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Genotypic Heterogeneity of *Streptococcus oralis* and Distinct Aciduric Subpopulations in Human Dental Plaque

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The genotypic heterogeneity of *Streptococcus oralis* isolated from the oral cavity was investigated using repetitive extragenic palindromic PCR. Unrelated subjects harbored unique genotypes, with numerous genotypes being isolated from an individual. *S. oralis* is the predominant aciduric bacterium isolated from noncarious tooth sites. Genotypic comparison of the aciduric populations isolated at pH 5.2 with those isolated from mitis-salivarius agar (MSA) (pH 7.0) indicated that the aciduric populations were genotypically distinct in the majority of subjects ($\chi^2 = 13.09$; $P = 0.0031$). Neither the aciduric nor the MSA-isolated strains were stable, with no strains isolated at baseline being isolated 4 or 12 weeks later in the majority of subjects. The basis of this instability is unknown but is similar to that reported for *Streptococcus mitis*. Examination of *S. oralis* strains isolated from cohabiting couples demonstrated that in three of five couples, genotypically identical strains were isolated from both partners and this was confirmed by using *Salmonella enteritidis* repetitive element PCR and enterobacterial PCR typing. These data provide further evidence of the physiological and genotypic heterogeneity of non-mutans streptococci. The demonstration of distinct aciduric populations of *S. oralis* implies that the role of these and other non-mutans streptococci in the caries process requires reevaluation.

The initiation of dental caries is associated with the ability of dental plaque to produce acid from ingested foods on a caries-prone tooth surface. At caries-prone sites, smooth tooth surfaces, interproximal sites, pits and fissures where plaque accumulates (1, 3, 10), and certainly within carious lesions, the local pH is acidic, and the bacteria present in these sites must be aciduric, exhibiting an ability to replicate in the prevailing or transient acidic environment. Dental plaque contains many species of acidogenic and aciduric microorganisms. The acidogenic bacteria most closely associated with the dental caries process are mutans streptococci (*Streptococcus mutans* and *Streptococcus sobrinus*), lactobacilli, and perhaps *Actinomyces* spp. There has been considerable discussion as to the role of other bacteria, in particular the role of the non-mutans streptococci (NMS), in the initiation and progression of dental caries. In an attempt to obtain understanding of the potential pathogenic role of these, van Houte and colleagues studied the acidogenicity of NMS isolated from sound and carious tooth sites (29, 35, 36). In these studies, the NMS were heterogeneous with respect to acidogenicity. Thus, from infected dentine within carious lesions and from plaque on sound surfaces in the mouths of caries-active subjects, NMS which were more acidogenic than NMS were isolated from sound tooth surfaces in caries-free subjects. The clonality of these strains has not been reported nor was the aciduricity of the isolates investigated. However, this was the first detailed and focused report of heterogeneity amongst individual NMS species of a determinant expected to be a significant feature of any microorganism involved in the initiation of dental caries. The acidogenic NMS were also more numerous than mutans streptococci, and it was proposed that these organisms may play a significant role in the caries process.

The aciduricity of bacteria isolated from the dental plaque biofilm has been investigated in a number of studies with a variety of in vitro techniques, primarily by determining the ability of isolates to metabolize carbohydrates and survive at acidic pH levels (11, 12, 15, 18, 23, 31, 32). These studies have demonstrated that lactobacilli and mutans streptococci are the most aciduric dental plaque bacteria, while NMS and *Actinomyces* spp. are less aciduric. However, the strains tested in those experiments were all isolated from conventional selective and nonselective culture media, which may have influenced the phenotypes of the strains isolated and subsequently examined. The predominant aciduric component of dental plaque has not been extensively investigated. In a preliminary report, we indicated that the predominant aciduric bacteria isolated from dental plaque taken from noncarious surfaces were NMS, with *Streptococcus oralis*, *Streptococcus parasanguinis*, and *Streptococcus intermedius* being the most frequently isolated (8). In this paper, we extend these observations and report the genotypic characterization of aciduric *S. oralis* strains from saliva and interproximal dental plaque samples. Representatives of the aciduric isolates from each subject were genotyped by using repetitive extragenic palindromic PCR (REP-PCR [2]) and were compared to those isolated from conventional culture media (pH 7.0). The stability of the *S. oralis* populations was assessed over periods of up to 12 weeks, and the transmissibility of strains was determined by comparing the *S. oralis* genotypes in the plaque flora of cohabiting couples.

