

Comparison of the microbiota of supra- and subgingival plaque in health and periodontitis

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Abstract

Background, aims: The purpose of the present investigation was to compare the microbial composition of supra and subgingival plaque in 22 periodontally healthy (mean age 32 ± 16 years) and 23 adult periodontitis subjects (mean age 51 ± 14 years).

Methods: A total of 2358 supra and separately subgingival plaque samples were collected from the mesial aspect of all teeth excluding 3rd molars in each subject. Samples were examined for the presence and levels of 40 bacterial taxa using whole genomic DNA probes and checkerboard DNA-DNA hybridization. Clinical assessments including dichotomous measures of gingival redness, bleeding on probing, plaque accumulation and suppuration, as well as duplicate measures of pocket depth and attachment level, were made at 6 sites per tooth. Mean counts ($\times 10^5$), % DNA probe count and % sites colonized for each species were determined separately for supra and subgingival samples in each subject and then averaged across subjects in the 2 clinical groups. Significance of differences between healthy and periodontitis subjects was determined using the Mann-Whitney test and adjusted for multiple comparisons.

Results: Mean total DNA probe counts ($\times 10^5$, \pm SEM) for healthy and periodontitis subjects in supragingival plaque were 72.1 ± 11 and 132 ± 17.5 , respectively ($p < 0.01$), and in subgingival plaque 22.1 ± 6.6 and 100.3 ± 18.4 , ($p < 0.001$). *Porphyromonas gingivalis*, *Bacteroides forsythus* and *Treponema denticola* could be detected in supragingival plaque samples of both healthy and periodontitis subjects. *Actinomyces* species were the dominant taxa in both supra- and subgingival plaque from healthy and periodontitis subjects. 4 *Actinomyces* species accounted for 63.2% of supragingival and 47.2% of subgingival plaque in healthy subjects and 48.1% and 37.8% in periodontitis subjects respectively. Increased proportions of *P. gingivalis*, *B. forsythus*, and species of *Prevotella*, *Fusobacterium*, *Campylobacter* and *Treponema* were detected subgingivally in the periodontitis subjects. *P. gingivalis*, *B. forsythus* and *T. denticola* were significantly more prevalent in both supra- and subgingival plaque samples from periodontitis subjects.

Conclusions: The main differences between supra and subgingival plaque as well as between health and disease were in the proportions and to some extent levels of *Actinomyces*, “orange” and “red” complex species.

Key words: microbiology; periodontal health; periodontal disease; supragingival plaque; subgingival plaque; periodontal pathogens; bacteria; DNA probes

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The microbial composition of subgingival plaque at periodontally diseased sites has been extensively studied (for review, see Haffajee & Socransky (1994), Zambon (1996)). A series of cultural studies of subgingival plaque taken from subjects with different forms of periodontal

disease and health reported a shift in the subgingival microbiota as the periodontium progressed from health through gingivitis to periodontitis (Moore & Moore 1994). Species thought to be compatible with health included members of the genera *Actinomyces*, *Strepto-*

coccus and *Veillonella*. Gram-negative species as well as species of *Eubacterium*, *Peptostreptococcus* and *Lactobacillus* were frequently detected in higher proportions in diseased sites. An extensive investigation of the subgingival microbiota in healthy, well-maintained elder

Table 1. Mean baseline (\pm SD) clinical characteristics of the subject groups

	Health (n=22)	Periodontitis (n=23)
Age (years)***	32 \pm 16	51 \pm 14
Number of missing teeth*	1.0 \pm 1.6	2.5 \pm 2.3
% males	36	61
Mean pocket depth (mm)***	2.3 \pm 0.3	2.8 \pm 0.4
Mean attachment level (mm)***	1.7 \pm 0.3	2.7 \pm 0.8
% sites with:		
plaque	66 \pm 23	70 \pm 24
gingival erythema*	33 \pm 21	56 \pm 33
bleeding on probing*	15 \pm 10	29 \pm 19
suppuration*	0 \pm 0	0.5 \pm 1.2

Significantly different at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; Mann-Whitney test.

and periodontitis subjects was performed using DNA probes and the checkerboard technique (Haffajee et al. 1998). The results indicated that the levels of *P. gingivalis*, *B. forsythus*, *Treponema denticola* and *Selenomonas noxia* were significantly elevated in periodontitis subjects, while the prevalence and levels of the other 36 subgingival taxa evaluated did not differ significantly among the three subject groups. These studies and others (Dahlén et al. 1992, Dahlén et al. 1995, Tanner et al. 1996, Ali et al. 1997) indicated that similar species may be found in subgingival plaque samples taken from periodontally healthy and diseased subjects, although the proportions and levels of specific species differed quite markedly.

Periodontal pathogens can be detected in supragingival plaque and in healthy subjects. Gmür & Guggenheim

(1994) used monoclonal antibodies to detect specific species in supragingival plaque samples of 21 periodontally healthy trainee dental hygienists. They found that the prevalence of *A. actinomycetemcomitans*, *B. forsythus*, *Campylobacter rectus* and *Prevotella nigrescens* was 33%, 48%, 43% and 100% respectively. However, the detected cell numbers were generally $<1\%$ of the sampled microbiota. Other investigators have also detected *A. actinomycetemcomitans*, *B. forsythus* and *C. rectus* in supragingival plaque samples taken from periodontally healthy sites (Lai et al. 1987, 1992, Gmür et al. 1989, Asikainen et al. 1991, Zimmer et al. 1991). These data indicate that suspected periodontal pathogens occur at a subset of supra and subgingival sites and in healthy subjects, albeit at low numbers and in low proportions. How-

ever, existing data do not provide a clear picture of other species in the oral microbiota or the relationships among species at supra and subgingival sites of healthy and periodontitis subjects.

Knowledge of the extent and colonization patterns of host compatible and pathogenic species should help to define the ecologic shifts that occur when moving from health to periodontal disease. Further, studies of the composition of the supra and subgingival microbiota in periodontally healthy subjects should help to define the nature of a desired post-therapy microbiota. Thus, the purpose of the present study was to compare, using checkerboard DNA-DNA hybridization, the microbial composition of supra and subgingival plaque in periodontally healthy and periodontitis subjects.

