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Cultivable Oral Microbiota of Domestic Dogs

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Bacteria were isolated from the dental plaques of nine dogs and a sample of pooled saliva from five other dogs and were then identified by comparative 16S rRNA gene sequencing. Among 339 isolates, 84 different phylotypes belonging to 37 genera were identified. Approximately half of the phylotypes were identified to the species level, and 28% of these were considered members of the indigenous oral microbiota of humans. The 16S rRNA gene sequences of the remaining 44 phylotypes were not represented in GenBank, and most of these phylotypes were tentatively identified as candidate new species. The genera most frequently isolated from saliva were *Actinomyces* (26%), *Streptococcus* (18%), and *Granulicatella* (17%). The genera most frequently isolated from plaque were *Porphyromonas* (20%), *Actinomyces* (12%), and *Neisseria* (10%). A comparison of the DNA sequences from this study with sequences of the human microbiota available in GenBank showed that, on average, canine and human microbiotas differed by almost 7% in the 16S rRNA gene. In conclusion, this study has shown that the cultivable oral microbiotas of dogs and humans show significant differences.

Oral diseases are common in dogs, as they are in humans, causing suffering and often leading to tooth loss. From extensive research with humans, it has become clear that an understanding of the microbial ecology of the mouth is fundamental to elucidating the etiology of most oral diseases (3), yet the oral ecology and microbiology of the dog largely remain uncharacterized. The aim of this study was to investigate the microbiota of the canine oral cavity that can be cultured in vitro in order to provide basic information for improvement of canine oral health care.

The canine oral microbiota was investigated by cultivating bacteria from the plaque and saliva of dogs and identifying the isolates obtained by comparative 16S rRNA gene sequencing. Although many previous workers have also investigated the cultivable oral microbiota of dogs, most have relied upon conventional identification methods, and their studies have been aimed at gaining a better understanding of the human microbiota. In particular, the dog has often been used as an animal model for human oral diseases, such as periodontal disease (28), and it has also received attention because of the bacteria that it may transfer to people, for example, through bites (1, 9). In this respect it has been recognized that the dog has a distinct oral microbiota, as it is widely believed that bites from dogs are less dangerous than human bites in terms of wound infection potential (12).

The large research effort directed at characterizing the human oral microbiota provides an excellent data set for comparison with the results from the present study. Comparison of species that fill similar habitats in different hosts may provide useful information regarding human and animal oral diseases by providing a basis for uncovering the universal characteristics of pathogenic and protective species.

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MATERIALS AND METHODS

Cohorts. All plaque samples were taken from dogs undergoing routine dental treatment at either the Waltham Centre for Pet Nutrition, Waltham-on-the-Wolds, or a United Kingdom veterinary referral clinic. Saliva samples were taken from dogs housed at the Waltham Centre for Pet Nutrition. None of the dogs involved in this study had taken antibiotics within the last 3 months. The dogs sampled were four miniature schnauzers (female, neutered), three cocker spaniels (male; one entire, two neutered), one Cairn terrier (female), and one collie cross (female, neutered). The Cairn terrier had Cushing's syndrome and was taking trilostane (Arnolds Veterinary Products Ltd., United Kingdom), a steroid synthesis inhibitor. The dogs housed at the Waltham Centre for Pet Nutrition were kept in environmentally enriched facilities.

Sample collection. Canine saliva was collected in two ways: a ball was used for play to make the dog salivate and then the saliva was syringed out of the mouth, or salivettes were used. After collection, 100% dextran was added to each sample to give a final concentration of 25% (vol/vol); samples from five dogs were pooled together and stored at −70°C. Plaque samples were taken from the gingival margin with a curette or dental probe. When a periodontal pocket was present, however, the sample was taken from the base of the pocket. Nine plaque samples were taken from the buccal surfaces of the upper second or third premolars of the nine dogs. In addition, a single supragingival plaque sample was taken from an upper second premolar, and a distal and mesial pooled sample was taken from the periodontal pocket on an upper second incisor, with both of these extra samples being taken from one of the nine dogs mentioned previously. Samples were taken prior to any other dental work or scoring and immediately transferred to a vial containing 1 ml of reduced transport fluid (25) for transportation to the laboratory.

