Journal of Clinical
Periodontology
ISSN 0303-6979

Distribution of selected bacterial species on intraoral surfaces

Donna L. Mager,
Laurie Ann Ximenez-Fyvie,
Anne D. Haffajee and
Sigmund S. Socransky
Department of Periodontology, The Forsyth Institute, Boston, MA, USA

Abstract

Background/aim: To examine the proportions of 40 bacterial species in samples from 8 oral soft tissue surfaces and saliva in systemically healthy adult subjects and to compare these microbiotas with those of supra- and subgingival plaque.

Methods: Microbial samples were taken from 8 oral soft tissue surfaces of 225 systemically healthy subjects using a ''buccal brush''. Saliva was taken by expectoration. Forty-four of these subjects provided additional supra- and subgingival plaque samples. Samples were individually evaluated for their content of 40 bacterial species using checkerboard DNA–DNA hybridization. The percentage of total DNA probe count was determined for each species, at each sample location and averaged across subjects. The significance of differences among the proportions of the 40 test species at different sample locations was sought in the 225 and 44 subjects separately using the Quade test and adjusted for multiple comparisons. Cluster analysis was performed using the proportions of the 40 species at the different sample locations using the minimum similarity coefficient and an average unweighted linkage sort. The proportions of each species were averaged across subjects in the resulting cluster groups and the significance of differences was tested using the *t*-test and ANOVA.

Results: Microbial profiles differed markedly among sample locations in the 225 subjects, with 34 of 40 species differing significantly. Proportions of *Veillonella parvula* and *Prevotella melaninogenica* were higher in saliva and on the lateral and dorsal surfaces of the tongue, while *Streptococcus mitis* and *S. oralis* were in significantly lower proportions in saliva and on the tongue dorsum. Cluster analysis resulted in the formation of 2 clusters with >85% similarity. Cluster 1 comprised saliva, lateral and dorsal tongue surfaces, while Cluster 2 comprised the remaining soft tissue locations. *V. parvula, P. melaninogenica, Eikenella corrodens, Neisseria mucosa, Actinomyces odontolyticus, Fusobacterium periodonticum, F. nucleatum* ss *vincentii* and *Porphyromonas gingivalis* were in significantly higher proportions in Cluster 1 and *S. mitis, S. oralis* and *S. noxia* were significantly higher in Cluster 2. These findings were confirmed using data from the 44 subjects providing plaque samples. The microbial profiles of supra- and subgingival plaque differed from the other sample locations, particularly in the increased proportions of the *Actinomyces* species. Species of different genera exhibited different proportions on the various intraoral surfaces, but even within the genus *Streptococcus*, there were differences in colonization patterns. *S. oralis, S. mitis* and *S. constellatus* colonized the soft tissues and saliva in higher proportions than the samples from the teeth, while the other 4 streptococcal species examined colonized the dental surfaces in proportions comparable to the soft tissue locations and saliva.

Conclusions: Proportions of bacterial species differed markedly on different intraoral surfaces. The microbiota of saliva was most similar to that of the dorsal and lateral surfaces of the tongue. The microbiotas of the soft tissues resembled each other more than the microbiotas that colonized the teeth both above and below the gingival margin.

The mean surface area of the adult oral cavity is approximately 215 cm² (Collins & Dawes 1987). The teeth, keratinized and nonkeratinized soft tissues comprise about 20%, 30% and 50% of this surface area respectively. While a great deal is known about the microbial composition of hard tissue biofilms, surprisingly little is known about the microbiotas that colonize approximately 80% of the surface area of the oral cavity. Most

studies that examined the microbiota on oral mucous membranes have employed relatively time-consuming cultural techniques. For this reason, the composition of the oral soft tissue microbiota is less well understood than that of dental plaque, where more studies, subjects, samples and species have been examined often by means of more rapid microbiological techniques.

Studies that have sought the presence of specific bacterial species on different oral soft tissue surfaces have been motivated, primarily, because of possible associations of soft tissue residents with various clinical conditions including oral malodor, dental caries and periodontal diseases. A number of studies have shown that soft tissue surfaces harbor periodontal pathogens (van Winkelhoff et al. 1986, Dahlen et al. 1992, Loos et al. 1992, Bosy et al. 1994, De Boever & Loesche 1995, Muller et al. 1995, 1997, Quirynen et al. 1995, 1998, Matto et al. 1996a,b, 1998, Lee et al. 1999). For example, van Winkelhoff et al. (1986) characterized the microbiotas of the tongue and tonsils using culture and phase contrast microscopy. They showed that spirochetes, motile organisms and black-pigmented species such as *Prevotella intermedia* colonized the tongue. Further, there was an association between periodontal breakdown and the presence of these organisms on the tongue, suggesting that the tongue could serve as a significant ecological habitat for periodontal pathogens. Dahlen et al. (1992) examined the tongue of periodontally diseased and nondiseased young adult Kenyan subjects for the presence of 7 putative periodontal pathogens. All test species including *Porphyromonas gingivalis, P. intermedia, Campylobacter rectus* and *Actinobacillus actinomycetemcomitans* could be detected in samples from both groups of subjects, although *P. gingivalis* was detected significantly more frequently in the tongue samples from the periodontally diseased subjects. In another study of *P. gingivalis*, Loos et al. (1992) analyzed saliva and microbial samples from the tongue dorsum, tonsil and attached gingiva of 8 adult subjects with untreated periodontitis and 1 patient with an untreated infected root canal. Their data indicated that *P. gingivalis* could colonize multiple ecological sites and that subjects could be infected with more than one

clonal type. Danser et al. (1996) studied the effect of SRP and periodontal surgery on the levels of *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedia* on the oral mucous membranes using indirect immunofluorescence. Improvement in clinical parameters after treatment was accompanied by a significant decrease in the subgingival prevalence of the 3 putative periodontal pathogens, but essentially no reduction in the prevalence of the test bacteria on the oral mucous membranes. The studies of Muller et al. (1995, 1997) and Asikainen et al. (1991) demonstrated that *A. actinomycetemcomitans* could be detected on the tongue dorsum, buccal mucosa and in saliva. Although no difference in the detection rate of *A. actinomycetemcomitans* was found between periodontally healthy and diseased subjects, *A. actinomycetemcomitans* was recovered more often on soft tissues and in saliva than in plaque samples (Asikainen et al. 1991).

Studies of oral malodor have contributed to our understanding of microbial colonization of oral soft tissues, particularly the dorsum of the tongue. These studies indicated that a wide range of species including *Treponema denticola, P. gingivalis, Bacteroides forsythus, Prevotella melaninogenica, P. intermedia, Fusobacterium, Streptococcus, Lactobacillus, Rothia, Capnocytophaga*, and *Actinomyces* spp. could be found in samples taken from the tongue (Bosy et al. 1994, De Boever & Loesche 1995). Further, sulfate-reducing bacteria, which may contribute to oral malodor, were found in samples taken from the tongue as well as the hard palate and vestibulum (Langendijk et al. 1999).

The above studies suggest that soft tissues may serve as reservoirs for infection or reinfection of the periodontium and may deserve therapeutic attention (Quirynen et al. 2001). However, to date there have been few investigations of a broad range of bacterial species in multiple hard and soft tissue samples taken from the same subjects. Thus, the purpose of the present investigation was to examine the proportions of 40 bacterial species in samples from 8 oral soft tissue surfaces and saliva in systemically healthy adult subjects. In addition, the microbiotas from the soft tissue surfaces were compared with samples of supra- and subgingival plaque.

Material and Methods

Subject population

Two hundred and twenty-five systemically healthy subjects > 18 years of age with at least 20 teeth were selected for the study. Periodontally healthy subjects had no pocket depth or attachment level measurements > 3 mm, but could have gingival inflammation. Periodontally diseased subjects had at least 4 sites with pocket depths > 4 mm and 4 sites with attachment level measurements > 4 mm. Subjects were excluded if they had received antibiotic therapy within the previous 3 months, had any systemic condition that could affect the host's periodontal status (e.g. diabetes, AIDS), had any condition that would require antibiotics for monitoring or treatment procedures (e.g. heart conditions, joint replacements), or had mucosal lesions, previous chemotherapy, radiation therapy or medications that cause xerostomia. Subjects were chosen from the patient pool at The Forsyth Institute. The purpose and nature of the study including the types of clinical measurements and sample collection were explained to all potential subjects. After reading and signing the consent form, the subjects were entered into the study.

