

# Ecology of Human Oral Lactobacilli

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Lactobacilli were found in saliva, on teeth, and on the dorsum of the tongue, the vestibular mucosa, and the hard palate in humans. Their proportions in saliva, expressed as percentage of the anaerobically cultivable flora, were 10- to 100-fold higher than those on the tooth surface, but were comparable to those on the epithelial surfaces. The adherence of *Lactobacillus casei* and *L. fermenti* to oral surfaces was compared with that of streptomycin-labeled *Streptococcus sanguis* and *S. salivarius* by using in vitro-cultivated cells. The affinity of both *Lactobacillus* species for the tooth surface was very low compared with that of *S. sanguis* but was somewhat higher than that of *S. salivarius*. The lactobacilli and both *Streptococcus* species adhered to a similar extent to the dorsum of the tongue, whereas the affinity of the lactobacilli and *S. salivarius* for the vestibular mucosa was about one-half of that of *S. sanguis*. The results suggest that the affinity of lactobacilli for oral surfaces significantly influences their proportional distribution in the mouth. The relatively low affinity of lactobacilli for the tooth surface suggests that their recognized association with carious lesions and mechanical appliances under certain conditions may be due primarily to mechanical retention rather than unique growth conditions.

Microorganisms indigenous to the human mouth vary considerably in their affinity for different oral surfaces. The proportions in which various *Streptococcus* sp., *Neisseria*, and *Veillonella* occur naturally on teeth or epithelial surfaces have been found to correlate positively with the relative affinity of the organisms for these surfaces. On the basis of these studies, it has been proposed that bacterial attachment per se constitutes an important factor in the colonization of oral surfaces by indigenous bacteria (16, 27, 41, 42).

Lactobacilli, in contrast to other indigenous oral bacteria, colonize the mouth only in relatively few numbers or are often undetectable. Because of their historical association with dental caries, much work has been devoted towards an understanding of the mechanisms which regulate their presence in the mouth. Attention mainly has been focused on factors which influence their growth, such as inhibitory salivary components (1, 18, 44), pH (37), or nutritional constituents (4, 36). The role of bacterial adherence in the ecology of lactobacilli, however, has received virtually no attention. In view of recent evidence concerning the importance of adherence as an ecological determinant for other indigenous bacteria, the influence of adherence on the oral colonization of lactobacilli was studied.

## MATERIALS AND METHODS

**Proportions of lactobacilli in various parts of the mouth.** Eleven- to 13-yr-old children and adults found to possess high salivary lactobacillus counts were selected for study. Pooled dental plaque was obtained from caries-free buccal and lingual tooth surfaces with sterile periodontal scalers. Concomitantly, samples were obtained from the dorsum of the tongue, the vestibular mucosa, and the hard palate by means of Calgiswabs (Colab Lab.). A few milliliters of saliva was also collected. All samples were placed in 1 ml of modified Ringer solution (41). The plaque samples were homogenized by sonic oscillation (42); the other samples and the saliva were mixed for 30 sec on a Vortex mixer. Appropriate dilutions of each sample were cultured on duplicate plates of Rogosa SL agar (Difco) for the enumeration of lactobacilli and on Trypticase soy agar (BBL) with 5% sheep blood for the enumeration of the total number of cultivable bacteria. All media were incubated anaerobically in Brewer jars filled with 80% N<sub>2</sub>, 10% H<sub>2</sub>, and 10% CO<sub>2</sub> for 3 to 4 days.

**Adherence of lactobacilli to oral surfaces.** During the investigation the participants, 22 to 38 years of age, consumed their normal diet. Oral samples obtained from adults were used as a source of *Lactobacillus* strains. Isolates, obtained from Rogosa SL agar which consisted of gram-positive, catalase-negative, nitrate-negative rods, were further characterized on the basis of criteria established by Rogosa et al. (33) and Davis (14).

*L. casei* strains 4PAL and 1PL-2 did not produce

gas, grew at 45 C, and formed acid from glucose, mannose, sucrose, mannitol, and salicin but not from arabinose, raffinose, or melibiose. Both strains hydrolyzed esculin, but neither strain produced ammonia from arginine. Strain 4PAL fermented sorbitol, whereas strain 1PL-2 did not. The final pH in 2% glucose broth was about 3.5. *L. fermenti* strains 2PLG and 4TG produced gas, grew at 45 C, formed acid from glucose, raffinose, mannose, and sucrose but were negative with arabinose, mannitol, sorbitol, melibiose, and salicin. Esculin was not hydrolyzed, but ammonia was produced from arginine. The final pH was about 4.2. *L. casei* and *L. fermenti* strains were chosen for the study of adherence because these species have been found to predominate among the oral human lactobacilli (8, 21, 33). Streptomycin-resistant strains of *S. sanguis* and *S. salivarius* have been previously described (42). The parent of the streptomycin-resistant *S. salivarius* strain HSL-1R was freshly isolated from the saliva of one of the subjects (J.H.).

