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Adherence of *Veillonella* Species Mediated by Extracellular Glucosyltransferase from *Streptococcus salivarius*

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The effect of extracellular products from *Streptococcus salivarius* on sucrose-dependent adherence to smooth surfaces by other oral bacteria was studied in vitro. Strains of *Streptococcus mitis, Streptococcus pyogenes,* and *Veillonella parvula* without innate ability to adhere to a steel wire were able to do so when incubated with sucrose and cell-free culture fluid from *S. salivarius* strains 9759, 25975, CNII, and MEPI. These culture fluids synthesized more adherent material and water-insoluble glucan than those from *Streptococcus mutans* C67-1 and seven other *S. salivarius* strains. Among the *S. salivarius* strains, glucosyltransferase (GT; dextransucrase, EC 2.4.1.5) activity varied more than 100-fold. Cells of *Veillonella* and *S. mitis* S3 that had been incubated in culture fluids from *S. salivarius* 25975 and 9759, respectively, and then washed adhered upon subsequent incubation with sucrose. This was due to adsorbed GT because (i) the adherence was sensitive to dextranase; (ii) it was observed only with the high-GT culture fluids; (iii) it was dependent on sucrose; and (iv) the washed *Veillonella* cells synthesized glucan, but not fructan, from sucrose. These results suggest that sucrose-dependent adherence of bacteria without such innate ability can be mediated by (i) entrapment in insoluble glucan synthesized by *S. salivarius* culture fluids, and (ii) prior adsorption of GT from *S. salivarius* culture fluids. The possibility that GT formed by high-yield strains of *S. salivarius* is distributed through the mouth by the action of salivary flow and contributes to sucrose-dependent adherence and plaque formation is considered.

Streptococcus salivarius commonly occurs in the human mouth. It is prominent on the dorsum of the tongue, in saliva, and on the mucosal surfaces of the cheek (22). On the tongue and in saliva, it constitutes about 40 to 60% of the facultatively anaerobic streptococci, and on the mucosal surfaces of the cheek it constitutes about 10 to 20% (16, 21, 49). Estimates of the concentration in saliva average about 10^8 organisms per ml (4, 42, 48, 53), with a range from 4×10^5 (3, 42) to 6×10^8 (48, 53). This variation is likely due to factors such as diet (3, 4), time of sampling, and age (49). In contrast, *S. salivarius* constitutes only about 1% of the facultative streptococci in human dental plaque (2, 22); however, this value is also diet dependent (3, 4). It has been proposed that its low prevalence in plaque is due to the relatively poor affinity of the organism for teeth and developing plaque (51, 52).

Certain strains of *S. salivarius* have proven to be cariogenic in hamster (15, 29, 32) and gnotobiotic rat (17, 23, 24, 31, 45) model systems. In fact, *S. salivarius* may have been the first organism shown to be cariogenic in animals (1). Compared with *Streptococcus mutans, S. salivarius* appears to be less cariogenic for smooth surfaces and fissures (14, 23, 24, 29, 32), and more cariogenic for root surfaces in rodents (16), but because of the low prevalence in human plaque, it has generally been assumed that *S. salivarius* does not play a direct or significant role in the development of plaque and human caries (16).

In this paper, we will describe experiments that show how *S. salivarius* might contribute indirectly to sucrose-dependent adherence and colonization. We found that many strains of *S. salivarius* excreted significant amounts of glucosyltransferase (GT; dextransucrase, EC 2.4.1.5) and that in the presence of sucrose this enzyme catalyzed the formation of insoluble glucan and adherent deposits. This allowed entrapment and colonization of several veillonellae and streptococci that normally do not colonize smooth surfaces. In addition, incubation of strains of *Veillonella* in *S. salivarius* culture fluids permitted them to adsorb GT and subsequently allowed them to adhere to steel wires. Since teeth and developing plaque are exposed to saliva and

since whole saliva can be expected to contain extracellular products, including GT, formed elsewhere in the mouth by *S. salivarius*, it becomes apparent how *S. salivarius* might play an indirect role in sucrose-dependent adherence and colonization in vivo.