Clinical monitoring

After initial screening for suitability and obtaining informed consent, subjects were clinically and microbiologically monitored. 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) at a baseline visit (Haffajee et al. 1983). The clinical parameters measured were gingival redness (0 or 1), pocket depth (mm), bleeding on probing (0 or 1), suppuration (0 or 1), attachment level (mm) and plaque accumulation (0 or 1). Saliva, oral mucosa and supra- and subgingival plaque samples for microbiological assessment were taken prior to the clinical measurements. The mean baseline clinical parameters for the 225 systemically healthy subjects are shown in Table 1. Two hundred and twenty-five subjects provided soft tissue samples and 44 of these subjects also provided samples of supra- and subgingival plaque taken separately from each tooth.

Table 1. Mean (±SD) baseline clinical characteristics of the 225 systemically healthy subjects and the subset of 44 subjects providing plaque samples

	Soft tissue samples only	Soft and hard tissue samples
N	225	44
age (years)	40±16	43±16
number of missing teeth	2.0±2.3	1.9±2.2
% males	42	41
mean pocket depth (mm)	2.7±0.7	2.7±0.5
mean attachment level (mm)	2.6±1.1	2.6±1.0
% of sites with		
plaque accumulation	65±43	87±61
gingival redness	53±31	62±38
bleeding on probing	23±21	29±22

Collection of samples

Subjects expectorated whole saliva into sterile Eppendorf microcentrifuge tubes. A 0.2 ml sample of whole saliva was vortexed with 0.15 ml sterile, filtered Tris EDTA buffer (TE; 10 mM Tris-HCl, 1 mM EDTA, pH 7.6). A 0.2 ml sample of this mixture was taken and 0.1 ml of 0.5 M NaOH added. Subsequently, microbial samples of 8 soft tissue sites were taken from each subject. Each mucosal sample was collected using a MasterAmp™ buccal swab brush (Epicentre Technologies, Madison, WI, USA). The soft tissue samples were obtained by gently stroking each site in an area large enough to yield sufficient numbers of microorganisms to examine. The size of the area and length of sampling time were location dependent. Soft tissue sites examined were 3 areas of the tongue, dorsum, lateral, and ventral surfaces, the floor of the mouth, buccal mucosa, hard palate, anterior vestibule and mucosa of the maxillary and mandibular lips, and the maxillary anterior attached gingiva. Sampling areas and times were as follows: $1 \, cm^2$ of the center of the dorsum tongue for 5 s, full length of both sides of the lateral tongue for 5 s each, and the entire ventral tongue for 10 s. The right and left sides of the floor of the mouth required 10 s each. The right and left buccal mucosas required sampling the entire area of both sides for 10 s each, without touching the teeth. The entire hard palate was sampled for 10 s. The vestibule/lip was sampled 10 s each for the maxillary and mandibular areas. Finally, the attached gingiva of the maxillary anterior was sampled for 10 s. Soft tissue samples were placed into individual tubes containing 150 µl of TE buffer to which 100 µl of 0.5 M NaOH was added.

Samples of supra- and subgingival plaque were obtained from up to 28 teeth in each of the subset of 44 subjects. After drying and isolation with cotton rolls, supragingival plaque was sampled from the mesiobuccal aspect of each tooth using sterile Gracey curettes. Each plaque sample was placed in individual tubes containing 150 µl of TE buffer. After removal of the supragingival sample and any remaining supragingival plaque, subgingival plaque samples were taken from the same sites (i.e., the mesiobuccal aspect of each tooth) using sterile Gracey curettes and placed in similar individual tubes also containing 150 µl of TE buffer. All samples from all subjects were individually analyzed for their content of 40 bacterial species using the checkerboard DNA–DNA hybridization technique. The 40 bacterial species studied are listed in Fig. 1.

Microbiological assessment

Samples were evaluated using a modification (Haffajee et al. 1997) of the checkerboard DNA–DNA hybridization technique (Socransky et al. 1994). The samples were lysed and the DNA placed in lanes on a nylon membrane using a Minislot device (Immunetics, Cambridge, MA, USA). After fixation of the DNA to the membrane, the membrane was placed in a Miniblotter 45 (Immunetics) with the lanes of DNA at 90° to the lanes of the device. Digoxigenin-labeled whole genomic DNA probes to 40 bacterial species were hybridized in individual lanes of the Miniblotter. After hybridization, the membranes were washed at high stringency and the DNA probes detected using antibody to digoxigenin conjugated with alkaline phosphatase and

chemifluorescence detection. Signals were detected using AttoPhos substrate (Amersham Life Science, Arlington Heights, IL, USA) and were read using a Storm Fluorimager (Molecular Dynamics, Sunnyvale, CA, USA), a computer-linked instrument that read the intensity of the fluorescent signals resulting from the probe–target hybridization. Two lanes in each run contained standards at concentrations of 10^5 and 10^6 cells of each species. The sensitivity of the assay was adjusted to permit the detection of 10^4 cells of a given species by adjusting the concentration of each DNA probe. Signals evaluated using the Storm Fluorimager were converted to absolute counts by comparison with the standards on the same membrane. Failure to detect a signal was recorded as zero.

Data analysis

Data available for all subjects were the counts of 40 bacterial species in saliva and from 8 different oral mucous membrane locations. In addition, samples of supragingival and separately subgingival plaque from all teeth, excluding third molars, were available from 44 subjects. For the plaque samples, the total DNA probe count for each species was computed at each sampled site in each subject and averaged to provide a single supra- and subgingival value for each species for each subject. The proportion (percentage of the DNA probe count) that each species comprised of the total DNA probe count at each sample location was computed. The significance of differences among the proportions of the 40 test species in saliva and at the 8 mucous membrane locations in the 225 subjects were sought using the Quade test and adjusted for multiple comparisons (Socransky et al. 1991). A similar analysis was performed comparing saliva, supra- and subgingival plaque and the 8 oral soft tissue locations in samples from the 44 subjects who provided plaque samples.

Cluster analysis was performed on the proportions of the 40 species at the different sample locations in the 225 subjects. Similarities were computed using the minimum similarity coefficient (Socransky et al. 1982) and sorted using an average unweighted linkage sort (Sneath & Sokal 1973). The proportions of each species were averaged across subjects in the different
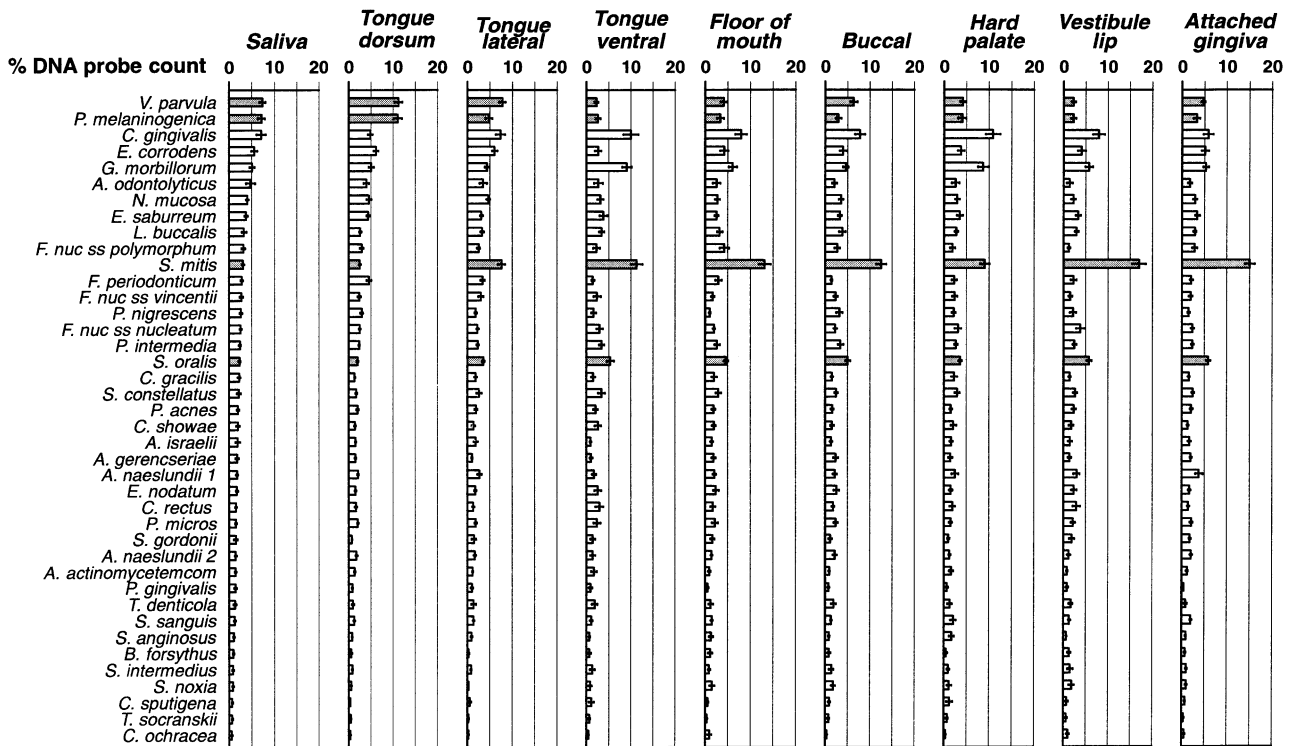
Fig. 1. Mean percentage DNA probe count ($\pm$ SEM) for samples from the 8 oral soft tissue locations and saliva taken from 225 systemically healthy adult subjects. Species are ordered according to their mean proportions in saliva. Significance of differences among sample locations was determined using the Quade test and adjusted for multiple comparisons (Socransky et al. 1991). The shaded bars represent species that showed particularly marked differences among sample locations.

cluster groups and the significance of differences was tested using the *t*-test and ANOVA. The analysis was repeated using the data from the 44 subjects who provided plaque samples.