MATERIALS AND METHODS

Isolation of *S. oralis* from saliva and interproximal plaque by using MSA and aciduric media. Paraffin wax (Ivoclar-Vivadent, Schaan, Liechtenstein)-stimulated saliva was collected from volunteers and decimally diluted in fastidious anaerobe broth (LabM; Salford, Lancs, England), and 100-μl aliquots were plated onto mitis salivarius agar (MSA) (Becton-Dickinson, Oxford, England) supplemented with 0.1% potassium tellurite (Becton-Dickinson) for the isolation of

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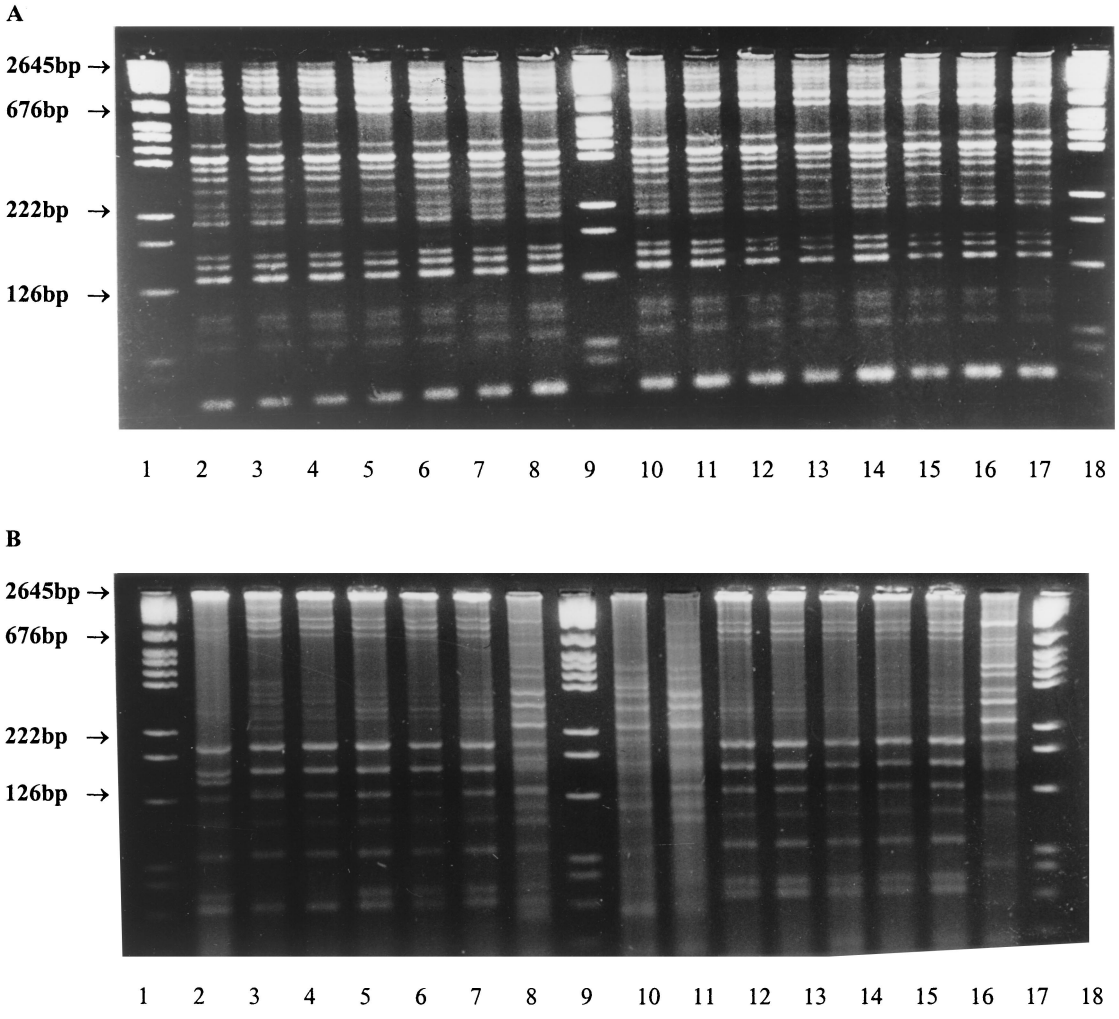

FIG. 1. REP-PCR patterns of *S. oralis* strains isolated from interproximal plaque samples using (a) MSA and (b) MPN methods from two subjects. Lanes 1, 9, and 18 contain molecular size markers.

viridans streptococci. Interproximal dental plaque was collected from a single caries-free site in each subject. The plaque samples were collected by using sterile wooden toothpicks, and each sample was placed in 1 ml of sterile PBSTC (1.58 g of $K_2HPO_4 \cdot 3H_2O$, 0.34 g of KH_2PO_4, 8 g of NaCl, 1.0 g of sodium thioglycollate, and 0.001 g of cetyltrimethylammonium bromide per liter of distilled water). The samples were dispersed by vortexing with sterile glass beads (BDH), decimally diluted in PBSTC, and plated onto MSA. The MSA plates were incubated anaerobically at 37°C for 3 days.