Sample processing. Canine plaque and saliva samples were serially diluted in phosphate-buffered saline and plated out onto Columbia agar base (Oxoid, Basingstoke, United Kingdom) containing 5% defibrinated horse blood (E&O Laboratories, Bonnybridge, United Kingdom) and anaerobe agar (Bioconnections, Leeds, United Kingdom) containing 5% defibrinated horse blood. The plates were incubated aerobically with 5% CO_2 or in an anaerobic chamber (Don Whitley Scientific Ltd., Shipley, United Kingdom) with an atmosphere containing 80% nitrogen, 10% hydrogen, and 10% carbon dioxide at 37°C. After 3 days and again after 7 to 10 days, the plates were examined and each morphotype was counted and subcultured to obtain pure cultures.

DNA sequencing. Identification of the isolates was carried out by partial 16S rRNA gene sequencing. The 16S rRNA gene was amplified from colony picks by PCR with global primers 27f and 1492r (19) to produce an amplicon of approximately 1,499 bases (all primers were from Sigma-Genosys, Poole, United Kingdom).

PCR was performed with 5 U *Taq* polymerase (BioTaq; Bioline, London, United Kingdom) per 100-μl reaction mixture by using the buffer supplied. The PCR mixture also contained 2.5 mM $MgCl_2$, 0.2 mM deoxynucleoside triphosphates, and each primer at 0.2 μM. PCR mixtures were subjected to the following thermal cycling conditions: 94°C for 5 min; 29 cycles of 94°C for 1 min, 54°C for 1 min, and 72°C for 1.5 min; and 72°C for 5 min.

The PCR products were cleaned and concentrated by using a QIAQuick PCR purification kit (QIAGEN, Crawley, United Kingdom). An ABI 310 genetic analyzer (PE Biosystems, Warrington, United Kingdom) was used for DNA sequencing of the amplicons from the initial PCR by using the 357f primer in the first instance. Additional sequencing was later performed by using a Beckman Coulter CEQ2000 automated DNA sequencer with the products of PCR with primers 27f, 342r, 357f, and 519r (19). Sequencing reactions and subsequent handling were performed as recommended by the manufacturers. The primers described by Lane (19) used in this study are named according to their position on the *Escherichia coli* 16S rRNA gene, followed by a letter to indicate the direction: forward (f) or reverse (r).

Sequence processing. When multiple-sequence reads were obtained for a single template, they were assembled by using BioEdit (available at http://www.mbio.ncsu.edu/BioEdit) and the contig assembly program (14). Sequences were aligned by using ClustalX (27), and all gaps were removed by using BioEdit. For short alignments of less than 400 bases, some gaps were sometimes allowed to remain in order to make maximum use of the available data. Distance matrices for the generation of phylogenetic trees were calculated from degapped alignments by using the DNAdist program of the Phylip software package (7) and the method of Jukes and Cantor (15) to correct for multiple substitutions. Unrooted neighbor-joining phylogenetic trees were produced from distance matrices by using the Neighbor program of the Phylip software package (7). To calculate the percent identity between pairs of sequences, degapped alignments were compared by using BioEdit without correcting for multiple substitutions.

Phylogenetic analyses. All isolates were initially identified by performing database searches with the 16S rRNA gene sequences obtained from a single read of approximately 300 to 400 bases. BLAST (2) searches of the GenBank nucleotide database and Ribosomal Database Project (5) searches were used for this purpose. The sequences were then allocated into operational taxonomic groups and used to produce phylogenetic trees, which were used to arrange the bacteria into phylotypes. The trees included the best database matches for each sequence to provide a visual representation of the relatedness of the isolated bacteria to named bacteria. Phylotypes were assigned according to the database search results and the distance separating known related species on trees, so that phylotype differences should approximately reflect species-level differences.

Once each of the isolated bacteria had been assigned to a phylotype, a second round of sequencing was performed to obtain more accurate sequence data for representatives of each phylotype. These data are termed high-fidelity sequences and are defined by having a minimum of double coverage for at least 600 bases. High-fidelity sequences for each phylotype were subjected to further database searching and tree analyses to obtain accurate phylogenetic data for the originating organism. Database searches, phylogenetic trees, and percent sequence homology to database sequences were used to identify each phylotype to the species level where possible. A difference of 3% or greater was taken to indicate that sequences were probably from distinct species (8, 11). Sometimes, more similar sequences were considered to be from distinct species if the sequences were particularly homogeneous with those of other described species of the genus. The methods described above were chosen and applied in order to minimize the possibility of erroneously flagging a sequence as originating from a previously undescribed species. These identifications are assumed to be indicative of the identity for each phylotype as a whole, since initial sequencing data showed their affinity.

