agarose gels for the M13 core primer products or 1.5% agarose gels for the T3B primer products (0.5 by 25 by 20 cm) for 5 h at 3 V/cm. Amplified products were detected by staining with ethidium bromide (2 μg/ml). Data evaluation was performed by direct visual comparison and by scanning (Flatbedscanner; Hewlett-Packard, Palo Alto, Calif.) and recording the banding patterns for calculation of correlations between the different isolates within a species by using the GelManager (BioSystematica, Prague, Czech Republic) software package. Absorbance profiles were corrected for gel-to-gel variation based on reference samples run on each gel. Patterns were then compared either by calculation of the correlation coefficient between absorbance profiles or by using a band position matching coefficient. Natural groupings of similar patterns were found by clustering the matrix and displaying the results as a dendrogram (GelManager) (data not shown).

RESULTS

PRAS biochemical tests. After anaerobic incubation for 2 days on BBA, colonies of most *B. tectum* strains were circular, entire, dome shaped, gray-white, and opaque, whereas colonies of *P. heparinolytica* and *P. zoogleoformans* were circular, entire, convex, smooth, translucent, and gray. Results of the biochemical reactions are summarized in Table 1. *B. tectum* isolates grew well in bile, hydrolyzed esculin, and fermented mannose. After 2 to 4 days of incubation, none of the strains produced indole, reduced nitrate, liquefied gelatin, or fermented melibiose, rhamnose, or xylose. Although the type strain, *B. tectum* ATCC 43331, was negative for lactose and maltose fermentation, 93% of the clinical isolates were positive for these reactions.

P. heparinolytica and *P. zoogleoformans* isolates are biochemically similar but could be differentiated by indole production. All *P. heparinolytica* strains produced indole, hydrolyzed esculin, and fermented arabinose, cellobiose, glucose, lactose, maltose, mannose, raffinose, and sucrose. Neither the type strain (ATCC 35895) nor any of the clinical isolates fermented rhamnose or trehalose, grew in bile, or reduced nitrate.

Based on a negative indole reaction, two isolates were identified as *P. zoogleoformans*. All of the *P. zoogleoformans* strains hydrolyzed esculin and fermented cellobiose, glucose, lactose, maltose, and sucrose. None of the strains grew in bile, reduced nitrate, or fermented either melibiose, rhamnose, or trehalose. Although one of the clinical isolates liquefied gelatin, the control strain, *P. zoogleoformans* ATCC 33285, did not.

RapID ANA II and API ZYM preformed enzymes. Individual key reactions from the RapID ANA II and API ZYM test kit systems were used to further characterize the clinical isolates (Table 2). With the API ZYM system, all *B. tectum* strains tested positive for acid phosphatase, alkaline phosphatase, and *N*-acetyl-β-D-glucosidase (NAG). Of 30 strains, 29 (97%) were positive for naphthol-AS-B1-phosphohydrolase, 27 (90%) were positive for α-D-glucosidase, and 26 (87%) were positive for β-D-galactosidase. All *B. tectum* strains were negative for β-D-glucosidase, α-D-mannosidase, butyrate esterase, caprylate esterase-lipase, myristate lipase, valine aminopeptidase, cys-

TABLE 2. RapID ANA II and API ZYM reaction profiles for *B. tectum*, *P. heparinolytica*, and *P. zoogleoformans* type strains and clinical isolates from dog and cat bite infections in humans