MATERIALS AND METHODS

Cultures. Table 1 lists the organisms studied and their origin. All but four of the *S. salivarius* strains were purchased from the American Type Culture Collection. The two strains isolated from human dental plaque were identified as *S. salivarius* according to *Bergey's Manual* (10). Upon receipt, all *S. salivarius* strains were streaked on mitis salivarius agar, and the plates were incubated overnight in an anaerobic GasPak (Baltimore Biological Laboratory [BBL]) at 37°C. A typical single colony was transferred to fluid thioglycolate medium containing 20% beef infusion and 5% $CaCO_3$. After growth at 37°C, the culture was stored at 4°C and transferred monthly. To check the purity of stock cultures, they were cultivated on Columbia agar with 5% defibrinated blood.

On mitis salivarius agar, all strains except 31067 were readily recognizable as *S. salivarius* because they formed relatively large, rough or mucoid colonies with diameters of 1 to 5 mm after overnight incubation. Some differences were apparent. For instance, strain 25975 had a rough ("frosty glass") appearance, and strain 9759 had a "fried egg" appearance due to exudate in the center of the colony. Strain 31067 formed small, flat, nonmucoid colonies, unlike the other strains.

The other *Streptococcus, Lactobacillus,* and *Veillonella* cultures were maintained as described above. For the experiments, cultures were grown at 37°C in brain heart infusion (BHI) in a 5% CO_2-air incubator. For the *Veillonella,* the media were boiled before use, and the cultures were incubated anaerobically (43). To check viability and purity, the *Veillonella* were streaked on V23 agar (43), and the plates were incubated for 24 h in 95% N_2-5% CO_2. The streptococci were similarly streaked on Columbia blood agar, and the plates were incubated in 5% CO_2-air. Gram strains were also done.

***S. salivarius* culture fluids.** Cultures were grown overnight into stationary phase in 100 ml of BHI (BBL) in a CO_2-air incubator. After removal of the cells by centrifugation (10,000 × *g*), the supernatant fluid was adjusted to pH 6.5 with KOH, passed through a membrane filter (0.45-μm pore size; Nalgene Labware Div., Nalge/Sybron Corp.), and stored at 4°C for future use. This filtration did not remove significant amounts of GT or fructosyltransferase (FT; levansucrase, EC 2.4.1.10) activity, and the filtrate retained these activities for at least 2 weeks (data not shown). The cells were washed once and then suspended in phosphate-buffered saline (PBS), pH 6.5, for the assays.

In vitro plaque formation. The method of McCabe et al. (35) was used, with the following modifications. BHI supplemented with 5% sucrose served as the test medium. The tubes of media were freshly inoculated with *Streptococcus* or *Veillonella* only for the first 3 days. Plaque accumulation was rated after a total of 7 days. To test the microbiological purity and viability of the plaques, a small piece was dispersed in saline and plated on Columbia blood or V23 agar.

In certain experiments, the medium was adjusted to pH 6.5 and supplemented with 1 ml of cell-free culture fluid from *S. salivarius* just before insertion of the wire. For the cell-free "plaque" formation studies, 0.02% thimerosal was added to insure sterility.

Enzyme assays. GT activities forming water-insoluble and -soluble glucan were determined by a

TABLE 1. *List of strains*

Organism	Designation		Source and/or description
	ATCC	Other	
Streptococcus salivarius	7073	NCTC 8618	ATCC; serological type I
	9222		Own collection
	9758		ATCC
	9759		Own collection; serological type I
	13419		ATCC; serological type II
	25975		ATCC
	27006	SS2	ATCC; cariogenic (15, 17)
	27945	NCTC 8606	ATCC; serological type I
	31067		ATCC
		CNII	S. A. Robrish; clinical isolate from human dental plaque
		MEPI	S. A. Robrish; clinical isolate from human dental plaque
S. mitis	9811		Own collection
	15909		Own collection
		S3	Own collection (18)
S. mutans		C67-1	J. D. de Stoppelaar (11)
S. pyogenes	14289		Own collection
S. sanguis	10556		Own collection
	10558		Own collection
		T175	J. D. de Stoppelaar
Veillonella alcalescens	17746	RV-12x	M. Rogosa (44)
	17747	HV-1	M. Rogosa (44)
V. parvula	17743	HV-19	M. Rogosa (44)
	17744	KON	M. Rogosa (44)
Lactobacillus acidophilus		CL35	J. London
L. casei		64H	J. London
L. coryniformis		M34	J. London

