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Bacteriology of Moderate (Chronic) Periodontitis in Mature Adult Humans

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A total of 171 taxa was represented among 1,900 bacterial isolates from 60 samples of sites affected with moderate periodontitis in 22 mature adult humans. The composition of the subgingival sulcus flora was statistically significantly different from that of the adjacent supragingival flora and the subgingival flora of 14 people with healthy gingiva, but was not significantly different from that of sulci affected with severe periodontitis in 21 young human adults. The sulcus floras of moderate periodontitis and severe periodontitis shared many of their predominant bacterial species, but there were differences in the relative proportions of some of these species. Similar relationships were found for seven taxa of treponemes that were cultured from the samples.

Moderate periodontitis (MP) with a slow rate of progression is the most common form of destructive periodontal disease and, in different degrees of severity, appears to affect the majority of the population over 35 years of age (7). The progressive tissue destruction probably occurs in discontinuous episodes. Although this clinical form of disease is less dramatic than localized juvenile periodontitis or severe generalized periodontitis (SP) in young adults, its medical significance is probably greater because of its widespread occurrence and consequent ultimate loss of teeth in many patients.

It is not known whether this form of periodontitis is more slowly progressive because of greater resistance in the patient population, or because of differences in the bacterial etiology, or both. To compare the bacterial flora of MP in mature adults with that of SP in young adults and that of mature adults with healthy gingiva, a systematic, statistically designed analysis of the floras was undertaken.

MATERIALS AND METHODS

Subjects. The human subjects were selected by the following criteria to represent adult MP: (i) age, 35 to 55 years; (ii) absence of present or past severe or chronic medical illness; (iii) presence of affected sites of 5- to 7-mm probeable depth and concomitant loss of periodontal attachment and visible gingival inflammation in all four quadrants of the dentition; and (iv) absence of antibiotic medication for at least 2 months before sampling.

Samples. Sixty MP samples were taken from 38 affected sites in 22 subjects. Samples were taken from proximal sites of 23 first molars, 10 second molars, 4 premolars, and 1 incisor. Of the 60 samples, 22 were of the residual supragingival flora adjacent to the affected sites. These supragingival samples were taken after superficial cleaning of the area with sterile toothpicks to serve as controls for determining possible contamination of subgingival samples next taken from the bottom of the affected sulci.

Healthy control samples. For comparative purposes, samples were taken in a comparable manner, after superficial supragingival cleaning, from the bottom of the gingival crevice of proximal sites on 21 teeth, representing all tooth types, from 14 people 30 to 70 years of age who had no clinical evidence of periodontal disease. These healthy (HP) samples were from a separate set of control subjects and did not include samples from the experimental gingivitis subjects described earlier (5). Some of these HP samples were compared with the experimental gingivitis and SP floras reported previously (5, 6). However, additional HP (control) samples have been analyzed and are included in the comparisons here.

Procedures. Sampling and bacteriological procedures were described in detail previously (2, 5). Briefly, samples were taken with sterile, nickel-plated Morse 00 scalers with detachable tips that were transferred immediately to prereduced, anaerobically sterilized broth under oxygen-free CO_2. Samples were dispersed by shaking with 100-μm-diameter glass beads, diluted in prereduced salts solution, and cultured in roll tubes of D-4 medium (5) and on petri plates of the same medium containing rabbit blood. Portions of the same dilutions also were inoculated to media (5) for the isolation of treponemes and mycoplasma. After 5 days of incubation, bacterial colonies were picked without selection, in a randomized pat-

tern, to obtain a representative cross section of the flora. Usually, 15 colonies from each sample were picked from plates and 15 were picked from roll tubes. All resulting colonies were streaked to check purity. If more than one morphotype or colony type was present, each was isolated and identified. Identification was made by electrophoretic analysis of the soluble cellular proteins (without sodium dodecyl sulfate) (4) and by chromatographic and biochemical procedures described previously (2), supplemented with appropriate tests as required for facultative or nutritionally demanding taxa. Undescribed species were assigned letter and number designations. *Actinomyces* strains that failed to react with available monovalent fluorescent-antibody conjugates were assigned to the species that they resembled phenotypically and denoted by (−). Spirochetes were enumerated from the highest dilutions that produced growth after incubation for 5 to 21 days, isolated, and identified (5).

