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Oral Ecology and Virulence of *Lactobacillus casei* and *Streptococcus mutans* in Gnotobiotic Rats

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Lactobacilli comprise a small percentage of the normal oral microbial flora of humans and are isolated commonly from saliva and frequently from an active caries lesion. We have compared the pathogenesis and colonization pattern of *Lactobacillus casei* with that of *Streptococcus mutans* strain 6715 in gnotobiotic rats. Of the two *L. casei* strains tested, *L. casei* strain ATCC 4646 caused slightly more caries than *L. casei* strain ATCC 11578. However, the level of caries induced by either *L. casei* strain was significantly lower ($P < 0.01$) than that observed in similar-aged rats monoassociated with *S. mutans* strain 6715. When groups of rats were infected with mixtures of *L. casei* strain ATCC 4646 and *S. mutans* strain 6715, or with *L. casei* followed by *S. mutans*, higher numbers of *L. casei* than *S. mutans* were found associated with the tongue and in saliva; *S. mutans* always predominated in plaque. The level of caries observed in these groups of rats was similar to that seen with rats monoassociated with *S. mutans* except when *L. casei* comprised greater than 1% of the plaque microflora. In this latter situation, the level of caries was significantly lower ($P \leq 0.05$) than that obtained in *S. mutans*-monoassociated rats. The results of this study suggest that *L. casei* colonizes sites in the oral cavity (including the tongue and saliva) other than the tooth surface in rats. The effect of *L. casei* in plaque toward reduction of *S. mutans*-induced dental caries in rats is discussed.

Lactobacilli represent a characteristic group of oral bacteria which numerically comprise a minor component of the oral microbiota and are detected in the oral cavity soon after birth. Although lactobacilli are easily detected in saliva by growth on selective media (2), they represent generally less than 1% of the bacterial flora present in saliva. Despite these low numbers of lactobacilli in the oral cavity, early findings that lactobacilli (i) could be correlated with caries formation (29, 34), (ii) were present in carious lesions and presumably on the teeth before caries initiation (3, 35), and (iii) were acidogenic and aciduric microorganisms (21) were presumptive evidence for the importance of this organism in human caries formation. The potential role of lactobacilli in human caries formation has gained support from animal studies which have implicated lactobacilli with carious lesion formation (1, 9, 10, 36, 37). Nevertheless, the failure to consistently isolate significant numbers of lactobacilli from human dental plaque led to numerous studies for other potentially cariogenic bacteria.

Streptococcus mutans is now considered by numerous investigators to be a major cause of carious lesions in humans (24, 26, 27) and experimental animals (11, 23, 30). This organism exhibits unique characteristics associated with its colonization and virulence, and a number of these are sucrose dependent. These traits include synthesis of soluble and insoluble glucan by extracellular and cell-associated glucosyltransferase (8, 13, 15, 16, 18, 19). Insoluble glucans facilitate *S. mutans* accumulation on tooth surfaces (15, 18, 19) and intercellular aggregation of *S. mutans* via cell surface receptors (15, 18, 19). Furthermore, carbohydrate metabolism by *S. mutans* leads to production of copious amounts of lactic acid which ultimately results in enamel dissolution and tooth decay (8, 16, 18). Finally, *S. mutans* is acidogenic and mildly aciduric (5, 6), and this fact, when coupled with its unique ability to colonize hard surfaces such as teeth, lends support for *S. mutans* preeminence in the etiology of caries formation. Despite this compelling evidence supporting an etiological

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role for *S. mutans* in dental caries, conflicting literature remains concerning the occurrence, numbers, and odontopathic potential of oral lactobacilli.

We report here studies with *Lactobacillus casei* in gnotobiotic rats which suggest that this species selectively colonizes soft oral tissue of the tongue and in saliva in rats. Further, when compared with *S. mutans*, *L. casei* poorly colonizes the tooth surface and has little odontopathic potential. We also present evidence that instead of contributing to the caries process, *L. casei*, if present in plaque, may reduce *S. mutans*-induced dental caries in rats.