Reaction profile	Result for:					
	B. tectum (n = 30)		*P. heparinolytica* (n = 16)		*P. zoogleoformans* (n = 2)	
	ATCC 43331	Clinical isolates (%)[a]	ATCC 39285	Clinical isolates (%)	ATCC 33285	Clinical isolates (%)
RapID ANA II						
Alkaline phosphatase	+	100	+	100	+	100
Glycosidases						
α-D-Galactosidase	−	7	+	100	+	0
β-D-Galactosidase	+	97	+	88	+	100
α-D-Glucosidase	+	100	−	94	+	100
β-D-Glucosidase	−	20	+	100	+	100
α-L-Fucosidase	+	93	+	100	+	50
NAG	+	100	+	100	+	100
α-L-Arabinosidase	−	0	−	38	−	0
β-D-Disaccharidase	−	7	+	100	+	50
Aminopeptidases						
Leucyl-glycine	+	100	+	94	+	100
Glycine	+	43	−	38	−	50
Proline	−	0	−	0	−	0
Phenylalanine	−	13	−	0	−	0
Arginine	−	73	−	25	−	50
Serine	−	40	−	63	−	50
Pyroglutamic acid	−	7	−	0	−	0
Indole	−	0	+	88[b]	−	0
Urease	−	0	−	0	−	0
API ZYM						
Phosphatases						
Alkaline phosphatase	+	100	+	100	+	100
Acid phosphatase	+	100	+	100	+	100
Naphthol-AS-B1-phosphohydrolase	−	97	+	81	−	100
Glycosidases						
α-D-Galactosidase	−	3	−	88	+	0
β-D-Galactosidase	+	87	+	94	+	100
α-D-Glucosidase	+	90	−	25	−	100
β-D-Glucosidase	−	0	+	69	−	0
α-L-Fucosidase	+	70	−	75	−	50
NAG	+	100	+	100	+	100
β-D-Glucuronidase	−	3	+	6	−	0
α-D-Mannosidase	−	0	−	0	−	0
Esterases/lipases						
Butyrate esterase	−	0	−	0	−	0
Caprylate esterase-lipase	−	0	−	6	−	0
Myristate lipase	−	0	−	0	−	0
Aminopeptidases						
Leucine	−	47	−	6	−	0
Cysteine	−	0	−	0	−	0
Valine	−	0	−	0	−	0
Trypsin	−	0	−	0	−	0
Chymotrypsin	−	0	−	0	−	0

[a] Percentage of strains positive or weakly positive for reaction.
[b] Isolates negative for indole production in the RapID ANA II test system were positive by an alternate method.

teine aminopeptidase, trypsin, and chymotrypsin. Although *B. tectum* ATCC 43331 was negative for β-D-glucosidase with both the RapID ANA II and the API ZYM systems, 6 of 30 clinical isolates (20%) were positive by the RapID ANA II system. All the *B. tectum* strains were positive for leucyl-glycine aminopeptidase, whereas no strain was positive for α-L-arabinosidase, proline aminopeptidase, indole, or urease with the RapID ANA II system.

All the *P. heparinolytica* strains were positive for alkaline phosphatase, acid phosphatase, and NAG with the API ZYM system. Of 16 strains, 13 (81%) were positive for naphthol-AS-B1-phosphohydrolase activity, 15 (94%) were positive for β-D-galactosidase, and 11 strains (69%) were positive for β-D-glucosidase. Although *P. heparinolytica* ATCC 39285 tested negative for α-D-galactosidase and α-D-fucosidase with the API ZYM system, 14 (88%) and 12 (75%) of the 16 clinical isolates, respectively, were positive. All isolates were positive for alkaline phosphatase, β-D-disaccharidase, α-L-fucosidase, α-D-galactosidase, β-D-glucosidase, and NAG with the RapID ANA II system. Although the type strain (ATCC 35895) and four of the clinical isolates were negative for α-L-fucosidase by the API ZYM system, in the RapID ANA II system, the type strain, as well as all the clinical isolates, tested positive. Finally, although the type strain was negative for α-D-glucosidase activity in both