Literature and database searches. To check if isolated bacterial species were part of the indigenous human oral microbiota, the following search was performed on the PubMed website (12 October 2004) by using the current valid name and all previous and invalid names: genus AND species AND (plaque OR saliva OR oral).

Database hits were followed up to determine whether the bacterium had been detected from the mouth of a human and, if so, whether it was considered to be a member of the indigenous oral microbiota of humans.

The closest BLAST sequence matches of human origin from GenBank to the phylotype sequences from this study were also identified. For each match, the sequence origin and citation recorded in GenBank were used in conjunction with a literature search as described above until a human origin match was identified. Cases in which a human had been infected by a dog (e.g., bite wounds) were excluded.

Nucleotide sequence accession numbers. The nucleotide sequences determined in this study have been deposited as a popset in GenBank with accession numbers AY827856 to AY827945.

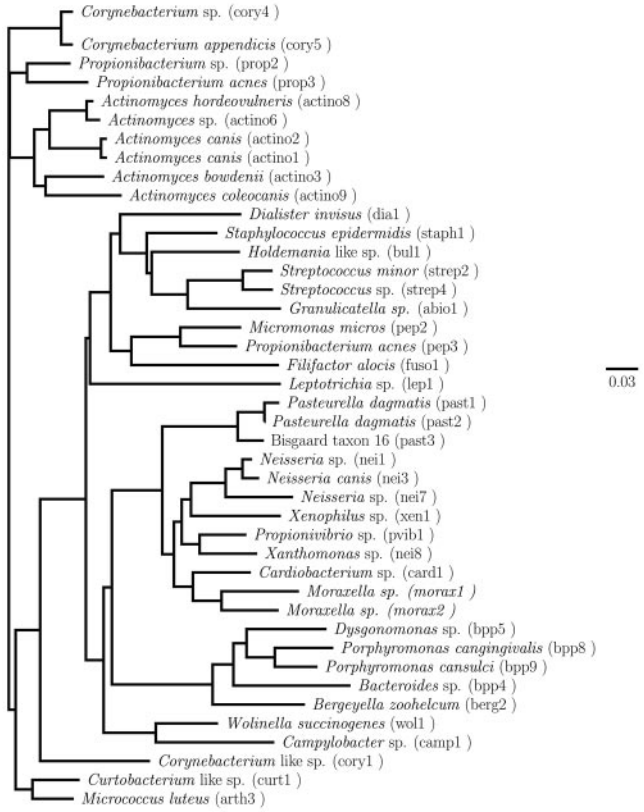

FIG. 1. Neighbor-joining tree showing all phylotypes for which high-fidelity sequences were obtained. Sequences are identified by their originating species or genus identification, with the phylotype given in parentheses. This tree is based on an alignment of 371 positions, which is significantly less than the length of the original sequences because of their diverse phylogenies, which introduced gaps into the alignment.

RESULTS

Phylogenetic diversity. A summary tree showing all of the phylotypes for which high-fidelity sequences were obtained is shown in Fig. 1. A total of 339 bacterial isolates were recovered by culture from the dental plaques of nine dogs and a pooled saliva sample. Among these isolates, 84 different bacterial phylotypes belonging to 37 genera were identified by 16S rRNA gene sequencing, and 34 isolates remained unidentified. In four cases, multiple phylotypes were identified as the same species, but the original phylotype designations were retained because they may represent distinct strains. Of the 84 phylotypes, species-level identifications were obtained for 40 phylotypes, as summarized in Table 1; the remainder could not be reliably identified to species level, and these are summarized in Table 2. Only 28% of the species identifications are considered members of the indigenous oral microbiota of humans based on a literature search. For 44 (52% of the total) phylotypes, there was no similar sequence available in GenBank to provide a species identification. These are tentatively identified as probable new species or genera, especially where it can be determined that GenBank does in fact hold a sequence for all valid members of the genus (Table 2).