In order to isolate sufficient *S. oralis* strains from each sample plated onto the MSA, 75 colonies not exhibiting extracellular polysaccharide production were picked from each sample and inoculated into 200 μl of Todd-Hewitt broth (Oxoid, Basingstoke, Hants, England) in flat-bottomed microtiter trays (Griffiths and Neilson, Billinghurst, Kent, England) and were grown anaerobically for 48 h. Each isolate was tested for sialidase activity (6) by transferring 50 μl of the Todd-Hewitt broth suspension to 20 μl of sialidase substrate [200 μg of 2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid (Sigma, Poole, Dorset, England) in 50 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) buffer, pH 7.5] in a flat-bottomed microtiter tray. The enzyme assays were incubated aerobically at 37°C for 3 h, and an increase in fluorescence, demonstrating sialidase activity, was measured by using a fluorimeter (Perkin-Elmer, Beaconsfield, Hants, England) at emission and excitation wavelengths of 380 and 460 nm, respectively. The remainder of the cultures were supplemented with glycerol to final concentrations of approximately 50% (vol/vol), and the microtiter plates were stored at −80°C.

The preliminary screening for sialidase-positive streptococci identified those isolates which were *S. oralis*, *Streptococcus mitis*, *S. intermedius*, or *Abiotrophia adjaciens* (17, 28, 39). In order to identify the *S. oralis* strains from amongst the collection of sialidase-positive strains, all of these isolates were tested for the production of β-fucosidase, β-*N*-acetylgalactosaminidase, and β-*N*-acetylglucosaminidase by using the appropriate fluorogenic substrate as previously described (4). Those strains which were negative for β-fucosidase, positive for the two hexosaminidase activities, and positive for sialidase activity were identified as *S. oralis*, and the identification was confirmed by using further physiological tests, including fermentation of a range of carbohydrates and hydrolysis of arginine and esculin (4).

Aciduric *S. oralis* strains were also isolated from each interproximal plaque sample. This isolation procedure employed was the most probable number (MPN) technique. The predominant aciduric bacteria in each sample capable of growth and proliferation in acidic media were isolated by using brain heart infusion (BHI) (Oxoid) adjusted to pH 5.2 by using citric acid and Na_2HPO_4 at final concentrations of 13 and 26 mM, respectively. Each plaque sample was decimally diluted in PBSTC to give a dilution series from 10^{-1} to 10^{-8}, and 12 replicates of each dilution, 15 μl of plaque dilution in PBSTC, and 135 μl of acidic BHI were set up in sterile flat-bottomed microtiter trays and incubated anaerobically for 5 days. The terminal pH of wells exhibiting growth was measured and found to be ±0.05 pH units of the original pH of the medium. Terminal wells, the lowest dilutions exhibiting bacterial growth were subcultured onto Columbia agar (Oxoid) supplemented with 5% (vol/vol) horse blood and were incubated anaerobically for 48 h. The colonies were examined by gram-staining, and those that appeared as gram-positive cocci were identified. Aciduric *S. oralis* strains were isolated from each subject, and these were stored in glycerol broth at −80°C until required for subsequent genotypic analysis (2). For the majority of the investigations, the MPN method was used as a convenient multiwell procedure for the isolation of aciduric *S. oralis* strains from samples, but the MPN method was used to quantitate the proportion of *S. oralis* in 18 interproximal samples. From these samples, the number of aciduric *S. oralis* strains as a percentage of the total bacterial count determined using the MPN method with BHI at pH 7.0 was calculated, and the proportion of *S. oralis* strains

TABLE 1. Genotypic heterogeneity of *S. oralis* strains isolated from interproximal plaque using MSA and aciduric media (MPN) of 15 adult subjects

Subject	Medium	No. of isolates	Subject-specific *S. oralis* genotype no.[a]														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	MSA	15	**14**	1													
	MPN	15	**10**		2	1	1	1									
2	MSA	15	6	1	5	2	1										
	MPN	5						1	1	1	1	1					
3	MSA	15	8	2	1	1	1	1	1								
	MPN	15								14	1						
4	MSA	15	15														
	MPN	15		10	2	1	1	1									
5	MSA	14	8	2	1	1	1	1									
	MPN	3							1	1	1						
6	MSA	16	4	1	1	1	1	1	1	1	2	1	1	1			
	MPN	20													15	4	1
7	MSA	15	**6**	2	2	1	1	1	1	1							
	MPN	7	**2**								1	1	1	2			
8	MSA	10	10														
	MPN	11		4	6	1											
9	MSA	10	**7**	2	1												
	MPN	9	**6**			1	1	1									
10	MSA	5	1	1	1	1	1										
	MPN	11						2	6	1	1	1					
11	MSA	20	**20**														
	MPN	20	**19**	1													
12	MSA	17	8	2	2	1	1	1	1	1							
	MPN	16								10	4	1	1				
13	MSA	13	5	3	2	1	1	1									
	MPN	16							3	2	11						
14	MSA	12	10	2													
	MPN	10			4	2	2	1	1								
15	MSA	11	5	2	2	1	1										
	MPN	6						1	1	1	1	1	1				

[a] Numbers in boldface indicate strains of the same genotype recovered from acidic media and MSA from each subject.

isolated from MSA was expressed as a percentage of the total bacterial count on nonselective media (9).