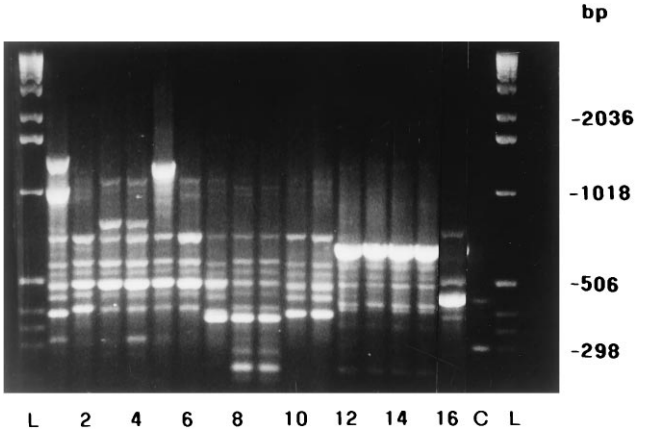

FIG. 1. PCR fingerprint profiles of *B. tectum* clinical isolates and type strain with the T3B primer. PCR fingerprinting was performed using the single, nonspecific primer, T3B, derived from t-DNA intergenic spacer region for amplification (35 cycles). Amplified products were separated on 1.5% agarose gels and stained with ethidium bromide. Lanes 1, 2, and 4 through 16, *B. tectum* clinical isolates; lane 3, *B. tectum* ATCC 43331; lanes L, DNA 1-kb ladder; lane C, control.

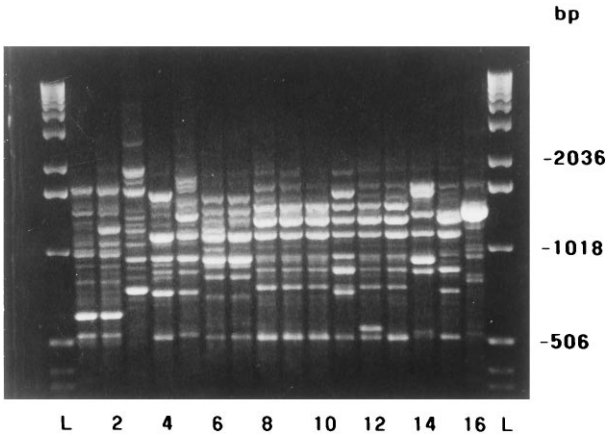

FIG. 2. PCR fingerprint profiles of *B. tectum* clinical isolates and type strain with the M13 core primer. PCR fingerprinting was performed using the single, nonspecific primer, M13 core, derived from the core sequence of M13 phage for amplification (35 cycles). Amplified products were separated on 1.2% agarose gels and stained with ethidium bromide. Lanes 1, 2, 4 through 13, 15, and 16, *B. tectum* clinical isolates; lane 3, *P. zoogleoformans* ATCC 33285; lane 14, *B. tectum* ATCC 43331; lanes L, DNA 1-kb ladder; lane C, control.

test systems, 15 of 16 strains (94%) were positive in the RapID ANA II system.

Both of the *P. zoogleoformans* strains were positive for the following reactions in both test systems: alkaline phosphatase, α-D-glucosidase, β-D-galactosidase, and NAG. In the RapID ANA II system, both isolates were also positive for β-D-glucosidase and leucyl-glycine aminopeptidase. In contrast, in the API ZYM system, neither clinical isolate was positive for β-D-glucosidase. Although *P. zoogleoformans* ATCC 33285 was negative for α-D-glucosidase with the API ZYM system, it was positive with the RapID ANA II system. Furthermore, although *P. zoogleoformans* ATCC 33285 was negative for naphthol-AS-B1-phosphohydrolase, α-D-glucosidase, and α-L-fucosidase with the API ZYM system, two, two, and one of the two clinical isolates were positive for these reactions, respectively. Furthermore, with both systems, *P. zoogleoformans* ATCC 33285 was positive for α-D-galactosidase, whereas neither of the two clinical isolates were positive.