Material and Methods

Subject population

22 periodontally healthy subjects (mean age 32 \pm 16 years) and 23 subjects with adult periodontitis (mean age 51 \pm 14 years), were selected for the study. Healthy subjects had at least 24 teeth and no sites with pocket depth or attachment level measurements greater than 4 mm. However, subjects in this group could exhibit gingival inflammation. The periodontitis subjects had ≥ 20 teeth and at least 4 sites with pocket depth >4 mm and/or 4 sites with

Table 2. Strains employed for the development of DNA probes

Actinomyces species		"Orange" complex		Other species and new DNA probes	
<i>Actinomyces gerencseriae</i>	23860	<i>Campylobacter gracilis</i>	33236	<i>Eubacterium sabureum</i>	33271
<i>Actinomyces israelii</i>	12102	<i>Campylobacter rectus</i>	33238	<i>Gemella morbillorum</i>	27824
<i>Actinomyces naeslundii</i> genospecies 1	12104	<i>Campylobacter showae</i>	51146	<i>Leptotrichia buccalis</i>	14201
<i>Actinomyces naeslundii</i> genospecies 2	43146	<i>Eubacterium nodatum</i>	33099	<i>Neisseria mucosa</i>	19696
"Purple" complex		<i>Fusobacterium nucleatum</i> ss <i>nucleatum</i>	25586	<i>Prevotella melaninogenica</i>	25845
<i>Actinomyces odontolyticus</i>	17929	<i>Fusobacterium nucleatum</i> ss <i>polymorphum</i>	10953	<i>Propionibacterium acnes</i> **	
<i>Veillonella parvula</i>	10790	<i>Fusobacterium nucleatum</i> ss <i>vincentii</i>	49256	<i>Selenomonas noxia</i>	43541
"Yellow" complex		<i>Fusobacterium periodonticum</i>	33693	<i>Streptococcus anginosus</i>	33397
<i>Streptococcus gordonii</i>	10558	<i>Peptostreptococcus micros</i>	33270	<i>Treponema socranskii</i>	S1
<i>Streptococcus intermedius</i>	27335	<i>Prevotella intermedia</i>	25611		
<i>Streptococcus mitis</i>	49456	<i>Prevotella nigrescens</i>	33563		
<i>Streptococcus oralis</i>	35037	<i>Streptococcus constellatus</i>	27823		
<i>Streptococcus sanguis</i>	10556	"Red" complex			
"Green" complex		<i>Bacteroides forsythus</i>	43037		
<i>Actinobacillus actinomycetemcomitans</i> *		<i>Porphyromonas gingivalis</i>	33277		
<i>Capnocytophaga gingivalis</i>	33624	<i>Treponema denticola</i>	B1		
<i>Capnocytophaga ochracea</i>	33596				
<i>Capnocytophaga sputigena</i>	33612				
<i>Eikenella corrodens</i>	23834				

All strains were obtained from the American Type Culture Collection (ATCC) except *Treponema denticola* B1 and *Treponema socranskii* S1 which were obtained from the Forsyth Institute. Microbial "complexes" were described by Socransky et al. (1998).

* ATCC strains 43718 and 29523; ** ATCC strains 11827 and 11828.

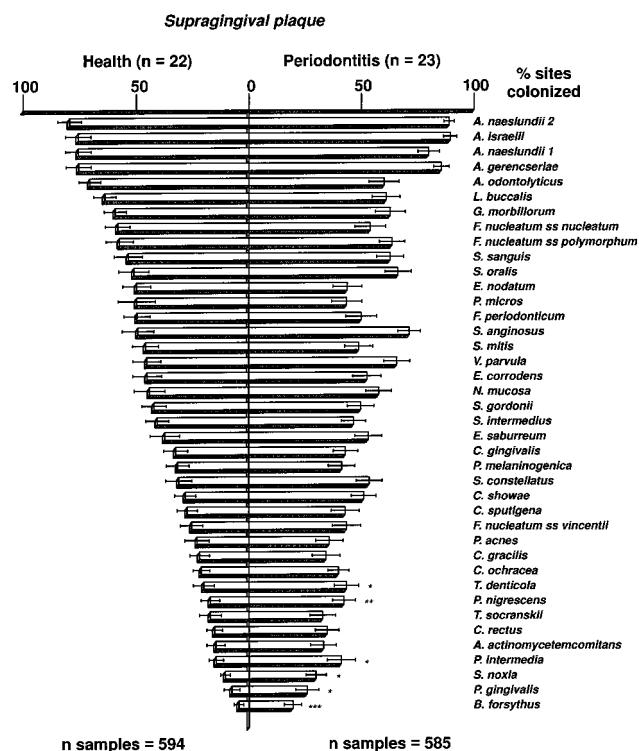


Fig. 1. Bi-lateral bar chart of the mean prevalence (% of sites colonized, \pm SEM) of individual species in supragingival plaque samples from 22 periodontally healthy and 23 periodontitis subjects. The prevalence was computed for each species in supragingival plaque samples in each subject in each clinical group and then averaged across subjects in the 2 groups. The data are ordered on the basis of the prevalence in the plaque samples of the healthy subjects. The significance of differences between periodontally healthy and periodontitis subjects was tested using the Mann Whitney test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ after adjusting for multiple comparisons.

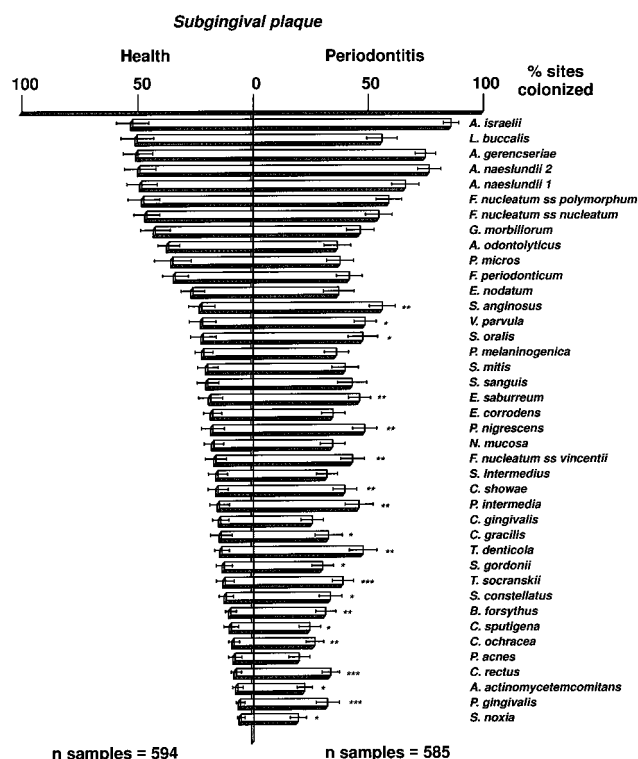


Fig. 2. Bi-lateral bar chart of the mean prevalence (% of sites colonized, \pm SEM) of individual species in subgingival plaque samples from 22 periodontally healthy and 23 periodontitis subjects. Methods of computation and data analysis were as described in Fig. 1.

attachment loss >4 mm. Exclusion criteria included pregnancy, lactation, periodontal or antibiotic therapy in the previous 3 months, any systemic condition which could influence the course of periodontal disease or which would require pre-medication for monitoring procedures. No subjects with localized juvenile periodontitis, rapidly progressive periodontitis or acute necrotizing ulcerative gingivitis were included in the study.