procedure modified from the one described earlier (8). A suitable volume of cell-free culture fluid or washed cells was incubated with 1.1 ml of 0.1 M potassium phosphate buffer, pH 6.5, containing 40 mM [*glucosyl*-^{14}C]sucrose (specific activity, 0.0053 Ci/mol) in a final volume of 2.2 ml. After 1 h at 37°C, the assay mixture was thoroughly agitated, and a 1.0-ml portion was mixed with 10 ml of 0.05 M phosphate buffer (4°C, pH 6.5) in a filtration manifold cup. Water-insoluble polymer was collected on a membrane filter (pore size, 0.45 μm; Metricel GN-6, Gelman Instrument Co., or type HA, Millipore Corp.) by vacuum filtration, washed with 10 ml of buffer and then with 5 ml of 80% ethanol, and counted in 5 ml of Aquasol (New England Nuclear Corp.). The channels ratio method was used to determine absolute activity.

To quantitate the total of water-soluble and -insoluble polymer, another 1.0-ml portion of the assay mixture was mixed with 10 ml of cold 80% ethanol in another cup on the manifold. After filtration and washing with 15 ml of 80% ethanol, the filter was similarly counted. The difference between total and water-insoluble glucan represents water-soluble glucan.

FT activities were assayed similarly, except that [*fructosyl*-^{14}C]sucrose, with a specific activity of 0.0042 Ci/mol, was used as the substrate.

GT and FT activities were converted into international units (micromoles of substrate converted into product per minute).

Adherence of *Veillonella* after incubation in *S. salivarius* culture fluid. Strains of *Veillonella* were cultivated for 24 h in BHI supplemented with 5% sucrose and CO_2. Sucrose is not metabolized by *Veillonella* (43), but it was added to mimic events after oral intake of sucrose. Cells from 10 ml of medium were collected by centrifugation, suspended in 10 ml of PBS (pH 6.8), and spun down again. The washed cells were suspended in 5 ml of culture fluid from *S. salivarius* 25975 and kept for 3 h at 22°C. The cells were collected by centrifugation, suspended in 10 ml of PBS, and spun down again. The pellet was suspended in 10 ml of BHI with 5% sucrose and a pH of 6.5 and incubated for 24 h at 37°C with a wire inserted in the medium. After two further incubations of the wire in fresh medium, the extent of "plaque" accumulation was rated (35). In the control experiment, the cells were incubated in uninoculated BHI rather than in *S. salivarius* culture fluid.

A similar experiment was done with *Streptococcus mitis* S3 and culture fluid from *S. salivarius* 9759.

Adsorption of GT and FT by *Veillonella*. *Veillonella parvula* KON was grown anaerobically (95% N_2-5% CO_2) in a modified Jordan medium (13) supplemented with 5% sucrose. After 20 h of incubation at 37°C, cells from 20 ml of medium were collected by centrifugation, suspended in 10 ml of PBS, and recentrifuged in 5-ml portions. One pellet was suspended in 5 ml of *S. salivarius* 25975 culture fluid and the other was suspended in 5 ml of uninoculated BHI. These suspensions were kept at 22°C for 3 h. The cells were spun down, suspended in 5 ml of PBS, and spun down again. The washed cells were resuspended in 5 ml of PBS, and a 1.0-ml portion of this suspension was incubated with 1.5 ml of H_2O and 2.5 ml of 40 mM [*glucosyl*-^{14}C]sucrose. After 1 and 2 h at 37°C, 1.0-ml portions were used to determine total and insoluble glucan synthesis as described above. Similar assays were done to quantitate fructan synthesis.

Other materials. A *Penicillium funiculosum* (NRRL 1768) dextranase (α-1,6-glucan 6-glucanohydrolase; EC 3.2.1.11) preparation from Merck Sharp & Dohme Research Laboratories containing 50% mannitol was used (lot L-675,659-0-25). When received in 1971, it had a specific activity of 5,000 international units/mg (dry weight) (7; this corresponds to 116 international units/mg [dry weight]). Consequently, it can be calculated that the purity was about 40% (7). This preparation was reassayed for the present study and found to have retained its activity.

Sucrose labeled in the glucosyl or fructosyl moiety was obtained from New England Nuclear Corp. Carrier sucrose (Ultra Pure) was purchased from Schwarz/Mann.