PCR fingerprinting. Fifteen of the 30 *B. tectum* clinical isolates and the type strain, ATCC 43331, were examined by PCR fingerprinting using the single primers, T3B and M13 core. Although the isolate profiles showed considerable overlap, based on the presence or absence or intensity of the bands, four distinct PCR fingerprint groups were distinguished with the T3B primer (Fig. 1). Type strain ATCC 43331 (lane 3) most closely resembled the group II isolates (lanes 2, 4, and 6). RMA 7743 (lane 5), distinguished by a unique band at ~1,150 bp, also shared other major bands common to the group II isolates. RMA 7323 (lane 1), with unique major bands at ~1,250 and 950 bp, shared some of the major bands common to the group III isolates (lanes 10 and 11). RMA 7327 (lane 7) differed from the group III isolates by the presence of a double band at ~390 and ~375 bp. Group I isolates (lanes 8 and 9) were differentiated on the basis of a primary band at ~375 bp and a less intense band at ~235 bp. Group IV isolates (lanes 12 to 15) were characterized by an intensely staining band at ~620 bp. RMA 7857 (lane 16) showed a unique PCR fingerprint profile with a major band at ~420 bp and no overlap of major bands with the other groups.

Variation within the groups was more notable with the M13 core primer, emphasizing the unique clonality of the different isolates (Fig. 2). For the most part, both primers categorized these isolates into the same four groups. *B. tectum* type strain ATCC 43331 (lane 14) shared (i) a major band at ~1,560 bp in common with RMA 7743 (lane 11) and RMA 7857 (lane 4) of group II; (ii) a major band at ~1,100 bp common to isolates in group I (lanes 6 and 7), group II (lanes 4, 5, 11, and 15), and group IV (lanes 8 to 10, 12, and 13); and (iii) a major band at ~850 bp in common with the group II isolates. Two isolates, RMA 7323 (lane 16) and RMA 7327 (not shown), had unique PCR fingerprint profiles. RMA 7323 (lane 16) was characterized by an intense major band at ~1,290 bp in common with type strain ATCC 43331 (lane 14), as well as with RMA 7571 (lane 15) and RMA 7564 (lane 5) of group II. RMA 7327 (not shown) was characterized by a major band at ~1,390 bp in common with the group IV isolates. Group III isolate profiles (lanes 1 and 2), which showed limited overlap with the other groups, were characterized by a unique major band at ~620 bp.

Twelve of the 16 *P. heparinolytica* clinical isolates and the two type strains, *P. heparinolytica* ATCC 35895 and *P. zoogleoformans* ATCC 33285, were also examined by PCR fingerprinting using the single primers, T3B and M13 core, and compared to the type strains for these species. With the T3B primer, the PCR fingerprint profiles for the *P. heparinolytica* clinical isolates were identical to that of the *P. heparinolytica* type strain, ATCC 39895 (Fig. 3, lane 1). In contrast, the M13 core primer produced PCR fingerprint profiles (Fig. 4) which delineated the *P. heparinolytica* clinical isolates into two main groups based on the presence of a major band at ~1,380 bp and the presence (group I; lanes 1 to 5) or absence (group II; lanes 6 to 12) of a major band at ~1,220 bp. Within these groups, differences in the minor bands (~500 to 1,200 bp) distinguished the different clones. Clinical isolate RMA 7605 (lane 10), which shared common minor bands with members of group II, was notably different based on its heavier major band at ~1,425 bp. The M13 core-primed PCR fingerprint profiles for all of these clinical isolates were markedly different from the profiles of either *P. heparinolytica* ATCC 39895 (lane 13) or *P. zoogleoformans* ATCC 33285 (lane 14).