Clinical monitoring

Subjects were screened for suitability and, if accepted, signed informed consent. Clinical measurements were taken at 6 sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual) at all teeth excluding third molars (a maximum of 168 sites per subject) as previously described (Haffajee et al. 1983). Clinical assessment included plaque accumulation (0/

1), overt gingivitis (0/1), bleeding on probing (0/1), suppuration (0/1), probing pocket depth and probing attachment level. Pocket depth and attachment level measurements were taken twice by the same examiner and the average of the pair of measurements was used for analysis. Such measurements were recorded to the nearest millimeter using a North Carolina periodontal probe (Hu-Friedy, Chicago, IL). The baseline clinical features of the 45 subjects are presented in Table 1.

Microbial assessment of plaque samples

2358 samples of supra and subgingival plaque were collected from up to 28 supra and 28 subgingival sites from both the healthy and periodontitis subjects, and individually analyzed by checkerboard DNA-DNA hybridization (Socransky et al. 1994). After drying and isolation with cotton rolls, supragingival plaque was sampled from the me-

sio-buccal aspect of each tooth excluding third molars, using sterile Gracey curettes. Each plaque sample was placed in individual tubes containing 150 μ l of TE buffer (pH 7.6). After removal of the supragingival sample and any remaining supragingival plaque, subgingival plaque samples were taken from the same sites (i.e., the mesio-buccal aspect of each tooth) using sterile Gracey curettes and placed in similar individual tubes. 100 μ l of 0.5 M NaOH were added to each tube and the samples were dispersed in a vortex mixer. Samples were boiled for 10 min and neutralized using 800 μ l of 5 M ammonium acetate. The released DNA was then placed into the extended slots of a Minislot-30 apparatus (Immunetics, Cambridge MA), concentrated onto a 15 \times 15 cm positively-charged nylon membrane (Boehringer Mannheim, Indianapolis, IN) and fixed to the membrane by cross-linking under ultraviolet light followed by baking at

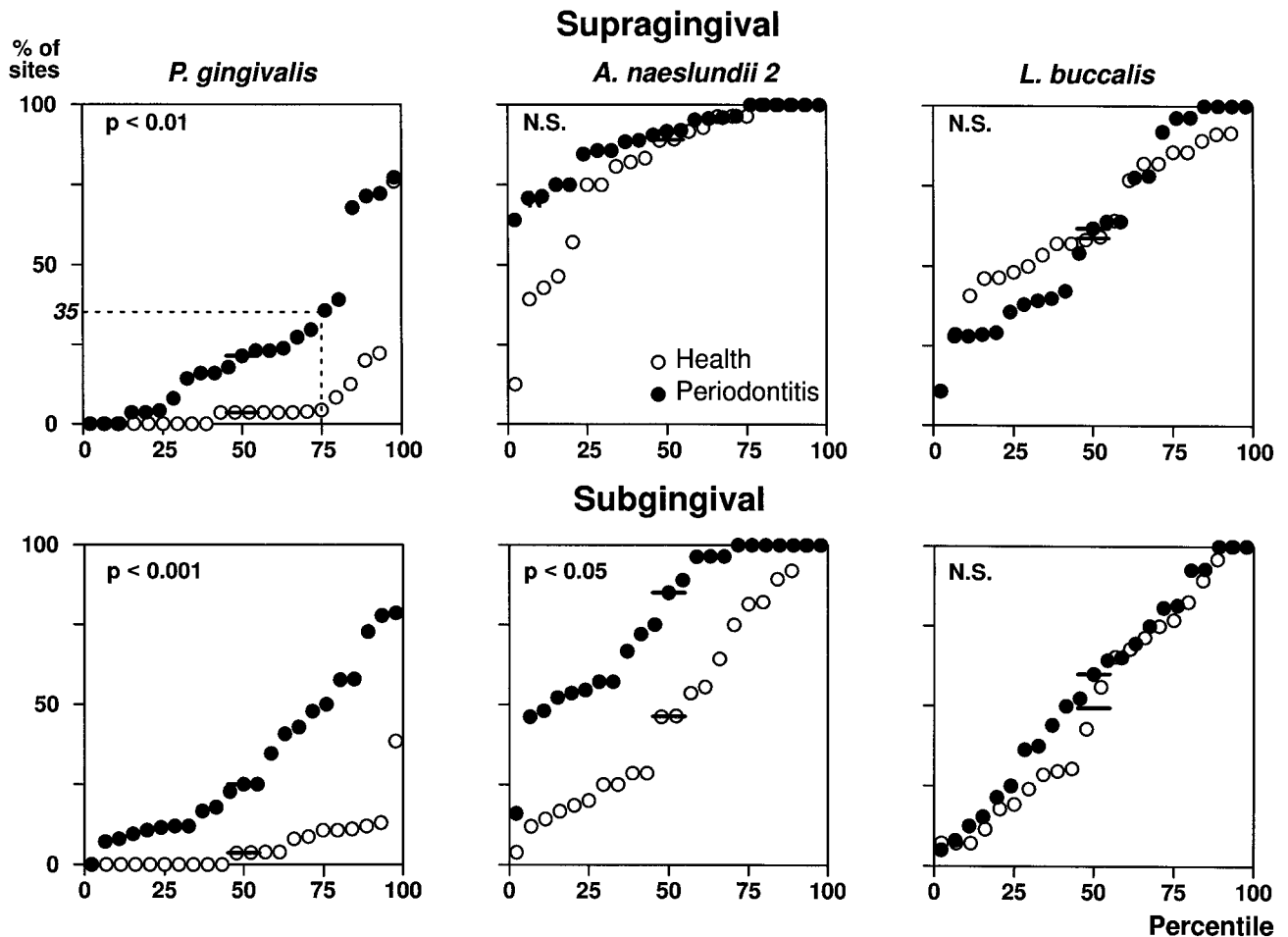


Fig. 3. Percentile plots of % of supra and subgingival sites colonized by *P. gingivalis*, *A. naeslundii* 2 and *L. buccalis* in periodontally healthy and periodontitis subjects. Each circle represents % of sites colonized in a subject by the indicated species. The top panels represent the supragingival plaque samples and the lower panels the subgingival samples. The y-axis represents % of sites colonized and the x-axis the percentiles. The pair of horizontal lines in each panel indicates the median values (50th percentile) for the diseased and healthy subjects. Significance of differences between distributions was tested using the Kolmogorov-Smirnov 2 sample test. The dotted line in the upper left panel indicates that 75% of periodontitis subjects have less than 35% of sites colonized by *P. gingivalis* in supragingival plaque. Conversely, 25% of periodontitis subjects exhibited >35% of sites colonized by this species.