Results
Comparison of soft tissue samples and saliva

Fig. 1 presents the mean percentage of the DNA probe count of samples from the 8 intraoral mucous membrane locations and saliva in the 225 subjects. The microbial profiles differed markedly among sample locations, with 34 of 40 species differing significantly among sample locations even after adjusting for 40 comparisons. In particular, proportions of *Veillonella parvula* and *P. melaninogenica* were higher in saliva and on the lateral and dorsal surfaces of the tongue, while *Streptococcus mitis* and *S. oralis* were in significantly lower proportions in saliva and on the tongue dorsum compared with the other sampled sites.

Cluster analysis was performed to seek similarities in microbial profiles among the 9 sample locations (Fig. 2).
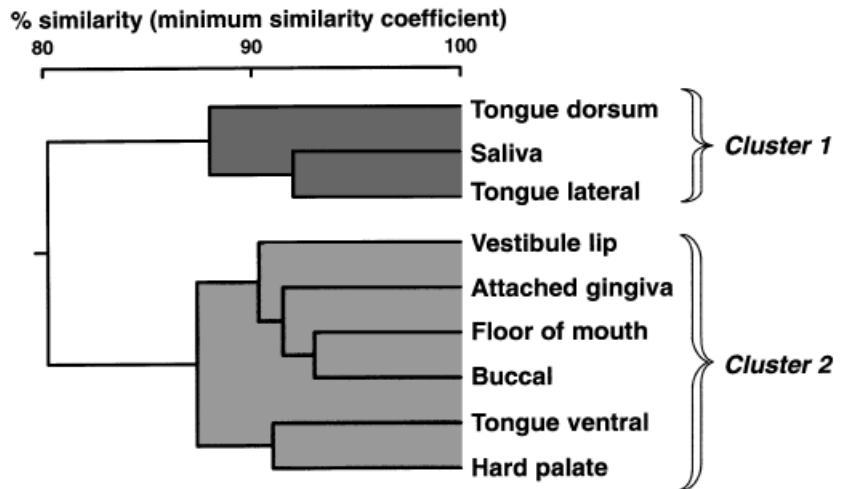

Fig. 2. Dendrogram of a cluster analysis of the mean species proportions from the 9 sample locations. A minimum similarity coefficient was employed and an average unweighted linkage sort. Two clusters were formed at >85% similarity.

Two clusters were formed with >85% similarity. One cluster comprised saliva, and the lateral and dorsal tongue surfaces, while a second cluster was made up of the remaining soft tissue locations. The species that differentiated the 2 cluster groups are shown in Fig. 3. *V. parvula, P. melaninogenica, Eikenella corrodens, Neisseria mucosa, Actinomyces odontolyticus, Fusobacterium periodonticum, F. nucleatum* ss *vincentii* and *P. gingivalis* were in significantly
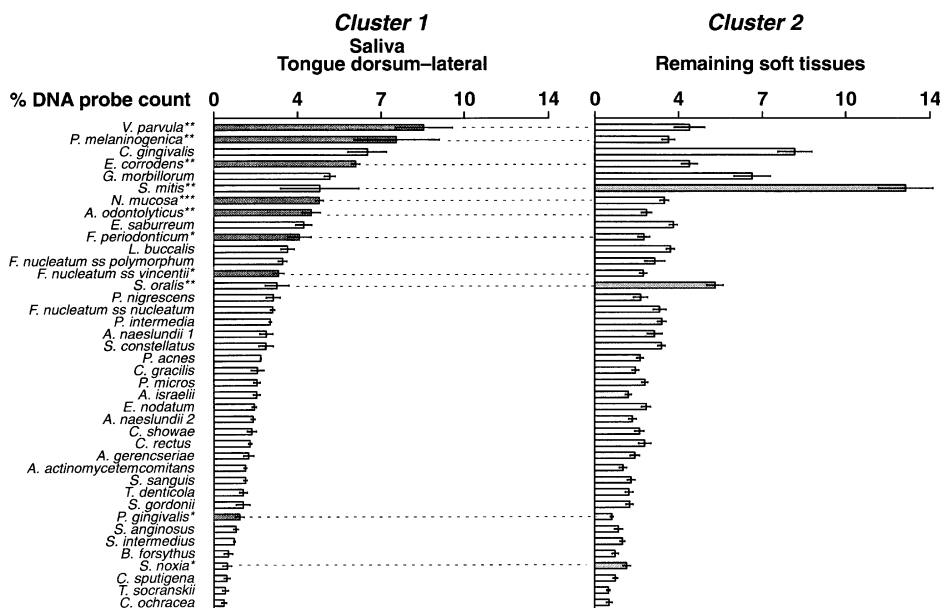
Fig. 3. Mean microbial proportions ($\pm$ SEM) of the 2 clusters in Fig. 2. The left panel indicates mean percentage of the 40 bacterial species of the saliva and lateral surface and dorsum of the tongue cluster (Cluster 1), while the right panel presents the mean proportions of the 40 bacterial species at the 6 remaining sampled surfaces (Cluster 2). Significance of differences between cluster groups was determined using the *t*-test: $*p<0.05$; $**p<0.01$; $***p<0.001$. The shaded bars represent the species that were significantly higher in proportion in a given cluster.

higher proportions in Cluster 1 (saliva–lateral–dorsal tongue) compared with Cluster 2 (the remaining soft tissue locations). In contrast, the mean proportions of *S. mitis, S. oralis* and *Selenomonas noxia* were significantly higher in Cluster 2 than Cluster 1.

Comparison of soft tissue samples, saliva and samples of supra- and subgingival plaque

The mean percentage of the DNA probe count of samples from the 8 intraoral mucous membrane locations, saliva and supra- and subgingival plaque in a subset of 44 of the 225 subjects is presented in Fig. 4. Once again there were marked differences in microbial profiles among the different sample locations, with 35 of 40 test species differing significantly among groups. In particular, supra- and subgingival plaque samples harbored significantly higher proportions of *Actinomyces* species compared with saliva or the soft tissue locations. Saliva and the dorsum of the tongue exhibited the highest proportions of *P. melaninogenica*. These sample sites as well as the lateral surface of the tongue harbored high proportions of *V. parvula*. As shown in Fig. 1, the other soft tissue locations tended to exhibit high proportions of streptococcal species as well as *Gemella*

morbillorum. Cluster analysis of the 11 sample locations reaffirmed the 2 clusters observed in Fig. 2 and indicated that samples of supra- and subgingival plaque differed markedly in composition from the other locations (Fig. 5). The species that differed among cluster groups are presented in Fig. 6. Similar to the individual locations, the hard tissue biofilm samples (Cluster A) were dominated by *Actinomyces* species, Cluster B (saliva–lateral–dorsal tongue) by *P. melaninogenica* and *V. parvula*, and Cluster C (remaining soft tissue locations) by *S. mitis, S. oralis, Streptococcus constellatus, Capnocytophaga gingivalis* and *G. morbillorum.*

Proportions of specific species at different intraoral locations

Several of the oral species examined exhibited different proportions on different intraoral surfaces (Fig. 7). For example, *A. naeslundii* genospecies 2 was found in high proportions in supra- and subgingival plaque samples but in low proportions in the soft tissue samples and saliva. *Eubacterium saburreum* and to some extent *S. mitis* showed the opposite pattern of colonization. These species were found in low proportions on the hard tissues but in high proportions on the soft tissue surfaces. *P. melaninogenica* was found

in high proportions on the lateral and dorsal surfaces of the tongue and in low proportions on the hard tissues and hard palate. The periodontal pathogen *B. forsythus* was found in highest proportions in subgingival plaque, while *Leptotrichia buccalis* was found in similar proportions in all sample locations. Even within a genus such as *Streptococcus*, there were differences in colonization patterns (Fig. 8). *S. oralis, S. mitis* and *S. constellatus* colonized the soft tissues and saliva in higher proportions than the samples from the teeth. The other 4 streptoccocal species colonized the dental surfaces in proportions comparable to the soft tissue locations and saliva.

