INFECTION AND IMMUNITY, June 1990, p. 1628–1633
0019-9567/90/061628-06$02.00/0

Vol. 58, No. 6

Microflora Associated with Experimental Root Surface Caries in Humans

BENTE NYVAD[1]* AND MOGENS KILIAN[2]

Department of Oral Anatomy, Dental Pathology and Operative Dentistry[1] and Department of Oral Biology,[2] Royal Dental College, Vennelyst Boulevard, DK-8000 Århus C, Denmark

Received 27 September 1989/Accepted 26 February 1990

This study describes the microflora from actively progressing root surface caries lesions, in which mineral loss had been determined by quantitative microradiography. The caries lesions were produced experimentally in root surface specimens from human molars inserted in lower partial dentures carried for 3 months by six elderly individuals. A total of 780 bacterial isolates were identified from 13 plaque samples, collected with a punch technique, and six dentin samples. The composition of the microflora showed distinct individual differences. The microflora from plaque samples associated with the highest mineral loss was dominated by either *Actinomyces viscosus* or a combination of mutans streptococci (serotypes c, d, and f) and *Lactobacillus* species (*L. casei* and *L. brevis*). Plaque from root surfaces with less pronounced mineral loss harbored a more complex microflora comprising gram-positive rods, mutans streptococci, *Streptococcus mitis* biovar 1, *Veillonella* spp., gram-negative rods, and low numbers of lactobacilli. In the latter samples, individual variations in the proportions of mutans streptococci (serotypes c, d, and g), *Actinomyces* species (*A. viscosus* and *A. naeslundii*), and *Veillonella parvula* biotypes were observed. These findings suggest that certain species or combinations of species are more cariogenic than others and that dominance of single acidogenic species in particular is conducive to high caries activity.

Microbiological studies have failed to come to an agreement on the typical microflora associated with root surface caries. Early studies focused on *Actinomyces* species, in particular *A. viscosus* (48, 49), because *Actinomyces* spp. and other filamentous bacteria had been identified in carious root dentin by both cultural and histological methods (23, 48). Later studies of the distribution of *Actinomyces* species on carious and noncarious root surfaces have not been able to confirm this association. In fact, there is evidence that plaque from noncarious root surfaces contains higher proportions of *Actinomyces* spp. than plaque from root lesion sites (1, 4, 18). Many recent studies have concluded that mutans streptococci may play an important role in the development of root surface caries due to the higher isolation frequency and/or higher proportion of these species on carious root surfaces (1, 4, 18, 25, 49). However, in some studies the percentages of various suspected cariogenic microorganisms have been too low or too variable to incriminate any particular species in the etiology of root surface caries (11–13, 15). These discrepancies between different studies may be ascribed to the lack of standardized experimental conditions.

Previous microbiological studies of root surface caries in vivo have been hampered by the lack of a precise definition of the demineralization activity of the lesions studied. Furthermore, the plaque samples in question may not have originated from the same lesion depth because of different sampling methods. None of the studies have used a sampling technique that takes into account possible variations in the composition of the microflora across the individual lesion (39). Finally, all the studies except one (49) used selective medium for enumeration of target species. However, such methods may detect only a small fraction of the total cultivable microflora (12).

The aim of this study was to characterize the predominant microflora from experimentally induced, actively progressing root surface caries lesions developed within a 3-month period in vivo. The study was planned so that possible inter- and intraindividual variations in the composition of the microflora could be compared with observed differences in the degree of demineralization across the lesions. The plaque samples were collected by a punch technique in order to obtain standardized samples from minute, well-defined areas of the root lesions.

MATERIALS AND METHODS

Participants. Six elderly persons (three males and three females, 59 to 80 years of age [mean age, 70]), all of whom had a partial denture in the lower jaw, volunteered for the study. The root caries index of the participants, i.e., the proportion of carious and filled root surfaces of the total number of root surfaces with gingival recession per individual (23), varied between 5% (subject 6) and 79% (subject 1), with a mean root caries index of 26%. Two of the participants (subjects 1 and 5) used drugs daily (glucocorticoid aerosols or cyclic antidepressants) and exhibited active root caries lesions (16) on 52 and 13% of the exposed root surfaces, respectively, when admitted to the study. None of the remaining participants showed active root caries lesions. In two participants (subjects 3 and 5), the stimulated salivary secretion rate was less than 1 ml/min.