RESULTS

Effect of *S. salivarius* culture fluids on colonization. Because teeth and developing plaque are exposed to metabolites formed elsewhere in the mouth by *S. salivarius*, the effect of such metabolites on adherence, colonization, and "plaque" formation was evaluated. Three streptococci and two veillonellae without innate ability to form plaquelike deposits were used as test organisms. The results (Table 2) showed that plaquelike deposits were formed on the steel wires when these five organisms were supplemented with sucrose and cell-free culture fluid from *S. salivarius* 9759. This was accompanied by colonization of the test organisms, because abundant growth occurred after dispersion and plating of a small piece of the plaquelike deposits. In addition, turbidity developed when

TABLE 2. *Effect of 10% cell-free culture fluid from S. salivarius 9759 on in vitro "plaque" formation by Streptococcus and Veillonella*

Organism	"Plaque" formation[a]	
	With culture fluid	Without culture fluid
S. pyogenes 14289	3	0
S. mitis 15909	4	0
S. mitis S3	4	0
V. parvula HV-19	>6	0
V. parvula KON	>6[b]	0
S. mitis 9811	3	1
S. sanguis T175	>6	2
S. sanguis 10556	5	2
S. sanguis 10558	5	4

[a] Assessed after 7 days, on a scale from 0 to 6, as described in the text. A rating of >6 indicates that a grade 6 plaque was obtained before the end of the test period.

[b] With a 10% supplement of culture fluids from *S. salivarius* 7073, 9222, and 31067, the "plaque" formation of *V. parvula* KON was rated as 0, <1, and <1, respectively.

the "plaque"-coated wires were transferred to medium not supplemented with *S. salivarius* culture fluid because of outgrowth of bacteria colonizing the wires. The presence of viable cells in the *Veillonella* "plaques" was also evident from the gas formed. The five control cultures that were never supplemented with *S. salivarius* culture fluid did not form adherent, plaquelike deposits, and the wires often became sterile after several transfers. One strain of *S. mitis* and three strains of *Streptococcus sanguis* that formed some primary "plaque" with sucrose were also examined. These organisms all formed more "plaque" when supplemented with culture fluid from *S. salivarius* 9759 (Table 2). Similar results were obtained when culture fluids from *S. salivarius* 25975, CNII, and MEPI were used as supplements (data not shown). These phenomena were sucrose dependent, because no adherent deposits were observed when sucrose was omitted. Comparatively little or no adherent deposits developed when culture fluids from strains 7073, 9222, and 31067 were used as supplement for *V. parvula* KON (Table 2). Consequently, this deposit-forming activity varies among different *S. salivarius* culture fluids.

Adherent polymer formation by cell-free culture fluids from *S. salivarius*. The previous results could have been due to entrapment of the nonadhering veillonellae and streptococci in adherent polymer synthesized by the *S. salivarius* culture fluids. Because *S. salivarius* is not widely recognized as a producer of adherent polymer from sucrose, 11 strains were evaluated for this capability. Four of these (9759, 25975, CNII, and MEPI) formed heavy deposits (Table 3), but the appearance was not always the same (Fig. 1). Culture fluids from the other seven strains formed much smaller or no visible deposits. The tenaciousness of these deposits became evident when they were not readily dislodged by a forceful stream of water from a faucet.

The amount of plaque formed in the presence of homologous cells is also shown in Table 3. All strains formed at least some plaque when the inoculum contained cells, but there was still considerable variation between strains. Several plaques resembled in size those made by *S. mutans* (35). Because the inoculation protocols were different (see Materials and Methods), it cannot be concluded that cells stimulated plaque formation. Greater variability between replicate tests was observed when cells were present. This might have been due to occasional selection of more strongly adhering cells upon serial transfer of the wire (18, 50). The strains that formed heavy deposits on the wires also adhered well to glass when tested according to the method of Olson et al. (40; data not shown).

TABLE 3. *Formation of adherent deposits by various strains of S. salivarius*

Strain	"Plaque" formation[a]	
	Without cells[b]	With cells[c]
7073	0–<1	1–5
9222	0	2
9758	<1	>6
9759	6	>6
13419	<1	4
25975	>6	>6
27006	1	4–5
27945	0	<1–4
31067	0	2–4
CNII	>6	>6
MEPI	>6	>6

[a] Assessed as described in the text. When duplicate tests did not agree, the range is indicated. A rating of >6 indicates that a grade 6 "plaque" was obtained before the end of the test period.

