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Subgingival and Tongue Microbiota during Early Periodontitis

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Abstract

Periodontal infections have a microbial etiology. Association of species with early disease would be useful in determining which microbes initiate periodontitis. We hypothesized that the microbiota of subgingival and tongue samples would differ between early periodontitis and health. A cross-sectional evaluation of 141 healthy and early periodontitis adults was performed with the use of oligonucleotide probes and PCR. Most species differed in associations with sample sites; most subgingival species were associated with subgingival samples. Few species were detected more frequently in early periodontitis by DNA probes. *Porphyromonas gingivalis* and *Tannerella forsythia* (*Tannerella forsythensis*) were associated with early periodontitis by direct PCR. In conclusion, the microbiota of tongue samples was less sensitive than that of subgingival samples in detecting periodontal species, and there was overlap in species detected in health and early periodontitis. Detection of periodontal pathogens in early periodontitis suggests an etiology similar to that of more advanced disease.

Keywords

microbiology; tongue; subgingival; health; early periodontitis

INTRODUCTION

While periodontal diseases affect over half of the adults in the United States (Albandar *et al.*, 1999), with appropriate interceptive treatment, periodontitis can be prevented. It has been difficult to identify those young adults who could develop periodontitis from those who remain periodontally healthy. Rather than universal prophylactic treatment, it would be preferable to identify and selectively treat individuals at high risk for disease before significant periodontal attachment loss occurs. Early periodontitis is detected clinically by loss of periodontal attachment, which, in younger adults, is strongly associated with gingivitis (Tanner *et al.*, 2005). Gingivitis, with or without early attachment loss, however, cannot indicate which individuals will develop periodontitis. The primary etiology of periodontitis is the presence of specific bacteria in the subgingival plaque biofilm. Thus, it seems likely that identification of certain species could aid in the risk assessment for early periodontitis.

The composition of the subgingival microbiota of chronic periodontitis in adults has been described by culture (Moore and Moore, 1994; Tanner *et al.*, 1998; van Winkelhoff *et al.*, 2002), immunological (Riviere *et al.*, 1996; Clerehugh *et al.*, 1997; Machtei *et al.*, 1999), and

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molecular methods (Socransky *et al.*, 1998; Paster *et al.*, 2001; Kumar *et al.*, 2003; de Lillo *et al.*, 2004). Culture analysis of subgingival plaque samples of early periodontitis detected *Tannerella forsythia* (*Tannerella forsythensis*), *Campylobacter rectus*, and *Selenomonas noxia* associated with progressing, compared with non-progressing, subjects, whereas *Porphyromonas gingivalis* was associated, by whole genomic DNA probes, with progressing periodontitis (Tanner *et al.*, 1998). These species have also been associated with moderate and advanced periodontitis (Moore and Moore, 1994; Socransky *et al.*, 1998). Molecular PCR cloning and sequencing methods have identified several species that are rarely or not detected by culture methods (Paster *et al.*, 2001), some of which show strong associations with adult periodontitis (Kumar *et al.*, 2003). Several periodontal species, including *P. gingivalis* and *Prevotella melaninogenica*, have been identified from tongue samples of adults (Van der Velden *et al.*, 1986; Lee *et al.*, 1999; Mager *et al.*, 2003). The aims of this study were to compare the microbiota detected in subgingival and tongue samples and to evaluate associations between recognized species and uncultivated phylotypes with early periodontitis. Bacterial species that showed positive associations with early attachment loss could be of value in assessing periodontal risk, particularly if from easily obtained tongue samples.

MATERIALS & METHODS

We conducted a cross-sectional cohort study comparing the microbiota of subgingival and tongue samples and the microbiota of periodontally healthy and early periodontitis individuals.

Study Population and Clinical Measurements

Periodontally healthy and early periodontitis individuals (Table) underwent clinical monitoring as previously described (Tanner *et al.*, 2005). Individuals were medically healthy, aged between 20 and 40 yrs, had not taken antibiotics in the previous 6 mos or had any previous periodontal therapy. Study participants had at least 24 teeth, and minimal periodontal attachment loss. Participants' rights were protected by approval of the study protocol from the IRBs of all participating institutions, and participants who signed an informed consent were accepted into the study. Participants completed medical and dental histories and a questionnaire that included race/ethnicity and history of cigarette smoking. Comprehensive clinical measurements were taken as previously described (Tanner *et al.*, 2005). Healthy participants had a mean periodontal attachment level of ≤1.5 mm and no sites with > 2 mm attachment loss. Early periodontitis 1 individuals also had a mean periodontal attachment level of ≤1.5 mm but at least 1 site with 2 mm attachment loss. Early periodontitis 2 individuals had > 1.5 mm to ≤2.0 mm mean periodontal attachment loss.