Root surface specimens. Specimens were prepared from the root surface adjoining the cemento-enamel junction of surgically removed human third molars, as described in detail elsewhere (40). Briefly, the teeth were rinsed in running tap water, and residual organic material on the root surfaces was removed by immersing the teeth in 5% sodium hypochlorite for 3 h. Subsequently the teeth were rinsed in distilled water. Mesial or distal surfaces were cut into square blocks (5 by 5 by 2 mm) and embedded in light-curing composite resin (Silux; 3M Co., St. Paul, Minn.) with the exposed root cementum surface in a recessed position about

* Corresponding author.

1 mm below the surface of the composite. This design facilitated undisturbed accumulation of bacterial deposits on the root surfaces after the specimens had been fixed with sticky wax in recessions in the buccal flanges of the dentures. Prior to mounting, the specimens were cleaned by sonification (MSE-MULLARD ultrasonic disintegrator; Measuring & Scientific Equipment Ltd., London, Great Britain) in an ice bath for 15 min at an amplitude of 16 μm.

Experimental conditions. Each of the six participants carried one root surface specimen in the mouth for 3 months. During this period the participants were instructed to wear the dentures day and night and to clean the dentures, except for the experimental sites, with tap water only. No dietary instructions were given, but a nonfluoride toothpaste was provided for cleaning of the natural teeth.

Sampling procedures. Prior to microbiological analysis, the specimens were removed from the dentures and vigorously flushed with 5 ml of sterile saline from a syringe. Subsequently, three plaque samples and one dentin sample were collected from each specimen. The individual plaque samples were obtained with a blunt, sterile hypodermic needle (diameter, 0.9 mm; Terumo Europe N.W., Leuven, Belgium), which was mounted on a 10-ml Luer syringe (Pharma-Plast; Værløse, Denmark). The needle was pushed perpendicularly into the softened root surface until it met resistance from the underlying sound dentin, and the sample was removed by a backward stroke of the plunger. The plaque samples were collected at 1-mm intervals on a straight line along the middle of the specimens and numbered consecutively (P1 to P3). This design was adopted because parallel quantitative microradiographic analyses were to be performed on the same specimens (40). The dentin samples (D) were collected with a small sterile spoon excavator (double-ended excavator no. 153/154; A/S C. G. Brincker, Hvidovre, Denmark) after surface plaque had been removed by ultrasonic treatment (MSE-MULLARD ultrasonic disintegrator) in chilled (4°C) sterile brain-heart infusion (BHI) broth (Difco Laboratories, Detroit, Mich.) for 30 s at an amplitude of 6 μm. The dentin samples originated from an area 1 to 2 mm away from the plaque samples.

Microbiological procedures. All 24 plaque and dentin samples were immediately transferred to 1 ml of chilled (4°C) nutrient BHI broth (Difco). As regards the plaque samples, the broth was initially deposited in the sterile syringe used for sampling in order to rinse the microbial deposits out of the needle. The microorganisms were then dispersed by sonification (MSE-MULLARD ultrasonic disintegrator) in a crushed-ice bath for 30 s (amplitude, 6 μm), and 0.1-ml portions of appropriate 10-fold dilutions were plated in duplicate on tryptone-yeast extract-blood-agar plates (22). The plates were incubated for 4 days at 36°C in an anaerobic glove box (model 1024; Forma Scientific, Marietta, Ohio) containing 80% N_2, 10% H_2, and 10% CO_2.

The predominant microflora from each sample was examined by subcultivation of 50 isolates from a representative section of agar plates yielding 30 to 300 discrete colonies. Following purification by serial subcultivation, the isolates were characterized by their cell morphology and Gram stain and catalase reactions, and further examined accordingly.

Streptococci were divided into species by the criteria described by Kilian et al. (28) on the basis of the following tests: fermentation of amygdalin, inulin, mannitol, D-(+)-melibiose, D-(+)-raffinose, and sorbitol, hydrolysis of D-arginine and esculin, production of acetoin and extracellular polysaccharides, and immunoglobulin A1 (IgA1) protease activity. Furthermore, representative isolates, including selected mutans streptococci, from each individual were characterized by serological methods (41).