Genotyping of *S. oralis*. Individual *S. oralis* strains were genotyped by using repetitive extragenic palindromic-PCR (REP-PCR) which, as previously described, results in amplicon patterns which are unique for each independent isolate of *S. oralis* and all other species of viridans streptococci (2). For confirmatory purposes, enterobacterial PCR (ERIC-PCR) and *Salmonella enteritidis* repetitive element PCR (SERE-PCR) were also used as previously described (2).

Visualization of amplicons. The amplification products of REP-PCR and ERIC-PCR were analyzed by using 2% Metaphor agarose (Flowgen, Staffordshire, England) containing 0.5 μg of ethidium bromide per ml and were separated electrophoretically on 20- by 25-cm gels at 140 V for 3 h in Tris-borate-EDTA buffer. SERE-PCR products (15 μl) were resolved on 0.8% agarose (Sigma) containing 0.5 μg of ethidium bromide per ml by electrophoretic separation at 140 V for 3 h. To all samples, 3 μl of tracking dye (0.25% bromophenol blue, 0.25% xylene cyanol FF, 30% glycerol) was added, and molecular size marker (pGEM DNA Markers; Promega, Southampton, England) were included on all gels, in three to four separate lanes, to facilitate comparison of tracks between gels. Gels were examined on a transilluminator and were photographed by using Polaroid type 665 positive-negative film (Sigma).

Computer-assisted analysis of the DNA patterns. All of the patterns of the isolates from an individual were compared by using GelCompar version 4.0 (Applied Maths, Kortrijk, Belgium). The individual bands in each of the patterns produced by the different PCR methods were analyzed by applying the Dice coefficient to the peaks. For clustering, the unweighted pair group method using mathematical averages (UPGMA) was used, and a band position tolerance of 1.5% was used for comparison of the DNA patterns. The analysis of the patterns was undertaken in accordance with the instructions of the manufacturer. Differences in the frequency of recovery of genotypes were compared statistically using the χ^2 test (Fisher's exact test).

Genotypic heterogeneity of *S. oralis* isolated from individuals. Interproximal plaque samples were taken from 15 subjects, and *S. oralis* strains were isolated from each sample by using MSA and the multiwell method as described above. The genotypes present in each subject, isolated on each medium, were compared by cluster analysis, and the similarity of the strains from the two culture conditions in each subject was determined.

Stability of *S. oralis* genotypes. The stability of the *S. oralis* populations in individual mouths was determined in two experiments. In the first, an oral rinse was obtained from each of five unrelated adults on three separate occasions: at baseline and 4 and 12 weeks later. The *S. oralis* component of the flora was isolated from each of the oral rinses by using MSA only. *S. oralis* isolates present in each sample were identified, and individual strains were genotyped by using REP-PCR. The genotypes of these populations were compared by cluster analysis, and the stability of the population present at baseline was determined over the 12-week period.

In the second experiment, to assess the stability of the aciduric *S. oralis* population in interproximal plaque, samples were taken from 10 adult subjects on two separate occasions 4 weeks apart. The predominant aciduric bacteria in each sample were isolated using the multiwell method as described above. The strains, primarily viridans streptococci, isolated in this way were identified. Those isolates identified as *S. oralis* were genotyped by using the REP-PCR method described above. The stability of the aciduric *S. oralis* populations in each subject was evaluated by cluster analysis.

Transmission of *S. oralis* between subjects. Interproximal plaque samples were taken from five cohabiting couples at the same time, and the *S. oralis* populations were isolated from each sample by using both the multiwell and MSA methods. The genotypes of *S. oralis* in each sample were determined, and the genotypes of each couple were compared by using cluster analysis in order to ascertain the presence of strains, which were apparently indistinguishable from each other, in the interproximal samples of each couple. The presence of such strains would indicate transmission of *S. oralis* between couples.