Microbial Analyses

Sample-taking—Supragingival plaque was removed with a Gracey curette, after which samples were taken with a fresh sterile curette from 4 subgingival sites, usually distolingual, on 1st or 2nd molars, and the samples were pooled. Tongue samples were also taken with a Gracey curette by stroking the posterior 1/3 of the dorsum of the tongue. Samples were placed in 100 μL of Tris-EDTA buffer and stored frozen at −70°C.

DNA Probe Analysis—Bacterial samples were analyzed by the PCR-based, reverse-capture oligonucleotide checkerboard assay (Socransky *et al.*, 1994; Paster *et al.*, 1998), as described in the modified procedure (Becker *et al.*, 2002). Probe species (Fig. 1) were selected to include frequently encountered periodontally related species or clones of novel unidentified, including not-yet-cultivated, phylotypes from PCR-cloning studies of human dental plaque (Paster *et al.*, 2001) and the tongue (Kazor *et al.*, 2003). The detection limit for the assay was about 10^3 cells.

Multiplex PCR Analysis—To improve detection sensitivity, particularly of *P. gingivalis*, we performed a direct PCR assay (Tran and Rudney, 1999) using 2 μL of sample. Amplicons were visualized by agarose gel electrophoresis. The detection limit for *P. gingivalis* was about 10^2 cells, and for *T. forsythia*, 10^3 cells.

Reactions from both assays were scored as species detected or not detected.

Statistical Analyses

Species Association with Subgingival and Tongue Samples—We analyzed data from paired subgingival and tongue samples taken at the same visit from each individual. Contingency tables evaluated species detection in subgingival and tongue samples. Differences in species prevalence in tongue and subgingival samples were evaluated by McNemar's Chi-square test, and similarities of species detection in tongue and subgingival samples were evaluated by the Chi-square test for association.

Species Associations with Early Periodontitis—Individuals were grouped into healthy and two early periodontitis disease categories (Table). Comparisons of species prevalence across clinical groups were done by the Mantel-Haenszel chi-square for trend.

Significance levels were not adjusted for the multiple comparisons over species.

RESULTS

Clinical Characteristics of Study Population

The clinical and demographic characteristics of 141 healthy and early periodontitis individuals (Table) are from the previously described study population of 221 individuals (Tanner *et al.*, 2005). Although samples were taken from all study participants, there was insufficient DNA from some participants to get an amplicon from PCR, resulting in differences in numbers of individuals with microbial data from the clinical study population. Most participants were never-smokers and Caucasian (65%); 21 were African-American. There was a positive association between early periodontitis and gingival index, percent sites bleeding on probing, pocket depth, participant age, gender, and cigarette smoking (Table).

Microbiology

Reverse-capture Oligonucleotide Probe Data

Subgingival and tongue samples: There were differences in species detection in 121 paired subgingival and tongue samples (Fig. 1). Many *Streptococcus* and *Streptococcus*-like species (*Gemella* and *Granulicatella*) were detected more frequently from, and were associated with, tongue samples ($p < 0.05$, McNemar's Chi-square test). *Eubacterium sulci*, *Actinomyces odontolyticus*, *Campylobacter concisus*, and *Prevotella melaninogenica* were also associated with tongue samples.

Most of the Gram-negative taxa and not-yet-cultivated phylotypes were detected more frequently from subgingival samples ($p < 0.05$, McNemar's Chi-square test), including Treponema species, *T. forsythia*, *Porphyromonas endodontalis*, *A. actinomycetemcomitans*, *Prevotella oris*, TM7 phylum, *Dialister invisus*, and *Fusobacterium nucleatum* subsp. *animalis.* Subgingivally associated Gram-positive species included *Streptococcus mutans*, *Streptococcus sanguinis*, *Streptococcus anginosus*, *Actinomyces naeslundii* II, *Actinomyces gerencseriae*, and *Filifactor alocis* ($p < 0.05$, McNemar's Chi-square test). Detection of *F. nucleatum* subsp. *polymorphum* and *Prevotella denticola* subgingivally was associated with detection on the tongue ($p < 0.05$, Chi-square test for associations). *P. gingivalis* was detected in less than 5% of samples when DNA probes were used.