Discussion

It has been shown that oral bacteria demonstrate specific tropisms toward the different biological surfaces in the oral cavity such as the teeth, mucosa and other bacteria (Gibbons 1989). The nonshedding surfaces of the teeth offer a far different habitat than the continually shedding surfaces of the oral mucosa. Owing to the repeated shedding of epithelial cells, there is less time for a complex biofilm to develop on soft tissue surfaces; thus, a premium is placed on potent mechanisms of adhesion. Clearly, the
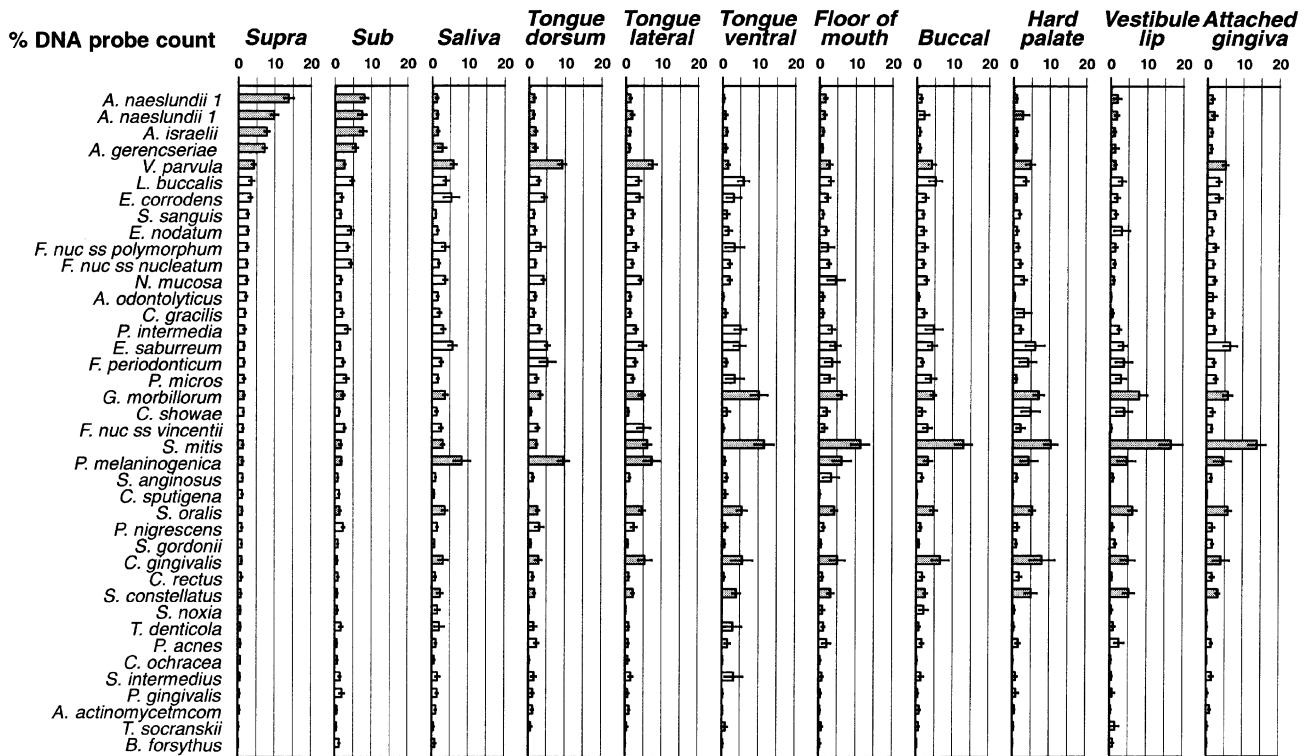
Fig. 4. Mean percentage DNA probe count ($\pm$SEM) for samples from the 11 intraoral locations in the subset of 44 of the 225 subjects who provided samples of supra- and subgingival dental plaque. Species are ordered according to their mean proportions in supragingival plaque. Significance of differences among sample locations was determined using the Quade test and adjusted for multiple comparisons (Socransky et al. 1991). The shaded bars represent species that showed particularly marked differences among sample locations.
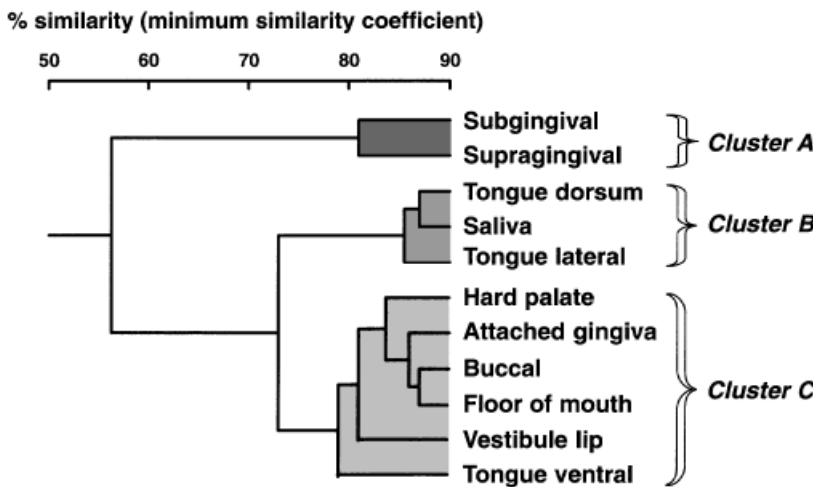

Fig. 5. Dendrogram of a cluster analysis of the mean species proportions from the 11 sample locations. A minimum similarity coefficient was employed and an average unweighted linkage sort. Three clusters were formed at >75% similarity. Cluster B was more similar to Cluster C than Cluster A.

differences in habitat and colonization for the 40 bacterial species studied suggest that different intraoral surfaces and bacterial species have different receptors and adhesion molecules that dictate the development of biofilms on different oral surfaces.

The purpose of the present investigation was to compare the proportions of 40 bacterial species in samples from 8 intraoral soft tissue locations, saliva and supra- and subgingival plaque. The results indicated that the major difference in colonization patterns was

between samples from the hard tissues and the various intraoral soft tissue locations and saliva. *Actinomyces* species colonized hard tissues at far higher proportions than soft tissues, while *P. melaninogenica, V. parvula* and *S. mitis* were found in higher proportions on soft tissue surfaces. Supra- and subgingival plaque most closely resembled each other in microbial composition and the microbiota of saliva resembled that of the dorsum and lateral surfaces of the tongue. However, clear differences were found in the microbiotas that colonized the different intraoral soft tissues. Indeed, sites that might be expected to be nearly identical in microbial profile were found to be distinctly different. For example, the microbiota of the keratinized attached gingiva might be expected to closely resemble that of the keratinized hard palate, yet it was more similar to that of the nonkeratinized floor of the mouth.