Statistical analysis. Good's analyses for coverage (1) and Good's L test (5), based on minimum percent similarity (S. S. Socransky, A. C. R. Tanner, and M. J. Goodson, J. Dent. Res. Spec. Issue A **60**:486, abstr. 705, 1981), were used to compare flora compositions.

RESULTS AND DISCUSSION

There were 1,900 bacterial isolates from sites affected with MP. The isolates were distributed among 171 taxa in 29 bacterial genera. Fifty-nine described species accounted for 67% of the residual supragingival flora, and 62 described species accounted for 65% of the subgingival sulcus flora. The remaining 52 supragingival and 74 subgingival taxa were undescribed bacterial species. (A total of 5.4% of the isolates failed to survive through identification [4.9% of the isolates from plates and 5.6% of the isolates from roll tubes].) Treponemes (considered separately) included seven cultured taxa, only two of which have been described. Mycoplasma were cultured from 4 of 17 residual supragingival samples and 5 of 29 sulcus samples.

There were 111 taxa, excluding treponemes, among 694 isolates from 22 supragingival MP samples and 136 taxa among 1,206 isolates from 38 subgingival MP samples. The statistical coverage values (1) were 94.81 ± 0.78% (standard deviation) and 96.43 ± 0.45%, respectively, indicating that the 111 taxa accounted for ca. 95% of the cultivable cells in the supragingival flora and 136 taxa accounted for about 96% of the cultivable cells in the subgingival samples. The 30 taxa that predominated in the residual supragingival samples are listed in Table 1. The 28 taxa that predominated in the MP-affected sulci and that were more numerous in the subgingival flora than in the supragingival samples (based on percentages of flora) are listed in Table 2.

As we found in SP in young adults (6), the composition of the residual supragingival flora was significantly different by L analysis (5) from that of the subgingival sulci in MP ($P = 0.027$). Of 171 taxa detected in the MP flora, 35 occurred only in the supragingival flora and therefore are unlikely agents of tissue destruction in the sulcus. Four of these species are listed in Table 1. Other named species that were detected only in the supragingival flora were *Clostridium putrificum*, *Eikenella corrodens*, *Lactobacillus jensenii*, *Propionibacterium granulosum*, *Streptococcus intermedius* II, *Streptococcus morbillorum*, and *Streptococcus mutans*.

TABLE 1. Predominant supragingival MP taxa that were less numerous (as % of isolates) in the adjacent subgingival MP flora[a]

Taxon	% of flora / % of samples positive		
	Sub-MP	Sup-MP	Sub-HP
Streptococcus sanguis II	2.0/34	5.6/45	6.3/71
Veillonella parvula	2.4/37	5.5/55	4.3/57
Capnocytophaga ochracea	0.4/11	4.3/50	4.0/33
Streptococcus sanguis I	1.9/34	4.2/64	2.4/38
Actinomyces NV[b]	2.8/21	3.7/50	3.7/33
Streptococcus anginosus	2.8/45	3.2/41	1.2/24
Actinomyces naeslundii (-)[c]	1.5/26	3.0/50	5.9/67
Actinomyces naeslundii I	1.7/18	3.0/36	5.3/43
Capnocytophaga gingivalis	0.4/13	2.0/27	0.4/14
Actinomyces viscosus II	0.1/3	2.0/36	0.6/14
Selenomonas D-4[d]	0.3/11	1.7/23	1.2/10
Actinomyces odontolyticus I	0.7/18	1.6/41	1.6/19
Actinomyces odontolyticus (-)[c]	0.9/18	1.6/32	1.0/19
Rothia dentocariosa	0.0/0	1.4/5	0.3/5
Leptotrichia buccalis	0.1/3	1.3/32	0.1/5
Actinomyces WVA 963	0.3/11	1.3/27	2.5/29
Coccus SM1	0.6/16	1.2/23	1.8/19
Actinomyces israelii (-)[c]	0.7/16	1.2/23	0.7/24
Bacteroides denticola	0.2/5	1.0/18	0.4/14
Veillonella dispar	0.2/8	1.0/9	0.3/5
Actinomyces israelii II	0.1/3	0.9/14	0.0/0
Bacillus circulans	0.0/0	0.9/5	0.3/10
Bacteroides oris	0.6/11	0.9/18	0.3/10
Fusobacterium D-3[d]	0.1/3	0.9/14	0.0/0
Streptococcus D-7[d]	0.2/3	0.9/9	7.8/43
Bacteroides D-31[d]	0.1/3	0.7/14	0.0/0
Eubacterium saburreum	0.0/0	0.6/14	0.4/14
Selenomonas D-1[d]	0.0/0	0.6/18	0.0/0
Capnocytophaga sputigena	0.2/3	0.6/18	0.7/19
Bacteroides gracilis	0.4/8	0.6/18	1.5/29