Health and early periodontitis samples: Subgingival samples from 141 individuals were compared between clinical categories (Fig. 2). Species detected more frequently in early periodontitis than in health included *Treponema denticola*, *F. alocis*, *P. endodontalis*, *Bacteroidetes* oral clone AU126, *F. nucleatum* subsp. *animalis*, and *Fusobacterium nucleatum* subsp. *polymorphum*, although none significantly, except *Actinomyces odontolyticus*.

Multiplex PCR Analysis of *P. gingivalis* and *T. forsythia*—The multiplex PCR assay detected more *P. gingivalis*-positive samples than did the DNA probe assay, a similar proportion of *T. forsythia* samples in 124 paired subgingival and tongue samples (Fig. 3). The detection frequency for *P. gingivalis* was similar from subgingival and tongue samples. *T. forsythia* was detected more frequently from subgingival as compared with tongue samples (McNemar Chi-square, $p = 0.001$). Detection of *T. forsythia* from the tongue was not associated with subgingival detection within individuals.

P. gingivalis from tongue and subgingival sample sites was significantly associated with early periodontitis (Mantel-Haenszel Chi-square, $p < 0.001$). *T. forsythia* detection subgingivally was also associated with early periodontitis ($p = 0.03$). There were insufficient *T. forsythia*-positive tongue samples to test their association with early periodontitis.

DISCUSSION

We compared the microbiota of tongue and subgingival samples of adults showing early stages of periodontitis. Clinically, there was a strong association of early periodontitis with gingival inflammation, as has been observed in other populations of young adults with periodontitis and in the source population of the current study (Tanner *et al.*, 2005). The microbiota of early periodontitis included several species associated with moderate to advanced periodontitis. The association of *P. gingivalis* with early periodontitis was of particular interest, because, using culture techniques, we had not associated this species with early periodontitis (Tanner *et al.*, 1998). By immunoassays, however, *P. gingivalis* was detected in 3- to 5-mm pockets more than in shallower sulci (Hamlet *et al.*, 2001), and in early periodontitis more frequently than in health (Riviere *et al.*, 1996). In the latter study, however, higher levels of *P. gingivalis* were detected in gingivitis than in early periodontitis, which is consistent with the elevated gingival inflammation in early periodontitis observed in the current study. *P. gingivalis* was also detected more frequently and in higher levels in adolescent individuals with early periodontitis as compared with health (Clerehugh *et al.*, 1997), and with deeper pocket and bleeding sites in a population of 11- to 13-year-old children (Ellwood *et al.*, 1997). In all these studies, *P. gingivalis* was detected in only a proportion of early periodontitis individuals, which may reflect the detection sensitivities of assays, or the involvement of additional species in the initiation of periodontitis.

In contrast to *P. gingivalis*, *T. forsythia* was associated with early periodontitis by culture (Tanner *et al.*, 1998). Furthermore, *T. forsythia*, but not *P. gingivalis*, was associated with, and predictive of, progressing attachment loss in older individuals with minimal periodontitis (Tran *et al.*, 2001), by the PCR assay used in the current study. *T. forsythia* was detected more frequently from adolescents with progressing attachment loss than from non-progressing individuals (Hamlet *et al.*, 2004). The current study also detected *T. forsythia* in health, consistent with its detection in periodontally healthy children, adolescents (Sirinian *et al.*, 2002), and adults (Gmür *et al.*, 1999). While a different health-associated *T. forsythia*-like phylotype has been reported (Leys *et al.*, 2002; Kumar *et al.*, 2003), *T. forsythia* and *P. gingivalis* were infrequently detected from individuals without periodontal bone loss, when culture methods were used (van Winkelhoff *et al.*, 2002). Analysis of these data suggests that

while *T. forsythia* colonizes periodontally healthy individuals, it colonizes more frequently and at higher levels in periodontitis.

The oligonucleotide probes facilitated assay of uncultivated phylotypes and fastidious species likely underrepresented by culture. Few differences were detected between health and early periodontitis, probably reflecting the small differences between clinical categories inherent in our design to characterize the earliest stages of periodontal loss. Species or phylotypes detected more frequently from early periodontitis, previously associated with moderate to advanced periodontitis by molecular methods (Paster *et al.*, 2001; Kumar *et al.*, 2003), included *F. alocis*, *T. denticola*, *Peptostreptococcus micros*, *P. endodontalis*, and *Bacteroidetes* clone AU126. *T. denticola* was also detected, by immunofluorescence, more frequently from early periodontitis than health (Riviere *et al.*, 1996). *F. alocis* and *P. endodontalis*, while both previously identified from periodontal sites by culture (Petit *et al.*, 1993; Tanner *et al.*, 1998), are fastidious species that likely require detection by molecular methods for association with periodontitis (Kumar *et al.*, 2003). The Obsidian Pool and TM7 phylotypes belong in bacterial groups with no known cultivated representatives. Their detection in health and early periodontitis indicates the complexity of the oral microbiota not previously recognized from culture studies.

