Quantitative Comparisons of Potentially Cariogenic Microorganisms Cultured from Noncarious and Carious Root and Coronal Tooth Surfaces

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Potentially cariogenic microorganisms cultured from noncarious and carious root and coronal (enamel) surfaces were quantitatively compared in patients 22 to 84 years of age (mean, 52 years). We collected 150 plaque specimens from 26 in situ teeth with initial root lesions and from 25 extracted teeth with advanced root lesions. The frequencies of isolation of Streptococcus mutans, Actinomyces viscosus, and Lactobacillus spp. were, respectively, 94, 72, and 51% at the noncarious root site; 98, 71, and 54% at the root lesion; 84, 61, and 44% at the noncarious enamel site; and 100, 66, and 90% at the enamel lesion. The streptococci made up the largest mean proportion of the total anaerobic cultivable microflora, ranging from 31.2% at the noncarious enamel site to 37.6% at the root lesion, while S. mutans varied between 18% at the noncarious enamel and root surfaces and approximately 24% at both the enamel and root lesions. The proportion of actinomyces ranged from 12.3% at the root lesion to 23.6% at the noncarious root site, while A. viscosus varied from 7.8% at the root lesion to 15.1% at the noncarious root site. The largest mean proportion of lactobacilli (4.2%) was recovered at the enamel lesion site. Proportions of Candida spp. made up less than 0.1% at all sites. Proportions of microorganisms did not differ significantly between noncarious enamel and root sites, but the noncarious coronal and root sites had higher (P < 0.05) proportions of actinomyces than did the root lesion. Also, enamel lesions had a greater (P < 0.05) percentage of Lactobacillus spp. than did root lesions. The number of streptococci recovered from the root lesion was greater (P < 0.01) than the number of actinomyces at the same site. S. mutans was recovered from initial root lesions in greater numbers (P < 0.001) than were actinomyces and lactobacilli. The number of S. mutans recovered at the initial root lesions was greater (P < 0.01) than that recovered from the advanced root lesions.

Preventive dentistry programs, improved treatment measures, and increased availability of dental care have enabled more adults to retain their natural dentition for a longer period (11). Improved tooth longevity, coupled with increasing numbers of elderly persons, will result in an increased number of individuals at risk for oral diseases associated with aging, including an increased risk for both coronal (enamel) and root surface (cemental) dental caries. This will necessitate continued and perhaps more rigorous research efforts directed towards caries etiology and control, particularly in view of the increasing importance of root caries.

Different intraoral environmental factors and possible age-related differences in immunity, life-style, and diet are generally considered to be important variables which may result in microbiological differences in the etiology of enamel and cemental caries. The isolation and identification of microorganisms recovered from human root caries has not yielded consistent results. For example, studies have reported the presence of Actinomyces spp. and Streptococcus mutans; yet, their predominance has not been established (10, 19, 21). These studies, however, did not compare the prevalence of these microorganisms with those recovered from noncarious root surfaces. A recent report from this laboratory (2) compared the effects of a fluoride treatment on the cultivable cariogenic microflora from incipient or shallow root lesions and the adjacent noncarious root surfaces. S. mutans was predominant at the root lesion sites, while Actinomyces viscosus was predominant at noncarious root surface sites.

This investigation describes the predominant cultivable cariogenic microflora from both sound and carious human root surfaces as well as from the adjacent sound and carious enamel surfaces. Carious root surfaces included both initial and advanced lesions. These characterizations have provided insight into the microbial ecology of sound and carious root surfaces.

MATERIALS AND METHODS

Patient selection. A total of 31 patients (11 males and 20 females), 22 to 84 years of age, with a mean age of 52, participated in this study. We sampled 51 teeth; 25 teeth from 16 subjects were extracted before sample collection, and in situ samples from 26 teeth were obtained from 15 patients. Of the 25 extracted teeth with root surface caries, 13 were from the maxillary arch; of these, 15% were molars, 23% were premolars, 8% were cuspids, and 54% were incisors. The 12 mandibular teeth included 17% molars, 25% premolars, 25% cuspids, and 33% incisors. Of the 26 in situ teeth, 8 were maxillary (62% premolars, 25% cuspids, 13% incisors), and 18 were mandibular (17% molars, 55% premolars, 11% cuspids, 17% incisors). We obtained 150 plaque specimens from 50 root and 10 enamel carious lesions and from 47 root and 43 enamel noncarious sites. Data obtained from the pretreatment examinations of the six patients in a previous report (2) were included in this study.