[b] Inoculated with 1 ml of cell-free fluid upon each transfer of the wire.

[c] Three inoculations on consecutive days.

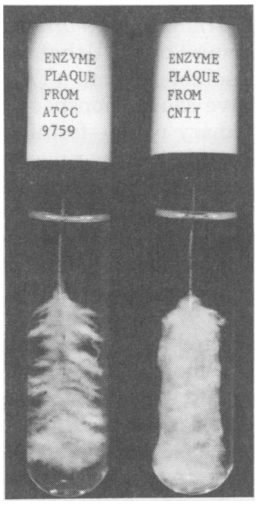

FIG. 1. *"Plaque formation" by cell-free culture fluids from S. salivarius 9759 and CNII. The wires were transferred daily into BHI supplemented with 5% sucrose and 10% cell-free culture fluid. Total incubation time was 1 week.*

GT and FT synthesis. The heavy deposits formed in the previous experiment were reminiscent of those formed by *S. mutans* (35). Sucrose-dependent adherence to smooth surfaces by *S. mutans* is associated with the formation of water-insoluble glucan (20, 26). Consequently, insoluble glucan formation by cell-free culture fluids from *S. salivarius* was measured. Because *S. salivarius* is often characterized as a levan, rather than as a glucan, producer (9, 27), extracellular FT was also measured. For comparison,

a cariogenic strain of *S. mutans*, C67-1 (11), was included. The results are summarized in Table 4. All 11 *S. salivarius* strains examined excreted a GT that formed a water-insoluble product. Among strains, the amounts varied more than 100-fold. Four strains (9759, 25975, CNII, and MEPI) formed appreciably more glucan than the others and *S. mutans* C67-1. Only the *S. mutans* C67-1 assay showed a significant amount of water-soluble glucan after 1 h. Comparison of Tables 3 and 4 shows that only culture fluids with high GT activity (9759, 25975, CNII, and MEPI) formed copious amounts of adherent deposits, which suggests that these characteristics are associated.

Extracellular FT was detected in all strains, and the fructan produced was water soluble. Five of the 11 *S. salivarius* strains had more GT than FT in their culture fluid. Whereas GT was found only in an extracellular form, significant amounts of FT were also associated with the cells (data not shown).

Adsorption of GT by nonprimary plaque formers. The previous two experiments showed that several strains of *S. salivarius* excrete a significant amount of a GT that forms an adherent, water-insoluble glucan (Tables 3 and 4). This could have caused nonspecific entrapment of the nonprimary plaque-forming streptococci and veillonellae in the experiments summarized in Table 2, but it was also possible that the nonprimary plaque formers adsorbed a product from the culture fluid that allowed them to adhere. This possibility was studied by incubating washed *Veillonella* cells in culture fluid from *S. salivarius* 25975. After 3 h, the cells were washed and tested for in vitro "plaque" formation. All four veillonellae subsequently adhered and colonized the wire (Fig. 2). The amount of "plaque" was relatively small (graded ≤ 1), compared with that observed before (Table 2). This was due, in part, to the fact that the tubes were inoculated only once, rather than three times. *S. mitis* S3 behaved similarly after incubation in culture fluid from *S. salivarius* 9759 (Fig. 3). The "plaques" were removed from the wires by overnight incubation in an endodextranase solution (0.1 mg/ml, pH 6.5). Occasionally, 10 times less dextranase was also effective. In another experiment, "plaque" formation was prevented by inclusion of dextranase (10 μg/ml) in the culture medium (data not shown). GT activity was lost and no "plaque" was formed after the supernatant fluids had been boiled for 10 min. If the GT of strains 25975 and 9759 is indeed responsible for the observed adherence, then one would expect that little or no "plaque" would accumulate with culture fluids low in GT activity. When this was tested with culture fluids from strains 7073, 9222, and 31067, we found that *V. parvula* KON did not adhere. Jointly, these observations strongly suggest that the colonization was mediated by GT adsorbed from the *S. salivarius* culture fluid. Three strains of *Lactobacillus, L. acidophilus* CL35, *L. casei* 64H, and *L. coryniformis* M34, were tested with 25975 culture fluid, but they did not colonize.