Gram-positive rods were separated into two groups on the basis of their ability to grow on Rogosa SL agar (Difco). Lactobacilli were subsequently divided into species, as suggested by Sharpe (46), according to reactions in the following tests: production of CO_2 from glucose, deamination of D-arginine, hydrolysis of esculin, and fermentation of amygdalin, D-(+)-cellobiose, mannitol, D-(+)-melezitose, D-(+)-melibiose, D-(+)-raffinose, rhamnose, and sorbitol. All the remaining gram-positive rods were identified by using the API ZYM kit no. 2520 (API System S.A., Montalieu-Vercieu, France) with the addition of tests for the presence of β-xylosidase (27) and catalase activities, and growth under aerobic conditions supplemented with 10% CO_2 (26). Strains belonging to *Bifidobacterium* were distinguished on the basis of fermentation in D-(+)-cellobiose, D-(+)-raffinose, and sorbitol (44).

Gram-negative anaerobic cocci were assigned to *Veillonella* spp. The remaining gram-negative isolates, which constituted a minor proportion of the cultivable flora, were not characterized further.

Saliva samples. At the end of the experiment, each participant contributed a sample of paraffin-stimulated saliva. Appropriate dilutions of the saliva samples were plated in duplicate on TYCSB-agar, which is selective for mutans streptococci (53). After 4 days of incubation in the anaerobic glove box (36°C), the concentration of mutans streptococci in saliva was calculated. This calculation was adjusted after the identity of 4 to 10 representative isolates from each participant had been determined by the physiological and serological methods described above. The detection limit for mutans streptococci was ≥10 CFU/ml.

Statistical analysis. Significances between selected bacterial groups in plaque and dentin samples were assessed by the Mann-Whitney *U* test (45).

RESULTS

Clinical and microradiographic observations. All the subjects developed soft root surface caries lesions with a yellowish to light brownish tan color within the 3-month period. Due to the soft consistency, the surface zone of the lesions was damaged by the punch sampling procedure. The quantitative data describing the mineral loss (Table 1) were therefore obtained from a neighboring serial section. However, the mineral distribution did not differ between the two sections, and each individual lesion exhibited a uniform depth in the central parts of the specimens. The mineral loss showed a linear relationship with time, indicating that the lesions were actively progressing (40).

Plaque and dentin samples. A total of 780 isolates from 19 plaque and dentin samples were characterized. After initial screening of the isolates, it became obvious that in five of the subjects the relative proportions of the predominant bacterial groups did not differ between parallel plaque samples. This tendency was further supported by the fact that the streptococcal population did not differ at the species level between two plaque samples from the same individual. It was therefore decided to analyze only two plaque samples per individual, and only one of these samples was characterized for the entire microflora. However, in one individual (subject 2) the proportions of gram-positive cocci and rods varied considerably between samples, and in this case all three plaque samples were analyzed.

Table 1 shows the distribution of the predominant cultiva-

TABLE 1. Distribution of predominant facultatively anaerobic microflora of 3-month-old experimental root caries plaque samples[a]

Organism	Subject 1 (13.0)				Subject 2 (10.6)					Subject 3 (7.6)				Subject 4 (7.0)				Subject 5 (6.6)				Subject 6 (4.1)			
	% of total			CFU/ml (S)	% of total				CFU/ml (S)	% of total			CFU/ml (S)	% of total			CFU/ml (S)	% of total			CFU/ml (S)	% of total			CFU/ml (S)
	P1	P2	D		P1	P2	P3	D		P1	P2	D		P1	P2	D		P1	P2	D		P1	P2	D	
Streptococcus spp.																									
S. gordonii (biovar 2/3)			2							2	2	10										4	6		
S. mitis (biovar 1)	4	2	4			4				24	40	12				2		6	8	4		8	16	18	
S. mitis (biovar 2)	4				2		2																2		
S. anginosus								2																	
S. mutans (serotype c)		2		1.5×10^7				6		6	2	10	5×10^6	22	14	8	2.5×10^6		2	6	2.5×10^6	4	10	18	5×10^6
S. mutans (serotype f)					58	76	57	19	2×10^6																
S. sobrinus (serotype d)					34	2	8	21	3×10^6									4	22		1.5×10^6				
S. sobrinus (serotype g)																		2							
Mutans streptococci (not typable)	2																					2	2		
Unidentified streptococci[b]					6	2	2	7											2	33					
Total	10	4	6		100	84	69	55		32	44	32		22	14	10		12	34	43		18	36	36	
Gram-positive rods																									
A. viscosus	82		88			2				16		4		4		2								2	
A. naeslundii	4		4			6								62		28		40		12		2		2	
B. dentium												32										30		6	
Propionibacterium spp.																								23	
Unidentified gram-positive rods										4															
L. acidophilus																				12		2			
L. salivarius																				6					
L. casei subsp. *casei*							15	21																	
L. casei subsp. *rhamnosus*								15				2													
L. brevis							13	8				4				6						6		2	
Unidentified lactobacilli																				4					
Total	86		92			8	28	44		20		42		66		36		40		34		40		35	
V. parvula	4		2							36		20		12		26		12		10		16		27	
Gram-negative rods										8		6				20		22				10		2	
Unclassified						8	2	1		4						8		14		13		16			

