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Bacteriocins as Factors in the In Vitro Interaction Between Oral Streptococci in Plaque

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The effect of bacteriocins on the composition of dental plaque flora was studied in vitro with bacterial plaque formed by oral streptococci on glass rods suspended in broth medium. Cell-free preparations containing mutacin SW31, a bacteriocin produced by *Streptococcus mutans*, killed sensitive cells present in the plaque selectively, but did not affect resistant cells. Similar preparations from a non-bacteriocinogenic mutant exerted only a slight effect. Mixed growth of bacteriocin-producing *S. mutans* SW31 with the sensitive *S. sanguis* Ny101 resulted in a nearly single-strain plaque of the former. Mutacin could be detected in the plaque substance as well as in the surrounding medium. A non-bacteriocinogenic mutant, which was shown not to be altered in functions affecting growth in plaque, allowed substantial growth of *S. sanguis* Ny101. Sequential inoculation of the bacteriocinogenic plaque former *S. sanguis* P3A3 and the sensitive *S. mutans* OMZ61 showed that the cells of the latter are killed rapidly in established plaques when inoculated with the bacteriocinogenic strain. A trypsin-sensitive antagonistic substance could be detected in the plaque, but not in the surrounding medium. The results indicate that bacteriocins can be active in plaque in vitro and suggest that bacteriocins could play a role in determining the composition of plaque in vivo.

The presence of dental plaque on teeth has long been known to be associated with dental caries and periodontal disease.

Streptococci are prominent organisms in plaque and in carious lesions (2, 3, 21). *Streptococcus mutans* is thought to be important in the initiation of carious lesions (8, 22, 30).

Quite strong evidence indicates that adherence of microorganisms is a regulator of plaque flora in vivo (10, 11, 17, 23). In addition, the involvement of factors such as nutrient requirements (4) and the production of antagonistic substances such as peroxide (7, 16), acid (7, 9, 25), and bacteriocins have been proposed mainly from in vitro experiments. Bacteriocins are produced widely by oral streptococci in vitro (12, 19, 28, 29), and their production was shown to be a species-specific character, with potential significance in dental plaque (31). Whether bacteriocins are actually produced in vivo is unknown. Although a number of objections against an ecological role of bacteriocins in dental plaque have been raised by some authors (7, 20, 27), these objections are based on indirect evidence.

This paper reports on the production and effect of two bacteriocins on the microbial composition of in vitro plaque formed on glass rods.

MATERIALS AND METHODS

Microorganisms. *S. mutans* SW31, *S. mutans* P14B4, and *S. sanguis* P3A3 were isolated from human dental plaque. *S. mutans* OMZ61 and *S. sanguis* Ny101 were obtained from H. van der Hoeven, Institute of Preventive and Community Dentistry, Nijmegen. To facilitate identification in mixed culture, streptomycin- and erythromycin-resistant mutants were selected from some of the strains. The strains were subcultured each fortnight on TY agar, consisting of 3% tryptone soya broth (Oxoid) and 1% yeast extract (Oxoid) solidified with 1.5% agar (Difco), in B&T jars (Searle Co.) filled with 90% N_2 and 10% CO_2.

Preparation of mutacin SW31. *S. mutans* SW31 was inoculated from an overnight culture into a medium consisting of 2% tryptone (Oxoid), 1% Lab Lemco powder (Oxoid), 0.8% sucrose, 0.2% NaCl, 0.2% $NaHCO_3$, and 0.04% Na_2HPO_4, and was grown in static culture for 40 h at 37°C. All subsequent steps were carried out at 4°C. Cells were removed by centrifugation for 15 min at 12,000 × *g*. The clear supernatant was brought to 35% saturation by slowly adding powdered ammonium sulfate under constant stirring. After 60 min, the precipitate was pelleted by centrifugation for 20 min at 18,000 × *g*. The pellet was dissolved in 1/100 of the original volume of 0.05 M potassium phosphate buffer (pH 7.0), dialyzed overnight against a large excess of the same buffer, and heated for 15 min at 70°C. The resultant preparation could be stored at 4°C without

loss in activity for at least 2 weeks and was used in the experiments. Further purification resulted in highly labile preparations not suitable for the present work.

