Methanogenic *Archaea* and human periodontal disease

Paul W. Lepp  
*Stanford University*

Mary M. Brinig  
*Stanford University & Veterans Affairs Palo Alto Health Care System*

Cleber C. Ouverney  
*Stanford University & Veterans Affairs Palo Alto Health Care System, cleber.ouverney@sjsu.edu*

Katherine Palm  
*Stanford University*

Gary C. Armitage  
*University of California - San Francisco*

*See next page for additional authors*

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Authors
Paul W. Lepp, Mary M. Brinig, Cleber C. Oouverney, Katherine Palm, Gary C. Armitage, and David A. Relman
Methanogenic Archaea and human periodontal disease

Paul W. Lepp1,†, Mary M. Brinig‡, Cleber C. Ouvrney†,§, Katherine Palm♦, Gary C. Armitage†, and David A. Relman1,‡

Departments of 1Microbiology and Immunology and 3Medicine, Stanford University, Stanford, CA 94305; 5Veterans Affairs Palo Alto Health Care System, Palo Alto, CA 94304; and 4Department of Stomatology, University of California, San Francisco, CA 94143

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Archaea have been isolated from the human colon, vagina, and oral cavity, but have not been established as causes of human disease. In this study, we reveal a relationship between the severity of periodontal disease and the relative abundance of archaeal small subunit ribosomal RNA genes (SSU rDNA) in the subgingival crevice by using quantitative PCR. Furthermore, the relative abundance of archaeal small subunit rDNA decreased at treated sites in association with clinical improvement. Archaea were harbored by 36% of periodontitis patients and were restricted to subgingival sites with periodontal disease. The presence of archaeal cells at these sites was confirmed by fluorescent in situ hybridization. The archaeal community at diseased sites was dominated by a Methanobrevibacter oralis-like phylotype and a distinct Methanobrevibacter subpopulation related to archaea that inhabit the gut of numerous animals. We hypothesize that methanogens participate in syntrophic relationships in the subgingival crevice that promote colonization by secondary fermenters during periodontitis. Because they are potential alternative syntrophic partners, our finding of larger Treponema populations sites without archaea provides further support for this hypothesis.

Materials and Methods

Subject Enrollment. Subjects were enrolled at the University of California, San Francisco (UCSF), School of Dentistry in the Ratcliff Center for Clinical Research (Division of Periodontology). The use of human subjects in this investigation was approved by the Stanford University Administrative Panel on Human Subjects in Medical Research and the UCSF Committee on Human Research. Subjects were at least 25 years old, were missing no more than 14 teeth, had a clinical diagnosis of generally healthy gingiva or chronic periodontitis, and were free of other oral soft tissue disease. Periodontal status of each subject was determined by measuring clinical attachment loss (CAL) to the nearest millimeter at the mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual sites around each tooth. Mean full-mouth CAL values were used to place patients in the following categories: healthy (mean CAL <0.6 mm), slight periodontitis (0.6 mm ≤ mean CAL <1.6 mm), moderate periodontitis (1.6 mm ≤ mean CAL <2.5 mm), and severe periodontitis (mean CAL ≥2.5 mm; Table 1). Subjects were excluded if they were diabetic, HIV-positive, pregnant, lactating, or had taken antibiotics in the previous 3 months, because these factors have been implicated in altering oral bacterial composition. Subjects completed a survey regarding age, gender, race, and habits of oral hygiene.

Sample Collection. Subgingival plaque samples were collected from 6–12 periodontal pockets from each subject by using Hartzell R-1, R-2 curettes. Supragingival plaque was removed from tooth surfaces before sampling. Separate sterile curettes were used for each plaque sample. Sampling included both clinically healthy and diseased sites. Clinical assessments at each site included the presence or absence of bleeding on probing (BOP), probing depth (PD), and CAL. Clinical assessments and sample collections were performed by one researcher (G.C.A.). Each site was classified as healthy (no BOP, CAL ≤1 mm, PD ≤3 mm), having gingivitis (BOP, CAL ≤1 mm, PD ≤4 mm), slight periodontitis (BOP, CAL 2–3 mm, and PD ≥4 mm), moderate periodontitis (BOP, CAL 4–5 mm, and PD ≥4 mm), or severe periodontitis (BOP, CAL ≥6 mm, and PD ≥4 mm, Table 2). In addition, a sample was taken from the dorsum of the tongue with a sterile plastic spatula. Less than 1 mg of plaque material from each sampled site was placed in a 1-ml O-ring microcentrifuge tube containing 200 μl of γ-irradiated H2O. A 100-μl aliquot was moved to another tube for fluorescent in situ hybridization (FISH) after vortexing. The remaining aliquot was frozen immediately and kept at −80°C before further processing.