The present investigation demonstrated that *Actinomyces* spp. (especially *A. naeslundii* genospecies 1 and 2) colonized teeth at far greater proportions than the soft tissues. Members of this species are known to be a
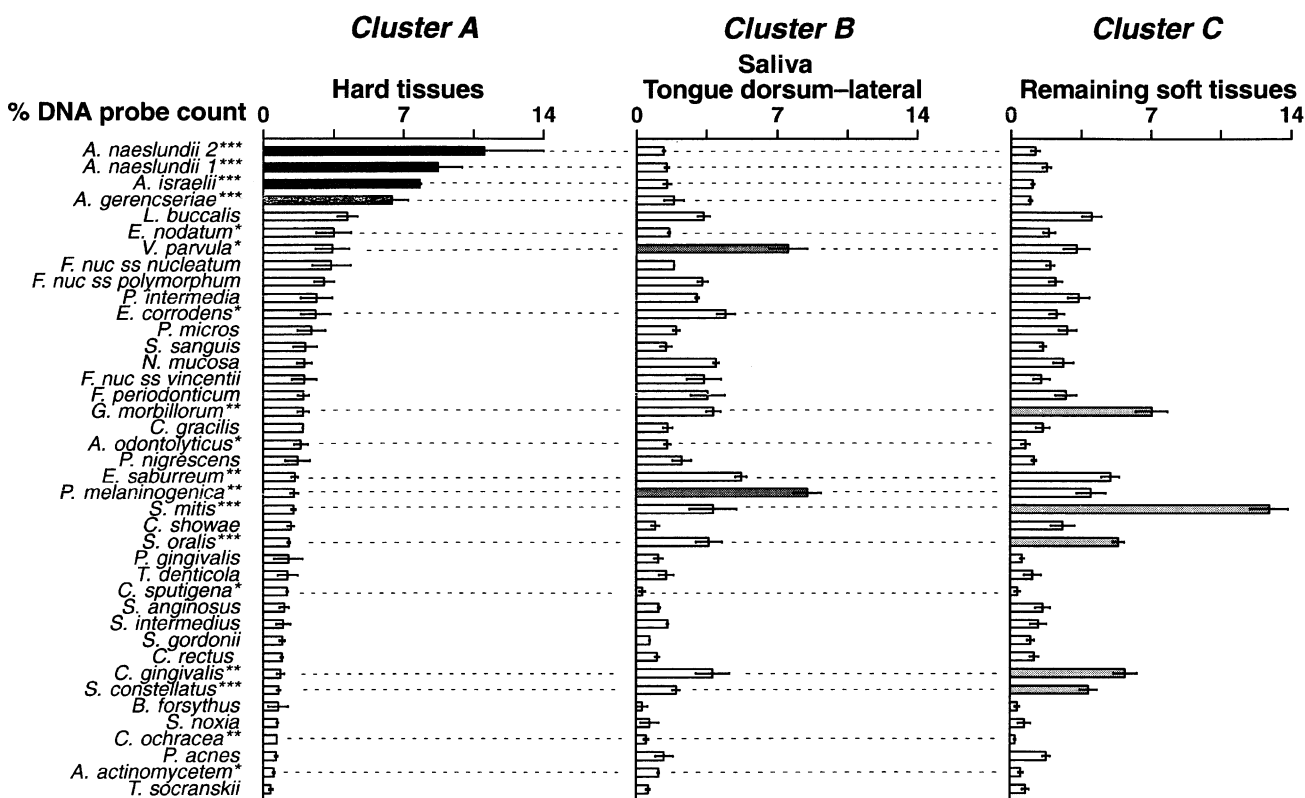
Fig. 6. Mean microbial proportions (±SEM) of the species in the 3 clusters in Fig. 5. The left panel (Cluster A) indicates mean percentage of the 40 bacterial species in supra- and subgingival plaque samples; the middle panel presents the mean microbial proportions of species in Cluster B (saliva, lateral surface and dorsum of the tongue), while the right panel presents the mean proportions of the 40 bacterial species at the 6 remaining sampled surfaces (Cluster C). Significance of differences among cluster groups was determined using ANOVA: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. The shaded bars represent the species that were significantly higher in proportion in a given cluster.

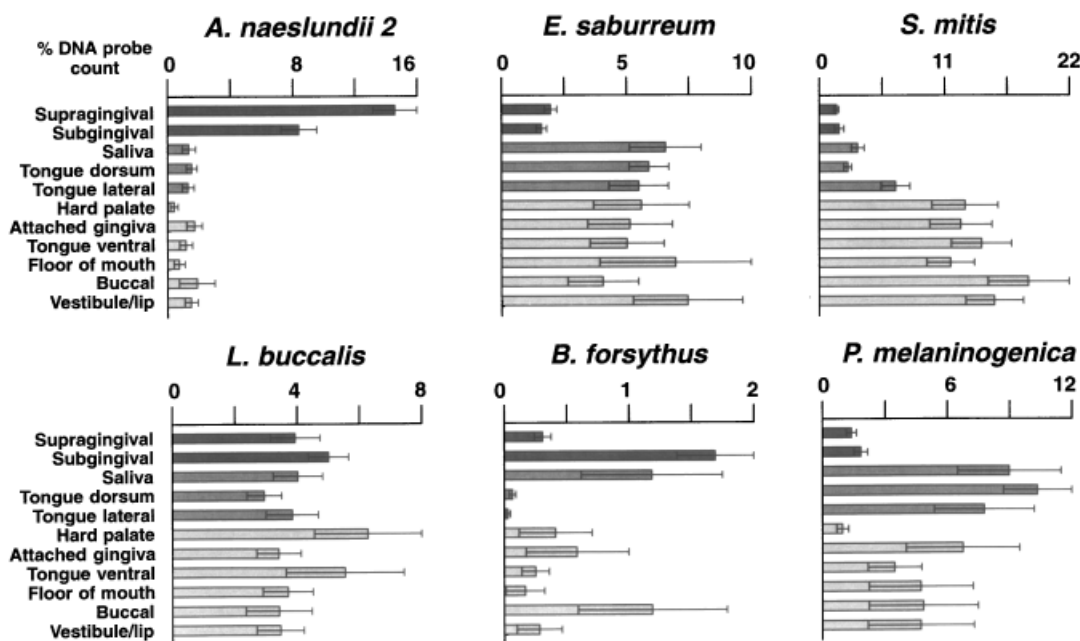*Fig. 7.* Mean proportion (±SEM) of 6 selected species in samples from the 11 intraoral locations. Significance of differences among sample locations was determined using the Quade test and adjusted for multiple comparisons (Socransky et al. 1991). The bars are shaded according to the cluster groups in Fig. 5.

Fig. 8. Mean proportion ($\pm$SEM) of 7 *Streptococcus* species in samples from the 11 intraoral locations. Significance of differences among sample locations was determined using the Quade test and adjusted for multiple comparisons (Socransky et al. 1991). The bars are shaded according to the cluster groups in Fig. 5.
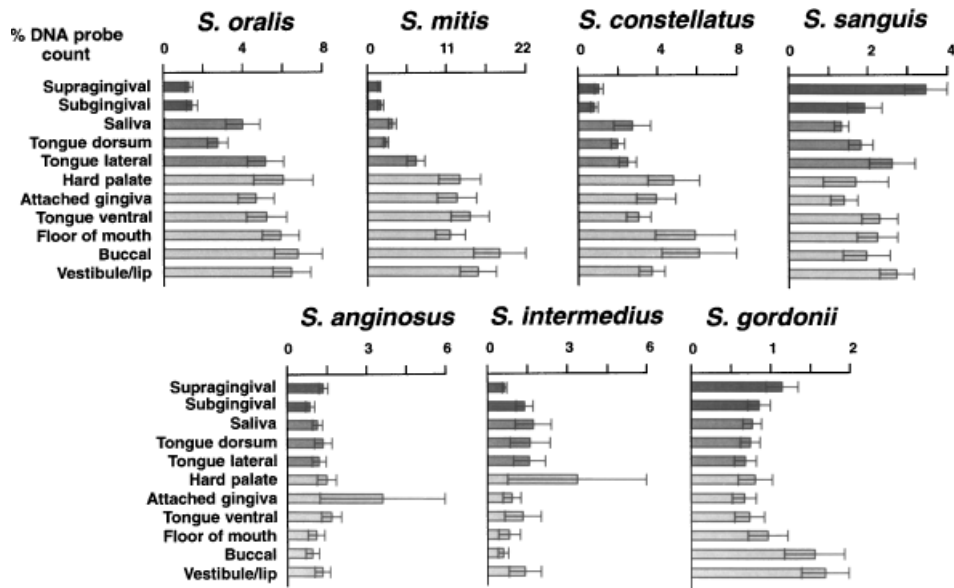
heterogeneous group, which can be prominent in dental plaque. Liu et al. (1991) showed that some strains of *A. naeslundii* bind to collagen, a molecule present in the matrices of both cementum and dentin. This ability to bind to collagen may be an important factor in actinomyces colonization at gingival and subgingival sites. Strains of *Actinomyces* spp. differ in their abilities to attach to salivary components (Gibbons 1989). Stromberg & Boren (1992) suggested that receptor specificities of bacterial cell-surface adhesins might determine the abilities of different *Actinomyces* strains to colonize different oral sites. Hallberg et al. (1998) found that 102 strains of *Actinomyces* isolated from teeth, buccal mucosa and tongue in 8 individuals could be classified into 3 major groups based on binding and coaggregation properties. *A. naeslundii* genospecies 1 was found to be prevalent on teeth, *A. naeslundii* genospecies 2 was the dominant *Actinomyces* species on both teeth and buccal mucosa, while *A. odontolyticus* was the dominant species on the tongue. In the present investigation the findings were similar, with the exception that *A. naeslundii* genospecies 1 was more dominant on the buccal mucosa than *A. naeslundii* genospecies 2. Hallberg et al. (1991) found that the colonization patterns of the 3 species correlated well with the binding specificities of each species to beta-linked galactosamine,

acidic proline-rich protein structures and beta-linked galactosamine-inhibitable coaggregation with selected strains of *Streptococcus* species. The authors suggested that surface-associated adhesion molecules, as well as surface structures (namely fimbriae), may mediate the intraoral colonization and distribution of *Actinomyces* species.