Determination of properties of mutacin SW31. The apparent molecular weight of mutacin SW31, prepared by ammonium sulfate precipitation, was calculated from its elution pattern on a Sephadex G-100 column (1.5 by 45 cm) prepared in 0.05 M potassium phosphate buffer (pH 7.0). The column was previously calibrated, using blue dextran 2000, bovine serum albumin, and cytochrome *c* as standards.

The isoelectric point of mutacin SW31 was determined by use of an LKB 8100-1 Ampholine column with carrier ampholytes of pH 3 to 10, operated at 14 W for 72 h. Fractions were collected and assayed for mutacin activity, protein (absorption at 280 nm), and pH.

The effect of various enzymes on mutacin activity was tested by incubating the bacteriocin (final concentration, 250 arbitrary units per ml) for 15 min at 37°C with 0.2 mg of the enzymes per ml in 0.05 M potassium phosphate buffer (pH 7.0) containing 0.01 M magnesium sulfate. Then the bacteriocin activity was tested as described below. No inhibition zones were observed in experiments with the enzymes alone.

Bacteriocin activity testing procedures. Production of bacteriocin in agar medium was tested by the stab inoculation technique. Cells of an overnight broth culture were stab inoculated into agar plates and incubated for 48 h at 37°C under an N_2-CO_2 atmosphere. After this time, overnight cultures of the indicator strain, diluted 1,000-fold into fresh medium, were sprayed over the plates, which were incubated for another 24 h. Clear zones in which growth was absent appeared around inoculated spots of producer strains.

To establish whether an inhibition zone was due to the presence of a bacteriocin, plates were sprayed after the initial growth with a filter-sterilized solution of trypsin (1 mg/ml), incubated for 1 h at 37°C, and subsequently sprayed with a solution of trypsin inhibitor (2 mg/ml). After standing at 37°C for about 1 h to allow the surface of the agar to dry, the plates were sprayed with the indicator strain.

The presence of bacteriocins in broth cultures was tested after removal of the cells by centrifugation for 15 min at 12,000 × *g*. Samples (0.1 ml) of the supernatant were pipetted into wells (9-mm diameter) punched in TY agar plates, previously seeded with about 10^5 colony-forming units of the indicator strain. The plates were subsequently incubated at 37°C for 24 h, after which bacteriocin activity resulted in clear zones around the wells.

Quantitation of cell-free bacteriocin preparations was carried out by pipetting 0.1-ml amounts of serial twofold dilutions into the wells. *S. sanguis* Ny101 was used as the indicator strain. After overnight incubation, the approximate surface area of the inhibition zones was plotted against $\log_2$ of the dilution factor. The apparent dilution at the dissection point of the resulting straight line with the dilution axis was assumed to contain 1 arbitrary unit of activity per 0.1 ml.

Assay of bacteriocin in plaque samples was carried out by a modification of the well diffusion test. Rods with adherent plaque were transferred to sterile TH-sucrose broth, which consisted of 3.64% Todd-Hewitt broth (Oxoid) supplemented with 2% sucrose, and incubated for another 6 h at 37°C. Then the rods were carefully rinsed with 5 ml of saline (0.85% NaCl), and the plaque was scraped into about 0.5 ml of soft (0.8%) TY agar at 70°C and kept for 15 min at this temperature. Samples (0.1 ml) of plaque suspension were pipetted into wells as described. After overnight incubation, clear zones around the wells indicated bacteriocin activity. Alternatively, the rods with adherent plaque were treated with chloroform vapor for 15 min and then treated as described, but omitting the temperature step. Culturing of temperature- or chloroform-treated plaque suspensions showed complete absence of surviving cells.

Determination of the killing effect. *S. sanguis* Ny101 was grown into mid-exponential phase in TY medium at 37°C in static culture. Cells were centrifuged, washed, and resuspended in 0.05 M potassium phosphate buffer (pH 7.0) at a concentration of 8.0×10^8 cells/ml. The suspension was split into two portions and incubated at 37°C. After 5 min, mutacin SW31 was added (final concentration, 100 arbitrary units per ml) to one portion; the other received only buffer. At the start and at times indicated by arrows in Fig. 1, samples were withdrawn and further incubated with trypsin (0.2 mg/ml). At intervals, portions were taken from all incubations, rapidly diluted more than 100-fold into ice-cold TY medium, and plated on TY agar.