MATERIALS AND METHODS

Microorganisms and culture conditions. Two American Type Culture Collection (ATCC) *Lactobacillus casei* strains, designated 11578 and 4646, and *S. mutans* strain 6715, which is resistant to streptomycin (20, 33), were used in these studies. All strains were maintained in brain heart infusion (BHI) agar stabs containing excess calcium carbonate at 4°C. For infection studies, each test strain was grown to log phase in BHI broth at 37°C in an atmosphere of 5% CO_2 in nitrogen. For studies with *S. mutans* strain 6715, 18-h cultures were used; for studies with *L. casei*, 30-h cultures were used. In studies involving infection with mixtures of *L. casei* and *S. mutans*, the number of colony-forming units (CFU) of bacteria in each culture was determined (optical density at 660 nm) and appropriately diluted in BHI broth for infection. Equal portions of the inoculating mixture were plated on mitis-salivarius agar (Difco Laboratories, Detroit, Mich.) containing streptomycin (2 mg/ml; Sigma Chemical Co., St. Louis, Mo.) (MS+S), Rogosa SL Agar (Difco), and blood agar (BHI agar base [Difco] plus 5% horse blood [Colorado Serum Co. Laboratories, Denver]) agar to determine precise numbers of *S. mutans*, *L. casei*, and total CFU present, respectively.

Experimental designs. For virulence assessment of *L. casei* strains ATCC 4646 and ATCC 11578 and *S. mutans* strain 6715, weanling germfree Fischer rats (age 20 days) were challenged with a known inoculum size (with the aid of a micropipette, 50 μl) of the test strain and fed a caries-promoting diet containing 5% sucrose (30). Colonization was confirmed by plating oral swab samples from individual rats 2 days after infection. Groups of rats were removed from the experiment at either 45 or 90 days of age, and caries was assessed by the Keyes procedure as described previously (30–32).

In experiments involving infection with mixed cultures, groups of weanling rats were inoculated at age 20 days with the appropriate bacterial mixture, and a portion of each group of rats was removed from the experimental design at 5- to 10-day intervals. Individual saliva, tongue, and mandibles were aseptically collected for microbial analysis (described below). In another experimental series, *L. casei* strain ATCC 4646 was introduced into 20-day-old rats followed by superinfection 5 days later with *S. mutans* strain 6715. At the termination of each experiment and after microbial analysis, mandibular molars were hemisectioned and scored for caries by the Keyes procedure as described previously (30–32).

Microbial analysis of saliva, tongue, and plaque. For saliva collection from older rats (age 35 days or older), anesthesized rats were given pilocarpine to stimulate saliva flow. Individual saliva samples were collected over a timed 20-min interval into sterile tubes as previously described (28). For young animals (age 30 days or younger), saliva was obtained with the aid of sterile capillary pipettes, which facilitated collection from small rats. This method involved complete sedation of the animal with pentobarbital sodium. After pilocarpine injection, a sterile capillary pipette was inserted into the mouth in the region of the salivary glands, and saliva was collected by capillary action and transferred to a sterile tube. Volumes of 0.4 to 1.0 ml were routinely collected in this manner, which was similar (volume/time) to that obtained from older animals. For microbial analysis of saliva, 10-fold dilutions of individual samples were made and plated in triplicate on MS+S, Rogosa SL, and blood agars. Plates were incubated for 48 h at 37°C in 5% CO_2 in nitrogen. The number of lactobacilli was determined by counts on Rogosa SL agar, whereas numbers of *S. mutans* were assessed by using MS+S agar; total bacteria were determined by counts on blood agar.

For microbial analysis of tongue and mandibular molar plaque after saliva collection, rats were sacrificed by cervical dislocation; the lower jaws were then aseptically removed and defleshed for microbiological analysis as described previously (31). The complete tongue was aseptically dissected and placed in a tube containing 2 ml of 0.067 M phosphate buffer, pH 7.2. Adherent bacteria were removed by sonic treatment for 10 s of full power (Branson Sonifier cell disrupter; Branson Instrument Co., Plainview, N.Y.). Appropriate 10-fold dilutions of mandibular plaque or tongue microflora were plated on Rogosa SL, MS+S, and blood agars as described above.

Statistics. The caries scores from each experimental group of rats were statistically reduced by computing means and standard errors. Differences among means were evaluated by analysis of variance and multiple mean comparisons, using the Duncan test (7). The number of *L. casei* and *S. mutans* CFU per sample per group of rats was expressed as the mean ± standard error. The significance of difference between means was determined by the Student *t* test.