Types of root lesions sampled. The initial lesions of this study comprised both grade I (incipient) and grade II (shal-
low) lesions, while the advanced lesions comprised about equal numbers of grade III (cavitation) and grade IV (pulpal) lesions, as described in detail in a recent report from this laboratory (2). Basically, incipient lesions have a soft surface without a surface defect, while shallow lesions have a surface defect of less than 0.5 mm. Grade III lesions present with cavitation greater than 0.5 mm but no pulpal involvement, while grade IV lesions present with pulpal or root canal involvement (2). Samples from the initial lesions were from teeth in situ, while samples from advanced lesions were from extracted teeth. Advanced lesions were sampled postextraction rather than preextraction to obtain samples from precisely defined areas of each tooth. Considerable care was taken to avoid disturbing or contaminating the advanced lesions during the extraction process. Bleeding was controlled by vasoconstriction with local anesthetic solutions containing epinephrine.

Sampling procedures. All plaque specimens from both in situ and extracted teeth were collected with a sterile spoon excavator (double-ended excavator no. 17; Hu Friedy Co., Chicago, Ill.). From each tooth sampled, plaque was collected from a carious root lesion, a noncarious root site adjacent to the root lesions, a noncarious enamel site just above the root lesion, and, if present, a smooth-surface carious enamel lesion (Fig. 1). The root and enamel control samples were taken within 0.5 to 2 mm of the root lesion. Not all of the coronal lesions, however, occurred adjacent to or even near root lesion. Also, lesions which crossed the cementoenamel junction were not sampled; i.e., only lesions which were contained wholly within the coronal (enamel) or root (dentin) surfaces were sampled (Fig. 1). Plaque samples from in situ teeth were deposited in 2 ml of sterile transport-diluting fluid (0.1% peptone in 0.85% saline, pH 7.2) and transported to the mycobiology laboratory.

Extracted teeth were placed in sterile vials containing a cotton pledget dampened with the sterile transport-diluting fluid. All of the specimens were transported to the microbiology laboratory immediately after extraction, transferred to a sterile petri dish, and placed under a dissecting microscope. The appropriate samples were collected from the sites previously described (Fig. 1) and placed in 2 ml of sterile transport-diluting fluid.

The plaque deposits were initially dispersed for 5 s with a vortex shaker (Vortex-Genie; Scientific Industries, Bohemia, N.Y.) followed by sonication (Sonipen model W-10; Ystrem Systems, Technic International, Inc., Bergenfield, N.J.) for 30 s at an output setting of 6. The plaque suspensions were then serially diluted 10-fold and plated onto enriched or selective media for the enumeration of the total cultivable microflora as well as of specific microbial populations. All of the laboratory procedures were completed within 2 h of sample collection or tooth extraction.

Plaque microbial assessments. Total anaerobic microflora was counted in CFUs recovered from brain heart infusion agar (Difco Laboratories, Detroit, Mich.). Counts of total streptococci and counts for individual streptococcal species (e.g., of S. mutans, S. sanguis, and S. salivarius) were obtained on mitis salivarius agar (Difco) plate计urite, while Lactobacillus counts were assessed on Rogosa SL agar (Difco). All media were incubated anaerobically at 37°C for up to 78 h in an atmosphere of 80% N₂-10% CO₂-10% H₂. The mitis salivarius agar plates were removed from the anaerobic atmosphere after 24 h and maintained for at least 24 h more in room atmosphere before counting. Yeast populations were determined on Sabouraud dextrose agar (Difco) incubated aerobically for 24 h at 37°C. Actinomyces spp. were initially counted after anaerobic incubation on the selective GMC (gelatin-metronidazole-cadmium) medium of Kornman and Loesche (13), which is a variation of the enriched gelatin agar of Syed (20). Because of problems with microorganisms other than actinomyces growing on GMC medium, population estimates later were obtained from CFAT (cadmium-fluoride-acroflavin-tellurite) medium, the selective medium of Zyliber and Jordan (22), after incubation in an atmosphere of 90% air-10% CO₂ for 96 h at 37°C.

Characterization of filamentous bacteria. Gram-positive, non-spore-forming, and non-acid-fast bacilli were differentiated by the method of Slack and Gerencser (18). These microorganisms were first analyzed for their relationship to oxygen by incubation under anaerobic and aerobic conditions at 37°C on brain heart infusion agar. Catalase tests and esculin hydrolysis analyses were also performed (18). To determine glucose fermentation products, cells were grown in peptone-yeast extract-glucose broth (Difco) (9) in an anaerobic atmosphere at 37°C for 4 to 7 days. At the end of the incubation period, the supernatant was analyzed for volatile and nonvolatile fatty acids by gas-liquid chromatography. A Packard gas-liquid chromatograph (model 838; Packard Instrument Co., Inc., Rockville, Md.), equipped with an H₂ flame ionization detector, was used with a glass column (ca. 0.4 by 182.9 cm) containing 10% diethyleneglycol adipate (DEGA, no. SOP-295; Analabs, Inc., New Haven, Conn.) on Chromosorb W-HP (no. GPC-019; Analabs). N₂ was the carrier gas (35 ml/min), and temperatures of 140°C for the column and 180°C for the detector were used. Volatile fatty acids were applied to the column as ether solutions; nonvolatile acids were methylated and extracted into chloroform before application.