[a] Plaque (P1, P2, P3), dentin (D), and saliva (S) samples were obtained from each subject. The total mineral loss (in volume percent micrometers [10^3]) in each subject's lesion is indicated in parentheses after the subject number. Mineral loss data are from Nyvad et al. (40). Values indicate percentage of total microflora for plaque and dentin samples or CFU for saliva samples.

[b] See text.

ble microflora in the 19 samples, expressed as a percentage of the total anaerobic flora. The subjects are ranked according to decreasing mineral loss in the lesions. It appears from the table that variations in the composition of the microflora between different subjects are more pronounced than variations from one sample to the next within the same subject. No significant differences ($P = 0.05$) could be recorded in the proportions of mutans streptococci, *Actinomyces* spp., lactobacilli, total streptococci, or total gram-positive rods between plaque and dentin samples.

The composition of the microflora showed distinct individual differences at both genus and species levels. The microflora from the two individuals with the highest mineral loss was dominated either by *A. viscosus* (subject 1) or by a combination of mutans streptococci (serotypes c, d, and f) and lactobacilli (*L. casei* and *L. brevis*) (subject 2). The latter subject was unique in that one sample comprised streptococci only. Individuals with a smaller mineral loss harbored a more complex microflora comprising gram-positive rods, mutans streptococci, *Veillonella* spp., *Streptococcus mitis* biovar 1 (IgA1 protease negative), gram-negative rods, and traces of lactobacilli. Within this group, individual variations in the proportions of mutans streptococci (serotypes c, d, and g), *Actinomyces* spp. (*A. viscosus* and *A. naeslundii*) and *Veillonella* biotypes were observed. Surfaces from two individuals (subjects 3 and 6) were colonized by *Bifidobacterium dentium*. All the lesions contained *Actinomyces* spp., mutans streptococci, and *S. mitis* biovar 1 in various proportions irrespective of the caries progression rate of the individual.

Twenty-six isolates of streptococci from two subjects could not be identified at the species level on the basis of previously published identification criteria. In subject 2 the colonial morphology of these strains was reminiscent of mutans streptococci, but the strains did not react with any of the mutans typing sera. Besides production of extracellular polysaccharides, these strains fermented mannitol and inulin but not melibiose, raffinose, or sorbitol. The unidentified streptococci in subject 5 fermented mannitol, melibiose, raffinose, and sorbitol and showed a variable reaction in the esculin hydrolysis test. However, none of these isolates fermented inulin or produced extracellular polysaccharides, and they failed to react with antisera against mutans serotypes and Lancefield groups D, H, K, and O.

Just under 5% of the total cultivable microflora remained unclassified because of either loss of viability or contamination (Table 1).

Saliva samples. All the subjects harbored high concentrations of mutans streptococci in saliva, ranging from 2.5×10^6 to 1.5×10^7 CFU/ml (Table 1). *S. mutans* (serotype c or f) was present in all the subjects, whereas only two subjects carried *S. sobrinus* (serotype d).

DISCUSSION

This study has for the first time described the composition of the microflora associated with actively progressing root surface caries lesions developed within a short standardized period (3 months) and with a known mineral loss. This experimental design has distinct advantages because it allows a correlation between the composition of the microflora and the caries activity. Furthermore, the experimental approach in this study overcomes a significant diagnostic problem by applying quantitative microradiography rather than crude clinical and/or radiographical criteria for estimation of mineral loss.

This study has shown that no particular species or group of microorganisms is uniquely associated with the initiation and progression of root surface caries. Among the six subjects examined, two different patterns of microflora were observed. One pattern was characterized by dominance by *A. viscosus* or a combination of mutans streptococci and lactobacilli. This pattern, which is in accordance with the microflora observed in previous studies of enamel (2, 37) and root caries (12, 18, 49) plaque, was associated with the highest mineral loss. The other pattern, found in association with root surfaces showing less pronounced mineral loss, was characterized by a more complex microflora comprising lactate-producing as well as lactate-metabolizing species.