Differences in species colonization patterns between subgingival sites and mucous membranes have been recognized for many decades, and have been attributed to different adherence patterns to host tissues. With the recognition that individual species can have distinct colonization preferences, detection of the periodontal (subgingival) pathogen *P. gingivalis*, on the tongue surface and other mucous membranes (Van der Velden *et al.*, 1986), was a notable observation. Our finding, however, that the majority of subgingival species were detected more frequently from subgingival sites than on the tongue suggests that subgingival samples would be more reliable than tongue samples for the detection of periodontal pathogens.

While other studies have not examined the species set of this report from paired samples, associations were consistent with literature reports, particularly a recent report comparing the microbiota of several intra-oral sites by DNA probes (Mager *et al.*, 2003). Subgingivally associated species from both reports included *A. actinomycetemcomitans*, *A. naeslundii* II, *A. gerencseriae*, and *T. forsythia*. While *S. mutans* is most frequently associated with dental caries, it was detected subgingivally in health (Tanner *et al.*, 1998) and after periodontal therapy (van der Reijden *et al.*, 2001). Several "subgingival" species, in addition to *P. gingivalis*, were detected at similar or higher frequencies from tongue than from subgingival samples. Tongue-associated species included *C. concisus*, a pathogen of gastric enteritis (Vandamme *et al.*, 1989). Other tongue-associated species—*S. salivarius*, *S. parasanguinis*, *Megasphera* phylotype, and *Rothia mucilaginosa*—were included as "positive controls" of the tongue samples (Kazor *et al.*, 2003).

We used two PCR-based methods to analyze the microbiota of samples. Both assays avoided problems associated with the anaerobic sample-handling needed for culture analysis and could detect uncultivated taxa with only sequence data for identification. The oligonucleotide probe assay allowed for the simultaneous detection of multiple species, but direct PCR of samples was more sensitive for *P. gingivalis*. The detection frequency was similar for *T. forsythia*, but there was only reasonable agreement between methods (data not shown), likely resulting from the differences in primers between the assays. The strongest disease associations were from the direct PCR assay. Whether species other than *P. gingivalis* and *T. forsythia* would show stronger associations with early periodontitis if assayed by direct PCR would require further analysis.

In conclusion, this cross-sectional analysis indicated that *P. gingivalis* from tongue and subgingival samples and *T. forsythia* from subgingival samples were associated with adults with early periodontitis. Several other taxa previously associated with moderate to advanced periodontitis were detected more frequently from early periodontitis than from health, although associations were not significant. This suggests similarities in the microbial etiology in early and advanced periodontitis. The microbiota of tongue and subgingival samples differed, reflecting site-specificity in oral species, although all species were detected in both sites.

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References

Albandar JM, Brunelle JA, Kingman A. Destructive periodontal disease in adults 30 years of age and older in the United States, 1988–1994. J Periodontol 1999;70:13–29. [PubMed: 10052767]

Becker MR, Paster BJ, Leys EJ, Moeschberger ML, Kenyon SG, Galvin JL, et al. Molecular analysis of bacterial species associated with childhood caries. J Clin Microbiol 2002;40:1001–1009. [PubMed: 11880430]

Clerehugh V, Seymour GJ, Bird PS, Cullinan M, Drucker DB, Worthington HV. The detection of *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis* and *Prevotella intermedia* using an ELISA in an adolescent population with early periodontitis. J Clin Periodontol 1997;24:57–64. [PubMed: 9049799]

de Lillo A, Booth V, Kyriacou L, Weightman AJ, Wade WG. Culture-independent identification of periodontitis-associated *Porphyromonas* and *Tannerella* populations by targeted molecular analysis. J Clin Microbiol 2004;42:5523–5527. [PubMed: 15583276]

Ellwood R, Worthington HV, Cullinan MP, Hamlet S, Clerehugh V, Davies R. Prevalence of suspected periodontal pathogens identified using ELISA in adolescents of differing ethnic origins. J Clin Periodontol 1997;24:141–145. [PubMed: 9083896]

Gmür R, Marinello CP, Guggenheim B. Periodontitis associated bacteria in supragingival plaque of dental hygienists: stability of carrier state and clinical development. Eur J Oral Sci 1999;107:225–228. [PubMed: 10424388]