When strains were isolated from couples which were indistinguishable by REP-PCR, these strains were subjected to additional strain genotyping by using

TABLE 2. Number of different genotypes recovered from saliva samples of subjects at baseline, 1 month, and 6 months

Subject	Time (h)	Subject-specific genotype no.[a]																											
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1	0	1	1	3	1	1	1	1	2	1	1	**4**																	
	1											**5**	1	1	1	1	1	1											
	6																		1	1	1	3	1	1	1	1	1	1	1
2	0	2	1	1	1	2	1	1	1	5	1	1																	
	1												1	1	2	4	1	1	1										
	6																			2	1	1	1	4	1	1	1	1	1
3	0	1	1	10	1	1	1	1	1																				
	1									1	2																		
	6											2	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
4	0	1	1	1	1																								
	1					1	1	1	1	3	1	1	1	1	1	2	1	1											
	6																		6	1	1	1	1	1	1				
5	0	1	**1**	1	1	4	1	1	1	1	1	1	1	1															
	1		**1**												1	1													
	6																1	1	1	1									

[a] Numbers in boldface indicate strains of the same genotype recovered on different occasions from the same subject.

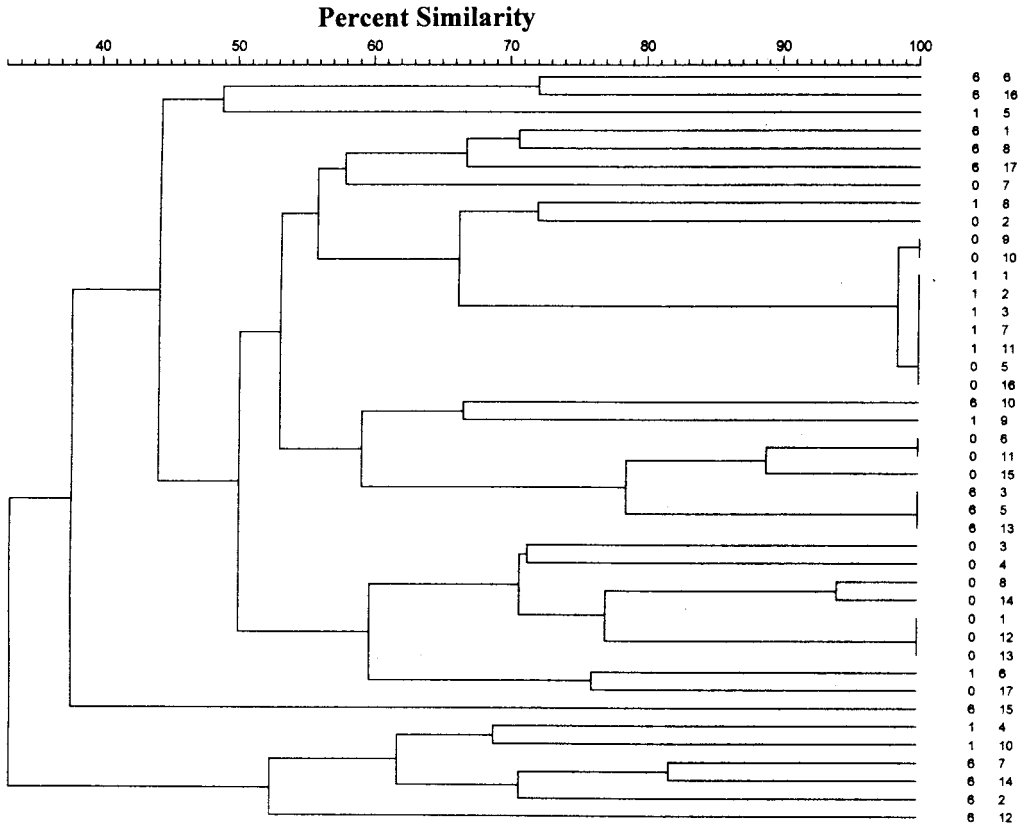

FIG. 2. Dendrogram illustrating genotypic relationships between *S. oralis* strains isolated from the saliva of a single subject. Strain identification is rendered time (0, 1, or 6 months) and strain number. Isolates 1, 2, 3, 7, and 11 from the 1-month sample were indistinguishable from isolates from the 6-month sample. Individual REP-PCR amplicons were marked, and the individual bands were analyzed by using the Dice coefficient and clustering using the UPGMA method.

SERE-PCR and ERIC-PCR methods. This was undertaken since strains which are different by REP-PCR are clearly different, but strains which exhibit the same pattern by only one typing method may be shown to be different when subjected to other typing systems. Therefore, indistinguishable strains should yield similar, but different, band patterns when examined with other typing methods.