[a] There were 1,206 subgingival MP isolates, 694 supragingival MP isolates, and 679 subgingival HP isolates.

[b] *Actinomyces* NV is an electrophoretically distinct group that reliably cross-reacts with *A. naeslundii* I and *A. viscosus* I antisera.

[c] Taxa marked (-) are serologically negative but have the phenotypic characteristics of the species.

[d] Undescribed species are designated by letters or numbers.

TABLE 2. Predominant subgingival MP taxa that were less numerous (as % of isolates) in the adjacent supragingival MP samples or in subgingival samples from people with healthy periodontia (HP)[a]

Taxon	% of flora / % of M2 samples positive		
	Sub-MP	Sup-MP	Sub-HP
Fusobacterium nucleatum[b]	10.8/76	6.3/68	4.3/48
Peptostreptococcus micros[b]	4.3/50	3.2/32	1.9/14
Eubacterium nodatum[b]	3.6/42	0.4/14	0.0/0
Bacteroides intermedius 4197[b,c]	3.1/37	2.0/18	0.0/0
Eubacterium timidum[b]	3.0/39	0.9/18	0.9/14
Wolinella recta	2.7/39	1.2/23	0.3/10
Eubacterium brachy[b]	2.7/32	0.0/0	0.0/0
Lactobacillus D-2[b,d]	2.1/21	1.0/32	0.9/14
Eubacterium D-11[b,d]	2.1/18	0.0/0	0.0/0
Actinomyces naeslundii III	2.0/13	0.6/14	1.2/14
Bacteroides gingivalis	1.7/13	0.0/0	0.0/0
Actinomyces israelii I	1.6/16	0.0/0	1.3/14
Peptostreptococcus anaerobius	1.5/24	1.4/14	0.0/0
Eubacterium D-8[b,d]	1.5/21	0.1/5	0.0/0
Bacteroides intermedius 8944[b,c]	1.4/13	0.3/9	0.4/14
Fusobacterium RD[d]	1.4/26	0.0/0	0.0/0
Selenomonas sputigena[b]	1.3/24	0.4/9	0.6/14
Wolinella HS[d]	1.2/24	0.9/14	0.1/5
Lactobacillus minutus[b]	1.2/21	0.6/18	0.3/10
Eubacterium D-6[b,c]	0.7/16	0.6/14	0.1/5
Eubacterium alactolyticum[b]	0.5/13	0.1/5	0.0/0
Staphylococcus epidermidis	0.5/13	0.4/14	0.4/14
Bacteroides pneumosintes[b]	1.1/5	0.1/5	0.3/10
Bacteroides loscheii	0.6/11	0.0/0	0.3/10
Fusobacterium D-2[b,d]	0.6/11	0.3/9	0.0/0
Haemophilus aphrophilus	0.5/11	0.0/0	0.4/5
Bacteroides oralis	0.5/8	0.1/5	0.3/10
Selenomonas D-6[d]	0.5/8	0.1/5	0.0/0

[a] There were 1,206 subgingival MP isolates, 694 supragingival MP isolates, 679 subgingival HP isolates.

[b] Species among the most likely etiologic agents of SP (6).