Dominance of single acidogenic species, as here observed in association with the highest caries activity, is likely to occur when the microbial community is influenced by a strong selection pressure, for example, as a result of continuous exposure to high concentrations of fermentable carbohydrates (for a review, see reference 6). Thus, an increase in sucrose consumption has been shown to result in an increase in the population of mutans streptococci and lactobacilli in human dental plaque (9, 17, 38, 47). However, *A. viscosus* may prefer a glucose-rich substrate, as evidenced by the propagation of high-glucose-affinity variants of *A. viscosus* in mixed chemostat cultures (51). Similarly, glucose rather than sucrose has been shown to select for *A. viscosus* in conventional rats (50).

The high root caries activity of the *A. viscosus*-dominated plaque was interesting. Because they have a moderate to low pH-lowering potential, *Actinomyces* species are not considered highly cariogenic (14). However, recent studies have shown that *A. viscosus* and *A. naeslundii* originating from root caries lesions are able to synthesize significant amounts of glycogen at low pHs, i.e., between 5 and 6, especially from glucose (30, 31). The subsequent slow degradation of these polymers (31) may lead to extended periods of acid production, which may increase the cariogenic potential of plaque dominated by these species. This virulence factor may be particularly important in root caries since the critical pH for dissolution of root mineral has been estimated to be significantly higher (pH 6.7) than that of enamel (pH 5.4) (21).

Plaque samples from root surfaces with lower caries activity contained a complex microflora comprising various acidogenic species and a relatively high proportion of *Veillonella* spp. (10 to 36%). *Veillonella* spp. have been shown to reduce the cariogenic potential of dental plaque formed by *S. mutans* or *S. sanguis* in gnotobiotic rats when present together with these organisms (35, 52). The reduced caries activity was ascribed to the formation of a food chain in which *Veillonella* spp. converted lactic acid into acids with a lower dissociation constant (34). This food chain has also been suggested to mitigate the harmful effect of acid production in dental plaque of a population of Tanzanian children who remained caries-free in spite of relatively high numbers of potentially cariogenic organisms (29). However, longitudinal microbiological studies of enamel caries in North American populations have generally failed to demonstrate an inverse correlation between *Veillonella* levels and caries activity (3, 5, 36).

In the present study, *Veillonella* spp. were practically absent in plaque samples from root surfaces with the highest mineral loss. The proportion of *Veillonella* spp. in dental plaque has been shown to increase after short-term exposure to sucrose (9, 38). However, according to Rogosa (43), growth of *Veillonella* spp. is inhibited at pHs below 5.5 to 6.

The absence of *Veillonella* spp. in subjects 1 and 2 in this study may therefore be the result of an ecological pressure induced by the low pH in rapidly progressing caries lesions. This hypothesis, which is consistent with the finding that a decrease in the proportion of *Veillonella* spp. may be associated with increasing demineralization activity (32, 38), may explain the lack of a clear-cut relationship between plaque levels of *Veillonella* spp. and caries. Because of the low acidurance of *Veillonella* spp., the potential caries-modifying effect of these species may be limited to an environment of low to moderate cariogenicity characterized by typical pHs above 6.

All the root caries plaque and dentin samples contained mutans streptococci, irrespective of the caries activity. The distribution of serotypes was reflected in saliva and confirmed that *S. mutans* (serotypes c, e, and f) may be isolated more frequently from active caries lesions than *S. sobrinus* (serotypes d and g) (19, 33, 42). Two individuals were colonized by at least three different serotypes on the same root surface. The relative proportions of the various serotypes in the caries lesion may be important to the overall acidogenic potential, since different serotypes exhibit different acidogenic (10), aciduric (8, 20), and biochemical characteristics (for a review, see reference 7). However, the few subjects examined did not reveal a relationship between particular serotypes and the rate of mineral loss.

It was surprising that none of the plaque samples from actively progressing root caries lesions in the present study contained *S. sanguis*. Besides the mutans streptococci, *S. mitis* biovar 1 was the only *Streptococcus* species encountered in significant proportions. This observation is of interest since the streptococci in this study were identified according to new taxonomic principles which allow more precise definition of the various taxa (28). Recent studies have confirmed that *S. mitis* biovar 1 is a typical member of some cariogenic communities on the root surface (G. H. W. Bowden, Abstr. Annu. Meet. Int. Assoc. Dent. Res. 1989, S40, p. 863). These findings indicate that *S. mitis* biovar 1 may possess hitherto unrecognized cariogenic properties.