Nucleic Acids Extraction. Nucleic acids were extracted from each 100-μl plaque sample by adding an equal volume of 0.1% blue dextran (Sigma) and 2× volume of cell lysis buffer (100 mM Tris-HCl, pH 7.4/20 mM EDTA/5 M guanidine isothiocyanate/2%/ Triton X-100). Proteinase K (Sigma) was added to a final concentration of 250 μg/ml, and the sample was incubated

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Abbreviations: CAL, clinical attachment loss; FISH, fluorescence in situ hybridization; SSU, small subunit.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AY374553 and AY374554).

†To whom correspondence should be addressed. E-mail: pwlepp@cmgm.stanford.edu.

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at 65°C for 30 min. Samples were then agitated in a FastPrep FP120 instrument (Oebiogene, Carlsbad, CA) at 4.0 m/s for 30 s with 0.1 g each of 0.1-mm, 0.5-mm, and 1-mm diameter baked zirconia/silica beads (Biospec, Bartlesville, OK). An equal volume of 99% benzyl alcohol was added, and the sample was vortexed before centrifugation at 7,000 g. The nucleic acids were precipitated by the addition of 1 volume of 99% benzyl alcohol, and the sample was centrifuged at 7,000 g for 1 min. The supernatant was removed and the pellet was redissolved in 50 μl of 1× TE buffer, pH 8.4

Phylogenetic Analysis. Initial alignment of amplified sequences was performed with the automated 16S rDNA sequence alignment of the ARB software package (www.arb-home.de) against a database of 12,569 complete and partial rRNA sequences. Ambiguously and incorrectly aligned positions were aligned manually on the basis of conserved primary sequence and secondary structure. Identity matrices were generated from either 572 or 998 masked (unambiguously aligned) positions, depending on the primer pair used for sequence library construction. Following the procedure of Kros et al. (18), sequences with ≥99% identity were considered as a single phylogroup. The phylogenetic associations of all representative sequences were determined by using a maximum-likelihood algorithm (19). These associations were confirmed by using a parsimony algorithm (19) and a neighbor-joining algorithm (20) of kimura two-parameter corrected evolutionary distances. Sequences were deposited in the GenBank database (accession numbers AY374553 and AY374554).

FISH. Polyribonucleotide probe (polyprobe) was generated from Microcon-100 (Millipore, Bedford, MA) purified PCR amplicons of cloned SBGA-1 16S rRNA that contained a T7 RNA polymerase promoter by using a protocol modified from that of DeLong et al. (21). The polyprobe was transcribed by using a modification of the manufacturer’s recommended protocol for RNAMax (Stratagene). Each 25-μl transcription-labeling reaction consisted of 1 μl of extracted DNA, buffer (10 mM Tris-HCl, pH 8.5/1.5 mM MgCl2/50 mM KCl/200 μM of each dNTP/0.05% Triton X-100), 400 nM each primer, and 1.25 units of AmpliTaq (Applied Biosystems, Foster City, CA). Archaeal 16S rDNA genes were amplified from the following cycle conditions: 35 cycles of 94°C (30 s), 58°C (30 s), and 72°C (30 s) followed by a 3-min extension at 72°C. Primer specificity and sensitivity were determined by using cloned 16S rDNA genes from Halobacterium salinarum (pHS16S; ATCC 33171), Sulfolobus acidocaldarius (pSc; ATCC 33909), and Escherichia coli B/r (pEc) (17) (see Supporting Text, Table 3, and Figs. 5–7, which are published as supporting information on the PNAS web site). PCR products of the appropriate size from patient samples were cloned by using the TOPO-TA kit (Invitrogen) as per manufacturer’s instructions and screened for the appropriately sized inserts. Inserts were sequenced on an ABI 377 sequencer (Applied Biosystems, Foster City, CA) using M13(-20F and M13(–27)R primers for duplicate coverage with ABI PRISM BigDye Terminators v2.0 reagents (Applied Biosystems).