RESULTS

Comparison of virulence patterns of *L. casei* and *S. mutans*. Both strains of *L. casei* tested readily colonized the oral cavity of monoassociated gnotobiotic rats (Table 1). Although similar numbers of *L. casei* strains ATCC 4646 and ATCC 11578 were recovered from mandibles of 45-day-old monoassociated rats, the level was significantly lower ($P \leq 0.05$) than that obtained with *S. mutans*-monoassociated animals. This difference in colonization was re-

flected in the caries activity, since *S. mutans*-monoassociated rats (age 45 days) had significantly higher ($P < 0.01$) caries activity than gnotobiotic rats monoassociated with the test *L. casei* strains. When the experiment was prolonged, the number of recoverable *L. casei* and *S. mutans* from mandibular molars of 90-day-old gnotobiotic rats increased. Although greater caries activity was observed in 90-day-old rats monoassociated with the test *L. casei* strains at age 20 days, the level was still significantly lower ($P < 0.01$) than the rampant decay observed in *S. mutans*-monoassociated rats.

Oral colonization of rats infected with mixtures of *L. casei* and *S. mutans*. Groups of rats which had been infected with approximately equal numbers of *L. casei* strain ATCC 4646 and *S. mutans* strain 6715 at age 20 days were assessed at intervals of 5 days for relative numbers of bacteria in saliva and mandibular plaque and caries activity (Table 2). *L. casei* quickly became the predominant organism in saliva, whereas *S. mutans* reached significant levels in plaque which were generally 100- to 1,000-fold higher than *L. casei* levels. The caries pattern obtained in these rats was similar to that obtained with animals monoassociated with *S. mutans* strain 6715 (Tables 1 and 2).

In the next series of experiments, incremental $\log_{10}$-higher ratios of *L. casei* to *S. mutans* were used for infection, and relative numbers of these bacteria were assessed in saliva and plaque and on tongue in order to determine more precisely the oral sites colonized by *L. casei* (Tables 3 and 4). Within 5 days after infection, *L. casei* became the dominant organism on tongue and in saliva, usually outnumbering *S. mutans* by 3- to 10-fold. However, *S. mutans*, despite its low inoculation ratio, reached significantly higher numbers in plaque ($P < 0.01$) than *L. casei* (Table

TABLE 1. *Virulence of L. casei strains ATCC 4646 and 11578 and S. mutans strain 6715 in monoassociated gnotobiotic rats*[a]

Day of sacrifice (age)	Strain	Plaque microflora[b] (CFU/mandible × 10^5)	Mean caries score[c]					
			Buccal		Sulcal		Proximal	
			Enamel	Dentinal slight	Dentinal slight	Dentinal extensive	Dentinal enamel	Dentinal slight
45	*L. casei* 4646	8.2 ± 0.9	6.0 ± 0.5	1.6 ± 0.2	3.2 ± 0.5	0.2 ± 0.1	0.0	0.0
	L. casei 11578	7.8 ± 1.0	4.6 ± 0.3	0.9 ± 0.2	1.3 ± 0.4	0.0	0.0	0.0
	S. mutans 6715	219.3 ± 2.5	23.1 ± 0.5	17.4 ± 0.6	20.5 ± 0.2	15.2 ± 0.5	8.0 ± 0.1	5.0 ± 0.3
90	*L. casei* 4646	37.3 ± 3.1	9.6 ± 1.2	5.5 ± 0.8	9.9 ± 1.0	7.0 ± 1.3	2.1 ± 0.3	1.4 ± 0.6
	L. casei 11578	35.1 ± 2.5	7.4 ± 0.6	2.5 ± 0.7	4.9 ± 1.1	1.6 ± 0.8	0.6 ± 0.2	0.3 ± 0.1
	S. mutans 6715	156.5 ± 4.3	25.6 ± 0.7	23.4 ± 0.9	26.0 ± 0.4	24.9 ± 0.7	7.9 ± 0.2	7.4 ± 0.2

[a] Groups of weanling germfree rats (age, 20 days) were infected orally with the test strain (2×10^7 to 4×10^7 CFU/ml of either *L. casei* 4646, *L. casei* 11578, or *S. mutans* 6715).

[b] Values are the mean (± standard error of the mean) from 50 to 80 rats per inoculum group (Rogosa SL and MS+S agars).

[c] Values expressed as the mean ± standard error of the mean of 50 to 80 rats per group.