In vitro plaque formation. The method used was a modification of that described by Jordan and Keyes (18). Plaque was formed on glass rods (12 by 0.4 cm) suspended from silicone rubber stoppers into test tubes (19 by 1.5 cm), filled to 2 cm from the top with TH-sucrose broth. The medium was inoculated with 0.2 ml of an overnight culture grown in TH broth. When mixed-culture plaque was required, 0.2 ml of each separately grown strain was used. After 24 h at 37°C, rods with adherent plaque were transferred to test tubes filled with freshly inoculated medium. This was repeated daily until the plaque was used for analysis.

Analysis of in vitro plaque. Rods were removed from the medium and carefully dried with tissue. Wet weights of plaque material were measured by weighing rods plus plaque and then scraping off the total plaque substance into 6 ml of sterile saline and reweighing the rods. The bacterial composition was determined after dispersion of the plaque material by two bursts of ultrasonic oscillation for 20 s with a microtipped Branson Sonifier at 75 W and subsequent culturing of serial dilutions of the suspension on TY agar supplemented with streptomycin or erythromycin (both 0.1 mg/ml) and incubation for 48 h under N_2/CO_2 atmosphere.

Mutagenic procedures. Non-bacteriocinogenic mutants of *S. mutans* SW31 were obtained according to a modified method of Adelberg et al. (1). Cells from 10 ml of a mid-exponential-phase culture, growing anaerobically in TH broth, were collected

on a membrane filter (0.45 μm; Millipore Corp.), washed, and resuspended in an equal volume of 0.05 M tris(hydroxymethyl)aminomethane-maleic acid buffer (pH 6.0) containing 100 μg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine per ml and incubated at 37°C for 30 min. Then cells were filtered, washed twice with buffer, and resuspended in 50 ml of TH broth. After growth at 37°C for about 4 h, the cell suspension was diluted to give about 50 colonies per plate when plated on TH agar. Plates were incubated anaerobically for 48 h and subsequently sprayed with a suspension of *S. sanguis* Ny101 (about 10^5 colony-forming units per plate). Absence of a clear zone around the primary colonies after another 24 h of incubation indicated loss of bacteriocin-producing ability. Such colonies were picked, purified, and further tested for bacteriocin production.

Chemicals. Trypsin (80,000 U/g) and trypsin inhibitor were obtained from Merck (Darmstadt, West Germany), streptomycin from Mycopharm (Delft, The Netherlands), erythromycin lactobionate from Abbott (Brussels, Belgium), and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine from Fluka (Buchs, Switzerland).

RESULTS

Production and properties of mutacin SW31. *S. mutans* SW31 produces in agar and liquid media an antibacterial substance with the characteristics of a bacteriocin. The highest activity in agar medium was obtained with TH agar; activity in liquid medium was only produced when sucrose was incorporated in the medium. The molecular weight of the mutacin after ammonium sulfate precipitation was about 40,000 as determined from Sephadex G-100 gel filtration, and the isoelectric point was about 8.0. In Table 1 some additional properties are given.

Sensitive cells were rapidly killed, but could be rescued by the action of trypsin (Fig. 1), which rapidly destroyed the bacteriocin and not the bacteriocin receptors presumed to be present in the cell envelope, since cells pretreated with trypsin were not protected from killing (not shown). The action spectrum (Table 2) was characterized by the relatively narrow range of susceptible strains when compared with other mutacins (13, 29); however, it was similar to that of an *S. mutans* GS5 mutacin described by Paul and Slade (24).

Growth of in vitro plaque. Plaque wet weights increased linearly with time during incubation for several days (Fig. 2 and 3). However, different strains had quite different rates of plaque formation, and the number of colony-forming units per weight unit of plaque substance varied significantly and ranged between 10^9 and 6×10^{10} per g (wet weight) in single-strain cultures. The number of colony-forming units per gram (wet weight) increased in time for some strains, whereas it decreased slightly for others (Fig. 2). This value may be influenced by factors such as the composition and amount of plaque matrix, the proportion of dead cells in the plaque, and incomplete dispersion of plaque material. The latter was routinely monitored by microscopic observation of plaque suspensions. With the strains used in the present investigation, clumping, although not completely absent, did not greatly influence the results.

TABLE 1. *Effect of various treatments on mutacin SW31 activity*[a]

Stable	Labile[b]
pH 1-10 (10 min, 37°C)[c]	
70°C, 15 min	120°C, 15 min (50% loss)
DNase	Trypsin
RNase	Pronase P
Phospholipase C	Lipase
Phospholipase D	

[a] Abbreviations: DNase, Deoxyribonuclease; RNase, ribonuclease.