TABLE 1. Phylotypes identified to species level

Phylotype[a]	16 rRNA sequence length (bp)	Species	% Identity
Actino1α	643	*Actinomyces canis*	96.7
Actino2α	697	*Actinomyces canis*	99.4
Actino3α	697	*Actinomyces bowdenii*	99.7
Actino5	396	*Actinomyces hordeovulneris*	98.4
Actino8α	684	*Actinomyces hordeovulneris*	96.1
Actino7	440	*Actinomyces hordeovulneris*	99.0
Actino9α	605	*Actinomyces coleocanis*	96.6
arth3α●	814	*Micrococcus luteus*	98.7
berg1	868	*Bergeyella zoohelcum*	94.8
berg2α	611	*Bergeyella zoohelcum*	95.5
bpp11	334	*Porphyromonas macacae*	95.4
bpp3●	399	*Prevotella heparinolytica*	96.9
bpp6	1,083	*Porphyromonas gulae*	99.9
bpp7	538	*Porphyromonas canoris*	96.7
bpp8α	655	*Porphyromonas cangingivalis*	99.1
bpp9α	611	*Porphyromonas cansulci*	98.5
Camp2●	374	*Campylobacter curvus*	96.7
Capno1α	362	*Capnocytophaga cynodegmi*	97.4
clos2	304	*Clostridium perfringens*	100.0
cory5α	885	*Corynebacterium appendicis*	98.0
dia1α●	758	*Dialister invisus*	99.8
fuso1α●	808	*Filifactor alocis*	99.0
fuso4	384	*Filifactor villosus*	98.1
gem1	908	*Gemella palaticanis*	97.9
Haem2	274	*Haemophilus haemoglobinophilus*	96.6
lam1	336	*Lampropedia hyalina*	97.0
nei3α	663	*Neisseria canis*	99.5
nei4	1,089	*Neisseria canis*	98.6
nei5	346	*Neisseria weaveri*	100.0
past1α	718	*Pasteurella dagmatis*	98.5
past2α	822	*Pasteurella dagmatis*	98.3
pep1●	333	*Peptostreptococcus anaerobius*	98.7
pep2α●	703	*Micromonas micros*	96.0
prop3α●	696	*Propionibacterium acnes*	99.8
rhod1●	417	*Dietzia psychralcaliphila*	98.7
staph1α●	638	*Staphylococcus epidermidis*	99.6
strep2α	634	*Streptococcus minor*	98.3
strep3●	403	*Streptococcus bovis*	99.2
wol1α●	857	*Wolinella succinogenes*	98.5
past3α	839	Bisgaard taxon 16	97.7

[a] α, a high-fidelity sequence was used for this phylotype identification; ●, an indigenous member of the human oral microbiota.

TABLE 2. Phylotypes not represented in GenBank[a]

Phylotype[b]	16S rRNA sequence length (bp)	Genus	Species by BLAST search	% Identity
Actino4α	358	*Actinomyces*	*A. slackii*	98.8
Actino6†α	658	*Actinomyces*	*A. hordeovulneris*	94.9
Actino10*	369	*Actinomyces*	*A. suimastitidis*	98.9
bpp4α	833	*Bacteroides*	*B. uniformis*	91.5
Camp1α†	625	*Campylobacter*	*C. rectus*	95.1
Capno2†	1065	*Capnocytophaga*	*C. gingivalis*	89.4
card1†α	890	*Cardiobacterium*	*C. valvarum*	93.0
clos3	470	*Clostridium*	*C. leptum*	85.5
clos1	421	*Clostridium*	*C. hathewayi*	94.4
clos4*	175	*Clostridium*	*C. litorale*	93.1
cory2	838	*Corynebacterium*	*C. falsenii*	95.4
cory1α	683	*Corynebacterium*	*C. bovis*	79.6
cory3	851	*Corynebacterium*	*C. ciconiae*	93.1
cory4α	737	*Corynebacterium*	*C. macginleyi*	94.1
cory6	227	*Corynebacterium*	*Corynebacterium* sp.	94.2
arth2*	358	*Curtobacterium*	*C. flaccumfaciens*	95.2
curt1α	639	*Curtobacterium*	*Curtobacterium* sp.	94.0
bpp5†α	702	*Dysgonomonas*	*Bacteroides* sp.	92.4
fuso2	420	*Eubacterium*	*E. oxidoreducens*	85.0
Fuso3†	1101	*Fusobacterium*	*F. nucleatum*	96.1
abio1†α	621	*Granulicatella*	*G. balaenopterae*	91.2
Haem1	843	*Haemophilus*	*H. haemoglobinophilus*	93.3
Haem3*	410	*Haemophilus*	*H. paraphrophilus*	95.5
bul1†α	655	*Holdemania*	*Solobacterium* sp.	84.6
lep1†α	852	*Leptotrichia*	*S. moniliformis*	85.6
Morax1α	728	*Moraxella*	*M. osloensis*	89.4
Morax2α	654	*Moraxella*	*M. cuniculi*	95.1
nei1†α	619	*Neisseria*	*N. dentiae*	96.0
nei7†α	892	*Neisseria*	*N. elongata*	94.4
pep3†α	696	*Peptostreptococcus*	*Helcococcus* sp.	89.5
bpp1	402	*Porphyromonas*	*P. catoniae*	90.1
bpp10	335	*Porphyromonas*	*P. endodontalis*	85.1
bpp2	413	*Prevotella*	*P. ruminicola*	82.5
prop2†α	654	*Propionibacterium*	*F. spumicola*	91.7
pvib1†α	607	*Propionivibrio*	*P. dicarboxylicus*	93.2
arth1	473	*Rothia*	*R. nasimurium*	92.7
bpp12†	902	*Stenotrophomonas*	*S. maltophilia*	91.8
lac1	580	*Streptococcus*	*S. infantarius*	90.4
Strep1*	343	*Streptococcus*	Several spp.	99.4
Strep4α	703	*Streptococcus*	*S. bovis*	91.8
prop1†	592	*Tessaracoccus*	*T. bendigoniensis*	93.5
nei8α	876	*Xanthomonas*	*Xanthomonas* sp.	95.0
xen1α	572	*Xenophilus*	*X. azovorans*	94.8
xen2	855	*Xenophilus*	*X. azovorans*	92.9