On the basis of this study, it may be concluded that root surface caries can develop in the presence of a broad spectrum of bacterial species. In spite of the reservations required because of the few subjects examined, it appears that some species or combinations of species may result in higher caries activity than others. Of particular interest was the finding that dominance of single acidogenic species may reflect a very high caries activity. In contrast, a high complexity of the microflora indicates a low cariogenic potential.

ACKNOWLEDGMENTS

We are grateful to Jørgen Henrichsen for serotyping the mutans streptococci.

The study was supported by a grant from the Danish Medical Research Council (no. 12-7585).

LITERATURE CITED

1. **Billings, R. J., L. R. Brown, and A. G. Kaster.** 1985. Contemporary treatment strategies for root surface dental caries. Gerodontics **1**:20–27.
2. **Bowden, G. H., J. M. Hardie, A. S. McKee, P. D. Marsh, E. D. Fillery, and G. L. Slack.** 1976. The microflora associated with developing carious lesions of the distal surface on the upper first premolars in 13–14-year-old children, p. 223–241. *In* H. M. Stiles, W. J. Loesche, and T. C. O'Brien (ed.), Microbial aspects of dental caries, vol. 1. Information Retrieval Inc., Washington, D.C.
3. **Boyar, R. M., and G. H. Bowden.** 1985. The microflora associated with progression of incipient carious lesions in teeth of children living in a water-fluoridated area. Caries Res. **19**: 298–306.
4. **Brown, L. R., R. J. Billings, and A. G. Kaster.** 1986. Quantitative comparisons of potentially cariogenic microorganisms cultured from noncarious and carious root and coronal tooth surfaces. Infect. Immun. **51**:765–770.
5. **Burt, B. A., W. J. Loesche, and S. A. Eklund.** 1985. Stability of selected plaque species and their relationship to caries in a child population over 2 years. Caries Res. **19**:193–200.
6. **Carlsson, J.** 1986. Metabolic activities of oral bacteria, p. 74–106. *In* A. Thylstrup and O. Fejerskov (ed.), Textbook of cariology. Munksgaard, Copenhagen.
7. **Coykendall, A., and K. B. Gustafson.** 1986. Taxonomy of *Streptococcus mutans*, p. 21–28. *In* S. Hamada, S. M. Michalek, H. Kinyono, L. Menaker, and J. R. McGhee (ed.), Molecular microbiology and immunobiology of *Streptococcus mutans*. Elsevier Science Publishers, Amsterdam.
8. **Denepitiya, L., and I. Kleinberg.** 1984. A comparison of the acid-base and aciduric properties of the various serotypes of the bacterium *Streptococcus mutans* associated with dental plaque. Arch. Oral Biol. **29**:385–393.
9. **Dennis, D. A., T. H. Gawronski, S. Z. Sudo, R. S. Harris, and L. E. A. Folke.** 1975. Variations in microbial and biochemical components of four-day plaque during a four-week controlled diet period. J. Dent. Res. **54**:716–722.
10. **De Soet, J. J., F. A. Toors, and J. de Graaff.** 1989. Acidogenesis by oral streptococci at different pH values. Caries Res. **23**: 14–17.
11. **Ellen, R. P., D. W. Banting, and E. D. Fillery.** 1985. *Streptococcus mutans* and *Lactobacillus* detection in the assessment of dental root surface caries risk. J. Dent. Res. **64**:1245–1249.
12. **Ellen, R. P., D. W. Banting, and E. D. Fillery.** 1985. Longitudinal microbiological investigation of a hospitalized population of older adults with a high root surface caries risk. J. Dent. Res. **64**:1377–1381.
13. **Ellen, R. P., E. D. Fillery, and D. W. Banting.** 1980. Comparison of selective broth and plating methods for isolation of *Streptococcus mutans* from root surface dental plaques. J. Clin. Microbiol. **11**:205–208.
14. **Ellen, R. P., and H. Onose.** 1978. pH measurements of *Actinomyces viscosus* colonies grown on media containing dietary carbohydrates. Arch. Oral Biol. **23**:105–109.
15. **Emilson, C. G., B. Klock, and C. B. Sanford.** 1988. Microbial flora associated with presence of root surface caries in periodontally treated patients. Scand. J. Dent. Res. **96**:40–49.
16. **Fejerskov, O., and B. Nyvad.** 1986. Pathology and treatment of dental caries in the aging individual, p. 238–262. *In* P. Holm-Pedersen and H. Löe (ed.), Geriatric dentistry. Munksgaard, Copenhagen.
17. **Folke, L. E. A., T. H. Gawronsky, R. H. Staat, and R. S. Harris.** 1972. Effects of dietary sucrose on quantity and quality of plaque. Scand. J. Dent. Res. **80**:529–533.
18. **Fure, S., M. Romaniec, C. G. Emilson, and B. Krasse.** 1987. Proportions of *Streptococcus mutans*, lactobacilli and *Actinomyces* spp. in root surface plaque. Scand. J. Dent. Res. **95**: 119–123.
19. **Hamada, S., N. Masuda, T. Ooshima, S. Sobue, and S. Kotani.** 1976. Epidemiological survey of *Streptococcus mutans* among Japanese children. Jpn. J. Microbiol. **20**:33–44.
20. **Harper, D. S., and W. J. Loesche.** 1983. Effect of pH upon sucrose and glucose catabolism by the various genogroups of *Streptococcus mutans*. J. Dent. Res. **62**:526–531.
21. **Hoppenbrouwers, P. P. M., F. C. M. Driessens, and J. M. P. M. Borggreven.** 1987. The mineral solubility of human tooth roots. Arch. Oral Biol. **32**:319–322.
22. **Jensen, S. B., H. Löe, C. R. Schiött, and E. Theilade.** 1968. Experimental gingivitis in man. IV. Vancomycin induced changes in bacterial plaque composition as related to development of gingival inflammation. J. Periodont. Res. **3**:284–293.
23. **Jordan, H. V., and B. F. Hammond.** 1972. Filamentous bacteria isolated from human root surface caries. Arch. Oral Biol. **17**:1333–1342.