Statistical analyses. Proportions of microorganisms recov-
TABLE 1. Frequency of isolation of microorganisms from four tooth sites

<table>
<thead>
<tr>
<th>Microorganism(s)</th>
<th>Enamel sites</th>
<th>Root sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Noncarious (n = 43)</td>
<td>Carious (n = 10)</td>
</tr>
<tr>
<td>Total streptococci</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>S. mutans</td>
<td>84</td>
<td>100</td>
</tr>
<tr>
<td>S. sanguis</td>
<td>44</td>
<td>30</td>
</tr>
<tr>
<td>S. salivarius</td>
<td>23</td>
<td>40</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>65</td>
<td>70</td>
</tr>
<tr>
<td>Total actinomyces</td>
<td>98</td>
<td>100</td>
</tr>
<tr>
<td>A. viscosus</td>
<td>61</td>
<td>66</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>44</td>
<td>90</td>
</tr>
<tr>
<td>Candida spp.</td>
<td>20</td>
<td>50</td>
</tr>
</tbody>
</table>

The frequencies of isolation of the microorganisms characterized at the four sampling sites are presented in Table 1. The frequency of isolation of streptococci ranged from 90% at noncarious enamel sites to 100% at both enamel and root lesion sites. Likewise, the frequency of isolation of actinomyces ranged from 94% at root lesion sites to 100% at enamel lesion sites. Isolation frequencies of the specific caries-associated organisms S. mutans, A. viscosus, and Lactobacillus spp. were, respectively, 84, 61, and 44% at noncarious enamel sites and 100, 66, and 90% at enamel lesion sites. Isolation frequencies of these same microorganisms were, respectively, 94, 72, and 51% at noncarious root sites and 98, 71, and 54% at root lesion sites. Candida spp. were isolated at frequencies ranging from 12% at noncarious and carious root sites to 90% at enamel lesion sites. Frequencies of isolation of streptococci other than S. mutans ranged from 21% for S. salivarius to 85% for Streptococcus spp., both at noncarious root sites (Table 1).

Microbial populations associated with dental caries activity are compared graphically in Fig. 2 as the mean percent of the total anaerobic cultivable microflora recovered at the respective sample sites. The streptococci made up the largest proportion, ranging from 31.2% at noncarious enamel sites to 37.6% at root lesion sites. S. mutans varied between 18% at the noncarious enamel and root sites and 24% at the enamel and root lesion sites. Total proportions of actinomyces ranged from 12.3% at root lesion sites to 23.6% at noncarious root sites, while A. viscosus varied from 7.8% at root lesion sites to 15.1% at noncarious root sites. The highest proportion of Lactobacillus spp. (4.2%) was recovered from enamel lesions.

Statistical comparisons between sample sites for differences within microbial populations revealed no significant

![Microorganisms](http://iai.asm.org/downloaded.png)

FIG. 2. Proportions of total streptococci, S. mutans, total actinomyces, A. viscosus, and Lactobacillus spp. recovered from noncarious and carious enamel and root sites. The numbers above the standard errors are the n values.
differences between noncarious enamel and noncarious root sites, but both the noncarious enamel and root sites had higher ($P < 0.05$) actinomyces proportions than did root lesion sites. In addition, enamel lesions had a greater ($P < 0.05$) percentage of Lactobacillus spp. than did root lesions (Fig. 2). Comparisons between microbial groups within each sample site showed, as expected, that the proportions of total streptococci were greater ($P < 0.05$ to $P < 0.001$), while those of lactobacilli were lower ($P < 0.001$), than the proportions of other microbial populations at all sites except at enamel lesion sites. The only significant difference ($P < 0.05$) among microbial populations at enamel lesion sites was between the proportions of both total streptococci and S. mutans compared with that of Lactobacillus spp.