[c] There are two genetically distinct species among strains designated *B. intermedius* (3). These were differentiated by electrophoretic pattern and are designated here by the VPI number of the DNA homology reference strain.

[d] Undescribed species are designated by letters or numbers.

Of the 136 taxa that were detected in the subgingival MP flora, 37 occurred in equal or greater concentrations (as a percentage of the flora) in the subgingival samples from people with healthy gingiva and therefore can be considered to be unimportant as direct causative agents of periodontitis. Most of these species, including all three described species of the genus *Capnocytophaga*, were considered to be unimportant in SP for the same reason (6). Several of the species are listed in Table 1. Other described taxa in this group were "*Actinomyces meyerii*," *Arachnia propionica*, *Bacterionema matruchotii*, *Campylobacter concisus*, *Propionibacterium acnes*, *Staphylococcus haemolyticus*, *Streptococcus intermedius* IV, and *Streptococcus mitis*.

Fourteen additional species were more numerous proportionately (as a percentage of the flora) in the residual supragingival MP flora than in the subgingival MP flora. Although these species might contribute to the disease, their distribution suggests that the sulcus is not their major site of proliferation and that they are less active in the sulcus, so they are unlikely to be the primary agents of periodontitis. In addition to some taxa listed in Table 1, named species in this group included *Bacteroides disiens*, *Fusobacterium naviforme*, and *Staphylococcus saprophyticus*.

The remaining 85 subgingival taxa appeared more frequently in the subgingival MP flora (as a percentage of 1,206 isolates) than in the HP subgingival flora or in the adjacent residual supragingival MP flora. Of these 85 species, 57 each occurred at less than 0.5% of the subgingival MP flora. Although they cannot be ruled out as possible etiological agents, their presence in low numbers and in only 1 to 4 of the 22 patients suggests that they are not of primary, or frequent, importance. In most diseases and environments, the impact of individual species is roughly in proportion to their concentration. However, because MP is slowly and erratically progressive, it is possible that this list includes clinically significant species that might increase during periods of active disease.

Of these 57 species, 26 were represented by a single isolate. They included *Lactobacillus acidophilus*, *Neisseria flava*, *Propionibacterium avidum*, and *Streptococcus parvulus*. Thirteen others occurred in only a single sample; the named species among these were *Acinetobacter calcoaceticus* at 0.2% of the sulcus flora, *Actinomyces viscosus* (−) at 0.4%, *Bifidobacterium dentium* at 0.3%, and *Haemophilus paraphrophilus* at 0.2%. Named species among the 18 other taxa that each represented less than 0.5% of the MP subgingival flora but occurred in two to four samples from affected sites included the following: *Actinobacillus actinomycetemcomitans*, 0.3%; *Bacteroides buccae*, 0.4%; *Bacteroides buccalis*, 0.2%; *Bacteroides capillosus*, 0.2%; *Bacteroides melaninogenicus* (genospecies 2381), 0.2%; *Bacteroides zoogleoformans*, 0.3%; and *Lactobacillus catenaforme*, 0.2%. All 57 species together comprised 9.79% of the MP subgingival isolates. The 28 taxa that predominated in the MP subgingival flora and that remain most highly suspect are listed in Table 2.

Although the taxa listed in Table 2 include the

most likely agents of MP, we do not believe that all of them are clinically significant in the etiology of the disease. Species that increase in the sulci of the diseased population could all be agents of a single mixed-culture disease or could include one or a few agents of the disease and others that were commensals that arose only in response to the changed environment.

Comparisons of the data in Table 2 with the developing flora in experimental gingivitis reported previously (5) indicate that most of the taxa listed in Table 2 did not arise simply as a result of the disease. *Fusobacterium nucleatum*, *Peptostreptococcus micros*, *Eubacterium timidum*, *Eubacterium brachy*, *Lactobacillus* D-2, *Actinomyces naeslundii* III, *Peptostreptococcus anaerobius*, *Eubacterium* D-8, *Bacteroides intermedius* 8944, *Fusobacterium* RD, *Selenomonas sputigena*, *Eubacterium* D-6, *Bacteroides pneumosintes*, and *Haemophilus aphrophilus* were each positively correlated with developing gingivitis, and a physiological role can be postulated for them. *F. nucleatum*, *P. micros*, *E. brachy*, *A. naeslundii*, *P. anaerobius*, *B. intermedius* 8944, and *B. pneumosintes* also are known to be pathogenic in other body sites.