There were even greater differences in colonization patterns within the genus *Streptococcus*. Other investigators have observed differences in colonization patterns of species in this genus. van Houte et al. (1971) examined the adherence of labeled *Streptococcus* species to dental plaque and oral epithelial surfaces in vivo. The proportions of labeled *S. sanguis* recovered from clean teeth or preformed dental plaques were much higher than those of labeled *Streptococcus salivarius* and similar to those observed for *S. sanguis* in naturally occurring oral biofilms. Proportions of labeled *S. salivarius* were higher than those of *S. sanguis* on the dorsum of the tongue but lower on the vestibular mucosa. Labeled *Streptococcus mutans* was found in lower proportions than *S. sanguis* and *S. salivarius* on the soft tissue surfaces. The authors indicated that the relative adherence of the *Streptococcus* species to the tongue and vestibular mucosa correlated with their proportions found naturally in these sites. Frandsen et al. (1991) investigated the colonization of

oral and pharyngeal surfaces by viridans streptococci. Samples taken from the buccal mucosa, tongue dorsum, oropharynx, supra- and subgingival plaque were evaluated culturally for 7 species of streptococci. Different species were associated with specific oral surfaces. For example, IgA1 protease activity was associated almost exclusively with streptococcal species colonizing a cleaned tooth or buccal mucosal surface. *S. mitis* biovar 1 and *S. sanguis* produced IgA1 protease that might facilitate colonization of sites such as buccal mucosa or the pellicle of a cleaned tooth. In contrast, habitats in which protease activity was unnecessary for colonization (mature plaque or tongue dorsum) were colonized by *S. mitis* biovar 2, *Streptococcus gordonii* and *S. oralis,* species that do not produce IgA1 protease. Our findings were similar to those of Frandsen et al. (1991), in that the most prominent streptococci detected on the buccal mucosa was *S. mitis*, although we did not distinguish between biovars 1 and 2.

Neeser et al. (1995) showed that *S. sanguis* OMZ 9 bound to human buccal epithelial cells in a sialic acid-sensitive manner, suggesting that *S. sanguis* may colonize both hard and soft tissues by binding to salivary glycoproteins with sialic acid residues. In accord with these findings, the results of the present investigation indicated that *S. sanguis*

colonized both teeth and soft tissues in comparable proportions.

Sklavounou & Germaine (1980) suggested that keratinization of epithelial cells was likely to be a significant factor in the adherence of oral streptococci. The results of the present investigation were in contrast to those findings. Cluster analysis using a minimum similarity coefficient found that keratinization of epithelial cells did not appear to be a significant factor in the adherence of oral bacteria, including streptococci, to the oral mucosa. For example, *S. mitis* colonized the hard palate and buccal mucosa at similar proportions, while *S. oralis* was found at similar proportions on the hard palate and floor of the mouth, and *S. constellatus* was at similar proportions on the attached gingiva and ventral tongue. In fact, the microbial profile of keratinized attached gingiva was more similar to that of the buccal mucosa than to that of the hard palate.

In accord with many studies in the literature (Krasse 1953, 1954, Gibbons et al. 1964, Beighton et al. 1987), the composition of the microbiota in saliva was most closely related to that of the dorsum of the tongue. Krasse (1953, 1954) examined the levels of *S. salivarius* in plaque, saliva, tongue, vestibule and buccal mucosa samples. *S. salivarius* was found at higher levels in samples of saliva and the tongue than in samples from the vestibule, buccal mucosa and teeth. Gibbons et al. (1964) found that *S. salivarius* and *P. melaninogenica* preferentially colonized the tongue and saliva compared with the teeth and the buccal mucosa. Other studies have found similarities between the microbiota recovered from the tongue and saliva samples. For example, Beighton et al. (1987) found that *S. mutans* levels in tongue samples were significantly correlated with levels of this species in saliva samples.

This investigation supports the findings of Asikainen et al. (1991) that *A. actinomycetemcomitans* may be found as often or more often on soft tissues and in saliva than in dental plaque. Indeed, several investigators have suggested that oral colonization of a subject by *A. actinomycetemcomitans* could be successfully determined by using oral mucosa or saliva samples (Muller et al. 1995, Eger et al. 1996, Zoller et al. 1996). Our data indicate that saliva or the tongue dorsum may be the best sites to sample the presence of *A. actinomy-cetemcomitans,* while subgingival samples may be superior for the detection of *B. forsythus.* Further, in accord with Umeda et al. (1998), our data suggest that saliva samples may be a good diagnostic indicator of the presence of periodontal pathogens including *P. gingivalis, P. intermedius, P. nigrescens* and *T. denticola.*

In the present investigation, the microbiota of the tongue was found to be colonized predominantly by Gram-negative species including *P. melaninogenica, V. parvula* and *C. gingivalis,* rather than streptococci as reported by Gordon & Gibbons (1966). However, the present study confirms numerous reports in the literature that the soft tissues, especially the tongue, may harbor periodontal pathogens including *P. gingivalis, T. denticola, A. actinomycetemcomitans* and *P. intermedia* (van Winkelhoff et al. 1986, Asikainen et al. 1991, Dahlen et al. 1992, Bosy et al. 1994, De Boever & Loesche 1995). These data suggest that the soft tissues may be an important reservoir for periodontal pathogens and could be a major factor in the recolonization of tooth surfaces after periodontal therapy.

There were limitations to the current investigation. The 40 bacterial species that were examined were those thought to be important in dental plaque. These species have been for many years in studies examining the composition of dental plaque as well as in studies comparing the therapeutic effects of different periodontal treatments. Thus, these species were useful for comparing the microbiotas of oral hard and soft tissues. This battery did not include species that may be important members of the soft tissue microbiota such as *S. salivarius* and *Streptococcus vestibularis.* Nonetheless, a large number of subjects, samples and species were examined providing a unique perspective on intraoral patterns of colonization. While differences in the composition of samples obtained from hard and soft tissues were expected, the magnitude of the effect of sample location on taxa such as the *Actinomyces* or streptococci as well as the differences in the microbial profiles obtained from different soft tissue surfaces were not anticipated. Although our results showed that the proportions of 34 of 40 bacterial species differed significantly, some of the species were found at such low proportions that they may have no clinical significance. For this reason, cluster analyses were performed to reveal similarities and dissimilarities in the colonization patterns of oral soft tissues. The similarity between microbiotas of saliva and the tongue was expected. However, the similarities between some of the tissues in Cluster 2 were surprising.

The present investigation demonstrated that all of the tested species could be found, on average, on all of the sampled surfaces. The major differences were in the proportions that colonized the different surfaces, suggesting that receptors, coaggregation or local habitat differences play major roles in defining community structure. Nevertheless, certain locations showed greater microbial similarities than others. The microbial composition of saliva was most similar to that found on the lateral and dorsal surfaces of the tongue, suggesting that these surfaces may be the major source of salivary bacteria. The microbiotas colonizing the remaining surfaces showed greater similarities to each other, but differences could be detected among surface locations. This was interesting given that both keratinized and nonkeratinized surfaces were represented in this group. The biofilms colonizing the teeth were somewhat similar to each other but quite different from the microbiotas on the oral soft tissue surfaces and in saliva. However, as pointed out above, tooth-colonizing species could be detected on the soft tissues. Thus, as discussed by many investigators, the soft tissues may act as reservoirs for tooth-borne pathogens and may require therapeutic attention. Soft tissues and saliva may also provide convenient sites for monitoring therapeutic interventions.