[b] Activity fully destroyed unless otherwise indicated.

[c] In acetate or phosphate buffer, adjusted to the desired pH and then neutralized with NaOH or HCl.

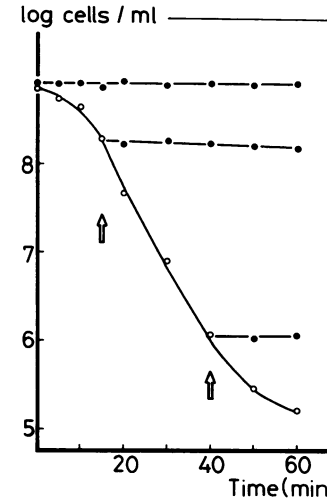

FIG. 1. *Killing effect of mutacin SW31. Washed cell suspensions of S. sanguis Ny101, containing 8.0 × 10^8 cells/ml in 0.05 M phosphate buffer (pH 7.0), were incubated at 37°C with 100 arbitrary units of mutacin SW31 (ammonium sulfate preparation) per ml. At the start and the times indicated by arrows, samples were withdrawn and further incubated with trypsin (0.2 mg/ml). At intervals, samples were rapidly diluted 100-fold into ice-cold TY broth and then plated in suitable dilutions. Symbols:* (○) *Treated with mutacin only;* (●) *treated with mutacin and then with trypsin.*

TABLE 2. *Action spectrum of mutacin SW31*

Indicator strain	No. tested[a]	No. sensitive[a]
S. mutans (serotypes *a–d*)	18	0
S. sanguis	14	12
S. salivarius	8	7
Other streptococci (serogroups A-C, E-G, K-P, and R-U, and *S. uberis*)	27	25
S. faecalis/faecium (serogroup D)	5	0
Actinomyces sp. (oral)	4	2
Other gram-positive bacteria[b]	25	0
Gram-negative bacteria[c]	19	0

[a] Determined by the well diffusion test as described in Materials and Methods.

[b] Genera include *Staphylococcus, Bacillus, Lactobacillus, Nocardia,* and *Bacterionema.*

[c] Genera include *Escherichia, Citrobacter, Fusobacterium, Neisseria, Proteus, Pseudomonas, Serratia,* and *Veillonella.*

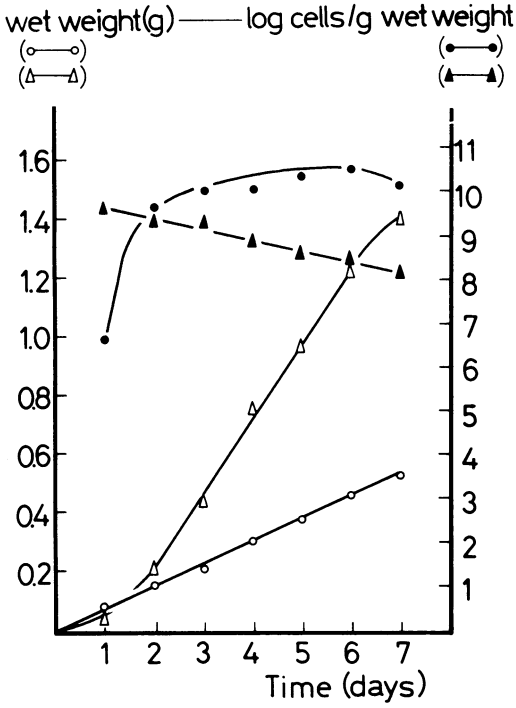

FIG. 2. *Growth of single-strain plaques on glass rods in TH-sucrose broth. Symbols:* (○, △) *Wet weight of plaque;* (●, ▲) *number of viable cells per gram (wet weight) of plaque;* (△, ▲) *S. mutans OMZ61;* (○, ●) *S. mutans P14B4.*

All *S. mutans* strains used and, in addition, *S. sanguis* P3A3 were plaque formers in single-strain cultures.

Production of bacteriocin in plaque. In TH-sucrose agar, both *S. mutans* SW31 and *S. sanguis* P3A3 produced large inhibition zones against the indicator strains used (Table 3). After treatment with trypsin, no or only small zones were observed. However, *S. mutans* SW31K2, a non-bacteriocinogenic mutant of *S. mutans* SW31, produced only a small, hazy, trypsin-resistant zone. Under the conditions used in the plaque experiments, mutacin SW31 could be detected in the liquid medium as well as in the plaque substance, whereas the *S. sanguis* P3A3 bacteriocin was detected in the plaque substance only. The mutant strain SW31K2 was non-bacteriocinogenic both in the liquid medium and in the plaque substance.