[a] The most likely estimated genus identification is shown based upon all analyses. The closest positively identified match from a BLAST search is also shown, along with the uncorrected percent sequence homology between the two sequences.

[b] †, sequences for all valid species of the genus were available for comparison (all subspecies were not necessarily available); α, a high-fidelity sequence was used for this phylotype identification; *, there was insufficient information to identify a phylotype to the species level or to identify it as novel.

Viable counts. Figure 2 shows the genera isolated from plaque and saliva samples as a proportion of the total viable count for each sample. Overall, *Actinomyces* species were the most abundant, composing 11.6% of the plaque microbiota and 25.5% of the saliva microbiota. No other genera were represented at over 5% in both plaque and saliva. *Granulicatella* and *Streptococcus* species were abundant in saliva at 16.5% and 18.2%, respectively, while *Porphyromonas* and *Neisseria* species dominated the plaque microbiota at 20.0% and 10.3%, respectively (along with the *Actinomyces* species mentioned previously). All 37 identified genera except for *Staphylococcus* were found in plaque samples, but only 10 genera were found in saliva samples (27%).

Comparison to human oral microbiota. To further investigate the relationship between the human and canine oral microbiota, the best-quality sequences from this study were used to query GenBank for clues about the relatedness of isolates from canines to isolates from humans.

The closest BLAST sequence matches of human origin to phylotypes from this study are summarized in Table 3. It is split into two parts depending upon whether the phylotype from this study was reliably identified to the species level or not. For identified phylotypes, the mean percent identity of the phylotype to the closest species sequence in GenBank was 98.3%, and that for the closest match of human origin was 93.3%. Of the human origin matches, 62.5% were from the oral cavity. In four cases (25%) the closest human origin match was the same as the phylotype species match; these were *Micrococcus luteus*, *Dialister invisus*, *Propionibacterium acnes*, and *Wolinella succinogenes*. For the less accurately identified phylotypes, the percent identities to the GenBank database sequences were generally lower, and only 25% of the closest human origin matches were from the oral cavity; half of these (two sequences) were the same as the closest overall BLAST match.