Zusammenfassung

Verteilung ausgewählter Bakterienarten auf introoralen Oberflächen
Zielsetzung: Untersuchung der Anteile von 40 Bakterienarten in Proben von 8 oralen Weichgewebsoberflächen und aus dem Speichel von allgemein gesunden Erwachsenen und Vergleich mit der Verteilung dieser Mikroorganismen in supra- und subgingivaler Plaque.
Methoden: Mikrobiologische Proben wurden von 8 oralen Oberflächen bei 225 allgemein

gesunden Personen mittels einer Bukkalbürste gewonnen. Speichel wurde durch Spucken gewonnen. Von 44 dieser Personen wurden zusätzlich supra- und subgingivale Plaqueproben entnommen. Die Proben wurden einzeln auf ihren Gehalt von 40 Bakterienarten mittels der Schachbrett-DNS-DNS-Hybridisierung untersucht. Der prozentuale Anteil an der Gesamt DNS-Sondenzahl wurde für jede Art an jeder Stelle bestimmt und pro Person gemittelt. Signifikante Unterschiede zwischen den Anteilen der 40 Testspezies an den unterschiedlichen Entnahmestellen wurde für die 225 und 44 Personen separat mittels des Quade-Tests nach Korrektur für multiple Testung bestimmt. Eine Clusteranalyse wurde mit den Anteilen der 40 Bakterienarten an den verschiedenen Entnahmestellen mittels eines Minimum-Ähnlichkeits-Koeffizienten und einer durchschnittlichen ungewichteten Verbindungsortierung durchgeführt. Die Anteile jeder Bakterienart wurden für jede Person in den sich ergebenden Clustergruppen gemittelt und die Signifikanz der Unterschiede mittels t-Test und ANOVA bestimmt.
Ergebnisse: Die mikrobiologischen Profile unterschieden sich deutlich zwischen den unterschiedlichen Entnahmestellen bei den 225 Personen, wobei sich für 34 von 40 Bakterienarten signifikante Unterschiede ergaben. Die Anteile von *V. parvula* und *P. melaninogenica* waren im Speichel und an den lateralen und dorsalen Flächen der Zunge höher, während *S. mitis* und *S. oralis* in signifikant geringeren Anteilen im Speichel und am Zungenrücken gefunden wurden. Die Clusteranalyse ergab 2 Cluster mit > 85% Ähnlichkeit; Cluster 1 bestand aus Speichel, seitlichen und dorsalen Zungenflächen, während Cluster 2 die übrigen Gewebeoberflächen umfasste. *V. parvula, P. melaninogenica, E. corrodens, N. mucosa, A. odontolyticus, F. periodonticum, F. nucleatum* ss *vincentii* und *P. gingivalis* wurden zu signifikant höheren Anteilen in Cluster 1 und *S. mitis, S. oralis* und *S. noxia* in Cluster 2 gefunden. Diese Ergebnisse wurden durch die Daten der 44 Patienten, von denen Plaqueproben genommen wurden, bestätigt. Die mikrobiologischen Profile der supra- und subgingivalen Plaqueproben unterschieden sich insbesondere hinsichtlich der erhöhten Anteile an *Actinomyces*-Arten von den anderen Entnahmestellen. Arten unterschiedlicher Genera zeigten unterschiedliche Anteile auf den verschiedenen intraoralen Oberflächen, aber selbst innerhalb des Genus *Streptococcus* bestanden Unterschiede hinsichtlich des Besiedlungsmusters. *S. oralis, S. mitis* und *S. constellatus* besiedelten die Weichgewebe und den Speichel zu höheren Anteilen als die Proben von Zähnen, während die anderen 4 untersuchten Streptokokkenarten die Zahnoberflächen zu ähnlichen Anteilen besiedelten wie die Weichgewebe und den Speichel.
Schlussfolgerungen: Die Anteile der Bakterienarten unterschieden sich deutlich auf den verschiedenen intraoralen Oberflächen. Die Mikroorganismen des Speichels waren denen der dorsalen und lateralen Flächen der Zunge am ähnlichsten. Die Mikroorganismen der Weichgewebsoberflächen ähnelten einander mehr als die Bakterien, die die Zähne ober- und unterhalb des Gingivarandes besiedeln.

Résumé

Répartition d'espèces bactériennes sélectionnées sur les surfaces intrabuccales

Le but de cet examen a été de calculer les proportions de 40 espèces bactériennes provenant d'échantillons de huit surfaces de tissu mou et de la salive chez des adultes sains et de comparer ces microbiotypes à ceux de la plaque sus- et sous-gingivale. Des échantillons microbiens ont été prélevés de huit surfaces buccales de tissu mou chez 225 patients sains au point de vue systémique utilisant une ''brosse buccale''. La salive a été prélevée par expectoration. Quarante-quatre des sujets ont également donné de la plaque sus- et sous-gingivale. Les échantillons ont été évalués individuellement pour leur teneur en 40 espèces bactériennes utilisant l'hybridisation ADN-ADN par damier. Le pourcentage du comptage par sonde ADN total a été déterminé pour chaque espèce, à chaque localisation et leur moyenne a été calculée parmi les patients. La signification des différences parmi les proportions des 40 espèces testées à différents endroits a été examinée chez les 225 et 44 sujets séparément utilisant le test de Quade et ajustée pour les comparaisons multiples. L'analyse par groupe a été effectuée à l'aide des proportions des 40 espèces aux différentes localisations des échantillons avec le coëfficient de similarité minimale et une relation de moyenne non-pondérée. La moyenne des proportions de chaque espèce a été calculée parmi les sujets dans les groupes résultant, et la signification des différences testées via le test-t et ANOVA. Les profils microbiens différaient extrêmement parmi les localisations de l'échantillonnage chez les 225 sujets avec 34 des 40 espèces différant significativement. Les proportions de *V. parvula* et *P. melaninogenica* étaient plus importantes dans la salive et sur les surfaces latérales et dorsales de la langue tandis que *S. mitis* et *S. oralis* se retrouvaient dans des proportions significativement inférieures dans la salive et sur le dos de la langue. L'analyse par groupe résultait dans la formation de deux groupes avec >85% de similarité : le groupe 1 comprenait la salive et les surfaces latérales et dorsales de la langue tandis que le groupe 2 comprenait les localisations des tissus mous restantes. *V. parvula, P. melaninogenica, E. corrodens, N. mucosa, A. odontolyticus, F. periodonticum, F. nucleatum* ss *vincentii* et *P. gingivalis* étaient en proportions significativement plus importantes dans le groupe 1 et *S. mitis, S. oralis* et *S. noxia* étaient significativement plus importants dans le groupe 2. Ces découvertes ont été confirmées par les données provenant des 44 sujets dont la plaque dentaire avait été prélevée. Les profils microbiens de la plaque sus- et sous-gingivale différaient des autres localisations d'échantillonnage particulièrement pour les proportions augmentées des espèces *Actinomyces*. Des espèces de différents genres montraient des proportions diférentes sur les surfaces intrabuccales distinctes mais même parmi le genre *Streptococcus*, il y avait des différences dans les modèles de colonisation. *S. oralis, S. mitis* et *S. constellatus* colonisaient les surfaces des tissus mous et la salive dans des proportions plus importantes que les échantillons provenant des dents tandis que les quatre autres espèces de streptocoques examinées colonisaient les surfaces dentaires dans des proportions comparables aux localisations des tissus mous et de la salive. Les proportions d'espèces bactériennes différaient énormément sur les sites intrabuccaux respectifs. Le microbiote de la salive était très semblable à celui des surfaces dorsales et latérales de la langue. Les microbiotes des tissus mous se ressemblaient les uns aux autres, davantage que les microbiotes qui colonisaient les dents tant en sus- qu'en sous-gingival.

References

Asikainen, S., Alaluusua, S. & Saxen, L. (1991) Recovery of *A. actinomycetemcomitans* from teeth, tongue, and saliva. *Journal of Periodontology* **62**, 203–206.

Beighton, D., Rippon, H. R. & Thomas, H. E. (1987) The distribution of *Streptococcus mutans* serotypes and dental caries in a group of 5- to 8- year-old Hampshire schoolchildren. *British Dental Journal* **162**, 103–106.

Bosy, A., Kulkarni, G. V., Rosenberg, M. & McCullock, C. A. G. (1994) Relationship of oral malodor to periodontitis: evidence of independence in discrete subpopulations. *Journal of Periodontology* **65**, 37–46.

Collins, L. M. C. & Dawes, C. (1987) The surface area of the adult human mouth and thickness of the salivary film covering the teeth and oral mucosa. *Journal of Dental Research* **66**, 1300–1303.

Dahlen, G., Manji, F., Baelum, V. & Fejerskov, O. (1992) Putative periodontopathogens in ''diseased'' and ''non-diseased'' persons exhibiting poor oral hygiene. *Journal of Clinical Periodontology* **19**, 35–42.

Danser, M. M., Timmerman, M. F., van Winkelhoff, A. J. & van der Velden, U. (1996) The effect of periodontal treatment on periodontal bacteria on the oral mucous membranes. *Journal of Periodontology* **67**, 478–485.

De Boever, E. H. & Loesche, W. J. (1995) Assessing the contribution of anaerobic microflora of the tongue to oral malodor. *Journal of the American Dental Association* **126**, 1384–1393.