Cell suspensions of the strains containing 2 to 5 × 10<sup>8</sup> colony-forming units per ml were prepared as outlined previously (42). Mixtures of one of the *Lactobacillus* strains and one of each of the *S. sanguis* and *S. salivarius* strains, which were included for comparative purposes, were obtained by mixing equal volumes of cell suspensions of each species.

In the study of the adherence to epithelial surfaces, 1 ml of the mixture was introduced into the mouth of the subjects and was held there for 10 min. All oral fluid was then expectorated. A sample of the fluid was incubated during the experimental period in a 37 C water bath, whereas another sample was retained for cultural studies. Simultaneously, samples were obtained from the dorsum of the tongue and vestibular mucosa by forceful swabbing with a Calgiswab. Saliva and samples from areas of the dorsum of the tongue and vestibular mucosa which had not been previously sampled were obtained at the end of a 120-min period. The proportions of lactobacilli and streptomycin-resistant streptococci in the mixture and the samples were determined on duplicate plates of Rogosa SL agar incubated anaerobically, and on Trypticase soy agar with 200 µg of streptomycin per ml and 5% sucrose, respectively, on the basis of colonial morphology (42).

In the study of adherence to the tooth surface, 1 ml of the mixture was held in the mouth for 10 min. All oral fluid was then expectorated and part of it was used for cultural study. After an additional 50 min, another saliva sample was collected. Directly thereafter dental plaque, which in certain instances was permitted to accumulate for a few days, was obtained with periodontal scalers or Calgiswabs from smooth buccal and lingual or approximal surfaces. Prior to sampling, each tooth surface was rinsed with 2 ml of sterile Ringer solution to remove adherent saliva. All samples were then treated, and the proportions of the organisms were determined, as described above.

The ability of lactobacilli to agglutinate with freshly collected, clarified, unstimulated, mixed saliva from the participating subjects was determined as described by Gibbons and Spinell (15). Other strains of *L.*

*casei*, *L. fermenti*, *L. acidophilus*, and *L. salivarius* studied were isolated from various oral surfaces and saliva of subjects.

The presence of streptomycin-labeled organisms (42), or of lactobacilli in the various oral sites studied, was determined prior to each experiment to exclude their influence on the experimental results. Because nonlabeled lactobacilli were used, it was important to determine the number of naturally occurring lactobacilli. Only two (J.H. and W.F.L.) of the subjects consistently harbored up to ca. 4,000 organisms per milliliter initially; the other subjects were negative. During the experiments, the numbers of lactobacilli in saliva or in swabbings obtained from the dorsum of the tongue ranged from zero to ca. 3,000 per milliliter; their numbers on the vestibular mucosa were negligible. On the other hand, the number of lactobacilli at the end of the 120-min period was about 10<sup>5</sup> to 10<sup>6</sup>/ml of saliva, 10<sup>4</sup> to 10<sup>5</sup> per tongue swab, and ca. 200 to 10<sup>4</sup> per swab obtained from vestibular mucosa and thus, far exceeded the number of resident organisms.

## RESULTS

**Distribution of lactobacilli.** The distribution of lactobacilli over various oral surfaces is shown in Table 1. Lactobacilli were found in saliva, dental plaque, and on the oral epithelial surfaces, including the dorsum of the tongue, the vestibular mucosa, and the hard palate. However, their proportions in saliva far exceeded those in dental plaque ( $P < 0.01$ ,  $t$  test). The proportions of lactobacilli in saliva were in the same order as those on the epithelial surfaces in children as well as in adults.

**Adherence of lactobacilli to epithelial surfaces.** Table 2 shows representative data from experiments in which the adherence of artificially introduced bacteria to the dorsum of the tongue or

TABLE 1. Percentage of lactobacilli of the anaerobically cultivable flora in saliva and on various human oral surfaces

Sample	10 Children	9 Adults
Dental plaque	0.005 <sup>a</sup> (0.00005-0.04)	0.003 (0.00005-0.03)
Saliva <sup>b</sup>	0.48 (0.07-1.4)	0.03 (0.002-0.4)
Dorsum of tongue	0.36 (0.001-1.9)	0.03 (0.001-0.15)
Vestibular mucosa	0.21 (0.001-0.5)	0.02 (< 0.0004-0.12)
Hard palate	0.23 (0.005-1.2)	Not Done

<sup>a</sup> Mean percentage; range shown between brackets.