Hamlet SM, Cullinan MP, Westerman B, Lindeman M, Bird PS, Palmer J, et al. Distribution of *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis* and *Prevotella intermedia* in an Australian population. J Clin Periodontol 2001;28:1163–1171. [PubMed: 11737515]

Hamlet S, Ellwood R, Cullinan M, Worthington H, Palmer J, Bird P, et al. Persistent colonization with *Tannerella forsythensis* and loss of attachment in adolescents. J Dent Res 2004;83:232–235. [PubMed: 14981125]

Kazor CE, Mitchell PM, Lee AM, Stokes LN, Loesche WJ, Dewhirst FE, et al. Diversity of bacterial populations on the tongue dorsa of patients with halitosis and healthy patients. J Clin Microbiol 2003;41:558–563. [PubMed: 12574246]

Kumar PS, Griffen AL, Barton JA, Paster BJ, Moeschberger ML, Leys EJ. New bacterial species associated with chronic periodontitis. J Dent Res 2003;82:338–344. [PubMed: 12709498]

Lee KH, Tanner AC, Maiden MF, Weber HP. Pre- and post-implantation microbiota of the tongue, teeth, and newly placed implants. J Clin Periodontol 1999;26:822–832. [PubMed: 10599911]

Leys EJ, Lyons SR, Moeschberger ML, Rumpf RW, Griffen AL. Association of *Bacteroides forsythus* and a novel *Bacteroides* phylotype with periodontitis. J Clin Microbiol 2002;40:821–825. [PubMed: 11880400]

Machtei EE, Hausmann E, Dunford R, Grossi S, Ho A, Davis G, et al. Longitudinal study of predictive factors for periodontal disease and tooth loss. J Clin Periodontol 1999;26:374–380. [PubMed: 10382577]

Mager DL, Ximenez-Fyvie LA, Haffajee AD, Socransky SS. Distribution of selected bacterial species on intraoral surfaces. J Clin Periodontol 2003;30:644–654. [PubMed: 12834503]

Moore WE, Moore LV. The bacteria of periodontal diseases. Periodontol 2000 1994;5:66–77. [PubMed: 9673163]

Paster BJ, Bartoszyk IM, Dewhirst FE. Identification of oral streptococci using PCR-based, reverse-capture, checkerboard hybridization. Methods Cell Sci 1998;20:223–231.

Paster BJ, Boches SK, Galvin JL, Ericson RE, Lau CN, Levanos VA, et al. Bacterial diversity in human subgingival plaque. J Bacteriol 2001;183:3770–3783. [PubMed: 11371542]

Petit MD, van Winkelhoff AJ, van Steenbergen TJ, de Graaff J. *Porphyromonas endodontalis*: prevalence and distribution of restriction enzyme patterns in families. Oral Microbiol Immunol 1993;8:219–224. [PubMed: 8247609]

Riviere GR, Smith KS, Tzagaroulaki E, Kay SL, Zhu X, DeRouen TA, et al. Periodontal status and detection frequency of bacteria at sites of periodontal health and gingivitis. J Periodontol 1996;67:109–115. [PubMed: 8667130]

Sirinian G, Shimizu T, Sugar C, Slots J, Chen C. Periodontopathic bacteria in young healthy subjects of different ethnic backgrounds in Los Angeles. J Periodontol 2002;73:283–288. [PubMed: 11922257]

Socransky SS, Smith C, Martin L, Paster BJ, Dewhirst FE, Levin AE. "Checkerboard" DNA-DNA hybridization. Biotechniques 1994;17:788–792. [PubMed: 7833043]

Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. J Clin Periodontol 1998;25:134–144. [PubMed: 9495612]

Tanner A, Maiden MF, Macuch PJ, Murray LL, Kent RL Jr. Microbiota of health, gingivitis, and initial periodontitis. J Clin Periodontol 1998;25:85–98. [PubMed: 9495607]

Tanner AC, Kent R Jr, Van Dyke T, Sonis ST, Murray LA. Clinical and other risk indicators for early periodontitis in adults. J Periodontol 2005;76:573–581. [PubMed: 15857098]

Tran SD, Rudney JD. Improved multiplex PCR using conserved and species-specific 16S rRNA gene primers for simultaneous detection of *Actinobacillus actinomycetemcomitans*, *Bacteroides forsythus*, and *Porphyromonas gingivalis.* J Clin Microbiol 1999;37:3504–3508. [PubMed: 10523542]