TABLE 2. *Distribution of L. casei strain ATCC 4646 and S. mutans strain 6715 in saliva and plaque and caries scores after mixed infection of gnotobiotic rats*[a]

Day of sacrifice (age)	Mean CFU (×10^4)				Mean caries scores[b]					
	Saliva[c]		Plaque[d]		Buccal		Sulcal		Proximal	
	L. casei	*S. mutans*	*L. casei*	*S. mutans*	Enamel	Dentinal slight	Dentinal slight	Dentinal extensive	Enamel	Dentinal slight
25	2.1 ± 0.5	0.2 ± 0.008	0.06 ± 0.003	20.5 ± 0.2	4.5 ± 1.0	0.3 ± 0.3	8.3 ± 1.5	0.5 ± 0.1	0.3 ± 0.3	0.0
30	14.3 ± 0.8	0.09 ± 0.005	0.24 ± 0.01	199.5 ± 8.2	11.5 ± 2.2	9.8 ± 0.6	12.7 ± 1.6	4.4 ± 0.8	1.3 ± 0.1	0.0
35	13.0 ± 1.0	0.28 ± 0.08	0.34 ± 0.05	312.2 ± 11.3	15.4 ± 0.6	11.2 ± 1.6	16.1 ± 1.7	6.4 ± 1.2	3.1 ± 0.3	0.8 ± 0.1
40	84.2 ± 2.4	0.13 ± 0.07	0.12 ± 0.05	720.0 ± 13.6	19.3 ± 2.1	14.5 ± 1.3	19.1 ± 2.3	9.3 ± 2.1	5.4 ± 0.4	3.2 ± 0.6
45	142.0 ± 10.5	0.57 ± 0.09	3.63 ± 0.32	2,550.0 ± 102.6	22.6 ± 1.1	16.7 ± 1.0	21.1 ± 1.6	14.3 ± 1.6	7.2 ± 0.4	4.5 ± 0.4

[a] Germfree rats infected at age 20 days with equal mixtures of *L. casei* strain ATCC 4646 (2.2×10^7 CFU/ml) and *S. mutans* strain 6715 (1.9×10^7 CFU/ml).

[b] Values are mean ± standard error of the mean of 10 to 15 rats per age group.

[c] Values are the mean number (± standard error of the mean) of CFU per milliliter of saliva from 10 rats per age group as determined on Rogosa SL and MS+S agars.

[d] Values are the mean number (± standard error of the mean) of CFU per mandible from 10 to 15 rats per age group (Rogosa SL and MS+S agars).

3). This dichotomy of the colonization pattern was even more evident in 35-day-old (data not shown) and 45-day-old (Table 4) gnotobiotic rats. *L. casei* ranged from 1×10^6 to 4×10^6 bacteria/ml of saliva and tongue samples, whereas *S. mutans* comprised approximately 1,000-fold-lower numbers in these samples (Table 4). *L. casei* did not contribute to the plaque microflora except in groups infected with the highest ratios (1,000:1 and 10,000:1) of this bacterium. At these latter ratios, *L. casei* represented 1.8 and 4.5% of the plaque microflora, respectively. However, even under conditions which should have greatly favored the establishment and colonization of *L. casei* on the tooth surface, *S. mutans* always competed more favorably for this niche (Tables 3 and 4).

Another situation which should favor *L. casei* colonization in the oral cavity would be first to infect young rats with this organism and, after its establishment in the oral cavity, to superinfect these animals with *S. mutans*. Table 5 presents the results of such an experiment. In this experiment, *L. casei* again reached high numbers on tongue and in saliva, outnumbering *S. mutans* by 50- to 300-fold. Although *S. mutans* became the principal organism in plaque, *L. casei* did comprise approximately 2.4% of the total plaque microflora in 45-day-old gnotobiotic rats.

Table 6 presents the caries patterns obtained in 45-day-old rats either infected with mixtures of *L. casei* and *S. mutans* or first infected with *L. casei* followed by superinfection with *S. mutans*. The level of caries in each experimental group was never higher than that obtained in rats monoassociated with *S. mutans*. In fact, when the final plaque flora consisted of greater than 99% *S. mutans* (10:1 and 100:1 ratios), the caries pattern was very similar to that of *S. mutans*-monoassociated animals. On the other hand, when *L. casei* comprised 1 to 5% of the plaque flora (groups of rats infected with either 1,000:1 or 10,000:1 ratios of *L. casei* to *S. mutans* or rats first infected with *L. casei* followed by *S. mutans*), the level of caries, in general, was