To ascertain whether any differences existed between initial lesions and the more advanced lesions of extracted teeth, a statistical analysis of the proportions of the caries-associated microbial populations recovered from these two classes of lesions was performed. The only difference within microbial populations was a higher ($P < 0.01$) proportion of S. mutans at the initial root lesion sites (33.9%) compared with 14.6% at the advanced root lesion sites (Fig. 3). S. mutans made up 81.1% of the streptococci at initial root lesion sites compared with 44.1% at the advanced root lesion sites (Fig. 3). Within the two root lesion classes, proportions of both total streptococci (41.8%) and S. mutans (33.9%) were higher ($P < 0.001$) than those of total actinomyces (11.5%), A. viscosus (7.8%), and lactobacilli (1.0%); the proportion of Lactobacillus spp. was lower ($P < 0.001$) than that of all other microorganisms at the initial root lesion sites. Likewise, the proportion of total streptococci (33.1%) was higher ($P < 0.01$ to $P < 0.001$), while the proportion of Lactobacillus spp. (1.8%) was lower ($P < 0.01$) than that of other microbial populations at advanced root lesion sites (Fig. 3).

**DISCUSSION**

The intent of this investigation was to determine the quantitative relationship among caries-associated microorganisms recovered from clinically noncarious and carious root and coronal tooth surfaces. Additionally, we hoped to gain insight into the microbial composition of advanced and initial root surface caries. Quantitative comparisons of caries-associated microorganisms recovered from the plaque of nondiseased and diseased sites of both coronal and root surfaces revealed the following important microbiological features of root surface caries. (i) Dental plaque from comparable or adjacent noncarious enamel and root surfaces comprised several caries-associated microbial populations in relatively high, but proportionately equal, numbers. (ii) The proportions of plaque actinomyces are significantly higher ($P < 0.05$) at the noncarious enamel and root sites than at the root lesion sites. (iii) Total streptococci and S. mutans are recovered in plaque from initial root lesions in greater numbers ($P < 0.001$) than either the actinomyces or lactobacilli. (iv) The number of S. mutans recovered from the plaque of initial root lesions is greater ($P < 0.01$) than that recovered from advanced root lesions. These findings suggest that S. mutans may be as closely associated with root surface caries as with coronal caries. This is indicated
by both the frequency of isolation and the proportionate levels of S. mutans recovered at all sample sites of teeth with carious root lesions. Although recovered less frequently and in lower proportions than S. mutans, A. viscosus and Lactobacillus spp., which also are associated with coronal caries, were recovered from both noncarious and carious root surfaces.

The data contained in this report should not be regarded as a precise definition of the cariogenic microflora associated with the tooth surfaces sampled. For example, we have isolated a variety of microorganisms, including Rothia dentocariosa, from both noncarious and carious sites (unpublished data) as well as dextranolytic strains of bifidobacteria from root lesions (12). Furthermore, the data presented here and in other studies of root caries (5, 8, 10, 19, 21) should not be considered entirely comparable because of differences in both the sampling and the culture methods. For example, in the present study both surface deposits of plaque and underlying carious dentin were included in a single sample from a carious root site, while Jordan and Hammond (10) cultured only the softened dentin from the deeper areas of root lesions in extracted human teeth. Also, the specimens they used were cultured on starch agar plus 5% sheep blood for the presence of filamentous bacteria. The use of various media to assess other microbial populations can be expected to show similar differences. Ellen et al. (5) demonstrated variations in population parameters associated with three different media used to quantify S. mutans.

A somewhat less recognized variable affecting recovery from carious lesions is the severity of the lesion itself, as illustrated in this report. Initial root lesions and the more advanced root lesions represent two different ecosystems. In initial root lesions, the microbiological population must, for the most part, be able to adhere to the root surface; S. mutans makes up more than 80% of the streptococci at the initial root lesion. As cavitation becomes more prominent, successional members of the microbiological community not dependent on surface attachment for growth begin to predominate; S. mutans comprises less than 45% of the streptococci at advanced lesion sites. Also, the accumulation of microbiological metabolic products and changes in other chemical and physical characteristics are some of the selective factors associated with advanced root lesions which would create differences between their microflora and that associated with initial root lesions. In this study, the plaque covering the sample sites of the extracted teeth with advanced root lesions was more dense than that covering the sample sites of the in situ teeth with initial lesions.

The findings from this study, however, compare favorably with those reported by Jordan and Hammond (10) and Sumney and Jordan (19) despite the fact that different sampling and culturing techniques were used. Jordan and Hammond (10) reported that several species of actinomyces were identified were strains of R. dentocariosa. Although only filamentous forms were described, streptococci, including some strains of S. mutans, were isolated from the root surface lesions in their study. Using a similar sampling technique but different culture media (5% sheep blood agar and mitis salivarius agar), Sumney and Jordan (19) sampled root lesions in extracted human teeth qualitatively for the presence of bacterial forms from the underlying layers of softened dentin. S. mutans was found to be a predominant organism, and all filamentous organisms appeared to be typical of the genus Actinomyces (19).