<sup>b</sup> Range number of lactobacilli per milliliter of saliva: children, 18,000-430,000; adults, 3,800-97,000.

TABLE 2. Percentage<sup>a</sup> of *Lactobacillus* species, streptomycin-resistant *S. sanguis*, and *S. salivarius* in saliva and on the dorsum of the tongue and vestibular mucosa

Subject	Strains	Mixture	Saliva		Dorsum of tongue		Vestibular mucosa		In vitro saliva
			10 min	120 min	10 min	120 min	10 min	120 min	10 to 120 min
J.H.	<i>L. casei</i> 1PL-2	69 <sup>b</sup>	41	14	31	24	24	13	36
	<i>S. sanguis</i> H7PR	19	25	53	41	38	54	74	14
	<i>S. salivarius</i> SuR	12	34	33	28	38	22	13	50
R.M.	<i>L. casei</i> 4PAL	69	48	4	49	36	37	15	42
	<i>S. sanguis</i> H7PR	17	17	93	24	46	26	58	12
	<i>S. salivarius</i> SuR	14	35	3	27	18	37	27	46
A.J.P.	<i>L. fermenti</i> 2PLG	23	25	8	24	30	15	4	38
	<i>S. sanguis</i> H7PR	37	29	83	40	51	48	91	17
	<i>S. salivarius</i> HSL1-R	40	46	9	36	19	37	5	45
D.M.S.	<i>L. fermenti</i> 4TG	43	22	31	51	78	40	25	41
	<i>S. sanguis</i> H7PR	37	32	51	20	17	50	57	23
	<i>S. salivarius</i> SuR	20	46	18	29	5	10	18	36
W.F.L.	<i>L. fermenti</i> 4TG	59	54	27	43	21	36	27	47
	<i>S. sanguis</i> H7PR	33	26	47	35	47	27	56	16
	<i>S. salivarius</i> SuR	8	20	26	22	32	37	17	37

<sup>a</sup> Expressed as percentage of the total of colony-forming units of all three species.

<sup>b</sup> Mean percentage.

the vestibular mucosa, as well as their oral clearance, was studied. In all, 15 experiments were performed by using mixtures of two different *L. casei* and *L. fermenti* strains in various combinations with one *S. sanguis* and three *S. salivarius* strains. To determine differences between the proportions of the organisms in two samples considered for comparison (e.g., the mixture and the saliva obtained at 10 min), first the individual ratio of each bacterial species was calculated by comparison of its mean percentage in one sample with its mean percentage in the other sample. Next, the mean of these ratios was calculated for all 15 experiments. The standard errors in the individual and mean ratios were obtained by propagation (43).

The bacterial proportions in the mixture were found to be different from those in the saliva obtained at 10 min (Table 2). Comparison of the mean ratios showed (Table 3) that the proportions of lactobacilli and *S. sanguis* in the 10-min saliva were decreased as compared with those in the mixture (i.e., mean ratio <1), whereas those of *S. salivarius* were increased, resulting in about a 3-fold relative increase of *S. salivarius*. A comparable change in bacterial proportions occurred in vitro, upon aerobic incubation at 37 C of mixtures with saliva from the subjects, within the few minutes used for plating of the samples. After 10 min of incubation, the proportional changes were slightly more accentuated, but no changes occurred thereafter for up to 120 min. Consequently, these changes were not attributed

to killing, but rather to cell clumping. It was found that most strains of *L. fermenti* tested, including strains 2PLG and 4TG and all strains of *L. salivarius*, aggregated strongly in the presence of whole, clarified saliva. *L. casei* strains were often nonreactive except for some strains, including 1PL-2, which aggregated only moderately. Also, all strains of *L. acidophilus* tested and many strains of the above species, including 4 PAL, could not be evenly dispensed in suspension and formed aggregates without saliva. Strong salivary aggregation has also been found to be a consistent feature of *S. sanguis* strains (*unpublished data*), whereas the three *S. salivarius* strains used were found to be nonreactive.