TABLE 3. *Distribution of L. casei strain ATCC 4646 and S. mutans strain 6715 in 25-day-old gnotobiotic rats after mixed infection at age 20 days with increasing numbers of L. casei*

Bacterial inoculation ratio[a] (*L. casei/S. mutans*)	Mean CFU (×10⁴)					
	Saliva[b]		Tongue[c]		Plaque[d]	
	L. casei	*S. mutans*	*L. casei*	*S. mutans*	*L. casei*	*S. mutans*
10:1	9.5 ± 1.0	0.10 ± 0.01	4.0 ± 1.0	0.10 ± 0.01	0.08 ± 0.02	73.0 ± 9.0
100:1	7.0 ± 1.8	0.37 ± 0.02	12.0 ± 1.5	0.11 ± 0.04	0.45 ± 0.10	101.0 ± 17.0
1,000:1	6.3 ± 2.0	0.08 ± 0.01	15.0 ± 2.5	0.36 ± 0.06	2.90 ± 0.14	91.0 ± 11.0
10,000:1	9.0 ± 1.0	0.06 ± 0.02	13.0 ± 1.0	0.25 ± 0.10	3.00 ± 0.16	86.0 ± 8.0

[a] Actual numbers of CFU in inoculating mixture of *L. casei/S. mutans* were: 10:1, 2.1×10^7 *L. casei* CFU/ml/1.5×10^6 *S. mutans* CFU/ml; 100:1, 3.5×10^7 *L. casei* CFU/ml/2.4×10^5 *S. mutans* CFU/ml; 1,000:1, 4.2×10^7 *L. casei* CFU/ml/2.8×10^4 *S. mutans* CFU/ml; 10,000:1, 1.2×10^8 *L. casei* CFU/ml/1.4×10^4 *S. mutans* CFU/ml.

[b] Values are the mean number (± standard error of the mean) of CFU per milliliter of saliva from 10 to 15 rats per inoculum group as determined on Rogosa SL and MS+S agars.

[c] Values are the mean number (± standard error of the mean) of CFU per tongue from 10 to 15 rats per inoculum group (Rogosa SL and MS+S agars).

[d] Values are the mean number (± standard error of the mean) of CFU per mandible from 10 to 15 rats per inoculum group (Rogosa SL and MS+S agars).

TABLE 4. *Distribution of L. casei strain ATCC 4646 and S. mutans strain 6715 in 45-day-old gnotobiotic rats after mixed infection*

Bacterial inoculation ratio[a] (*L. casei/S. mutans*)	Mean CFU (×10⁴)					
	Saliva[b]		Tongue[c]		Plaque[d]	
	L. casei	*S. mutans*	*L. casei*	*S. mutans*	*L. casei*	*S. mutans*
10:1	240.00 ± 15.90	0.15 ± 0.02	91.20 ± 11.50	0.12 ± 0.01	0.06 ± 0.01	4,970.00 ± 205.00
100:1	370.00 ± 27.30	0.43 ± 0.10	170.00 ± 38.60	0.44 ± 0.09	3.30 ± 0.16	2,240.00 ± 154.80
1,000:1	190.70 ± 9.70	0.31 ± 0.02	275.10 ± 24.90	0.20 ± 0.05	28.00 ± 1.12	1,540.90 ± 90.70
10,000:1	290.30 ± 18.70	0.11 ± 0.09	320.10 ± 15.00	0.37 ± 0.06	76.00 ± 1.20	1,610.00 ± 180.00

[a-d] See Table 3.

TABLE 5. *Distribution of L. casei strain ATCC 4646 and S. mutans 6715 in saliva, tongue, and plaque of rats infected with the former on day 20 followed by the latter on day 25*[a]

Day of sacrifice (age)	Mean CFU ($\times 10^4$)[b]					
	Saliva		Tongue		Plaque	
	L. casei	*S. mutans*	*L. casei*	*S. mutans*	*L. casei*	*S. mutans*
35	13.20 ± 1.0	0.11 ± 0.05	18.70 ± 3.60	0.40 ± 0.10	3.0 ± 0.03	1,204.30 ± 16.50
45	131.0 ± 9.5	0.55 ± 0.15	227.00 ± 26.0	0.80 ± 0.10	44.0 ± 0.03	1,810.00 ± 30.00

[a] Germfree rats (age 20 days) were infected with 2.1×10^7 *L. casei* CFU/ml followed by challenge with 1.8×10^7 *S. mutans* CFU/ml on day 25.
[b] See Tables 2 and 3 for details of methods.