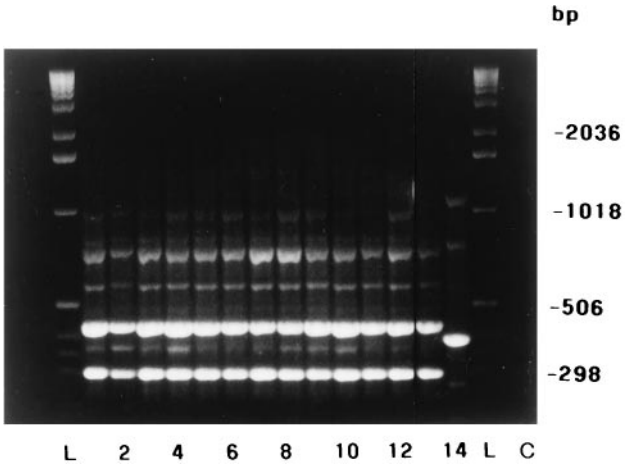

FIG. 3. PCR fingerprint profiles of *Prevotella* species clinical isolates and type strains with the T3B primer. PCR fingerprinting was performed using the single, nonspecific primer, T3B, derived from t-DNA intergenic spacer region for amplification (35 cycles). Amplified products were separated on 1.5% agarose gels and stained with ethidium bromide. Lane 1, *P. heparinolytica* ATCC 35895; lanes 2 through 13; *P. heparinolytica* clinical isolates; lane 14, *P. zoogleoformans* ATCC 33285; lanes L, DNA 1-kb ladder; lane C, control.

DISCUSSION

Although PRAS biochemical tests are the conventional method used to discriminate among various anaerobic bacterial species, additional tests are often required to adequately differentiate some species of anaerobic gram-negative rods (5). Phenotypic characteristics of veterinary species that are involved in bite wound infections in humans have not been included in the taxonomic schema commonly utilized by clinical laboratories. To more accurately identify some of these anaerobic species, we have characterized *B. tectum*, *P. heparinolytica*, and *P. zoogleoformans* clinical isolates using the standard PRAS biochemical tests, by detection of preformed enzymes with the RapID ANA II and API ZYM systems, and by PCR fingerprinting.

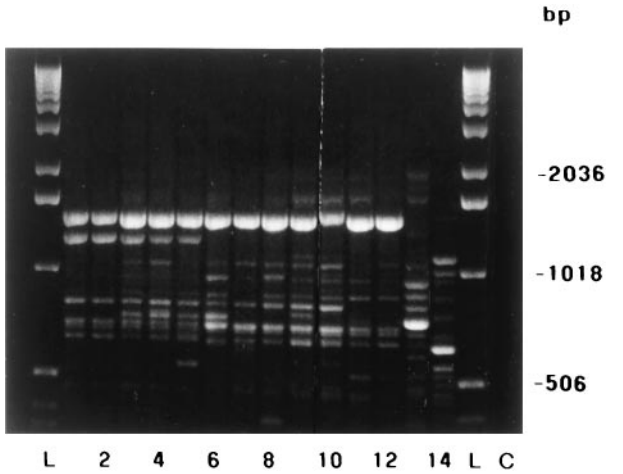

FIG. 4. PCR fingerprint profiles of *Prevotella* species clinical isolates and type strains with the M13 core primer. PCR fingerprinting was performed using the single, nonspecific primer, M13 core, derived from the core sequence of M13 phage for amplification (35 cycles). Amplified products were separated on 1.2% agarose gels and stained with ethidium bromide. Lanes 1 through 12, *P. heparinolytica* clinical isolates; lane 13, *P. heparinolytica* ATCC 35895; lane 14, *P. zoogleoformans* ATCC 33285; lanes L, DNA 1-kb ladder; lane C, control.

Problems in identifying *B. tectum* isolates arise due to its similarity to *P. bivia*. Profile numbers generated by various anaerobe test kits often misidentify *B. tectum* strains as *P. bivia*, *Prevotella melaninogenica*, or *Prevotella oralis* group species. Some of the weakly fermentative strains resemble *Bacteroides capillosis*. In this study, the key biochemical reactions used to differentiate *B. tectum* from *B. capillosis* and *P. bivia* were growth in 20% bile and esculin hydrolysis. Further characterization of our isolates performed by PCR fingerprinting techniques indicated that, within this species, at least four groups of *B. tectum* can be distinguished. Each of these groups produced fingerprint profiles distinct from those of two *P. bivia* clinical isolates with both of the primers (unpublished observations). However, these PCR fingerprint profiles showed no correlation with the different biochemical reaction profiles of these isolates. In another study, four *B. tectum* DNA homology groups and their corresponding biochemical reaction profiles for strains isolated from the normal oral flora of dogs and cats were described (8). Other than three strains that weakly fermented sucrose and thus matched their group E description, most of the biochemical profiles of our strains did not match their four groups. Whereas Love et al. (8) found that only 1% (1 of 73) of their *B. tectum* strains fit the group E description, we found that 10% (3 of 30) of our *B. tectum* clinical isolates separated as group E isolates based on their biochemical reactions. Furthermore, none of the strains in the Love et al. study acidified esculin, whereas 53% (16 of 30) of our isolates did. Although two of our strains fermented salicin, they did not otherwise match the group II designation. Finally, no correlation was observed between our PCR fingerprint groups and the previously described DNA homology groups as defined by their biochemical reaction profiles.