Tran SD, Rudney JD, Sparks BS, Hodges JS. Persistent presence of *Bacteroides forsythus* as a risk factor for attachment loss in a population with low prevalence and severity of adult periodontitis. J Periodontol 2001;72:1–10. [PubMed: 11210065]

van der Reijden WA, Dellemijn-Kippuw N, Stijne-van Nes AM, de Soet JJ, van Winkelhoff AJ. Mutans streptococci in subgingival plaque of treated and untreated patients with periodontitis. J Clin Periodontol 2001;28:686–691. [PubMed: 11422591]

Van der Velden U, van Winkelhoff AJ, Abbas F, de Graaff J. The habitat of periodontopathic micro-organisms. J Clin Periodontol 1986;13:243–248. [PubMed: 3457811]

van Winkelhoff AJ, Loos BG, van der Reijden WA, Van der Velden U. *Porphyromonas gingivalis*, *Bacteroides forsythus* and other putative periodontal pathogens in subjects with and without periodontal destruction. J Clin Periodontol 2002;29:1023–1028. [PubMed: 12472995]

Vandamme P, Falsen E, Pot B, Hoste B, Kersters K, De Ley J. Identification of EF group 22 campylobacters from gastroenteritis cases as *Campylobacter concisus.* J Clin Microbiol 1989;27:1775–1781. [PubMed: 2768465]

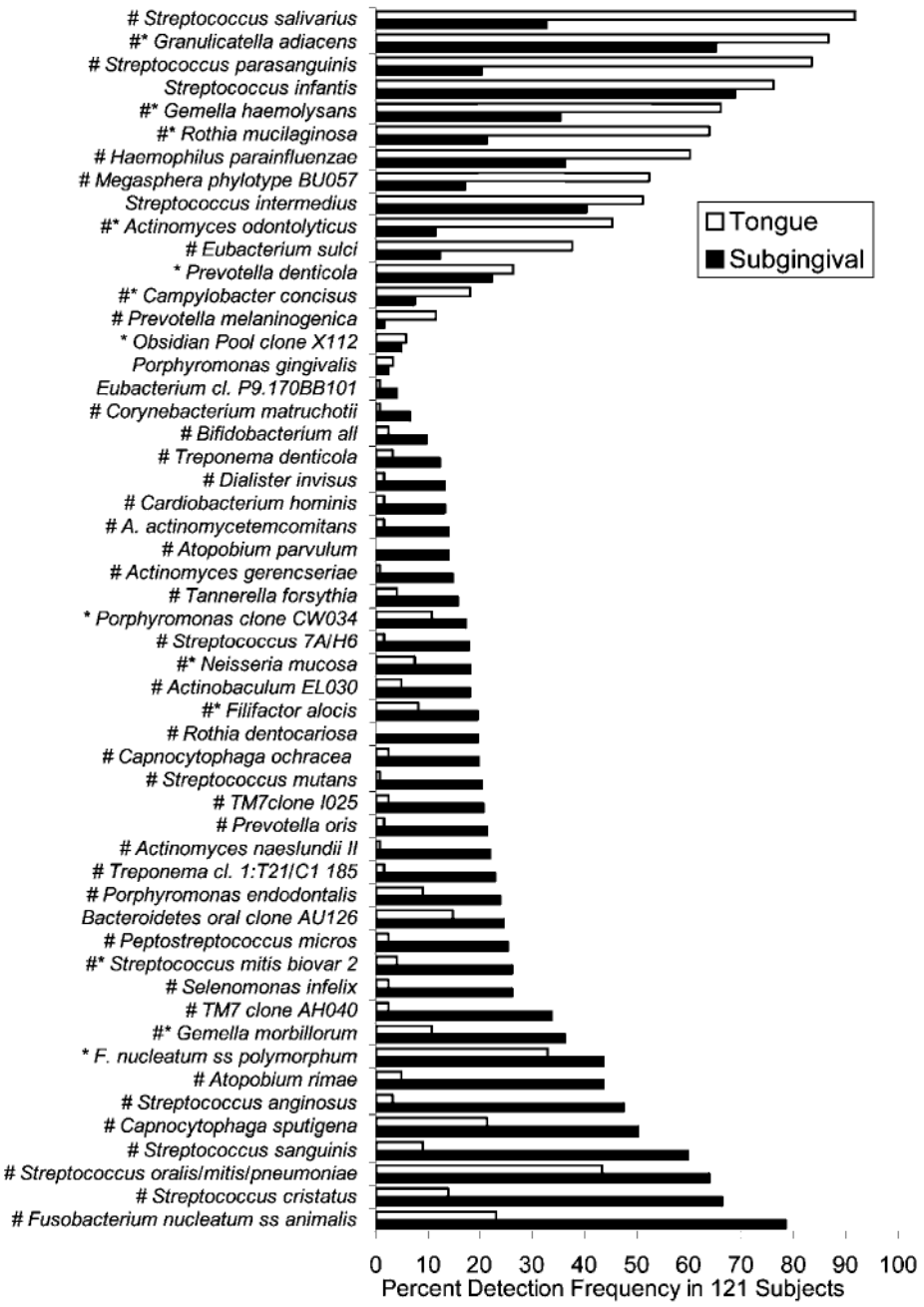

Figure 1.