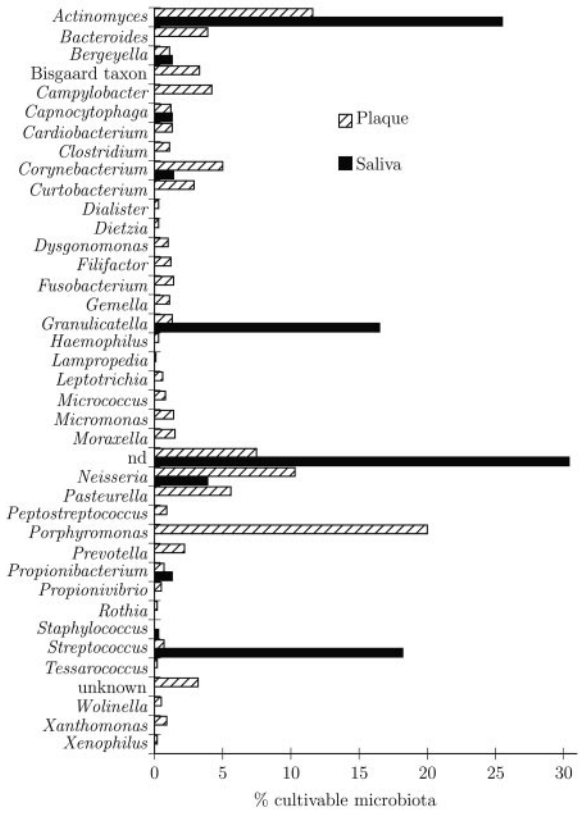

FIG. 2. Summary of genera isolated from plaque and saliva as the mean percentage of total cultivable microbiota (percentages were calculated per sample before averaging). nd, sequence data were insufficient or not obtained; unknown indicates that the sequences were not represented in GenBank.

DISCUSSION

The genera isolated from the oral cavities of dogs were typical of those found in human dental plaque (22) and included *Actinomyces*, *Porphyromonas*, *Fusobacterium*, *Neisseria*, and *Streptococcus*. Identification of the bacteria to the species level, however, often proved difficult. Initial sequencing revealed a large proportion of bacteria which appeared not to be represented in public databases, so further sequencing was carried out. This eliminated the possibility that poor sequence data would spoil the database matches and showed that many phylotypes really were not represented in the databases.

Marked differences between the plaque bacteria of different animals have been known for well over 20 years. In a review of the human oral microbiota, for example, Socransky and Manganiello (24) note that rodents lack *Peptostreptococcus*, *Bacteroides* (now *Porphyromonas* and *Prevotella*), *Treponema*, *Vibrio*, and *Leptotrichia* species; and beagle dogs seem to have a higher proportion of *Bacteroides melaninogenicus* (now reclassified as several species). Harvey et al. (13) also noted that some bacteria isolated from dogs and cats differ slightly from those found in humans, and this has led to a proposal for the renaming of such organisms. For example *P. gingivalis* isolates of canine and feline origin are catalase positive, but isolates of human origin are catalase negative. There has certainly been a boom in naming new species in recent years, as evidenced by the first publication dates of named species identified in this study ($n = 39$). Over half of the identified species were first described in the last 24 years (since 1981), and 28% were first described in the last 5 years (since 1999).

Sequence analyses and literature searches showed that most isolates obtained from dogs in this study are not normally found in the human oral cavity (22, 24), although the genera were similar to those found in humans. In addition, the proportions of certain cultivable genera in canine plaque did not match those typically found in human plaque; for example, *Streptococcus* species, which are common in human plaque (e.g., *Streptococcus sanguis*), were detected in only one canine sample, comprising less than 1% of the total microbiota; and the species was one not normally found in the human oral microbiota (*Streptococcus suis*). The dental plaque of humans typically contains streptococci at approximately 28% of the cultivable microbiota (24). The results from the present study confirm previous reports (29) of the low levels of *Streptococcus* species in dogs (less than 4% in supragingival plaque), so it seems that this is a fundamental difference between human and canine plaque. Streptococci, however, were found to be abundant in the pooled saliva sample (18.2%).

Poor representation of streptococci has also been observed in the dental plaque of certain marsupials (4), so perhaps dogs are not unusual in this respect. Since streptococci are considered so important in human dental plaque, particularly as primary colonizers, this difference raises the question of what organism may fill an equivalent niche in dogs and other animals. *Granulicatella* species may be able to play such a role, as they are closely related to streptococci and were isolated from several plaque samples in this study and were isolated at a high frequency from the pooled saliva sample (16.5%). Although it is not clear whether *Granulicatella* species are able to act as primary colonizers, an in vitro study by Pratten et al. (23) detected *Granulicatella adjaciens* in a 24-h-old biofilm grown on human dental enamel from a pooled saliva inoculum, but the organism was present at levels below the detection limit in the inoculum itself. It is also possible that in the present study streptococci were present in plaque samples at a proportion below the detection limit determined by dilutions producing confluent growth on agar. This could be checked by using selective media or by molecular methods; however, if streptococci are present in very low numbers, they are unlikely to be fulfilling the same function as they do in plaque of human origin.