RESULTS

Genotypic heterogeneity of *S. oralis* within individuals. In the majority of the 15 subjects, the aciduric *S. oralis* populations were distinct from the *S. oralis* population isolated by using MSA. Representative examples of REP-PCR patterns of multiwell- and MSA-isolated *S. oralis* strains for individual subjects are shown in Fig. 1. In only 4 of the 15 subjects were *S. oralis* strains isolated by the MSA and multiwell methods indistinguishable from each other by REP-PCR typing. The data are summarized in Table 1, which shows the number of strains examined per subject, the number of genotypes per subject amongst those strains examined, and those genotypes recovered from each subject on the MSA and from the aciduric medium. The null hypothesis that each subject harbored only one *S. oralis* population, i.e., that the same genotypes should be recovered from both media, was tested, and it was found that the genotypes recovered from the two media were significantly different ($\chi^2 = 13.09$; $P = 0.00031$). Therefore, it may be concluded that the *S. oralis* populations isolated from the aciduric media were distinct from those recovered from MSA.

The proportion of aciduric *S. oralis* in interproximal plaque samples. The aciduric *S. oralis* isolates represented 1.9% (± 0.7%) of the MPN of bacteria in the samples isolated in BHI at pH 7.0. The MPN of bacteria at pH 5.2 represented 3.8% (± 1.2%) of the total MPN of bacteria at pH 7.0. Thus, on average, the *S. oralis* strains isolated in the media at pH 5.2 represented approximately 50% of the bacterial count. The *S. oralis* isolates recovered from the MSA plates formed 11.2% (± 3.6%) of the total count from the nonselective media.

Stability of *S. oralis* genotypes. To determine the stability of the *S. oralis* populations, strains were recovered by using MSA from the saliva of five subjects sampled over a 12-week interval. There were considerable differences in the number of genotypes of *S. oralis* present in the *S. oralis* population of each subject. When the genotypes of each subject were compared, it was found that in only two subjects were any strains exhibiting the same REP-PCR pattern isolated at baseline and 4 weeks later. In no subject were strains which were identical to those isolated in the 12-week sample isolated in the baseline or 4-week samples (Table 2). A typical dendrogram of these relationships is shown in Fig. 2.

The stability of the aciduric *S. oralis* populations was determined in 10 subjects from whom interproximal plaque was sampled at baseline and after 4 weeks. In five subjects, identical genotypes were recovered on each of the two sampling times. However, in each subject, additional genotypes were recovered from the plaque samples at each sampling time. These data are summarized in Table 3 and illustrate the overall heterogeneity of aciduric *S. oralis* genotypes isolated from the interproximal plaque samples and the instability of the *S. oralis* populations in these subjects.

Transmission of *S. oralis*. *S. oralis* strains were isolated from five couples by using both MSA and the multiwell method. In

TABLE 3. Numbers of genotypes identified amongst the aciduric *S. oralis* strains recovered from the interproximal plaque of 10 subjects sampled at baseline and at 1 month later

Subject	Time (h)	Subject-specific genotype no.[a]																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	0	**7**	2	1	1	1	1	1										
	1	**8**						1	1									
2	0	**2**	1	1	1	2	3	1	1	1	1	1						
	1	**8**											1	6	1	1		
3	0	**8**	3	2	1	1	1	1										
	1	**12**							2	1	1	1						
4	0	**9**	3	2	1	1												
	1	**13**					1	1										
5	0	4	1	1	1													
	1					5												
6	0	**8**	2	1	1	1	1	1										
	1	**3**							3	2	1	1	1	1	1	1	1	1
7	0	2	3	1	1	1												
	1						2	2	1	1	1	1	1	1	1			
8	0	2	2	2	1	1	1	1	1	1								
	1										14	1	1	1				
9	0	2	2	2	1	1	1	1	1									
	1									6	1	2	1	3	1			
10	0	9	4	1	1	1	1											
	1							6	2	2	1	1	1	1	1			

[a] Numbers in boldface indicate strains of the same genotype recovered on different occasions from the same subject.

all, 40 to 62 isolates per couple were genotyped; a total of 258 isolates. On examination of the dendrograms of all strains from each couple, it was apparent that in three couples *S. oralis* strains which were indistinguishable by REP-PCR were isolated from each member of the couple (Fig. 3). In one couple, 3 of 16 strains from the acidic media from one partner were identical to 10 of 16 strains from the other. In the second couple, 15 of 20 strains from the acidic medium were identical to 1 of 7 strains from the acidic medium from the other partner, and in the third couple, 19 of 20 strains from the acidic medium were identical to 1 of 5 strains from the acidic medium from the other partner. When the identical strains from each couple were further examined by SERE-PCR and ERIC-PCR, it was apparent that the strains were indistinguishable by both methods (data not shown).