120°C for 20 min. Two lanes on each membrane had standards that consisted of a mixture at 10^5 and 10^6 cells of each bacterial species tested.

A list of the 40 bacterial strains employed for the preparation of DNA probes is presented in Table 2. All strains were purchased as lyophilized stocks from the American Type Culture Collection (ATCC, Rockville, MD) (exceptions are noted in the Table). The preparation of DNA probes and evaluation of the plaque samples using checkerboard DNA-DNA hybridization were described in detail previously (Ximénez-Fyvie et al. 2000).

Data analysis

Microbiological data available for each subject were the counts of each of the

40 test species from up to 28 supragingival and, separately, up to 28 subgingival plaque samples per subject. The analyses compared microbial data expressed as counts $\times 10^5$ (levels), % DNA probe count (proportion) and % sites colonized (prevalence). In order to compare the counts of each of the bacterial species, the data were expressed as counts $\times 10^5$ at each site, averaged within a subject and then averaged across subjects for supra and subgingival counts separately. In a similar fashion, the % DNA probe count and prevalence of each species were computed at each site, averaged across sites within each subject and then across subjects. Analyses compared supragingival plaque composition in healthy and diseased subjects as well as comparisons of subgingival plaque composition in

the 2 clinical groups. Significance of differences between groups for each species were sought using the Mann-Whitney test. Adjustments were made for multiple comparisons as described by Socrasky et al. (1991).

Results Prevalence

Mean prevalence (% of sites colonized) of the 40 test species in supra and subgingival plaque samples from healthy and periodontitis subjects is summarized in Figs. 1, 2, respectively. Members of the genus *Actinomyces* and *Leptotrichia buccalis* were detected in a higher % of both supra and subgingival sites than any other species in both subject populations. 6 species were significantly more prevalent in supragingival plaque

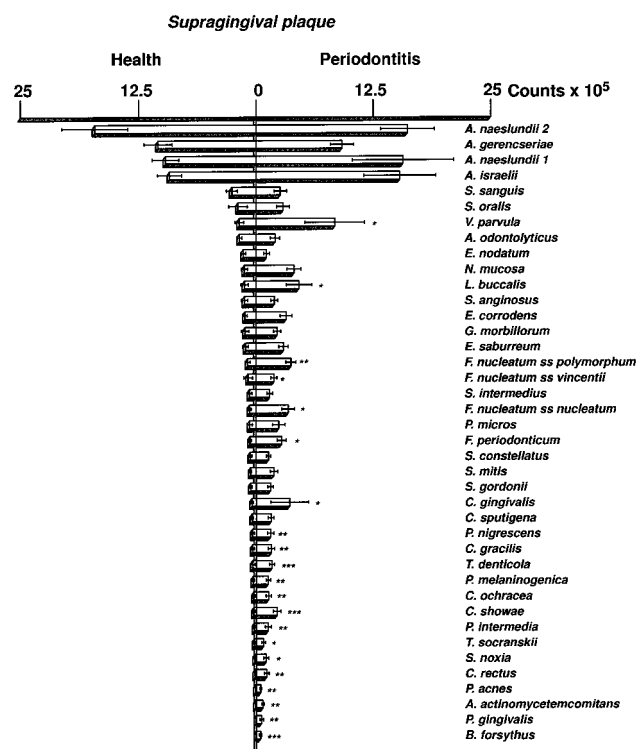


Fig. 4. Bi-lateral bar chart of the mean counts ($\times 10^5$, \pm SEM) of individual species in supragingival plaque samples from 22 periodontally healthy and 23 periodontitis subjects. The mean count was computed for each species in the supragingival plaque samples for each subject, and then averaged across subjects in the 2 clinical groups. The data are ordered on the basis of mean counts in the periodontally healthy subjects. The significance of differences between counts in periodontally healthy and periodontitis subjects was tested using the Mann Whitney test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ after adjusting for multiple comparisons.

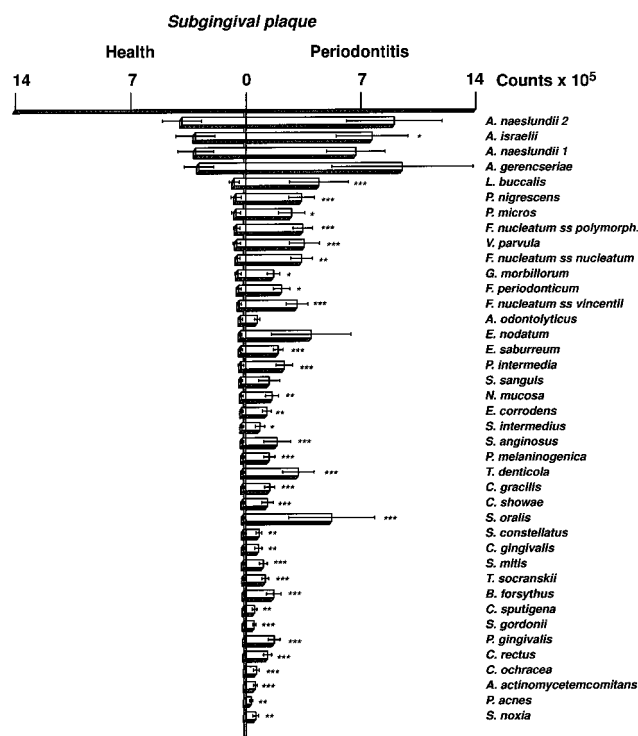


Fig. 5. Bi-lateral bar chart of the mean counts ($\times 10^5$, \pm SEM) of individual species in subgingival plaque samples from 22 periodontally healthy and 23 periodontitis subjects. Methods of computation and data analysis were as described in Fig. 4.

samples from periodontitis subjects than periodontally healthy subjects (Fig. 1). These included the “red” complex species, *B. forsythus*, *P. gingivalis* and *T. denticola* as well as *Prevotella intermedia*, *P. nigrescens* and *S. noxia*. The difference in prevalence of species between periodontally healthy and diseased subjects was more striking for the subgingival plaque samples (Fig. 2). 20 of 40 species were significantly more prevalent in periodontitis subjects than in subjects who were periodontally healthy even after adjusting for multiple comparisons. Many of these species were suspected periodontal pathogens including the “red” complex species, *A. actinomycetemcomitans* and *S. noxia*.