Another important genus found in the human oral cavity is *Fusobacterium*, whose species are able to adhere to most other oral genera (17). No *Fusobacterium* species were unequivocally identified from the samples used in this study; however, a *Fusobacterium nucleatum*-like species (phylotype fuso3) was detected, and two species of the closely related genus *Filifactor* were detected (phylotypes fuso1 and fuso4). These bacteria were detected at low levels in plaque (2.6% combined), in agreement with previous reports of a low prevalence of *Fusobacterium* species in the healthy gingiva of dogs (18). Phylotype fuso3 is a strong candidate for being a previously undescribed taxon, as its closest match, *F. nucleatum*, differs from fuso3 by 3.9% over 1,101 nucleotides and all valid *Fusobacterium* species are represented in GenBank (some subspecies are absent).

TABLE 3. Comparison of sequences found in the canine oral microbiota with sequences of human origin on GenBank

Level of identification and phylotype	Species	% Identity	Closest human species	% Identity
Species level				
actino3	*A. bowdenii*	99.7	*A. naeslundii*+[a]	96.4
actino1	*A. canis*	96.7	*Actinomyces* sp.+	91.7
actino2	*A. canis*	99.4	*Actinomyces* sp.+	92.9
actino9	*A. coleocanis*	96.6	*A. europaeus*	90.4
actino8	*A. hordeovulneris*	96.1	*A. nasicola*	93.3
berg2	*B. zoohelcum*	95.5	*Flavobacteriaceae* genomosp. C1	90.2
capno1	*C. cynodegmi*	97.4	*Capnocytophaga* sp.	94.3
dia1	*D. invisus*	99.8	*D. invisus*+	99.8
arth3	*M. luteus*	98.7	*M. luteus*+	98.7
nei3	*N. canis*	99.5	*Neisseria* sp.+	93.1
prop3	*P. acnes*	99.8	*P. acnes*+	99.8
bpp8	*P. cangingivalis*	99.1	*P. levii*	88.5
bpp9	*P. cansulci*	98.5	*Tannerella forsythensis*+	85.5
past1	*P. dagmatis*	98.5	*Haemophilus cf. paragallinarum*	89.9
strep2	*S. minor*	98.3	*Streptococcus* sp.+	89.5
wol1	*W. succinogenes*	98.5	*W. succinogenes*+	98.5
Mean		98.3		93.3
Less reliable than species level				
actino6	*A. hordeovulneris*	94.9	*A. nasicola*	94.2
bpp4	*B. uniformis*	91.5	*Bacteroides* sp.	98.2
bpp5	*Bacteroides* sp.	92.4	*Dysgonomonas mossii*	89.9
cory1	*C. bovis*	79.6	*C. accolens*	79.9
cory4	*C. macginleyi*	94.1	*C. macginleyi*	94.1
card1	*C. valvarum*	93.0	*C. valvarum*	93.0
prop2	*Friedmanniella spumicola*	91.7	*Luteococcus sanguinis*	91.8
abio1	*G. balaenopterae*	91.2	*G. elegans*	81.9
pep3	*Helcococcus* sp.	89.5	*H. sueciensis*	89.5
morax2	*M. cuniculi*	95.1	*M. cuniculi*	95.1
morax1	*M. osloensis*	89.4	*M. osloensis*	89.4
nei1	*N. dentiae*	96.0	*N. meningitides*	93.1
nei7	*N. elongata*	94.4	*N. elongata*+	94.4
strep4	*S. bovis*	91.8	*S. mitis*+	90.8
bul1	*Solobacterium* sp.	84.6	*Solobacterium* sp.+	84.6
xen1	*X. azovorans*	94.8	*Lautropia* sp.+	92.2
Mean		91.5		90.8

[a] +, bacteria of oral origin.

Classic human periodontal pathogens, including *Porphyromonas gingivalis*, *Tannerella forsythensis*, and *Actinobacillus actinomycetemcomitans* (10), were not detected in any of the samples investigated in this study. Similarly, of 132 known species identified from 2,522 clones by a recent thorough molecular analysis of the human oral microbiota (22), only 4 species from the present study were detected (*Peptostreptococcus anaerobius*, *Micromonas micros*, *Filifactor alocis*, and *Neisseria weaveri*). It seems unlikely that the difference can be wholly attributed to isolation failure in the present study because many closely affiliated organisms were detected. Several significant genera were detected in the pooled saliva but not in plaque samples, including *Propionibacterium* and *Lactobacillus*.