Table 1. Vital statistics for enrolled human subjects

<table>
<thead>
<tr>
<th>Health Status</th>
<th>Healthy</th>
<th>Slight</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean CAL, mm*</td>
<td>0.19 ± 0.01</td>
<td>1.59 ± 1.98 ± 0.02</td>
<td>3.83 ± 3.83 ± 0.04</td>
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</tr>
<tr>
<td>No. of patients</td>
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<td>12</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>8</td>
<td>0</td>
<td>10</td>
<td>18</td>
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<td>0</td>
<td>1</td>
<td>1</td>
<td>10</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>7</td>
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<tr>
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<td>0</td>
<td>1</td>
<td>2</td>
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<td>25</td>
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<tr>
<td>Female</td>
<td>5</td>
<td>0</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Age†</td>
<td>43.3 ± 13.6</td>
<td>34</td>
<td>42.6 ± 11.7</td>
<td>46.0 ± 12.2</td>
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Table 2. Vital statistics for patient samples

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<th>Sample Type</th>
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<th>Moderate</th>
<th>Severe</th>
<th>Healthy controls</th>
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<td>7</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Mean PD, mm*</td>
<td>NA</td>
<td>3.24 ± 0.08</td>
<td>4.04 ± 0.15</td>
<td>4.71 ± 0.12</td>
<td>6.37 ± 0.08</td>
<td>8.90 ± 0.12</td>
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<td>Mean CAL, mm†</td>
<td>NA</td>
<td>0.45 ± 0.50</td>
<td>1.00</td>
<td>2.47 ± 0.06</td>
<td>4.69 ± 0.05</td>
<td>7.96 ± 0.11</td>
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<tr>
<td>Male</td>
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<td>16</td>
<td>20</td>
<td>30</td>
</tr>
</tbody>
</table>