DISCUSSION

This study has demonstrated that the *S. oralis* population in an individual is heterogeneous, that the aciduric *S. oralis* strains may be genotypically distinct from those isolated from the same plaque sample using MSA, and that the *S. oralis* populations are unstable but are apparently readily transmissible between cohabiting couples. In previous studies, heterogeneity in populations of other dental plaque bacteria has been reported (9, 13, 14, 16, 20–22, 33, 37). Studies of the clonality of the phylogenetically related species *S. mitis* biovar 1 have reported diversity similar to that found in the present study for *S. oralis*. Howhy and Kilian (16) identified 106 *S. mitis* biovar 1 isolates from amongst 250 streptococcal isolates from three members of the same family. The 106 isolates represented 24 different genotypes by restriction endonuclease analysis with 6 to 13 types being isolated from each individual. Similarly, Fitzsimmons et al. (13) isolated 101 *S. mitis* biovar 1 strains from 40 neonates, and when examined by ribotyping, these isolates represented 93 unique types, again demonstrating the high degree of diversity within this species. It is apparent from the data presented in this report that *S. oralis* populations behave in a similar manner. This high degree of genotypic diversity is not restricted to these two species of viridans streptococci, as we demonstrated in a previous study that unrelated members of each of the human species of viridans streptococci exhibited considerable diversity with either REP-PCR, ERIC-PCR, or SERE-PCR (2).

The instability of the *S. oralis* populations reported here is in contrast to the apparent population stability of *S. mutans* (19) and *Prevotella intermedia* and *Prevotella nigrescens* (22) populations in human dental plaque but is more like the reported instability of *S. mitis* biovar 1 in infants (13). The rate of genotypic change must be rapid and may be reflected in the antigenic composition of the organism, providing a mechanism with which to avoid the host's immune system and consequent elimination. It may be expected that all oral bacteria, especially those on mucosal surfaces, including those colonizing the periodontal pocket, would behave in a similar fashion; however, they do not behave in this manner, as evidenced by the reported stability of *P. nigrescens* and *P. intermedia* populations (22). Other mechanisms may underlie the rapid changes in population structure. We found that after 500 in vitro divisions there was no change in the REP-PCR pattern of recently isolated individual *S. oralis* strains (unpublished observations). However, more extensive growth periods, involving 10,000 replications of *Escherichia coli*, resulted in significant changes in genotype associated with movement of transposons within the genome (24). It would seem unlikely that this mechanism for modifying the genotypes of these streptococci is responsible for the changes observed, given the slow doubling time of bacteria in dental plaque (3) and the relatively short interval between sampling times. Both *S. oralis* and *S. mitis* are highly competent and may undergo transformation in vivo by horizontal gene transfer (25), which would be expected to produce the changes in REP-PCR patterns seen here in *S. oralis*. It has also been suggested that the rapid genotypic change may be related to the generation and loss of REP-PCR priming sites during DNA replication (34).

It was demonstrated in this study that *S. oralis* strains with the same genotype were isolated from the dental plaque of cohabiting couples. This is not unexpected, since other viridans streptococci have been shown to be transmitted between couples and between mothers and their infants (7, 13, 16, 21, 27). Transmission is presumably mediated by salivary transfer between partners. It is of note that in each of the three couples where transmission was demonstrable, the strain which appeared to have been transmitted was predominant in one of the partners. The use of the other PCR-based genotyping methods to confirm the similarity of the common genotypes provides unequivocal support for the isolation of the same strains of *S. oralis* from each member of these three couples, since the PCR priming sites are different for each reaction (26, 38).

We have previously reported that *S. oralis* is amongst the most numerous aciduric bacterial species in dental plaque (8) and that mutans streptococci are rarely isolated from among the predominant aciduric bacteria in plaque associated with caries-free tooth surfaces. In this report, we have subjected the *S. oralis* populations to extensive genotypic analysis, and these results considerably extend these previous observations. We have shown that aciduric *S. oralis* populations which are genotypically distinct from those isolated from MSA (initial pH 7.0) exist in plaque. The presence of this distinct population is unexpected, since in other studies it has been demonstrated that although NMS can survive in acidic conditions, their survival was regarded as phenotypic adaptation (32). The present