TABLE 6. *Caries scores of gnotobiotic rats (age 45 days) infected with L. casei strain ATCC 4646 and S. mutans strain 6715*

Group	Mean caries scores[a]					
	Buccal		Sulcal		Proximal	
	Enamel	Dentinal slight	Dentinal slight	Dentinal extensive	Enamel	Dentinal slight
10:1[b]	23.4 ± 0.6	17.1 ± 0.7	20.6 ± 0.6	14.3 ± 1.0	7.5 ± 1.1	4.8 ± 0.6
100:1[b]	21.9 ± 0.8	17.2 ± 0.6	19.9 ± 0.7	13.1 ± 1.0	6.6 ± 0.7	4.7 ± 0.6
1,000:1[b]	19.5 ± 0.5[c]	14.1 ± 0.7[c]	18.5 ± 0.5[c]	12.3 ± 0.8[c]	6.0 ± 0.2[c]	4.6 ± 0.4
10,000:1[b]	17.7 ± 0.6[c]	13.5 ± 0.5[c]	17.7 ± 0.9[c]	11.8 ± 0.7[c]	5.7 ± 0.3[c]	3.9 ± 0.3[c]
L. casei (day 20)[d]	19.5 ± 0.9	14.6 ± 0.6[e]	17.5 ± 1.7[c]	12.9 ± 1.2[e]	5.8 ± 0.9[e]	3.5 ± 0.3[c]
S. mutans (day 25)[d]						
S. mutans only	22.4 ± 0.6	16.9 ± 0.7	21.1 ± 0.4	15.8 ± 0.4	8.0 ± 0.1	5.5 ± 0.2

[a] Values are the mean ± standard error of the mean of 10 to 15 rats per inoculum group.
[b] Germfree rats infected at age 20 days. See Tables 3 and 5 for actual number of CFU in inoculating mixture and Tables 4 and 5 for microbial analysis of saliva, tongue, and mandibular plaque.
[c] Significantly lower than obtained in *S. mutans*-monoassociated rats; $P \leq 0.01$.
[d] See Table 5 for actual number of CFU used.
[e] Significantly lower than obtained in *S. mutans*-monoassociated rats; $P \leq 0.05$.

significantly lower ($P \leq 0.05$) than that seen with rats only infected with *S. mutans*.

DISCUSSION

Due to the considerable literature on the potential role of lactobacilli in caries formation (1, 3, 8, 10, 29, 34–38), we have evaluated the ability of *L. casei* to induce dental caries, its site of colonization in the oral cavity, and its influence on *S. mutans*-induced dental caries in gnotobiotic Fischer rats provided a caries-promoting diet containing 5% sucrose (20, 30–32). Both strains of *L. casei* tested (ATCC 4646 and ATCC 11578) colonized tooth surfaces and induced caries formation on buccal and sulcal molar surfaces of 45-day-old rats (Table 1). However, the number of *L. casei* present in plaque and the level of caries induced were significantly lower ($P < 0.01$) than that obtained with similar-aged rats monoassociated with *S. mutans* strain 6715. The difference in the level of colonization and caries activity between *L. casei*- and *S. mutans*-monoassociated rats was still evident when the experiment was prolonged 45 days (90-day-old rats). These results were in agreement with previous studies in rodents with *L. casei* (36, 37), *L. acidophilus* (10), and other species of *Lactobacillus* (9) which demonstrated that lactobacilli induce dental caries. The present study, as well as investigations by Fitzgerald and co-workers (9), further support the contention that lactobacilli have less affinity for the tooth surface of gnotobiotic rats than do *S. mutans*.