Because of the proportional changes resulting from aggregation, the values for the 10-min saliva were considered the most representative with respect to the availability of organisms for adherence to the dorsum of the tongue and the vestibular mucosa as determined at 10 min. Comparison of the mean ratios showed (Table 3) that the affinity of the lactobacilli for the tongue was similar to that of both streptococcal types; their affinity for the vestibular mucosa was comparable to that of *S. salivarius*, but about one-half that of *S. sanguis*. No significant differences in relative adherence between the *L. casei* and *L. fermenti* strains were observed.

During the 10- to 120-min period, the number of introduced organisms on the tongue and vestibular mucosa, as well as in the saliva, was found to decrease about 100-fold. Comparison of

the proportions of each species on the tongue and vestibular mucosa at 10 and 120 min and calculation of the mean ratios (Table 3) indicated that all three species were released at a similar rate from the tongue, whereas from the vestibular mucosa the lactobacilli and *S. salivarius* were released at about twice the rate of *S. sanguis*.

The proportions of lactobacilli and *S. salivarius* in the 10-min saliva samples were about  $\times 1.5$  those of *S. sanguis*. However, as is shown by the mean ratios (Table 3), the proportions of *S. sanguis* in the 120-min saliva had increased, whereas those of the lactobacilli or *S. salivarius* had decreased, which resulted in *S. sanguis* proportions about  $\times 3$  higher than those of both other organisms. Therefore, if reattachment had taken place during the 10- to 120-min period, it might have favored the proportions of *S. sanguis* on the epithelial surfaces at the end of the experiment.

In contrast to the increase in *S. sanguis* in the saliva in vivo during the 10- to 120-min period, no such change was observed in vitro when 10-min saliva samples were incubated for the remainder of the period (Table 3). This suggests that changes observed in vivo were not due to differences in cell aggregation or viability between the three bacterial species but, rather, that the organisms introduced into the mouth adhered to a different extent and were cleared at a different rate from the oral surfaces.

In other experiments, the mouth was vigorously rinsed with 200 ml of sterile saline after expectoration of the saliva at 10 min. Sampling of the dorsum of the tongue and the vestibular mucosa just prior to and directly after the rinse indicated differences in the rate of bacterial clearance from the epithelial surfaces similar to those observed during the 10- to 120-min period in the experiments described above.

**Adherence of lactobacilli to the tooth surface.** Data from the experiments in which the affinity of *L. casei* and *L. fermenti* for the tooth surface was compared with that of *S. sanguis* and *S. salivarius* are shown in Table 4. The proportions of *S. sanguis* in plaque samples obtained 60 min after introduction of the mixture in the mouth were higher, whereas those of the lactobacilli and of *S. salivarius* were far lower than those in the 10- or 60-min saliva. The individual ratios of these proportions were first calculated for each plaque sample from each subject separately; then their mean ratio was determined for all subjects. Comparison of the mean ratios obtained for each of the three species demonstrated that if the 10 min-saliva was considered representative for the proportions of the organisms available for adherence, the affinity of *S. sanguis* was about  $\times 45$  higher than that of the lactobacilli and  $\times 118$  that of *S. salivarius*. When the 60-min saliva sample was used as a basis for comparison, the affinity of *S. sanguis* was about  $\times 11$  that of the lactobacilli and  $\times 24$  that of *S. salivarius*.

## DISCUSSION

Many investigations involving lactobacilli have demonstrated their presence on the teeth or in saliva. The present study shows that lactobacilli, if present in the mouth, can also be isolated in significant numbers from the dorsum of the tongue (*see also* 39), the vestibular mucosa, and the hard palate. This is in contrast to what is suggested by the study of Steinle, Madonna, and Bahn (38) and discussed by Burnett and Scherp (7). These investigators used an agar-replica method to determine the presence of oral "lactobacillus growth sites" and reported few or none on the soft oral tissues. It is apparent that the agar-replica technique is only

TABLE 3. Mean ratios of lactobacilli and streptomycin-resistant *S. sanguis* and *S. salivarius* after comparison of oral samples