Influence of mutacin SW31 on mixed-plaque composition. Mixed growth of *S. sanguis* OMZ61s and *S. sanguis* Ny101e ("s" and "e" indicate streptomycin and erythromycin resistance, respectively) resulted in plaques with fairly constant proportions of both strains in time. The strains produced no antagonistic substance to each other in plate tests. On addition of mutacin SW31 to the culture liquid, the amount of viable *S. mutans* OMZ61s (resistant to the bacteriocin in plate test) remained unchanged, whereas the viability of *S. sanguis* Ny101e (sensitive to mutacin SW31) decreased rapidly to below the limit of detection (Fig. 4). In control experiments with an identical prepa-

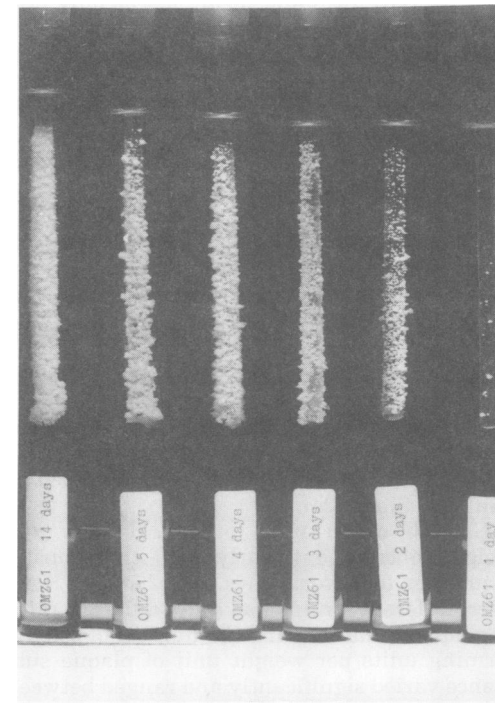

FIG. 3. *Growth of S. mutans OMZ61 on glass rods in TH-sucrose broth.*

TABLE 3. *Antagonistic activity of the strains used in the plaque experiments*

Strains tested		Width of inhibition zone (mm)[a]			
		Agar medium test[b]		In vitro plaque system[c]	
Producer	Indicator	Untreated	Trypsin treated	Liquid medium	Plaque substance
S. mutans SW31	*S. sanguis* Ny101s	10 (S)	2 (H)	5	5
S. sanguis Ny101s	*S. mutans* SW31	2 (H)	2 (H)	0	
S. mutans SW31K2	*S. sanguis* Ny101s	2 (H)	2 (H)	0	0
S. sanguis Ny101s	*S. mutans* SW31K2	2 (H)	2 (H)	0	
S. sanguis P3A3	*S. mutans* OMZ61s	10 (S)	0	0	4
S. mutans OMZ61s	*S. sanguis* P3A3	0		0	0

[a] Measured from the edge of the well or colony to the margin of growth.

[b] Stab inoculation test with TH-sucrose agar was used. Trypsin treatment was carried out as described in the text. (S) refers to zones with clear, sharp edges; (H) indicates hazy zones with diffuse edges.

[c] Well diffusion test was used for samples taken from the liquid medium after removal of the cells by centrifugation. Plaque samples were tested in an analogous way, as described in the text. In separate experiments it was shown that the material produced by *S. mutans* SW31 and *S. sanguis* P3A3 was destroyed by trypsin.