Large proportions of the cultivable microbiota from canine plaque were *Actinomyces* species (12%) and *Corynebacterium* species (5%). These genera have recently been implicated in canine periodontitis by Takada and Hirasawa (26), who suggest that they may play the same role in canine periodontitis that *P. gingivalis* plays in human periodontitis. This suggestion is based upon their findings that the proportion of these genera possessing a trypsin-like activity (TLA) is increased in periodontitis sites compared with the proportion in healthy sites and may explain in part the absence of *P. gingivalis* from plaque collected in this study if they are able to compete for a similar niche. TLA was not determined in the present study, however.

Dent and Marsh (6) conducted a study of the dental plaque of nine animal species, including several types of monkey, lemurs, a tiger, a genet, a giraffe, and four American cocker spaniels, to test the hypothesis that there may be a basic plaque microbial community common to all animals. The results for the dogs agree approximately with those from the present study and are summarized as follows (the results from this study are in parentheses): *Streptococcus* spp., 6.1% (0.7%; in saliva, 18.2%); *Veillonella* spp., 21.6% (not detected); *Neisseria* spp., 7.2% (10.3%); *Actinomyces* spp., 8.8% (11.6%); and *Fusobacterium nucleatum*, 5.8% (*Fusobacterium* spp., 1.4%). They proposed that representatives of the genera *Actinomyces*, *Bacteroides*, *Fusobacterium*, *Neisseria*, *Streptococcus*, and *Veillonella* may constitute the basic components of human and animal gingival margin plaque. The data from the present study support this hypothesis to some extent, although *Veillonella* species were not detected and *Streptococcus* and *Fuso-*

TABLE 4. Basic components of canine and human dental plaque[a]

Criteria for basic oral microbiota	Canine examples	Human examples
Phylum *Fusobacteria*	*Leptotrichia* spp.	*Fusobacterium nucleatum*
Class *Bacilli* (*Firmicutes*)	*Granulicatella* spp.	*Streptococcus sanguinis*
Genus *Neisseria*	*Neisseria canis*	*Neisseria mucosa*
Genus *Actinomyces*	*Actinomyces canis*	*Actinomyces naeslundii*
Phylum *Bacteroidetes*	*Porphyromonas cansulci*	*Porphyromonas gingivalis*

[a] Determined by use of a modification of the criteria suggested by Dent and Marsh (6).

bacterium species were detected rarely. In light of this, it may be appropriate to broaden the criteria for describing the basic plaque microbiota beyond the genus level to accommodate the data from this study and in recognition of the vast array of niches and organisms available, as shown in Table 4.

The rapidly growing sequence databases are a valuable resource which greatly simplified the identification of bacteria in the present study; however, 44 phylotypes were not identified to the species level because good matches could not be found. If the 6 phylotypes with below-average sequence quality are excluded, 38 phylotypes are considered likely new taxa (Table 2). GenBank includes a representative of every valid species for the genus of 12 of the candidate new species, so these most likely represent new species. In cases where the GenBank record is incomplete, no conclusion can be drawn because a named but unsequenced species may turn out to be a good match.

The results showed clearly that the cultivable canine oral microbiota is mainly composed of species distinct from those found in humans, with similar species from the two hosts differing by almost 7% in the 16S rRNA gene on average (Table 3). This is a surprisingly large difference which does not fit well with the generally accepted rate of molecular evolution of 1% per 50 million years (MY) (21) and the divergence time of humans and canines of approximately 80 million years ago (MYA) (16, 20). If it is assumed that the oral microbiota of dogs and humans originated from their common ancestor and formed isolated populations at the speciation event, either a divergence time of 175 MYA, a substitution rate of 2.2% per 50 MY, or some intermediate situation is required to fit the data.

In conclusion, it has been shown that the cultivable oral microbiota of dogs differs significantly from that of humans. In particular, the species found in either host are not likely to be found in the other, but it is thought that other members of the genus in question are likely to fill a similar niche in many cases. Superficial similarities and practical considerations may have encouraged the use of dogs for models of oral disease in humans, but the results of this study suggest that such experiments are unlikely to yield useful information regarding specific bacterial involvement in such processes. The large sequence divergence between bacterial 16S rRNA gene sequences from humans and dogs seems best explained by a higher than expected rate of molecular evolution in the bacteria of the oral cavity. If this can be confirmed and measured accurately, then oral community comparisons between animals could be used as a method for estimating host evolutionary divergence times in the absence of more reliable measures. It would be interesting to complement this work with similar studies with other animals to help establish an understanding of the basic oral microbiota, if such a concept really exists. Identification of pathogens in other animals would also be of great value if detailed comparisons could be made to identify the common features of oral pathogens.

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