Eger, T., Zoller, L., Muller, H-P., Hoffmann, S. & Lobinsky, D. (1996) Potential diagnostic value of sampling oral mucosal surfaces for *Actinobacillus actinomycetemcomitans* in young adults. *European Journal of Oral Science* **104**, 112–117.

Frandsen, E. V. G., Pedrazzoli, V. & Kilian, M. (1991) Ecology of viridans streptococci in the oral cavity and pharynx. *Oral Microbiology and Immunology* **6**, 129–133.

Gibbons, R. J., Kapsimalis, B. & Socransky, S. S. (1964) The source of salivary bacteria. *Archives of Oral Biology* **9**, 101–103.

Gibbons, R. J. (1989) Bacterial adhesion to oral tissues: a model for infectious diseases. *Journal of Dental Research* **68**, 750–760.

Gordon, D. F. Jr. & Gibbons, R. J. (1966) Studies of the predominant cultivable

micro-organisms from the human tongue. *Archives of Oral Biology* **11**, 627–632.

Haffajee, A. D., Cugini, M. A., Dibart, S., Smith, C., Kent, R. L. Jr. & Socransky, S. S. (1997) The effect of SRP on the clinical and microbiological parameters of periodontal diseases. *Journal of Clinical Periodontology* **24**, 324–334.

Haffajee, A. D., Socransky, S. S. & Goodson, J. M. (1983) Comparison of different data analyses for detecting changes in attachment level. *Journal of Clinical Periodontology* **10**, 298–310.

Hallberg, K., Hammarstrom, K. -J., Falsen, E., Dahlen, G., Gibbons, R., Hay, D. I. & Stromberg, N. (1998) *Actinomyces naeslundii* genospecies 1 and 2 express different binding specificities to *N*-acetyl-B-D-galactosamine, whereas *Actinomyces odontolyticus* expresses a different binding specificity in colonizing the human mouth. *Oral Microbiology and Immunology* **13**, 327–336.

van Houte, J., Gibbons, R. J. & Pulkkinen, A. J. (1971) Adherence as an ecological determinant for streptococci in the human mouth. *Archives of Oral Biology* **16**, 1131–1141.

Krasse, B. (1953) The proportional distribution of different types of Streptococci in saliva and plaque material. *Odontologisk Revy* **4**, 304–312.

Krasse, B. (1954) The proportional distribution of *Streptococcus salivarius* and other Streptococci in various parts of the mouth. *Odontologisk Revy* **5**, 203–211.

Langendijk, P. S., Hagemann, J. & van der Hoeven, J. S. (1999) Sulfate-reducing bacteria in periodontal pockets and in healthy oral sites. *Journal of Clinical Periodontology* **26**, 596–599.

Lee, K. H., Tanner, A. C. R., Maiden, M. F. J. & Weber, H. P. (1999) Pre- and post-implantation microbiota of the tongue, teeth, and newly-placed implants. *Journal of Clinical Periodontology* **26**, 822–832.

Liu, T., Gibbons, R. J., Hay, D. I. & Skobe, Z. (1991) Binding of *Actinomyces viscosus* to collagen: association with type 1 fimbrial adhesin. *Oral Microbiology and Immunology* **6**, 1–5.

Loos, B. G., van Winkelhoff, A. J., Dunford, R. G., Genco, R. J., De Graaff, J., Dickinson, D. P. & Dyer, D. W. (1992) A statistical approach to the ecology of *Porphyromonas gingivalis*. *Journal of Dental Research* **71**, 353–358.

Matto, J., Saarela, M., Alaluusua, S. & Jousimies-Somer, H. (1996a) Similarity of salivary and subgingival *Prevotella intermedia* and *Prevotella nigrescens* isolates by arbitrarily primed polymerase chain reaction. *Oral Microbiology and Immunology* **11**, 395–401.

Matto, J., Saarela, M., von Troil-Linden, B., Kononen, E., Jousimies-Somer, H., Torkko, H., Alaluusua, S. & Asikainen, S. (1996b) Distribution and genetic analysis of oral *Prevotella intermedia* and *Prevotella nigrescens*. *Oral Microbiology and Immunology* **11**, 96–102.

Matto, J., Saarela, M., Alaluusua, S., Oja, V., Jousimies-Somer, H. & Asikainen, S. (1998) Detection of *Porphyromonas gingivalis* from saliva by PCR by using a simple sample-processing method. *Journal of Clinical Microbiology* **36**, 157–160.

Muller, H. -P., Eickholz, P., Heinecke, A., Pohl, S., Muller, R. F. & Lange, D. E. (1995) Simultaneous isolation of *Actinobacillus actinomycetemcomitans* from subgingival and extracrevicular locations of the mouth. *Journal of Clinical Periodontology* **22**, 413–419.

Muller, H. -P., Heinecke, A., Borneff, M., Knopf, A., Kiencke, C. & Pohl, S. (1997) Microbial ecology of *Actinobacillus actinomycetemcomitans*, *Eikenella corrodens* and *Capnocytophaga* spp. in adult periodontitis. *Journal of Periodontal Research* **32**, 530–542.

Neeser, J. R., Grafstrom, R. C., Woltz, A., Brassart, D., Fryder, V. & Guggenheim, B. (1995) A 23 kDa membrane glycoprotein bearing NeuNAc alpha 2-3Gal beta 1-3GalNAc O-linked carbohydrate chains as a receptor for *Streptococcus sanguis* OMZ 9 on human buccal epithelial cells. *Glycobiology* **5**, 97–104.

Quirynen, M., Bollen, C. M. L., Vandekerckhove, B. N. A., Dekeyser, C., Papaioannou, W. & Eyssen, H. (1995) Full- vs. partial-mouth disinfection in the treatment of periodontal infections: short-term clinical and microbiological observations. *Journal of Dental Research* **74**, 1459–1467.

Quirynen, M., De Soete, M., Dierickx, K. & van Steenberghe, D. (2001) The intra-oral translocation of periodontopathogens jeopardises the outcome of periodontal therapy. A review of the literature. *Journal of Clinical Periodontology* **28**, 499–507.

Quirynen, M., Mongardini, C. & van Steenberghe, D. (1998) The effect of a 1-stage full-mouth disinfection on oral malodor and microbial colonization of the tongue in periodontitis patients. A pilot study. *Journal of Periodontology* **69**, 374–382.

Sklavounou, A. & Germaine, G. R. (1980) Adherence of oral streptococci to keratinized and nonkeratinized human oral epithelial cells. *Infection and Immunity* **27**, 686–689.

Socransky, S. S., Haffajee, A. D., Smith, C. & Dibart, S. (1991) Relation of counts of microbial species to clinical status at the site. *Journal of Clinical Periodontology* **18**, 766–775.

Socransky, S. S., Smith, C., Martin, L., Paster, B. J., Dewhirst, F. E. & Levin, A. E. (1994) Checkerboard DNA–DNA hybridization. *Biotechniques* **17**, 788–792.

Socransky, S. S., Tanner, A. C. R., Goodson, J. M., Haffajee, A. D., Walker, C. B., Ebersole, J. L. & Sornberger, G. C. (1982) An approach to the definition of periodontal disease syndromes by cluster analysis. *Journal of Clinical Periodontology* **9**, 460–471.

Sneath, P. H. A. & Sokal, R. R. (1973) *Numerical taxonomy. The principles and practice of numerical classification*. San Francisco: W.H. Freeman & Co.

Stromberg, N. & Boren, T. (1992) Actinomyces tissue specificity may depend on differences in receptor specificity for GalNAc-containing glycoconjugates. *Infection and Immunity* **60**, 3268–3277.

Umeda, M., Contreras, A., Chen, C., Bakker, I. & Slots, J. (1998) The utility of whole saliva to detect the oral presence of periodontopathic bacteria. *Journal of Periodontology* **69**, 828–33.

van Winkelhoff, A. J., van der Velden, U., Winkel, E. G. & De Graaff, J. (1986) Black-pigmented Bacteroides and motile organisms on oral mucosal surfaces in individuals with and without periodontal breakdown. *Journal of Periodontal Research* **21**, 434–439.

Zoller, E. T., Muller, H. -P., Hoffmann, S. & Lobinsky, D. (1996) Potential diagnostic value for sampling oral mucosal surfaces for *Actinobacillus actinomycetemcomitans* in young adults. *European Journal of Oral Science* **104**, 112–117.

Address:
Donna L. Mager
Department of Periodontology and
Molecular Genetics
The Forsyth Institute
140 The Fenway
Boston, MA 02115
USA