Wolinella recta, *Actinomyces israelii* I, *Wolinella* HS, *Lactobacillus minutus*, and *Fusobacterium* D-2 also showed increases over the healthy or experimental gingivitis floras, indicating that they might play a role in periodontitis.

Because periodontitis is believed to occur after gingivitis, species that were first detected in gingival index (GI) 2 sites (probably as a result of increased serum and blood) are especially suspect as etiological agents. These include *Eubacterium nodatum*, *B. intermedius* 4197, and *Selenomonas* D-6.

Bacteroides ginginvalis was not detected among the 1,341 supragingival or subgingival HP isolates or among the 3,035 experimental gingivitis isolates (5). It respresented 1.7% of the MP subgingival flora and 0.2% of the SP subgingival flora (6). Similarly, *Eubacterium* D-11 and *Eubacterium alactolyticum* were absent from the HP and experimental gingivitis populations. Of these three, only *E. alactolyticum* now is known to occur in clinical infections of other body sites. Because these species appear to be unique to periodontitis in the oral flora, they may seem to be the agents of the disease. However, because they did not occur in the early stages of disease (i.e., GI 2 sites), it is equally possible that they occur as the result of the disease and that tissue destruction must occur before their appearance. The observation (S. S. Socransky, personal communication) that *B. gingivalis* is isolated most reliably from very advanced SP may support the suggestion that the species arises as a result of the disease.

Because the incidence and frequency of *Staphylococcus epidermidis*, *Bacteroides loescheii*, and *Bacteroides oralis* differed little between MP and HP, these three species probably have no clinical significance.

Spirochetes were detected microscopically in 7 of 17 (41%) MP supragingival samples, 22 of 29 (76%) subgingival samples, and 0 of 9 HP subgingival samples. Frequency comparisons with 42 subgingival SP samples (6) are shown in Table 3. These data suggest that *Treponema vincentii*, *Treponema denticola*, treponemes A, A-1, and D, and the "large treponeme" may have clinical significance.

If we assume that certain species most commonly are the causative agents of MP as it occurs in the general population, we could eliminate from the lists in Tables 2 and 3 those that were detected in only a few of the patients. (In this context we are considering the total clinical MP population, and we are interested first in

TABLE 3. Selected flora of samples from moderate and severe periodontitis[a]

Taxa	% of samples positive[b]		
	RS-MP	Sub-MP	Sub-SP
Microscopic			
Total treponemes	41	76	88
"Large treponeme"	0	7	64[c]
Cultural			
Treponeme A[d]	65	62	55
Treponeme A-1	6	14	14[c]
Treponeme D	6	10	14[c]
Treponeme E	6	3	0
Treponeme F	6	0	2
Treponeme J	6	3	12
Treponeme K	0	0	2
Treponeme L	0	0	2
Treponeme N	0	0	2
Treponema denticola	0	17	21[c]
Treponema vincentii	0	14	0
Mycoplasma	23	17	55

[a] No spirochetes were detected in any of 9 subgingival samples from 8 people with healthy gingiva.

[b] RS, residual supragingival; sub, subgingival samples; MP, moderate periodontitis; SP, severe periodontitis.

[c] Taxa among the most likely etiologic agents of SP (6).

[d] Undescribed taxa are designated by letters and numbers.

agents that are most commonly responsible. All species that relate to the etiology should increase in this population as compared with the healthy population, and they may be expected to increase generally in proportion to their overall significance in the moderate periodontitis population. [This was the rationale for sampling relatively few isolates from more patients. Complete analysis of each bacterial isolate is both expensive and time-consuming, which are major limiting factors.]) Because only 30 isolates represented each sample (average coverage, 70%) and because disease activity is sporadic, suggesting that the flora might vary (or perhaps only that the resistance of the patient changes), we arbitrarily chose 5 (13%) of 38 subgingival samples as a minimum frequency that might indicate significant clinical importance. Of the species listed in Tables 2 and 3, *Selenomonas* D-6, *B. pneumosintes*, *B. loescheii*, *B. oralis*, *Fusobacterium* D-2, and *H. aphorphilus* failed to meet this criterion. Treponemes A-1 and D, the "large treponeme," *T. denticola*, and *T. vincentii* remained highly suspect. These five treponemes and 22 other bacterial species that were present in 13% or more of the subgingival samples remain as the most likely causative agents of MP.