TABLE 4. *Extracellular glucosyltransferase and fructosyltransferase activities of various S. salivarius strains*

Strain	Enzyme activity[a]		
	GT		FT[b] (total)[d]
	Insoluble[c]	Total[d]	
7073	1.4	1.8	7.3
9222	1.4	1.4	19.8
9758	2.0	2.1	2.6
9759	24.9	25.8	7.6
13419	1.9	1.6	25.2
25975	67.8	69.1	38.2
27006	4.3	4.4	1.4
27945	3.5	3.9	19.7
31067	0.5	0.5	1.4
CNII	151.7	156.7	11.2
MEPI	117.0	119.1	6.6
S. mutans C67-1	7.5	17.4	10.6

[a] After overnight growth in BHI. Activity is expressed in international units per liter of cell-free culture fluid. At least two cultures were assayed; average values are given.

[b] No insoluble fructan was detected anywhere of the (the sensitivity of the assay was 0.2 international units/liter).

[c] Polymer insoluble in 0.05 M potassium phosphate buffer, pH 6.5.

[d] Polymer insoluble in 80% ethanol.

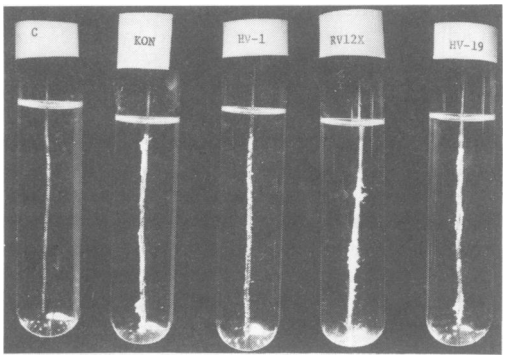

FIG. 2. *Colonization of V. parvula KON and HV-19 and V. alcalescens HV-1 and RV-12x after incubation in culture fluid from S. salivarius 25975. The control (C) shows that Veillonella that had been incubated in BHI did not colonize the wire.*

To further confirm and to quantitate GT adsorption, the *V. parvula* cells were assayed. The results (Table 5) showed that the cells had acquired the ability to form glucan from sucrose. The adsorption of GT was accompanied by a loss of activity from the *S. salivarius* culture fluid. However, this loss (76%) far exceeded the uptake (22%, Table 5). This might have been due to inactivation of GT upon adsorption on the *Veillonella* cells. The observed adherence was not associated with fructan formation because assays showed that the *Veillonella* cells did not have FT activity (Table 5).

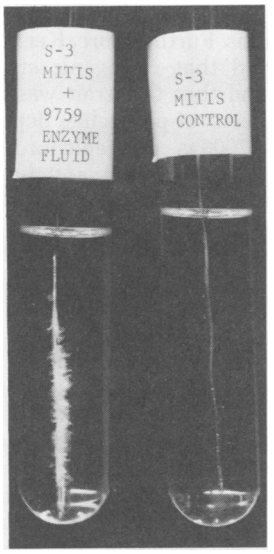

FIG. 3. *Colonization of S. mitis S3 after incubation in culture fluid from S. salivarius 9759.*

TABLE 5. *Adsorption of GT and FT by Veillonella parvula KON*

Fraction	Activity[a]	
	GT[b]	FT[c]
S. salivarius 25975 culture fluid		
Before incubation with *Veillonella*	1.22	0.20
After incubation with *Veillonella*	0.28	ND[d]
Veillonella cells[e]		
Before incubation	0.00	<0.0006
After incubation and washing	0.25	<0.0006
Wash fluid from *Veillonella* cells	0.03	ND

[a] Expressed in international units for each fraction as a whole.

[b] Both insoluble and total glucan formation were measured, but no significant differences were observed.

[c] Total fructan formation was measured.

[d] ND, Not done.

[e] Calculated absorbance (at 600 nm) of the *Veillonella* suspension during the incubation was 2.5.