Figs. 1, 2 fail to show the difference in prevalence of individual species in different subjects. Fig. 3 presents 6 percentile plots that indicate the prevalence of *P. gingivalis*, *Actinomyces naeshlundii* genospecies 2 and *L. buccalis* in both supra and subgingival plaque samples

for each subject. Fig. 3 indicates that *P. gingivalis* was not detected in 41% and 45% of supra and subgingival plaque samples respectively of periodontally healthy subjects (Fig. 3, open circles, left panels). In contrast, 50% of periodontitis subjects harbored *P. gingivalis* at more than 20% of sites both supra and subgingivally (Fig. 3, black circles, left panels). The distributions for *P. gingivalis* were significantly different between health and disease. The other 2 “red” complex species, *B. forsythus* and *T. denticola* as well as other suspected periodontal pathogens showed similar distributions. *A. naeshlundii* genospecies 2 was far more prevalent than *P. gingivalis*, particularly in supragingival plaque. All periodontitis subjects exhibited this species in $>60\%$ sites with median values approaching 90%. This species was significantly more prevalent in the subgingival samples of periodontitis subjects than periodontally healthy subjects. The distributions of *L.*

buccalis were similar in both health and disease for the supra and subgingival plaque samples. More prevalent species, such as other members of the genus *Actinomyces* exhibited patterns of colonization similar to *A. naeshlundii* genospecies 2 or *L. buccalis*.

Mean counts

Mean total DNA probe counts ($\times 10^5$, \pm SEM) in supra and subgingival plaque samples were 72.1 ± 11.1 and 22.1 ± 6.6 for the 22 healthy subjects (n sites=594, n samples=1188) and 132.7 ± 17.5 and 100.3 ± 18.4 for the 23 periodontitis subjects (n sites=585, n samples=1170). Both supra and subgingival plaque samples from healthy subjects presented significantly lower total bacterial counts than from periodontitis subjects ($p < 0.01$ supragingival, $p < 0.001$ subgingival).

Mean counts of the 40 individual bacterial species in supra and subgingi-

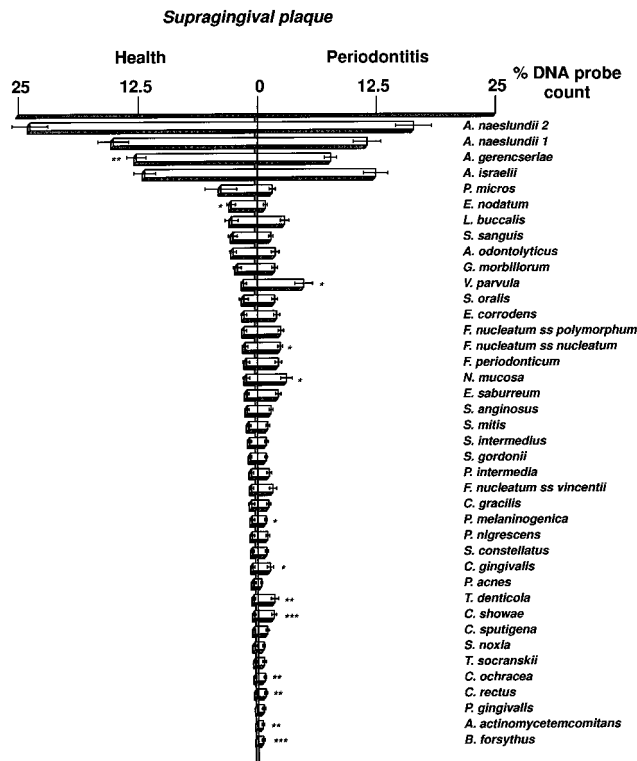


Fig. 6. Bi-lateral bar chart of the mean % DNA probe count (\pm SEM) comprised by each species in supragingival plaque samples from 22 periodontally healthy and 23 periodontitis subjects. The % DNA probe count was computed for each species in each supragingival plaque sample, averaged within a subject and then averaged across subjects in the 2 clinical groups. The data are ordered on the basis of the proportions in the periodontally healthy subjects. The significance of differences between periodontally healthy and periodontitis subjects was tested using the Mann Whitney test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ after adjusting for multiple comparisons.

val plaque samples taken from healthy and periodontitis subjects are summarized in Figs. 4, 5, respectively. The dominant species in supra and subgingival plaque samples from both healthy and periodontitis subjects were *Actinomyces naeslundii* genospecies 1, *A. naeslundii* genospecies 2, *Actinomyces gerencseriae* and *Actinomyces israelii*. A number of the species detected in significantly higher counts in supragingival samples from periodontitis subjects were known or putative periodontal pathogens, including *B. forsythus*, *P. gingivalis*, *T. denticola*, *A. actinomycetemcomitans*, *C. rectus*, *P. intermedia*, *P. nigrescens*, *S. noxia* and *Fusobacterium nucleatum* subspecies (Fig. 4). Differences in mean counts between samples of subgingival plaque from healthy and periodontitis subjects were much more dramatic. Periodontitis subjects had significantly higher counts of all of the test species in the subgingi-

val plaque samples compared with healthy subjects with the exception of *A. gerencseriae*, *A. naeslundii* genospecies 1 and 2, *Actinomyces odontolyticus*, *Eubacterium nodatum* and *Streptococcus sanguis* (Fig. 5). No species were detected in significantly higher counts in either supra or subgingival plaque samples from healthy subjects when compared with the corresponding samples from the periodontitis subjects.

Proportions

The proportions (mean % DNA probe count) of the 40 test species in supra and subgingival plaque samples from the healthy and periodontitis subjects are presented in Figs. 6, 7. *A. gerencseriae* and *E. nodatum* were in higher proportions in the supragingival plaque of periodontally healthy subjects than subjects with periodontitis. Eleven species were in higher proportions in the supra-

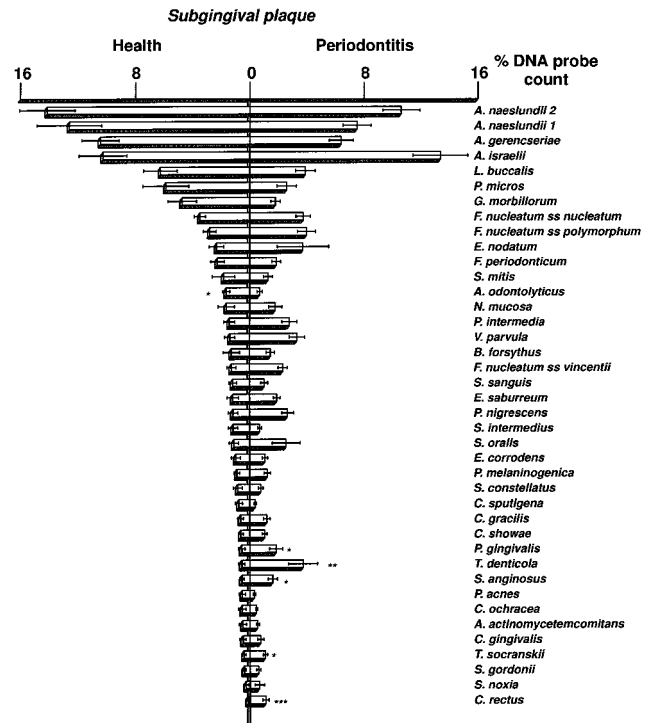


Fig. 7. Bi-lateral bar chart of the mean % DNA probe count (\pm SEM) comprised by each species in subgingival plaque samples from 22 periodontally healthy and 23 periodontitis subjects. Methods of computation and data analysis were as described in Fig. 6.