24. **Katz, R.** 1980. Assessing root caries in populations: the evolution of the root caries index. J. Public Health Dent. **40**:7–15.
25. **Keltjens, H. M. A. M., M. J. M. Schaeken, J. S. van der Hoeven, and J. C. M. Hendriks.** 1987. Microflora of plaque from sound and carious root surfaces. Caries Res. **21**:193–199.
26. **Kilian, M.** 1978. Rapid identification of *Actinomycetaceae* and related bacteria. J. Clin. Microbiol. **8**:127–133.
27. **Kilian, M., and P. Bülow.** 1976. Rapid diagnosis of *Enterobacteriaceae*. I. Detection of bacterial glycosidases. Acta Pathol. Microbiol. Scand. **84B**:245–251.
28. **Kilian, M., L. Mikkelsen, and J. Henrichsen.** 1989. A taxonomic study of viridans streptococci: description of *Streptococcus gordonii* sp. nov. and emended descriptions of *Streptococcus sanguis* (White and Niven 1946), *Streptococcus oralis* (Bridge and Sneath 1982), and *Streptococcus mitis* (Andrewes and Horder 1906). Int. J. Syst. Bacteriol. **39**:471–484.
29. **Kilian, M., A. Thylstrup, and O. Fejerskov.** 1979. Predominant plaque flora of Tanzanian children exposed to high and low water fluoride concentrations. Caries Res. **13**:330–343.
30. **Komiyama, K., R. L. Khandelwal, and D. E. Duncan.** 1986. Glycogen synthetic abilities of *Actinomyces viscosus* and *Actinomyces naeslundii* freshly isolated from dental plaque over root surface caries lesions and non-carious sites. J. Dent. Res. **65**:899–902.
31. **Komiyama, K., R. L. Khandelwal, and S. E. Heinrich.** 1988. Glycogen synthetic and degradative activities by *Actinomyces viscosus* and *Actinomyces naeslundii* of root surface caries and noncaries sites. Caries Res. **22**:217–225.
32. **Llory, H., A. Dammron, M. Gioanni, and R. M. Frank.** 1972. Some population changes in oral anaerobic microorganisms, *Streptococcus mutans* and yeasts following irradiation of the salivary glands. Caries Res. **6**:298–311.
33. **Masuda, N., N. Tsutsumi, S. Sobue, and S. Hamada.** 1979. Longitudinal survey of the distribution of various serotypes of *Streptococcus mutans* in infants. J. Clin. Microbiol. **10**:497–502.
34. **Mikx, F. H. M., and J. S. van der Hoeven.** 1975. Symbiosis of *Streptococcus mutans* and *Veillonella alcalescens* in mixed continuous cultures. Arch. Oral Biol. **20**:407–410.
35. **Mikx, F. H. M., J. S. van der Hoeven, K. G. König, A. J. M. Plasschaert, and B. Guggenheim.** 1972. Establishment of defined microbial ecosystems in germ-free rats. I. The effect of the interaction of *Streptococcus mutans* or *Streptococcus sanguis* with *Veillonella alcalescens* on plaque formation and caries activity. Caries Res. **6**:211–223.
36. **Milnes, A. R., and G. H. W. Bowden.** 1985. The microflora associated with developing lesions of nursing caries. Caries Res. **19**:289–297.
37. **Minah, G. E., and W. J. Loesche.** 1977. Sucrose metabolism in resting-cell suspensions of caries-associated and non-caries-associated dental plaque. Infect. Immun. **17**:43–54.