*Mean PD = probing (pocket) depth ± SE.
†Mean CAL ± SE.
lyzation was stopped by placing the reaction on ice and adding EDTA (pH 7.2) to a final concentration of 50 mM. Subgingival plaque samples were vigorously vortexed for 5 min. Samples were fixed in 0.5× PBS (145 mM NaCl/8.7 mM Na2HPO4/1.5 mM NaH2PO4, pH 7.4) and 3.7% (wt/vol) formalin overnight at 4°C, transferred to 4-mm-diameter Teflon slides (Erie Scientific, Portsmouth, NH), and air dried. Samples were dehydrated by successive passage in 50%, 80%, and 90% EtOH for 3 min each. Ten microliters of hybridization buffer [0.9 M NaCl/20 mM Tris-HCl, pH 7.4/1% SDS/1 mg/ml poly(A)/50% formamide], and 50 ng of polyprobe was added to each well. Conditions that optimized polyprobe specificity were determined by using cloned artificial targets for FISH with a range of archaeal or bacterial gene copy number was estimated for each sample from a standard curve generated from a 10-fold serial dilution of pHs16S or pEc16S, respectively (Supp. Figs. 6 and 7). The assay specificity and possible inhibition were tested with reactions possessing generally healthy gingiva or exhibiting various degrees of periodontitis (Fig. 2). The relative abundance of archaeal SSU rDNA in relation to total prokaryotic SSU rDNA was significantly higher (P<0.01) than in patients with moderate periodontitis. The mean CAL of patients with either severe or moderate periodontitis was significantly higher (P<0.01) than the baseline measured from cycles 3 to 15. Total archaeal or bacterial gene copy number was estimated for each sample from a standard curve generated from a 10-fold serial dilution of pHs16S or pEcl16S, respectively (Supp. Figs. 6 and 7). Archaela small subunit (SSU) rDNA was quantified by using primers SDArch0333aS15 and SDArch0958aA19 and probe S*Univ0515aA19 (5’-FAM-TTACCGCGGCGKTGGGAGCTAMRA-3’; ref. 23). Bacterial rRNA was quantified by using primers SDBact0008aS20 (5’-AGAGTTTGATCCTGGCGTCAG-3’; ref. 23) and S*Univ0515aA19 and probe SDBact0338aS18 (5’-FAM-GTCCGCTCGGATGGAGTT-TAMRA-3’; ref. 24). Assay specificity was validated in samples from healthy and diseased sites, as well as tooth scrapings, from 50 periodontitis patients. In addition, included 29 subgingival plaque samples and 20 tongue scrapings from healthy and diseased sites, as well as 2 tongue scrapings, from 50 periodontitis patients. We also measured the abundance of Bacteria-specific SSU rDNA to normalize for variations in microbial biomass between samples, as described (26). The Archaea- and Bacteria-specific assays had lower detection limits of 100 and 1,000 gene copies, respectively (Figs. 6 and 7). Archaela SSU rDNA was not detected in any of the 31 samples from the healthy control population. Archaela SSU rDNA was detected in 36% of the periodontitis patients. Archaela SSU rDNA was detected in 76.6% of periodontitis sites but was not detected in samples from healthy sites or tongue scrapings from Archaela-positive periodontitis patients. There was a significant correlation between the relative abundance of archaela SSU rDNA and the severity of disease within the Archaela-positive subset of patients (Fig. 1). Archaela SSU rDNA accounted for 18.5% of total prokaryotic DNA abundance. A large portion of this variation was due to slight differences in the slope of the real-time PCR standard curve, which was then amplified in the conversion of log to absolute gene copy number. To minimize interexperimetal variation, we constructed a composite standard curve that encompassed the standard curves from all individual experiments, similar to the procedure previously described (26). All samples and standards were analyzed in duplicate within each individual experiment. Samples with an intralexperimental coefficient of variation >1 were reanalyzed. Significant differences in archaeal, bacterial, and treponemal rRNA gene copy numbers between disease states were assessed by a two-tailed, unpaired t test. The hypothesis that the relative proportion of archaea increased with the degree of CAL was tested by one-way ANOVA. All errors are reported as standard error unless otherwise specified. Results The subjects enrolled in this study were classified as either possessing generally healthy gingiva or exhibiting various degrees of periodontitis (Table 1). The mean CAL, a measure of disease severity, in patients with severe periodontitis was significantly higher (P<0.01) than in patients with moderate periodontitis. The mean CAL of patients with either severe or moderate periodontitis was significantly higher (P<0.01) than the baseline measured from cycles 3 to 15. Total archaeal or bacterial gene copy number was estimated for each sample from a standard curve generated from a 10-fold serial dilution of pHs16S or pEcl16S, respectively (Supp. Figs. 6 and 7). Archaela small subunit (SSU) rDNA was quantified by using primers SDArch0333aS15 and SDArch0958aA19 and probe S*Univ0515aA19 (5’-FAM-TTACCGCGGCGKTGGGAGCTAMRA-3’; ref. 23). Bacterial rRNA was quantified by using primers SDBact0008aS20 (5’-AGAGTTTGATCCTGGCGTCAG-3’; ref. 23) and S*Univ0515aA19 and probe SDBact0338aS18 (5’-FAM-GTCCGCTCGGATGGAGTT-TAMRA-3’; ref. 24). Assay specificity was validated in samples from healthy and diseased sites, as well as tooth scrapings, from 50 periodontitis patients. In addition, included 29 subgingival plaque samples and 20 tongue scrapings from healthy and diseased sites, as well as 2 tongue scrapings, from 50 periodontitis patients. 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The relative abundance of archaela SSU rDNA in relation to total prokaryotic SSU rDNA was significantly higher (P<0.05) in severe and moderate periodontitis sites compared to slight periodontitis sites within the Archaela-positive subset of patients. There was a significant relationship between the degree of CAL and the relative abundance of archaela SSU rDNA (P<0.0001) within the periodontitis patient subpopulation. There was no discernible relationship between the data set may have been too small to
resolve statistically significant relationships involving these parameters.

As expected, bacterial rRNA gene copy numbers were significantly higher \((P < 0.001)\) in severely and moderately diseased periodontitis sites compared to the healthy sites of periodontitis patients. Similarly, the mean bacterial rRNA gene copy numbers were significantly higher at slight periodontitis and gingivitis sites compared to the healthy sites of periodontitis patients \((P < 0.015)\). The mean bacterial gene copy number was significantly lower \((P < 0.005)\) in samples from the healthy control group \((5.3 \times 10^6 \pm 2.4 \times 10^5\) gene copies per \(\mu l)\) compared to samples from healthy sites in periodontitis patients \((3.5 \times 10^6 \pm 9.5 \times 10^5\) gene copies per \(\mu l)\; Fig. 2).

The analysis of 77 samples from six patients obtained 12–18 months after treatment, and the comparison of these results with those obtained from these sites before treatment, revealed a significant decrease in the relative abundance of archaean SSU rDNA from a mean of 12.3 ± 4.6% to 0.0056 ± 0.0035% \((P < 0.001)\). This decrease was accompanied by a drop in the patients’ mean CAL from 3.8 ± 0.072 to 2.4 ± 0.19, indicating an improvement in disease status. The decrease in the relative abundance of archaean SSU rDNA was caused by a decline at each sampled site and not caused by a reduction in prokaryotic biomass or an increase in bacterial 16S rDNA copy number, the latter of which remained nearly constant at \(1.0 \times 10^7 \pm 1.8 \times 10^6\) copies before treatment and \(1.5 \times 10^7 \pm 2.3 \times 10^6\) copies after treatment \((P > 0.1)\).