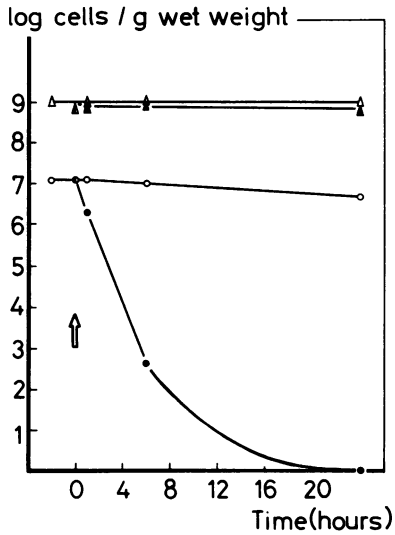

FIG. 4. *Influence of mutacin SW31 on a mixed plaque of S. mutans OMZ61s* (△, ▲) *and S. sanguis Ny101e* (○, ●). *At the time indicated by the arrow, the plaques were transferred to sterile media containing either 30 arbitrary units of mutacin SW31 per ml (closed symbols) or an equal amount of a preparation obtained from the non-bacteriocinogenic strain S. mutans SW31K2 (open symbols).*

ration of the supernatant taken from *S. mutans* SW31K2 cultures, only a slight effect on the viability of *S. sanguis* Ny101e was observed (Fig. 4). These results indicate that externally added bacteriocin diffuses into the plaque and remains active long enough to kill the sensitive cells present.

Mixed growth of bacteriocin-producing and -sensitive strains. Analysis of plaques after 4 days of mixed growth (Table 4) showed that the bacteriocinogenic *S. mutans* SW31 greatly outnumbered the sensitive *S. sanguis* Ny101s; however, when the non-bacteriocinogenic mutant SW31K2 was used, *S. sanguis* Ny101s made up a substantial part of the viable plaque flora. The pH of the incubation medium was identical with both combinations. The results might have been influenced by altered properties of the mutant strain other than loss of the production of an antagonistic substance, such as growth rate, plaque-forming ability, or clumping. Therefore, the parent and mutant strain were grown together in mixed plaque. Both strains were recovered in almost equal numbers. Furthermore, the amount of plaque wet weight was almost identical for both strains.

To investigate the role of bacteriocin in the establishment of strains in preexisting plaque, the two plaque-forming strains, *S. mutans* OMZ61 and *S. sanguis* P3A3, were successively inoculated at 2-day intervals (Fig. 5). *S. mutans* OMZ61 plaque was rapidly invaded by bacteriocinogenic *S. sanguis* P3A3. In the reverse experiment, *S. mutans* OMZ61s did not significantly contribute to the total plaque flora, although considerable amounts of *S. mutans* OMZ61s were found in the culture medium (Table 5). Analysis of the total number of viable cells in the plaque (Fig. 6) showed that *S. mutans* OMZ61s was not just overgrown by *S. sanguis* P3A3 but was actually killed, since 24 h after the addition of *S. sanguis* P3A3 the total viable cell count dropped to only 5% of the original value. The proportion of *S. mutans*

OMZ61s at this time was only 0.15% of the original viable count (Fig. 5).

DISCUSSION

Data so far obtained concerning the possible role of bacteriocins in microbial ecology are conflicting (15). This is particularly true for dental plaque, although bacteriocins are produced abundantly by oral streptococci (12, 19, 28, 29). Rogers (26) reported that bacteriocin production depends on specific nutritional requirements that will hardly be met in vivo. Since bacteriocins are proteins and thus potentially susceptible to the action of proteases, they would be inactivated rapidly in situ by plaque proteases (20). However, according to others (6, 12), many bacteriocin-like substances, although sensitive to trypsin, are only slightly affected by plaque proteases. A third objection was raised as a result of the observation that protection against bacteriocin action was induced in sensitive oral streptococci on growth in media containing sucrose (27, 31), presumably as a consequence of the diffusion barrier provided by the extracellular polysaccharides formed. However, this phenomenon was not observed with all strains (6, 31). The ultimate proof of the active role of bacteriocins in plaque would be delivered by a demonstration of a direct influence of bacteriocins on the plaque ecology.

The present results clearly indicate that bacteriocins are produced in plaque in vitro, kill sensitive cells, and thus regulate the microbial composition of the plaque.

The plaque matrix does not prevent the action of bacteriocins, but instead may serve as a stabilizing environment, as is suggested by the following observations: (i) incorporation of su-

TABLE 4. *Analysis of in vitro plaque after 4 days of mixed culture*

Combined strains	Cells/g		Plaque wt (g)[a]
	Wet wt	Ratio	
S. mutans SW31 *S. sanguis* Ny101s	1.8×10^9 7.1×10^2	2.5×10^6	0.17
S. mutans SW31K2 *S. sanguis* Ny101s	8.0×10^8 1.1×10^7	73	0.16
S. mutans SW31 *S. mutans* SW31K2s	7.4×10^8 4.9×10^8	1.5	0.17

[a] Plaque formed by single-strain cultures of *S. mutans* SW31 and *S. mutans* SW31K2 weighed 0.17 and 0.15 g, respectively.