gingival plaque samples of the periodontitis subjects including *B. forsythus*, *A. actinomycetemcomitans* and *T. denticola*. In subgingival samples, proportions of *P. gingivalis*, *Streptococcus anginosus*, *C. rectus* and the treponemes, *T. denticola* and *Treponema socranskii* were significantly higher in periodontitis subjects, while only *Actinomyces odontolyticus* was significantly higher in periodontally healthy subjects.

Species were grouped according to the complexes described by Socransky et al. (1998) (Table 2). The proportions these groups comprised in supra and subgingival plaque samples from healthy and periodontitis subjects are presented in Fig. 8. The areas of the pies were adjusted to reflect the mean total counts at each of the 4 sample locations. As described previously, the highest mean total counts were for the supragingival plaque samples from the periodontitis subjects while the lowest

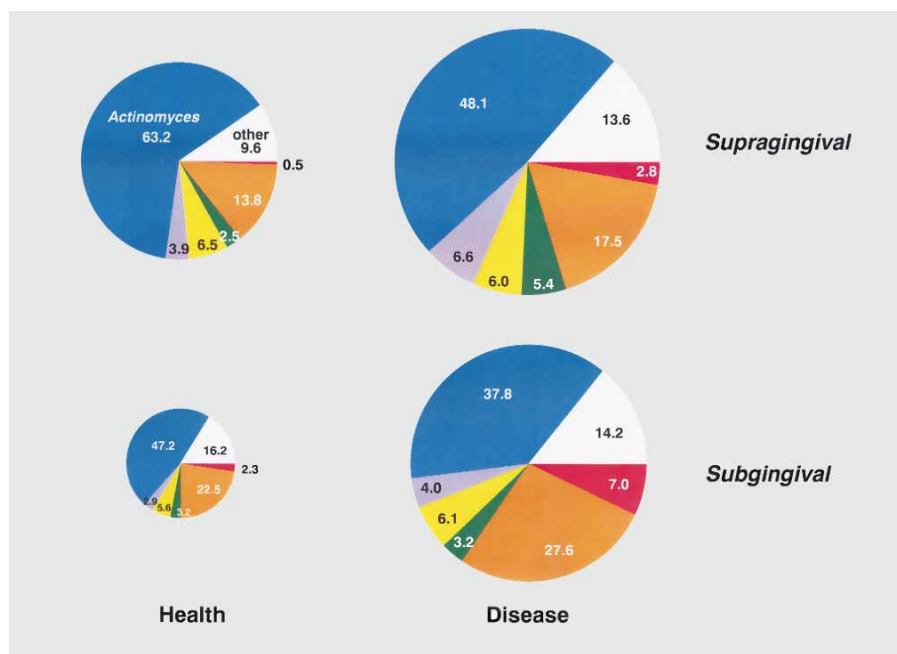


Fig. 8. Pie charts of the mean % DNA probe count of microbial groups in supra and subgingival plaque samples from 22 periodontally healthy and 23 periodontitis subjects. The species were grouped into 7 microbial groups based on the description of Socransky et al. (1998). The areas of the pies were adjusted to reflect the mean total counts at each of the sample locations. The significance of differences in mean percentages of the supra and subgingival complexes in health and disease was tested using the Kruskal Wallis test. The “red”, “orange” and *Actinomyces* species were significantly different at $p < 0.001$ and the “green” complex species differed at $p < 0.05$ after adjusting for 7 comparisons.

mean total counts were for the subgingival samples from the periodontally healthy individuals. *Actinomyces* species represented the highest proportion of organisms in both supra and subgingival plaque from healthy (63.2% supragingival, 47.2% subgingival) and periodontitis subjects (48.1% supragingival, 37.8% subgingival). The microbiota of healthy and diseased subjects differed primarily in that periodontitis subjects exhibited a higher proportion of “red” and “orange” complex species and a lower proportion of *Actinomyces* species in both supra and subgingival plaque than healthy subjects. The increase in proportions of “red” and “orange” complex species from either health to disease or supragingival to subgingival appeared to occur at the expense of the *Actinomyces* species. The relationship between “red” complex and *Actinomyces* species is demonstrated in Fig. 9. The mean ratio of *Actinomyces* species to “red” complex species decreased from 117:1 in supragingival plaque samples of healthy subjects to 5.4:1 in subgingival plaque samples from periodontitis subjects.

Discussion

The present investigation examined supra and subgingival plaque samples from periodontally healthy and periodontitis subjects in order to provide a more comprehensive picture of the microbial profiles in these different sample locations. This was made possible by the use of the checkerboard DNA-DNA hybridization technique which permitted the examination of 2358 plaque samples in 22 periodontally healthy and 23 periodontitis subjects for their content of 40 bacterial taxa. In addition, the recognition that bacterial species form distinct associations in dental plaque facilitated the orderly description of these ecosystems.

There was variability in the composition of the supra and subgingival plaque samples from site to site and subject to subject. However, on average, certain patterns of colonization could be discerned. All 40 taxa could be detected in some samples of supra and subgingival plaque obtained from both periodontally healthy and diseased subjects. However, there were differences in

mean levels, prevalence and proportion among the 4 sample locations (supra versus sub, health versus disease). The greatest numbers of organisms were detected in the supragingival plaque of the periodontitis subjects, while the lowest numbers were detected in the subgingival samples from the periodontally healthy subjects. *Actinomyces* species appeared to dominate all 4 sample locations, although proportions of these organisms were highest in supragingival samples from healthy subjects (63.2%) and lowest subgingivally in periodontitis subjects (37.8%). The decrease in the proportion of the *Actinomyces* species occurred as a result of an increase in the proportion of “red” and “orange” complex species. These 2 complexes were higher in subgingival than supragingival sites and higher in disease than health. Thus, the combined proportion of “red” and “orange” complex species was 14.3% in supragingival health, 20.3% in supragingival disease, 24.8% in subgingival health and 34.6% in subgingival disease.