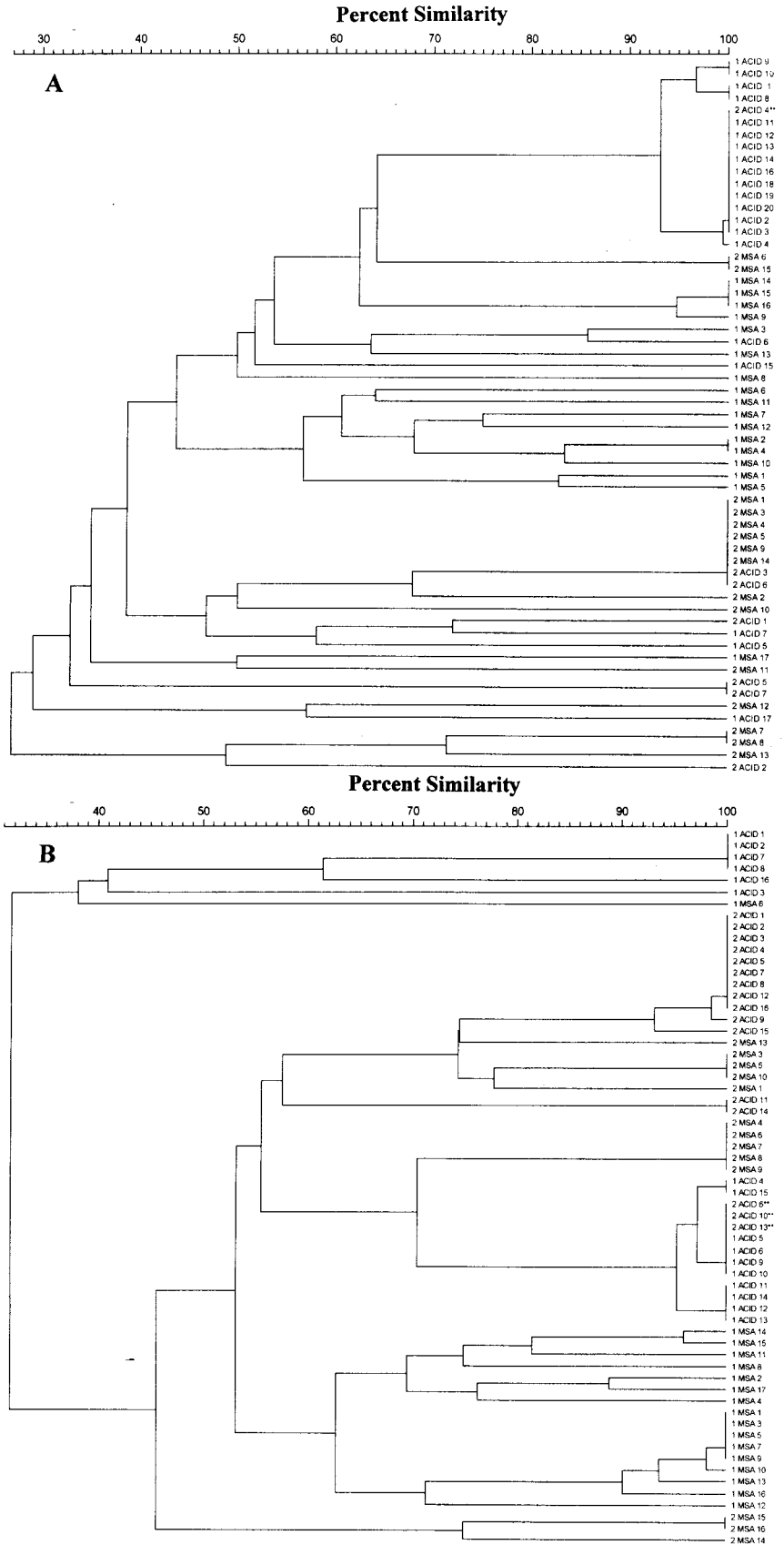

FIG. 3. Representative dendrograms illustrating genotypic relationships between *S. oralis* strains isolated from cohabiting couples A and B with partners identified as 1 and 2. In each couple, the first number indicates the individual partner, MSA and ACID indicate the culture method used to isolate the strains, and the last number indicates the isolate number. In couple A, strain 2 ACID 4 was identical to strains from the other partner, and in couple B, strains 2 ACID 6, 10, and 13 were identical to strains from the other partner. For comparison, individual REP-PCR amplicons were marked, and the individual bands were analyzed by using the Dice coefficient and clustering using the UPGMA method.

data support the presence of genetically distinct aciduric *S. oralis* in which there may be a genetic basis to their survival and proliferation under the acidic conditions.

NMS are heterogeneous with respect to acidogenicity and, by virtue of their numerical superiority, these acidogenic NMS may play a significant role in the caries formation process (29, 35, 36). The present data may also be viewed in the same manner. The predominant aciduric bacteria isolated from caries-free tooth surfaces are not mutans streptococci but are rather *S. oralis* and other viridans streptococci including *S. intermedius*, *S. parasanguinis*, and *Streptococcus anginosus*. The role of these bacteria in the initiation of caries is not yet known, although many NMS, including *S. oralis*, are reported to induce caries in laboratory rats (40). The data presented here clearly indicate that *S. oralis* is genotypically heterogeneous with respect to aciduricity, and our previous study indicated that this species was predominant amongst the aciduric dental plaque microflora.

This is the first report of genotypic heterogeneity amongst NMS relating to aciduricity. The significance of the aciduric *S. oralis* and other aciduric NMS in the caries process warrants further consideration, and it may be that the central role assigned previously to *S. mutans* will require reevaluation.

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