To investigate the diversity of *Archaea* in the human subgingival crevice, SSU rDNA was amplified with domain-specific primers and cloned independently from samples collected from six patients with periodontitis. For this purpose, we used the same archaeal primer set as that used in the 5‘ nuclelease assay, and a set that amplified a larger segment of the SSU rDNA; these primers were tested for both sensitivity and specificity (see Supporting Text). All 105 sequenced clones fell within the genus *Methanobrevibacter* of the *Euryarchaeota* division. Phylogenetic analysis by both maximum-likelihood and maximum parsimony algorithms produced identical topologies (Fig. 3). Analysis using a neighbor-joining distance method produced a topology that differed from the other two analyses only in its placement of *Methanobrevibacter cuticularis* at the root of the clade containing *Methanobrevibacter filiformis*, *Methanobrevibacter ruminantium*, and *Methanobrevibacter arboriphilicus*.

The clone libraries were dominated (81% of clones) by a phylotype (SBGA-1) with 99.8% identity to the 572 nucleotides of *Methanobrevibacter oralis* available from GenBank (Fig. 3). Using reverse primer SDArch1378aA20, we were able to extend the sequence from what was probably *Methanobrevibacter oralis* by an additional 436 nucleotides and demonstrate that this phylotype is clearly distinguishable from *Methanobrevibacter smithii*. Phytophage SBGA-1 shared 97.7% identity with *M. smithii* over 998 nucleotides (positions 349–1378, *E. coli* numbering). The remainder (19%) of the cloned sequences was composed of the phylotype SBGA-2. This phylotype shared 99.8% identity with a *Methanobrevibacter* sequence associated with the ciliate *Euplодinium maggi*, which inhabits the ovine rumen (27, 28). This phylotype was also closely related (99.5% identity) to, but distinct from, the human oral “phylotype 3” identified by Kulik et al. (1). Together these three phylotypes, along with phylotypes from a number of ruminants and swine, formed a clade that shared ancestry with *M. oralis* to the exclusion of *M. smithii* (Fig. 3). Although each of the phylotypes within this clade was distinguishable from the others, the nucleotide differences occurred in unpaired, nonhelical regions and may represent sequencing errors, interperon variability, or different strains of a single species. Phylotype SBGA-2 shared 98.6% sequence iden-
those used previously with polyribonucleotide probes (21). We optimal discrimination between SBGA-1 targets and nontarget samples. When we used cloned artificial targets for FISH, we specific RNA polyprobe for FISH that enabled us to characterize vol) at 65\(^\circ\) of treponemal rDNA at sites with archaeal rDNA was 6.2 no detected archaeal rDNA. In contrast, the relative abundance specimens from severe and moderate periodontitis sites that had \(P_{1.4\%}\); this difference was statistically significant (\(P < 0.05\)). Members of the genus \textit{M. oralis} are highly diverse in form and dimensions were largely consistent with those of \textit{M. oralis} (8), although the cell width observed in this study was approximately twice that previously reported.

Methanogenesis by \textit{M. oralis} is a hydrogen-consuming process. In syntrophic relationships, this process facilitates the growth of hydrogen-producing organisms, which include some of the known oral bacterial pathogens. If syntrophy is an important feature of more severely diseased periodontal pockets, one might expect to find other syntrophic partners in methanogen-negative, diseased sites. Treponemes are a potential hydrogen competitor, and are a monophyletic group for which group-specific primers can be designed. Therefore, we determined the relative abundance of \textit{Treponema} species rDNA within the same collection of plaque samples as used to investigate the relative abundance of archaeal rDNA (Fig. 8). We found that treponeme rDNA represented 12.4 \pm 3.8\% of the prokaryotic rDNA in a set of specimens from severe and moderate periodontitis sites that had no detected archaeal rDNA. In contrast, the relative abundance of treponemal rDNA at sites with archaeal rDNA was 6.2 \pm 1.4\%; this difference was statistically significant (\(P < 0.05\)). There was no significant difference in the relative abundance of treponemal rDNA found at sites with slight periodontitis or gingivitis, regardless of whether archaeal rDNA was detected or not; however, the number of sites with these disease classifications and detected archaea was small.