The most striking difference between

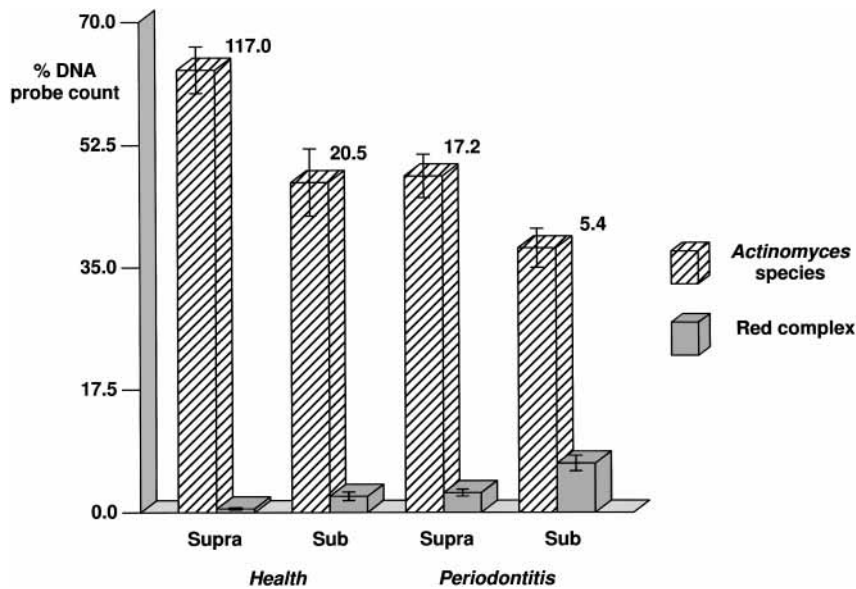


Fig. 9. Bar chart of the mean percent of the DNA probe count (\pm SEM) for the *Actinomyces* (*A. naeslundii* genospecies 1 and 2, *A. gerencseriae* and *A. israelii*) and "red" complex (*B. forsythus*, *P. gingivalis* and *T. denticola*) species in supra and subgingival plaque samples from 22 periodontally healthy and 23 periodontitis subjects. The numbers over the bars express the ratio of the *Actinomyces* species to the "red" complex species in each sample location.

periodontal health and disease was in the mean counts of the individual species. For example, in the supragingival samples, over half of the species were found at significantly higher mean counts in the periodontitis subjects compared with the healthy subjects. The difference between health and disease was even more pronounced for the subgingival plaque samples, where mean counts of 34 of the test species were significantly elevated in plaque samples from the periodontitis subjects. One might speculate that the reason for the differences in mean counts for the supragingival plaque samples was a higher level of inflammation and increased gingival crevice fluid flow in the periodontitis subjects. Further, spread of organisms from the subgingival to the supragingival environment of the periodontitis subjects might have contributed to the increased counts of species supragingivally in these subjects. The difference in mean counts of species subgingivally between health and disease was expected, since the subgingival environment in the 2 groups of subjects is quite different. The periodontitis subjects had deeper periodontal pockets which not only provided a larger area for the colonization of species, but a physically different habitat. Nonetheless, mean counts of 25 of 40 taxa were significantly higher

in the subgingival plaque samples of the periodontitis subjects, after adjusting for 40 comparisons, when only pockets ≤ 3 mm were compared in the 2 subjects groups (data not shown). This finding implies that pocket depth is not the sole determinant of differences in subgingival microbial composition between periodontally healthy and periodontitis subjects.

A previous publication compared the microbial composition of supra and subgingival plaque in periodontitis subjects (Ximénez-Fyvie et al. 2000). It was found that many suspected periodontal pathogens could be detected in supragingival plaque samples. The current investigation extends these findings by demonstrating that periodontal pathogens could be detected in both supra and subgingival plaque samples from periodontally healthy individuals. As pointed out earlier, detection of periodontal pathogens in supragingival plaque samples is not uncommon (Gmür & Guggenheim 1994). Periodontal pathogens have also been frequently detected in periodontally healthy subjects (Friskén et al. 1987, Lai et al. 1987, 1992, Mombelli et al. 1990, McNabb et al. 1992, Gmür & Guggenheim 1994, Ali et al. 1997). Detection of pathogenic species in healthy subjects prior to disease and in supragingival plaque samples has important

implications in the prevention and treatment of periodontal infections (Ximénez-Fyvie et al. 2000).

The data of the present investigation provided few surprises. The microbiota of all sample locations was, on average, dominated by the *Actinomyces* species, a greater proportion of "red" and "orange" complex species were observed in samples from diseased individuals and subgingival sites, periodontal pathogens were detected both in healthy subjects and in supragingival plaque samples and total counts of bacterial species were highest in supragingival samples from periodontitis subjects and lowest in subgingival plaque samples from periodontally healthy subjects. These and the detailed findings presented in the Figs. help to bring into focus the nature and composition of plaque samples from ecologically different intra-oral locations. As expected, certain members of the microbiota were common in bacterial plaque whether the samples were obtained from supra or subgingival sites in diseased or healthy subjects. This core group of organisms was typically built around the *Actinomyces*, but members of the genera *Streptococcus*, *Veillonella*, *Capnocytophaga*, *Eikenella*, *Leptotrichia* and *Neisseria* also appeared to play prominent roles. Members of the "orange" complex such as the *Fusobacterium*, *Prevotella* and *Campylobacter* species also appeared to be regular inhabitants of plaque particularly when gingival inflammation was present. Members of the "red" complex such as *B. forsythus*, *P. gingivalis* and *T. denticola* could be detected at a small proportion of sites at low levels in samples from supra and subgingival plaque in healthy subjects. These species were detected in higher levels and proportions in periodontally diseased subjects both above and below the gingival margin. It seems likely that periodontal pathogens colonize the supragingival plaque of periodontally healthy individuals for considerable periods of time prior to disease initiation. The development of gingival inflammation may foster proliferation of these organisms. Further, enlargement of the gingival tissues might lead to location of these species below the gingival margin, a habitat furthering their proliferation. The lateral wall of the periodontal pocket can provide a site of attachment and a source of nutrients. In addition, the organisms may benefit by entry into or between the epithelial

cells lining the periodontal pocket (Fives-Taylor et al. 1999, Holt et al. 1999). The improved environment may foster multiplication of these species which could account for the higher numbers of these organisms detected in disease.

The data obtained from the supra and subgingival plaque samples from the periodontally healthy subjects indicated that while there was a shift in the microbiota during disease, the shift was not so radical that "re-adjustment" back to health could not be reasonably accomplished in the majority of periodontitis subjects. Clearly, decreasing the proportion of "red" and "orange" complex species and decreasing the numbers of organisms in the subgingival space should be desired endpoints of infection control in periodontitis subjects (Fig. 8). The methods to achieve this goal are largely but not entirely in our hands.