The general finding that oral inoculation of gnotobiotic Fischer rats with mixtures of *L. casei* and *S. mutans* resulted in the establishment of the latter in plaque on the tooth surface and the former in saliva and on the tongue suggested that these two genera of oral bacteria have completely different sites and ecological determinants for colonization in this animal model. Numerous studies have been performed to establish the ecological niche of various oral bacteria (4, 12, 22, 25). Studies on host tropism of oral bacteria by Gibbons and co-workers (14, 15, 17, 39) have suggested that lactobacilli exhibit a poor ability to adhere to oral mucosal surfaces, especially in the case of human lactobacilli and rat epithelial cells. However, the number of human oral lactobacilli in saliva and on epithelial sur-

faces was shown to greatly exceed the number on tooth surfaces, thus suggesting that mechanical retention rather than unique growth conditions accounted for the presence of lactobacilli on tooth surfaces (39). Alternatively, lactobacilli may have a lower affinity for the tooth surface than *S. mutans*, whereas the reverse would be true for colonization of epithelial cells. In vitro studies comparing adherence of the *L. casei* and *S. mutans* strains to hard surfaces and epithelial cells will be necessary to clarify this point.

Recent studies in humans by van Houte and co-workers (38) indicated that the level of lactobacilli can reach high proportions (greater than 1% of the total cultivable flora) in plaque before caries development and thus could contribute to the initiation of caries. In our studies, when rats were mixedly infected with numbers of *L. casei* which greatly outnumbered *S. mutans* (1,000:1 and 10,000:1), *L. casei* represented greater than 1% of the flora present on molar surfaces within 5 days after infection. Although the *L. casei* strain used in the present investigation (ATCC 4646) did induce some caries in monoassociated rats (Table 1), it is difficult to conclude from the *L. casei* and *S. mutans* mixed-infection experiments whether *L. casei* did indeed contribute to the initiation of carious lesions. When *L. casei* represented less than 1% of the cultivable flora on molars, the level of caries observed was similar to that obtained in *S. mutans*-monoassociated rats (Tables 2, 4, and 6), whereas when *L. casei* represented greater than 1% of the flora, the level of caries was significantly lower ($P \leq 0.05$) (Tables 4–6). One explanation for these results would be that when *L. casei* represents greater than 1% of the plaque microflora, it occupies sites on the tooth surface which might otherwise be colonized by *S. mutans*. This would result in a net reduction in the number of *S. mutans* in early plaque, a situation which may lead to less caries formation. We cannot rule out, however, the possibility that significant numbers of *L. casei* in plaque actually reduce the cariogenic potential of *S. mutans*.

It should also be pointed out that fresh plaque isolates of *L. casei* may behave differently than the type culture *L. casei* strains used in this study. In this regard, Loesche and Straffon (27) showed that a small number of human subjects with low overall caries experience developed active carious lesions, with lactobacilli being the most prominent member of their plaque flora. Nevertheless, their studies, as well as those of numerous other investigators, clearly associate *S. mutans* with dental caries.

In the present study, we observed a contrasting pattern of colonization by *S. mutans* and *L. casei* for oral tissues. *S. mutans*, even when introduced at low ratios when compared with *L. casei*, quickly became established in plaque. On the other hand, *L. casei* outnumbered *S. mutans* on the tongue and in the saliva. We cannot explain elevated levels of lactobacilli as a result of passive removal from plaque on teeth because, if this occurred, one would also expect to see high levels of *S. mutans* in saliva; however, this did not occur. A more likely explanation would be that *S. mutans* colonizes plaque on teeth and initiates the caries process, whereas the lactobacilli become established at other oral mucosal surfaces, including the tongue. The continued desquamation of mucosal epithelium would lead to elevated levels of lactobacilli in saliva. As *S. mutans*-induced dental caries begins to develop, low levels of lactobacilli may begin to associate with plaque at the developing lesion; nevertheless, their numbers remain disproportionately low when compared with *S. mutans*. Evidence for this was provided by experiments where lactobacilli were superinfected into *S. mutans*-monoassociated rats (Table 5). Later in the experimental design, lactobacilli often reached higher levels in plaque; however, their numbers still comprised less than 5% of the total plaque flora. Although lactobacilli can adhere to molars of monoinfected rats (Table 1), these latter results provide evidence that *S. mutans* has a greater affinity and initially outcompetes lactobacilli for tooth surfaces of rats mixedly infected with these two organisms.

In conclusion, we have presented evidence that *L. casei* selectively colonizes the tongue and saliva and probably reaches plaque by a passive process from saliva. *S. mutans*, on the other hand, selectively and exclusively colonizes the tooth surface, reaches significant numbers in plaque, and initiates decay. *L. casei* has much less cariogenic potential than *S. mutans*, and under conditions where it reaches moderate numbers in plaque, it may actually reduce the level of *S. mutans*-induced dental caries.

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