Because there have been no previous reports of *B. tectum* strains isolated from humans, it was important to compare the PCR results of our isolates to those of the type strain, *B. tectum* ATCC 43331, originally isolated from a cat abscess. Although none of the clinical isolates were identical to the type strain, there was significant overlap of both major and minor bands with the clinical isolates by using both primers, indicating that they are likely to be of the same species. Nevertheless, the variation with the T3B primer does suggest that the group of isolates currently identified as *B. tectum* species is actually composed of more than one species or subspecies.

P. heparinolytica and *P. zoogleoformans*, bile-sensitive, saccharolytic, anaerobic, gram-negative rods which have been isolated from human and animal oral flora (1, 7), are common isolates in cultures from infected dog and cat bite wounds. Both species form a viscous, glutinous zoogleal mass in broth media. In contrast to the original description of *P. heparinolytica* (7), all of our clinical isolates, as well as the *P. heparinolytica* type strain, ATCC 35895, fermented raffinose. The key reaction differentiating *P. heparinolytica* isolates from *P. zoogleoformans* isolates is the indole reaction. Unfortunately, false-negative indole reactions can be obtained with these organisms by standard techniques. In fact, one of our isolates, RMA 7727, was originally identified as *P. zoogleoformans* based on negative indole reactions from three different test systems, i.e., a spot indole test with *p*-dimethylaminocinnamaldehyde, the PRAS biochemical (Kovács reagent) test, and the RapID ANA II test system (*p*-dimethylaminocinnamaldehyde). However, this strain showed a PCR fingerprint profile identical to those of *P. heparinolytica* ATCC 35895 and all other *P. heparinolytica* clinical isolates with the T3B primer and a PCR fingerprint profile comparable with those of the *P. heparinolytica* group II clinical isolates with the M13 core primer. Furthermore, this isolate showed 74.3% DNA homology with the *P. heparinolytica* type

strain, ATCC 35895, which compares well with other *P. heparinolytica* strains isolated from cats, dogs, and horses (1, 7a). The indole test, repeated using a 4- to 5-day-old culture in chopped meat broth, resulted in a positive reaction for this isolate, thus confirming the identification of this isolate as *P. heparinolytica.*

Although *Prevotella buccae* and *Prevotella oris* strains are also indole negative and have biochemical profiles similar to that of *P. zoogleoformans*, unlike either *P. heparinolytica* or *P. zoogleoformans*, they do not form a zoogleal mass in PRAS biochemical broth media. Furthermore, although both *P. zoogleoformans* and *P. buccae* ferment similar carbohydrates (6), *P. zoogleoformans* produces α-L-fucosidase and NAG whereas *P. buccae* does not. In the RapID ANA II system, nearly 100% of *P. oris* isolates are positive for α-L-arabinosidase, and thus, this is a key enzymatic reaction for differentiating such isolates from *P. zoogleoformans*, which is negative for this enzyme. Therefore, phenotypic characteristics and results from the RapID ANA II and API ZYM systems were important in differentiating these *Prevotella* species.

PCR fingerprinting using the T3B primer demonstrated the homogeneity of the *P. heparinolytica* isolates. Only the *P. zoogleoformans* type strain, ATCC 33285, showed a variant pattern. Analysis with the M13 core primer distinguished two groups and one variant fingerprint profile. The fact that none of the *P. heparinolytica* clinical isolates matched the M13 core-primed fingerprint profile for the ATCC type strain may be due to the nature of the primer used and the fact that the clinical isolates are of animal origin whereas the ATCC type strain is of human origin. The M13 core primer is designed to detect intraspecific differences. These differences are likely to be more pronounced between the human and animal strains.