L analysis (5) of all samples (supragingival and subgingival) from each person (11 people with two samples, 9 people with four samples) showed that the floras of people were significantly different ($P = 0.001$). This difference should help to pinpoint the bacterial species that occur in most patients and may represent frequent etiological agents of MP.

In addition, subgingival samples from each of 13 subjects (11 pairs, one set of 3 and one set of 4) showed that the subgingival floras of different people were significantly different ($P = <0.001$). Multiple supragingival samples from seven MP subjects also showed differences between people, but with a lower probability ($P = 0.052$). Because the number of multiple samples (required for L analysis) differed, we could not assume that there was greater person similarity in the supragingival flora than in the subgingival MP flora. To make the analyses comparable, we analyzed seven pairs of subgingival samples from the same seven subjects from whom paired supragingival samples were available. In this comparison, the subgingival flora differed with a probability of 0.049, similar to that observed with comparison of the supragingival flora ($P = 0.052$). Thus, with the numbers of samples now available, there is no evidence that either the supragingival or the subgingival flora is less variable among MP patients.

L analysis (5) showed that the composition of the MP subgingival flora was significantly different from the HP subgingival flora ($P = <0.001$). The MP subgingival flora was not significantly different from that of the SP subgingival flora (6) ($P = 0.165$). The MP and SP supragingival floras each were significantly different from the HP supragingival flora ($P = 0.003$), but not from each other ($P = 0.54$). To obtain a valid L analysis, it was necessary to combine all subgingival or supragingival samples from each person into a single sample (representing the flora of that person) and then compare the floras of the MP subjects with those of the HP or SP people. Comparison of all MP samples with all HP or SP samples would not be a valid test because the floras of different people within each population were significantly different and the multiple samples from each person could not be considered to be independent observations.

Direct comparisons of the MP and SP floras showed 136 taxa among 1,206 subgingival MP isolates (coverage, 96.43 ± 0.45%) and 146 taxa among 1,711 subgingival SP isolates from 46 samples (coverage, 97.83 ± 0.32%), indicating that the complexity of the two floras was similar. In addition, many of the same species predominated in both the MP and the SP subgingival floras. Species that occurred at about the same levels in subgingival MP and SP samples were *A. naeslundii* III, *B. intermedius* 4197, *B. intermedius* 8944, *B. loescheii*, *B. oralis*, *Fusobacterium* D-2, *F. nucleatum*, *Lactobacillus* D-2, *P. micros*, and *Staphylococcus epidermidis*. However, the proportions of other predominant species differed. Species that were more numerous in MP (as a percentage of the subgingival flora) than in SP were *A. israelii* I, *B. gingivalis*, *B. pneumosintes*, *E. brachy*, *Eubacterium* D-11, *Fusobacterium* RD, *H. aphrophilus*, *P. anaerobius*, *Selenomonas* D-6, *Selenomonas sputigena*, *Wolinella* HS, and *W. recta*. The predominant subgingival MP bacterial species that were in higher concentrations in SP than MP were *Eubacterium* D-8, *E. nodatum*, *E. timidum*, *L. minutus*, and *E. alactolyticum*. Other species that had increased importance in the SP subgingival flora were *Streptococcus anginosus* and *Streptococcus* D-39.

The most suspect species of the MP flora included four of six treponemes and 14 of 22 other bacterial species that also were the most likely causative agents of SP in young adults (6). Thus, it appears that although the ratios of several of the predominant species differ in the two disease classifications, the etiologies of SP and MP are very similar or the same and SP may be an exaggerated form of MP.

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