Samples compared	Mean ratio		
	<i>Lactobacillus</i>	<i>S. sanguis</i>	<i>S. salivarius</i>
Mixture and saliva (10 min)	0.74 $\pm$ 0.10 <sup>a</sup>	0.86 $\pm$ 0.11	2.33 $\pm$ 0.29 <sup>b</sup>
Saliva (10 min) and tongue (10 min)	1.08 $\pm$ 0.18	1.30 $\pm$ 0.26	0.87 $\pm$ 0.07
Saliva (10 min) and vestibular mucosa (10 min)	0.82 $\pm$ 0.11	1.91 $\pm$ 0.28 <sup>c</sup>	0.72 $\pm$ 0.09
Tongue (10 min) and tongue (120 min)	0.79 $\pm$ 0.10	1.21 $\pm$ 0.25	0.96 $\pm$ 0.11
Vestibular mucosa (10 min) and vestibular mucosa (120 min)	0.78 $\pm$ 0.16	1.52 $\pm$ 0.22 <sup>c</sup>	0.52 $\pm$ 0.13
Saliva (10 min) and saliva (120 min)	0.59 $\pm$ 0.13	2.76 $\pm$ 0.32 <sup>c</sup>	0.53 $\pm$ 0.09
Saliva (10 min) and in vitro saliva (10 to 120 min)	1.06 $\pm$ 0.17	0.85 $\pm$ 0.13	1.11 $\pm$ 0.11

<sup>a</sup> Standard error of mean ratio.

<sup>b</sup> Difference between *S. salivarius* and the lactobacilli or *S. sanguis* significant ( $P < 0.01$ , *t* test).

<sup>c</sup> Difference between *S. sanguis* and the lactobacilli or *S. salivarius* significant ( $P < 0.01$ , *t* test). All other differences not significant.

TABLE 4. Percentage<sup>a</sup> of lactobacilli and streptomycin-resistant *S. sanguis* and *S. salivarius* adhering to dental plaque

Subject	Strains	Mixture	Saliva		Plaque sample <sup>b</sup>			
			10 min	60 min	1	2	3	4
J.H.	<i>L. casei</i> 1PL2	75.7	37.2	11.6	1.8	4.3	2.9	3.3
	<i>S. sanguis</i> H7PR	18.0	23.5	72.8	97.2	94.2	95.1	94.2
	<i>S. salivarius</i> SuR	6.3	39.3	15.6	1.0	1.5	2.0	2.5
D.M.S.	<i>L. casei</i> 1PL2	75.7	24.0	11.7	1.3	8.4	6.7	
	<i>S. sanguis</i> H7PR	18.0	39.3	64.5	97.4	88.2	90.5	
	<i>S. salivarius</i> SuR	6.3	36.7	23.8	1.3	3.4	2.3	
A.J.P.	<i>L. fermenti</i> 2PLG	27.5	22.2	19.8	2.2	18.4	0.9	
	<i>S. sanguis</i> H7PR	42.7	35.0	50.3	93.4	74.4	98.4	
	<i>S. salivarius</i> SuR	29.8	42.8	29.9	4.4	7.2	1.7	
D.M.S.	<i>L. fermenti</i> 2PLG	22.4	12.3	21.1	5.9	27.8	6.9	
	<i>S. sanguis</i> H7PR	33.7	28.7	44.5	91.8	67.0	91.3	
	<i>S. salivarius</i> SuR	43.9	59.0	34.4	2.2	5.2	1.8	
A.J.P.	<i>L. fermenti</i> 4TG	51.9	20.4	9.3	0.8	1.5	2.2	2.1
	<i>S. sanguis</i> H7PR	25.9	18.8	57.0	97.9	97.4	97.8	94.9
	<i>S. salivarius</i> SuR	22.2	60.8	33.7	1.3	1.1	< 1.1	3.0
J.H.	<i>L. fermenti</i> 4TG	51.9	25.5	16.5	3.1	< 1.5	3.2	
	<i>S. sanguis</i> H7PR	25.9	14.8	57.9	94.2	97.7	95.4	
	<i>S. salivarius</i> SuR	22.2	59.7	25.6	2.7	2.3	1.4	

<sup>a</sup> Expressed as percentage of the total of colony-forming units of all three species.

<sup>b</sup> Each sample obtained from a different tooth surface.

suitable for the detection of concentrated masses of lactobacilli.

The proportions of lactobacilli in dental plaque obtained from smooth, nonretentive tooth surfaces were found to be strikingly lower than their proportions in either the saliva or on the epithelial surfaces. This observation is consistent with the finding of other investigators (19, 20, 26) that these organisms can be more often isolated from saliva than from dental plaque.