Microbiologists are faced with many challenges when identifying anaerobic bacterial species from the oral flora. Similar characteristics among the *Bacteroides* and *Prevotella* species isolated from human oral cavities, including colony morphology, Gram stain morphology, and fermentation patterns, cause problems for taxonomic categorization. Strains obtained from normal animal oral flora may differ from strains of the same species obtained from humans. Strains obtained from the oral flora of normal animals may differ from strains isolated from bite wounds in humans. Our study emphasizes that the source of isolation can be very important in the correct identification of an isolate. The human body can be considered a selective medium for certain oral animal strains that likely produce specific virulence factors allowing them to survive and flourish in the new host environment. Laboratory clinicians should be aware of the complications involved in identifying these organisms when culturing bite wounds inflicted by animals. We recommend that key reactions be kept in mind and that a combination of identification systems be used for the accurate identification of isolates from animal bite wounds in humans.

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REFERENCES

1. **Bailey, G. D., L. V. H. Moore, D. N. Love, and J. L. Johnson.** 1988. *Bacteroides heparinolytica*: deoxyribonucleic acid relatedness of strains from the oral cavity and oral-associated disease conditions of horses, cats, and humans. Int. J. Syst. Bacteriol. **38:**42–44.
2. **Cato, E. P., R. W. Kelley, W. E. C. Moore, and L. V. Holdeman.** 1982. *Bacteroides zoogleoformans* (Weinberg, Nativelle, and Prévot 1937) corrig., comb. nov.: emended description. Int. J. Syst. Bacteriol. **32:**271–274.
3. **Claros, M., G. Schönian, Y. Graser, T. Montag, A. C. Rodloff, D. M. Citron, and E. J. C. Goldstein.** 1995. Strain differentiation of '*Bacteroides fragilis* group' species and *Prevotella bivia* by PCR fingerprinting. Anaerobe **1:**209–217.
4. **Claros, M. C., D. M. Citron, S. Hunt Gerardo, E. J. C. Goldstein, G. Schönian, T. Montag, B. Hampel, and A. C. Rodloff.** 1996. Characterization of indole negative *Bacteroides fragilis* group species using PCR fingerprinting and resistance profiles. Clin. Infect. Dis. **23**(Suppl. 1)**:**S66–S72.
5. **Duerden, B. L., J. S. Brazier, S. V. Seddon, and W. G. Wade.** 1992. Medical and environmental aspects of anaerobes. Wrightson Biomedical Publishing Ltd., Petersfield, England.
6. **Durmaz, B., H. R. Jousimies-Somer, and S. M. Finegold.** 1995. Enzymatic profiles of *Prevotella*, *Porphyromonas*, and *Bacteroides* species obtained with the API ZYM system and Rosco diagnostic tablets. Clin. Infect. Dis. **20:** S192–S194.
7. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore.** 1977. Anaerobe Laboratory manual and update, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.

7a. **Love, D.** Personal communication.

8. **Love, D. N., J. L. Johnson, R. F. Jones, M. Bailey, and A. Caberley.** 1986. *Bacteroides tectum* sp. nov. and characteristics of other nonpigmented *Bacteroides* isolates from soft-tissue infections from cats and dogs. Int J Syst. Bacteriol. **36:**123–127.
9. **Okuda, K., T. Kato, J. Shiozu, I. Takazoe, and T. Nakamura.** 1985. *Bacteroides heparinolytica* sp. nov. isolated from humans with periodontitis. Int. J. Syst. Bacteriol. **35:**438–442.
10. **Welsh, J., and M. McClelland.** 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. **18:**7213–7218.
11. **Welsh, J., and M. McClelland.** 1991. Genomic fingerprints produced by PCR with consensus tRNA gene primers. Nucleic Acids Res. **19:**861–866.