38. **Minah, G. E., G. B. Lovekin, and J. P. Finney.** 1981. Sucrose-induced ecological response of experimental dental plaques from caries-free and caries-susceptible human volunteers. Infect. Immun. **34**:662–675.
39. **Nyvad, B., and O. Fejerskov.** 1987. Active and inactive root surface caries: structural entities?, p. 165–179. *In* A. Thylstrup, S. A. Leach, and V. Quist (ed.), Dentine and dentine reactions in the oral cavity. IRL Press, Oxford.
40. **Nyvad, B., J. M. ten Cate, and O. Fejerskov.** 1989. Microradiography of experimental root surface caries in man. Caries Res. **23**:218–224.
41. **Perch, B., E. Kjems, and B. Ravn.** 1974. Biochemical and serological properties of *Streptococcus mutans* from various human and animal sources. Acta Pathol. Microbiol. Scand. **82B**:357–370.
42. **Qureshi, J. V., M. Goldner, W. H. le Riche, and J. A. Hargreaves.** 1977. *Streptococcus mutans* serotypes in young schoolchildren. Caries Res. **11**:141–152.
43. **Rogosa, M.** 1964. The genus *Veillonella*. I. General cultural, ecological, and biochemical considerations. J. Bacteriol. **87**: 162–170.
44. **Scardovi, V., and F. Crociani.** 1974. *Bifidobacterium catenulatum*, *Bifidobacterium dentium*, and *Bifidobacterium angulatum*: three new species and their deoxyribonucleic acid homology relationships. Int. J. Syst. Bacteriol. **24**:6–20.
45. **Schefler, W. C.** 1969. Statistic for the biological sciences, p. 130–133. Addison-Wesley Publishing Co., Massachusetts.
46. **Sharpe, M. E.** 1981. The genus *Lactobacillus*, p. 1653–1679. *In* M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (ed.), The prokaryotes. Springer-Verlag, New York.
47. **Staat, R. H., T. H. Gawronski, D. E. Cressey, R. S. Harris, and L. E. A. Folke.** 1975. Effects of dietary sucrose levels on the quantity and microbial composition of human dental plaque. J. Dent. Res. **54**:872–880.
48. **Sumney, D. L., and H. V. Jordan.** 1974. Characterization of bacteria isolated from human root surface carious lesions. J. Dent. Res. **53**:343–351.
49. **Syed, S. A., W. J. Loesche, H. L. Pape, Jr., and E. Grenier.** 1975. Predominant cultivable flora isolated from human root surface caries plaque. Infect. Immun. **11**:727–731.
50. **van der Hoeven, J. S.** 1974. A slime-producing microorganism in dental plaque of rats, selected by glucose feeding. Caries Res. **8**:193–210.
51. **van der Hoeven, J. S., M. H. de Jong, A. H. Rogers, and P. J. M. Camp.** 1984. A conceptual model for the co-existence of *Streptococcus* spp. and *Actinomyces* spp. in dental plaque. J. Dent. Res. **63**:389–392.
52. **van der Hoeven, J. S., A. I. Toorop, and F. H. M. Mikx.** 1978. Symbiotic relationship of *Veillonella alcalescens* and *Streptococcus mutans* in dental plaque in gnotobiotic rats. Caries Res. **12**:142–147.
53. **van Palenstein Helderman, W. H., M. Ijsseldijk, and J. H. Huis in't Veld.** 1983. A selective medium for the two major subgroups of the bacterium *Streptococcus mutans* isolated from human dental plaque and saliva. Arch. Oral Biol. **28**:599–603.