The affinity of artificially introduced *L. casei* and *L. fermenti* for the tooth surface was found to be lower than that of *S. sanguis* but somewhat higher than that of *S. salivarius*. Their affinity for the dorsum of the tongue was comparable to that of both streptococci, whereas their affinity for the vestibular mucosa was similar to that of *S. salivarius* but lower than that of *S. sanguis*. This indicates that the low proportions in which lactobacilli naturally occur in dental plaque and their higher proportions on the epithelial surfaces relative to saliva are positively correlated with their relative affinity for these sites. The affinity of lactobacilli for oral surfaces may be, therefore, of significant influence on their proportional distribution, as has been previously proposed for *Streptococcus* species and oral *Neisseria* and *Veillonella* (16, 27, 41, 42).

Studies in which in vitro-cultivated organisms

of a single type have been introduced into the mouth have demonstrated the existence of mechanisms involved in their oral clearance (2, 3, 5, 6, 24, 40). Regarding their specificity, the experiments of Bloomfield (5, 6) suggested that the rapid disappearance of *Sarcina lutea* from the tongue was due to the bactericidal effect of saliva and mouth secretions, whereas that of *Escherichia coli* and *Staphylococcus albus* was the result of mechanical removal. The relative increase of *S. sanguis* over the lactobacilli and *S. salivarius* in saliva within 1 to 2 hr after their oral introduction, as observed in the present study, indicates that different bacterial types are not eliminated from the mouth at similar rates. It is unlikely that these proportional changes were due to differences between the organisms in in vivo growth rate in view of the short experimental period; rather, they are indicative of differences between the adherence of the organisms to oral surfaces.

Why lactobacilli are unable to colonize the human mouth consistently in high numbers is unknown. Their oral establishment and maintenance seem to depend on the presence of teeth and, more specifically, on carious lesions (22, 32, 35, 36, 38). Similarly, the teeth seem to constitute the main and indispensable oral site for *S. sanguis* (9, 11, 12, 13). In contrast, the dorsum of the tongue seems to be the main habitat of *S. salivarius* (10, 12, 25, 28, 36). Thus,

unlike *S. salivarius*, the lactobacilli and *S. sanguis* seem unable to maintain themselves in the mouth if the tongue or the other oral epithelial surfaces, or both, are the sole sites for colonization. However, in the present study it was observed that the affinity of *S. salivarius*, lactobacilli, and *S. sanguis* for the tongue was comparable. Differences in growth rate on the tongue between these organisms and between the lactobacilli, *S. sanguis*, and other organisms on other oral epithelial surfaces could be the determinant factor in their disappearance from the mouth when teeth are eliminated as their prime source. On the other hand, differences in adherence between species could exist which were too small to be noticed or which could not have been detected by the present short-term experiments, and these could be of important ecological consequence. The attachment of bacteria to the tongue may involve an initial reversible phase of attraction, followed by a time-dependent irreversible phase of adherence such as has been observed in the adherence of marine bacteria to surfaces (29). In this respect it is of interest that *S. salivarius* has recently been shown to possess a surface "fuzzy" coat which seems to mediate its adherence to oral epithelial surfaces (17). Indeed, the majority of indigenous bacteria attached to the vestibular mucosa (17) or to the dorsum of the tongue (*unpublished data*) in the human mouth appear to be attached by a morphologically similar fuzzy coat. It has not yet been ascertained if cells of the strains of *Lactobacillus* and *S. sanguis* used in this study possess an analogous surface structure.

The relatively low affinity of lactobacilli for teeth suggests that mechanical retention, either directly or indirectly via incorporation of cells in food, may play an important role in their colonization of teeth. The close association of these organisms with carious lesions and their low proportions found elsewhere on the tooth surface support that view. This distribution could also be due to the inhibitory influence of saliva, which has been proposed as a determinant factor in the ecology of lactobacilli (44). However, the numbers of oral lactobacilli can be dramatically increased by insertion of mechanical appliances such as orthodontic bands (4, 31, 34), dentures (36), or a palatal prosthesis (30). Under these conditions the organisms would still be expected to be exposed to saliva and, thus, the possible inhibitory influence of saliva can, at best, be assigned only a limited regulatory role.

The increase in lactobacilli associated with mechanical appliances has generally been explained on the basis of food retention providing

essential nutrients, e.g., carbohydrates (23, 36) or a low plaque pH favoring their growth (37). However, with few exceptions (34) it has not been recognized that these areas also provide retention sites for bacteria. Thus, the importance of carious lesions and appliances for the establishment of lactobacilli in the mouth may be simply in serving to mechanically retain these organisms, rather than in providing conditions uniquely suitable for their growth. This also suggests that the lactobacilli are not involved in the initiation of carious lesions on smooth, nonretentive tooth surfaces, although they could participate in their progression.

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