Species detected by oligonucleotide DNA probes in reverse-capture checkerboard assay from paired tongue and subgingival samples of 121 healthy and early periodontitis individuals. Species detected more frequently from tongue samples are at the **top** of the Fig., and those detected more frequently subgingivally are at the **bottom** of the Fig. #Species prevalence differs, tongue *vs.* subgingival, McNemar's Chi-square $p < 0.05$. *Species-associated tongue and subgingival Chi-square for associations, $p < 0.05$.

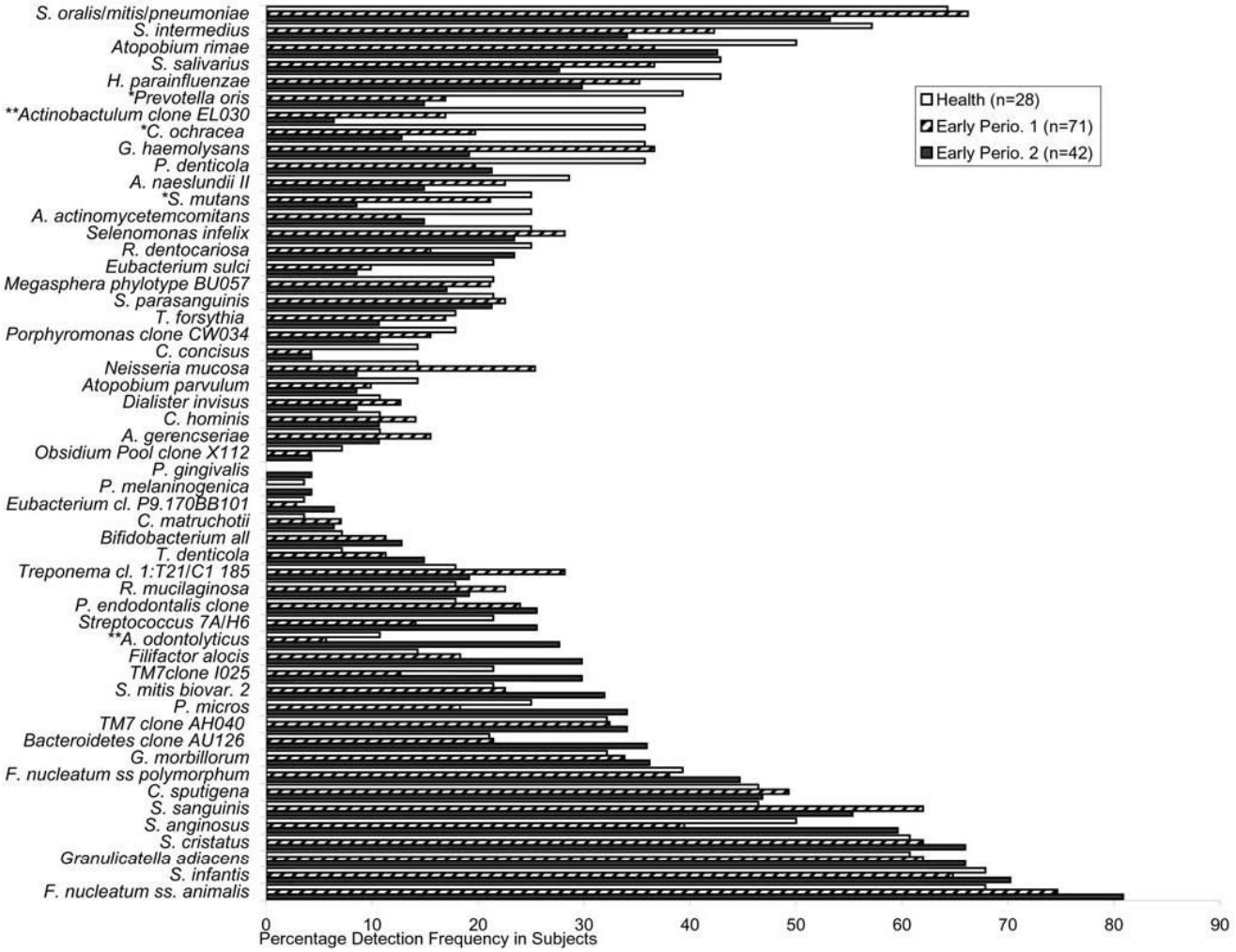

Figure 2.