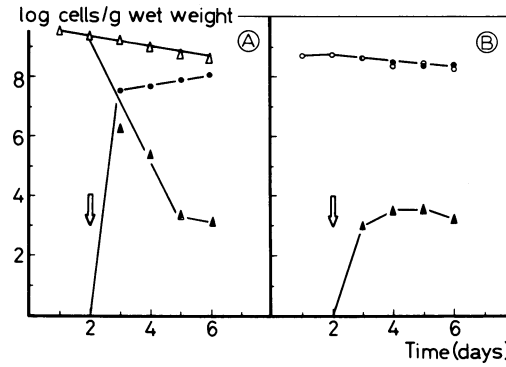

FIG. 5. *Effect of superinfection of preestablished plaque with bacteriocin-producing and nonproducing strains on the bacterial composition of the plaque. (A) Plaque preformed by S. mutans OMZ61s (△, ▲) was incubated with cultures of the bacteriocin-producing S. sanguis P3A3 (●) at the time indicated by the arrow. (B) S. sanguis P3A3 plaque (○, ●) was incubated with cultures of S. mutans OMZ61s (▲). Open symbols represent single-strain cultures of the strain indicated; closed symbols represent the number of cells in mixed cultures.*

TABLE 5. *Analysis of single-strain and mixed cultures of S. mutans OMZ61s and S. sanguis P3A3 after 4 days of incubation*

Primary strain[a]	Secondary strain[a]	Plaque				Medium (cells/ml of medium)		
		Cells/g (wet wt)			g (wet wt)	*S. mutans*	*S. sanguis*	Ratio[b]
		S. mutans	*S. sanguis*	Ratio[b]				
S. mutans OMZ61s		9.0×10^8			0.79	NT[c]		
S. sanguis P3A3			3.2×10^8		1.01		NT	
S. mutans OMZ61s	*S. sanguis* P3A3	2.5×10^5	4.0×10^7	1.6×10^2	0.88	$<10^3$	1.6×10^6	$>1.6 \times 10^3$
S. sanguis P3A3	*S. mutans* OMZ61s	3.1×10^3	3.3×10^8	1.1×10^5	0.73	4.2×10^5	2.0×10^6	4.8

[a] Plaques of the primary strains were transferred after 2 days of growth to media inoculated with the secondary strain and were then incubated for 2 more days.
[b] Ratio of *S. sanguis* P3A3 to *S. mutans* OMZ61s.
[c] NT, Not tested.

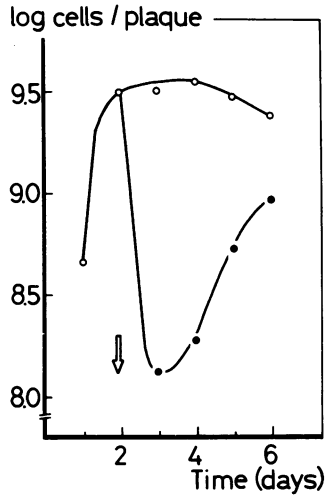

FIG. 6. *Total number of viable cells in preestablished S. mutans OMZ61s plaque alone* (○) *and after incubation with S. sanguis P3A3 added at the time indicated by the arrow* (●). *Data were derived from the experiment described in Fig. 5A.*

crose in the medium is required to obtain active mutacin SW31 in liquid culture, but may be replaced by glucose in agar medium; and (ii) antagonistic activity could be detected in plaques of *S. sanguis* P3A3 but not in the surrounding medium. The idea is further stressed by the observations made by other investigators (5, 24) who reported that mutacin activity is associated with the surface of the producer cells, from which it can be dissociated by mechanical treatment.

It is tempting to extend the present findings to natural dental plaques. Arguments against an ecological role for bacteriocins in dental plaque have been made because bacteriocinogenic and sensitive strains coexist at the same sites (19, 27). However, no confirmatory data were presented by the authors. In this connection, it is of interest that the mutual inhibition of streptococcal strains in small plaque samples is somewhat lower than among strains isolated from plaques obtained from different tooth surfaces (31). Histological investigation of plaque shows that bacteria often exist as discrete microcolonies (14). Thus, bacteriocins might play a significant role on a microecological scale in dental plaque in vivo.

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