**Discussion**

Members of the domain \textit{Archaea} are highly diverse in form and function, but curiously, disease causation is not among their demonstrated capabilities. We found that the relative abundance of archaeal SSU rDNA increased in relationship to the severity of periodontal disease within a cohort of patients. There was a corresponding decrease in the relative abundance of the archaeal SSU rDNA coinciding with an improvement in periodontal status after treatment. The etiology of a polymicrobial disease such as periodontitis is likely to be more complex than suggested by the traditional paradigm of disease involving a single virulent organism. Traditional approaches for establishing causation require the use of a relevant model system with the presumption that transfer of a purified microbial isolate will be sufficient to reproduce disease, as specified in Koch's postulates. Molecular criteria have been proposed for imputing a causative role when the putative factor cannot be easily isolated or purified (29).

\textit{Archaea} were detected in only a subset of patients with severe disease. The assay used was capable of detecting amounts of \textit{Archaea} representing as little as 0.001\% of the prokaryotic population, suggesting that the methodology was not a limiting factor in detection. Two hypotheses, which are not mutually exclusive, may be advanced to explain the presence of oral methanogens in only a subset of periodontitis patients. The first hypothesis is that host genetics may predispose some individuals to colonization by oral methanogens. However, a comparison of the prevalence of oral and colonic methanogens found that all individuals harboring oral methanogens also harbored colonic methanogens, but not vice versa (9), suggesting that host genetics is not a sufficient explanation for the exclusion of methanogens from the oral cavity. An additional study of monzygotic and dizygotic twins found that host genetics did not play a significant factor in differences in breath methane emission, a hallmark of colonic methanogens (30).

The second hypothesis proposes niche exclusion of methanogens by other hydrogen-metabolizing microbes in some patients. Sulfate-reducing bacteria (SRB) are potential competitors that have been reported to be harbored by \(\approx 64\%\) of periodontitis patients, and their presence has been correlated with pocket depth (31). Under standard conditions, sulfate-reducing bacteria should out-compete methanogens (32), assuming that the availability of sulfate is not limited. However, if the interactions between subgingival SRB and methanogens are similar to those in the colon, then the two groups may coexist within the same environment (33, 34). Recent research has indicated that both may coexist in the oral cavity (13).

Members of the genus \textit{Treponema} are also potential hydrogen competitors, and include a well-known periodontal patho-gen, \textit{Treponema denticola}. Previous work has demonstrated that \textit{T. denticola}, like \textit{Porphyromonas gingivalis} and \textit{Tannerella forsythensis}, is associated with severe periodontitis, as a member of the “red” polymicrobial disease complex (35). It has also recently been demonstrated that some \textit{Treponema} species are capable of homoacetogenesis, a hydrogen-consuming process (36). We found that the relative abundance of treponemal rDNA was significantly lower in sites with archaeal rDNA than in sites without archaeal rDNA, suggesting that some \textit{Treponema} species may compete with methanogens. Our results present the possibility that methanogens and treponemes may serve as alternative syntrophic partners with other members of the subgingival biofilm community, such as other members of the red complex. In this scenario, methanogenic \textit{Archaea} indirectly promote periodontal disease in some patients by serving as a hydrogen sink, thereby permitting the proliferation of one or more pathogenic secondary fermenters to levels beyond that which would be possible in the absence of the archaea.

The apparent restricted diversity exhibited by the oral \textit{Archaea} may reflect the adaptation of a small minority of organisms within this broad domain of life to this particular niche. The length and morphology of the cells labeled with the Cy-3 archaea-specific polyprobe were consistent with those of \textit{Methanobrevibacter oralis}. However, the cells observed in this study were nearly twice the width of those previously reported, which may reflect differences in growth rate or nutritional status (8). Members of the genus \textit{Methanobrevibacter} are strict anaerobes,
and previous studies have shown that mature subgingival plaque provides the highly reduced environment necessary for anaerobic growth (37). Although SBGA-1 was identified in all six of the patients from which archaeal clone libraries were created, phylotype SBGA-2 was recovered from only two of the six patients. Although this latter phylotype appears to be a minor constituent of the methanogenic population, the number of patients examined was not large enough to determine the true distribution of this phylotype.

We speculate that syntrophic interactions between Archaea and other members of the microbial flora may be an important feature of some polymicrobial diseases (4). The identity and role of the complementary syntrophic partner(s) should provide an important avenue for future research in eliciting the microbial mechanisms involved in chronic periodontitis and other polymicrobial diseases.

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