In a similar study, Syed et al. (21) determined the qualitative and quantitative composition of the plaque microflora associated with root surface caries from 21 plaque samples. Plaque associated with the root lesions of patients without active enamel caries was recovered with a sterile orthodontic wire and cultured on the nonselective medium MM10 sucrose-blood agar. Two groups of plaque samples were identified. Group I plaque samples had a high concentration (30%) of S. mutans and a low concentration (1%) of Streptococcus sanguis; group II plaque samples had a high concentration (48%) of S. sanguis and no S. mutans. Our data are similar to the group I findings of Syed et al. (21). However, since S. mutans was recovered in relatively high proportions (up to 24%) in all of our samples, while S. sanguis never exceeded 4%, our data differed from their group II findings. This inverse relationship between S. mutans and S. sanguis in carious teeth has been reported previously (3, 8, 14).

Results reported by Hill et al. (8), who examined seven root lesion plaque samples from natives of New Guinea, are in general agreement with our findings; i.e., streptococci were the most predominant microorganisms, while the actinomyces made up a large proportion of the remaining flora. Using horse blood agar for culturing, they found the streptococci to average 68% of the anaerobic flora compared with 26% for the actinomyces (e.g., A. viscosus ≤4%), while the lactobacilli (cultivated on Rogosa SL agar) composed ≤1%. Our study showed that the levels of streptococci and actinomyces were approximately half of those found by Hill et al. (8), while the level of A. viscosus in our study was twice theirs; proportions of lactobacilli were nearly the same in both studies. It is important to reiterate that our study used two different media (GMC and CFAT) to assess the populations of actinomyces. Both media supported the growth of A. viscosus, A. naeslundii, and A. odontolyticus, but A. israelii was only recovered from GMC medium. Although A. israelii could not be recovered from CFAT medium, there were no statistical differences between the estimates of total populations of actinomyces and A. viscosus obtained from either medium.

Ellen et al. (5), noting that previous reports concerning differences in root lesions, data on noncarious surfaces, reported that Streptococcus spp. made up less than 5% of the total plaque flora collected from noncarious root surfaces of high-caries-risk hospitalized subjects when cultured on MM10 sucrose-blood agar. S. mutans, monitored with a mannitol-containing broth, had a 63% detection frequency. These samples were taken from a narrow zone at the midline of the exposed root surface extending coronally from the gingival margin to the cementoenamel junction by using the cutting edge of a sterile lancet. Ellen et al. (5) suggested that their relatively low recovery of streptococci may have been influenced by the institutionalized nature or age of the study population, or both. However, the oral microflora of hospitalized patients, regardless of age, can change greatly, both qualitatively and quantitatively, after admission (4). A combination of parameters may influence this, including changes in diet, personal and oral hygiene habits, medication, and bed confinement, to name a few.

It has been conjectured that diet and environmental/bacterial interactions involved in root surface caries may be different from those associated with coronal caries. However, reports (7, 15) on the high number of cemental caries in narcotic addicts and alcoholics have attributed this increased caries activity to poor oral hygiene, a craving for sweets, and, consequently, an increased sugar intake. Similarly, the frequency of root surface caries was higher in those individ-
uals given sugar between meals in the classic Vipeholm study (6). It is obvious that dietary habits influence coronal caries activity and that high levels of acidogenic and aciduric bacteria such as \textit{S. mutans} and lactobacilli are associated with these lesions. Indeed, Banting et al. (1), in assessing the prevalence of root caries among their institutionalized patient group, found root surface caries to be positively associated with coronal caries and suggested that the coronal caries experience is an important variable for predicting which patients are likely to experience root caries. Ravald and Hamp (17) reported that new root surface caries in high-risk patients demonstrated significant correlations with (i) their previous root caries experience, (ii) high salivary lactobacillus counts, and (iii) low saliva secretion. Similarly, Ellen et al. (Abstr. Int. Assoc. Dent. Res. 1984, no. 430, p. 218) reported that, although \textit{S. mutans} and lactobacillus levels made up a minor percentage of the oral flora in chronically hospitalized patients, the recovery levels of these two organisms were highly correlated ($r = 0.932$) and that their isolation frequency was related to root caries development. Although we recovered different numbers of \textit{S. mutans}, our data also suggest that lactobacilli and, particularly, \textit{S. mutans} are closely associated with root caries. Such findings are highly supportive of the statement by Jordan and Hammond (10) that the "concept of a specific bacterial aetiology for different types of caries should not be interpreted too rigidly in all cases."

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LITERATURE CITED