Species detected by oligonucleotide DNA probes from pooled molar subgingival plaque samples from 28 healthy, 71 early periodontitis 1, and 42 early periodontitis 2 individuals. Health-associated species are at the **top** of the Fig., whereas species detected more frequently in early periodontitis 2 are at the **bottom** of the Fig. Mantel-Haenszel Chi-square for Trend: * $p \leq 0.05$, ** $p \leq 0.01$.

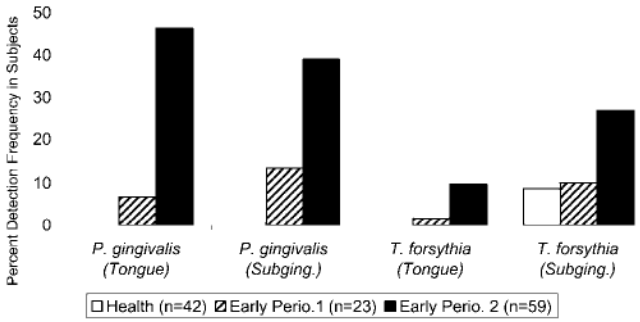

Figure 3.

Detection of *P. gingivalis* and *T. forsythia* in 124 paired tongue and subgingival samples of 23 healthy, 59 early periodontitis 1, and 42 early periodontitis 2 individuals, by means of the multiple PCR assay. There was no difference in *P. gingivalis* detection between tongue and subgingival samples, but *T. forsythia* was detected more frequently subgingivally ($p = 0.001$). *P. gingivalis* in tongue and subgingival samples ($p < 0.001$) and *T. forsythia* in subgingival samples ($p < 0.03$) were associated with early periodontitis.

Table

Clinical and Demographic Characteristics of the Study Population

	Health[a] (n = 28)	Early Periodontitis 1[b] (n = 71)	Early Periodontitis 2[c] (n = 42)	Significance (H, EP1, EP2)
Mean age (in yrs) ± SEM[d]	26.9 ± 1.03	30.5 ± 0.72	31.7 ± 1.04	p = 0.0051[e]
Gender, n (% male)	8 (29%)	33 (46%)	23 (55%)	p = 0.0367[f]
Never-smoker, n (%)	24 (86%)	46 (65)%	26 (62%)	
Smoking history:				
Former smoker, n (%)	2 (7%)	13 (18)%	7 (17%)	p = 0.0522[f]
Current smokers, n (%)	2 (7%)	12 (17)%	9 (21%)	(all groups)
Race/Ethnicity:				
Asian, n (%)	3 (23%)	7 (54%)	3 (23%)	
African-American, n (%)	0	11 (52%)	10 (48%)	
Hispanic, n (%)	2 (20%)	2 (20%)	6 (60%)	
Caucasian, n (%)	21 (23%)	48 (53%)	22 (24%)	p = 0.0409[g]
Other, n (%)	2 (33%)	3 (50%)	1 (17%)	(4 major groups)
Gingival Index (mean ± SEM)	0.50 ± 0.07	0.75 ± 0.05	1.30 ± 0.08	p < 0.0001[e]
Plaque Index (mean ± SEM)	0.51 ± 0.07	0.72 ± 0.06	0.93 ± 0.10	p < 0.0222[e]
Percent sites BOP* (mean ± SEM)	6 ± 1	13 ± 2	41 ± 4	p < 0.0001[e]
Pocket depth (mm, mean ± SEM)	1.98 ± 0.03	2.04 ± 0.02	2.53 ± 0.04	p < 0.0001[e]
Attachment level (AL) (mm, mean ± SEM)	1.14 ± 0.02	1.28 ± 0.02	1.70 ± 0.02	

[a] Health: mean AL ≤1.5 mm, no sites > 2 mm AL loss.

[b] Early periodontitis 1: mean AL ≤1.5 mm, ≥1 site more than 2 mm AL loss.

[c] Early periodontitis 2: mean AL > 1.5 mm.

[d] SEM = Standard Error of the Mean.

[e] Kruskal-Wallis test.

[f] Mantel-Haenszel Chi-square test.

[g] Chi-square.

* Bleeding on probing