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Zusammenfassung

Vergleich der Mikroflora der supra- und subgingivalen Plaque im gesunden Zustand und bei Parodontitis

Der Zweck der vorliegenden Untersuchung war es, die Zusammensetzung der supra- und subgingivalen Mikroflora von 22 parodontal gesunden (Altersdurchschnitt 32 ± 16 Jahre) mit der von 32 Patienten mit Erwachsenenparodontitis (Altersdurchschnitt 51 ± 14 Jahre) zu vergleichen. Die Insgesamt 2358 Plaqueproben wurden bei jeder Person an allen Zähnen (außer die 3. Molaren) von der Mesialfläche getrennt supra- und subgingivale entnommen. Die Proben wurden hinsichtlich des Vorkommens und der Anzahl von 40 bakteriellen Taxa mit einer Ganz-Genomischen-DNA-Sonde und der Schachbrett-DNA-DNA-Hybridisierung untersucht. An 6 Stellen pro Zahn wurden klinische Befunde einschließlich dichotomer Messung der Gingivarötung, der Sondierungsblutung, der Plaqueakkumulation und Suppuration als auch Zweifachmessung von Taschentiefe und Attachmentniveau durchgeführt. Für jede Person wurde für jede Spezies die mittlere Anzahl ($\times 10^5$), der Prozentsatz mittels DNA-Sondenbestimmung und der Prozentsatz der kolonisierten Taschen getrennt nach supra- und subgingival bestimmt. Die wurden anschließend für die 2 klinischen Gruppen Durchschnittswerte gebildet. Mit dem Mann-

Whitney-Test der für Mehrfachvergleiche adjustiert war wurde die Signifikanz der Unterschiede zwischen gesunden und kranken Personen bestimmt. Die durchschnittliche Gesamtbakterienzahl ($\times 10^5$, \pm SEM) betrug für die supragingivale Plaque 72.1 ± 11 für Gesunde und 132 ± 17.5 für Kranke ($p < 0.01$) und entsprechend für die subgingivale Plaque 22.1 ± 6.6 bzw. 100.3 ± 18.4 ($p < 0.001$). Sowohl bei Gesunden als auch bei Parodontitispatienten konnte in den supragingivalen Plaqueproben *Porphyromonas gingivalis*, *Bacteroides forsythus* und *Treponema denticola* nachgewiesen werden. Sowohl bei den Gesunden als auch bei den Kranken waren in der supra- und der subgingivalen Plaque die *Actinomyces* Spezies die dominierende Taxa. Vier *Actinomyces* Spezies stellten bei den Gesunden 63.2% der supragingivalen und 47.2% der subgingivalen Plaque. Bei den Parodontitispatienten waren die entsprechenden Prozentsätze 48.1% in der supra- und 37.8% in der subgingivale Plaque. Subgingival wurden bei den Parodontitispatienten erhöhte Anteile an *P. gingivalis*, *B. forsythus* und Spezies von *Prevotella fusobacterium*, *Campylobacter*, *Treponema* nachgewiesen. Bei den Parodontitispatienten zeigten sowohl in den supra- als auch subgingivalen Plaqueproben *P. gingivalis*, *B. forsythus* und *T. denticola* eine signifikant höhere Prävalenz. Die Hauptunterschiede zwischen supra- und subgingivalen Plaqueproben sowie Gesunden und Parodontitispatienten lagen in den Proportionen und zu einem gewissen Maß der Menge von *Actinomyces*- "orange"- und "rot"-Komplex-Spezies.

Résumé

Comparaison de la flore de la plaque dentaire sus- et sous-gingivale en présence de parodontite sain et de parodontite

Le but de l'étude présente a été de comparer la composition microbienne de la plaque dentaire sus- et sous-gingivale chez 22 patients sains âgés de 32 ± 16 ans et 32 adultes avec parodontite âgés de 51 ± 14 ans. Un total de 2358 échantillons séparés de plaque dentaire sus- et sous-gingivale ont été prélevés de la partie mésiale de toutes les dents sauf des dents de sagesse. Les échantillons ont été examinés pour la présence et les teneurs en 40 espèces de bactéries en utilisant les sondes ADN génomiques totales et l'hybridisation ADN-ADN en damier. Les examens cliniques comprenaient les mesures dichotomiques de la rougeur gingivale, du saignement au sondage, de l'accumulation de la plaque dentaire et de la présence de suppuration ainsi que des mesures dupliquées de la profondeur de poche et des niveaux d'attache sur 6 sites par dent. Les comptages moyens $\times 10^5$, le comptage de la sonde ADN et le % de sites colonisés par chaque espèce ont été déterminés séparément pour les échantillons de plaque dentaire sus- et sous-gingivale et mis en moyenne pour tous les sujets dans les deux groupes cliniques. Des différences significatives entre les sujets sains et avec parodontite

ont été déterminées en utilisant le test de Mann-Whitney et ajustées pour les comparaisons multiple. Les comptages par sonde ADN totaux moyens $\times 10^5 \pm$ EC pour les sujets sains et avec parodontite dans la plaque sus-gingivale étaient respectivement de 72.1 ± 11 et 132 ± 18 ($p < 0.001$) et pour la plaque sous-gingivale de 22.1 ± 7 , 100 ± 18 ($p < 0.001$). Le *porphyromonas gingivalis*, le *Bacteroides forsythus* et la *Treponema denticola* pouvaient être détectés dans les échantillons de plaque sus-gingivale des sujets sains et avec parodontite. Les espèces *Actinomyces* étaient les plus dominantes dans la plaque sus- et sous-gingivale tant chez les sujets sains que les sujets avec parodontite. 4 espèces *Actinomyces* apportaient 63% des échantillons de la plaque sus-gingivale et 47% de ceux de la sous-gingivale chez les sujets sains, et 48 et 38% chez ceux avec parodontite. Des proportions augmentées de *P. gingivalis*, *B. forsythus* et des espèces de *Prevotella*, *Fusobacterium*, *Campylobacter* et *Treponema* ont été identifiées en sous-gingival chez les sujets avec parodontite. *P. gingivalis*, *B. forsythus* et *T. denticola* avaient une fréquence globale significativement plus importante tant dans les échantillons de plaque sus- que sous-gingivale chez les patients avec parodontite. Les différences les plus importantes entre la plaque sus- et sous-gingivale ainsi qu'entre les sujets sains et malades étaient dans les proportions et à un certain point dans les teneurs en *Actinomyces* et dans les espèces complexes "orange" et "rouge".

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