DISCUSSION

It has now been well established that the mouth represents a complex ecosystem, where various microbial species occupy preferential habitats (22). It is likely that interactions between these microbial components occur and contribute to the overall ecology. In the present study, interactions between extracellular products from *S. salivarius,* sucrose, and several veillonellae were evaluated in vitro. *Veillonella* was chosen because (i) it is routinely found in plaque (2, 22), (ii) it does not metabolize sucrose, and (iii) it does not attach well to cleaned teeth or preformed dental plaque (33). The results obtained show that GT from *S. salivarius* combined with sucrose can mediate smooth-surface colonization of veillonellae and certain streptococci that form little or no adherent deposits from sucrose by themselves. The data suggest that at least two mechanisms play a role. The first one involves entrapment of cells in adherent glucan formed in the presence of *S. salivarius* culture fluids by a possibly nonspecific mechanism (Tables 2 through 4). With respect to the question of specificity, Slade reported recently that various gram-positive and gram-negative cells "produced significant adherence" on glass upon incubation with sucrose and GT from *S. mutans* B13 (47). The second mechanism entails prior adsorption of GT by *Veillonella* cells (Table 5); this adsorption resulted in relatively strong binding, because GT was present on the cell walls after washing. More recent evidence confirms that the cell-bound GT activity is difficult to remove (54). This phenomenon appears to exhibit a certain specificity, because several lactobacilli did not colonize after incubation with *S. salivarius* culture fluid. Indeed, Slade found that binding of GT from *S. mutans* B13 to various organisms was relatively selective, with homologous cells showing the greatest adherence with sucrose after a 1-h incubation with GT and three subsequent washings. Several of the gram-negative organisms tested also adhered, but more weakly (47). It would be of interest to establish whether these cells adsorb GT and what the nature of the binding is.

Obviously, colonization of steel wires cannot be directly equated with colonization of teeth, because, among other reasons, attachment of veillonellae and streptococci to enamel is affected by the presence of the salivary pellicle (28, 37, 41). However, the effect of this salivary component could not be evaluated by the meth-

ods used in the present study. Slade, in reviewing in vitro methods to study colonization, concluded that "hydroxyapatite and glass surfaces are similar in value as a model" (47). We found that the *S. salivarius* strains that formed plaque on a steel wire also adhered well to glass.

For our experiments (Table 5, Fig. 2) the veillonellae were cultivated in the presence of sucrose in an attempt to mimic a recent oral intake of sucrose. Even though the cells were washed subsequently, it is conceivable that some sucrose was carried over with the cells into the *S. salivarius* culture fluid, which might have caused entrapment of GT on the cells. McCabe and Smith reported that *S. mutans* cells bind homologous GT and glucan when incubated with soluble GT and sucrose (34), but it is not known whether *S. salivarius* GT behaves similarly. However, recent experiments have shown that *V. parvula* cells that have been grown in the same medium but without added sucrose also bind *S. salivarius* GT (54).

Our results show that all strains of *S. salivarius* examined excreted GT when grown in BHI with glucose, although the amounts varied more than 100-fold among strains. Four strains had more GT in their culture fluid than the cariogenic *S. mutans* C67-1 strain (Table 4). Similar observations were made when other strains of *S. mutans* were compared with *S. salivarius* strains 13419 (12) and 25975 (8). Insoluble glucan formation by certain strains of *S. salivarius* was noted originally by Niven et al. in 1941 (39) and has since been confirmed by others (8, 12, 24, 31, 46). It remains to be seen whether the low GT activity of strains such as 7073 has physiological significance. In vitro cell-free plaque formation was relatively weak with these strains (Table 3).

Notwithstanding the above, *S. salivarius* is better known as a levan producer, which appears to stem from its capacity to produce characteristically large, mucoid colonies on sucrose- or raffinose-containing agar (38). Our results indicate that glucose-grown cultures of all strains had extracellular and cell-bound FT activities and that the fructan formed was soluble. The extracellular activities varied about 30-fold among strains. Chassy et al. reported that sucrose-grown cultures of strain 25975 contained five times more FT than glucose grown cultures and that this was largely due to a 60-fold increase in the extracellular FT activity (8). Consequently, it appears that with sucrose in the medium levan production overshadows glucan production, even though some strains elaborate substantial amounts of GT. This may explain why *S. salivarius* is often characterized as a levan, rather than glucan, producer (9, 16, 27).

Because the adherence of *S. mutans* to smooth surfaces is associated with its capacity to form an adherent glucan containing α-1,3- as well as α-1,6- linkages (22), it is tempting to speculate that the same might be true for *S. salivarius*. Table 3 shows that the *S. salivarius* glucans have adherent properties. In addition, the finding that the artificial plaques were at least somewhat susceptible to α-1,6-dextranase action, combined with Dewar and Walker's observation that the 13419 glucan was partially hydrolyzed by an α-1,3-glucanase (12), supports this hypothesis. Furthermore, Kelstrup and Gibbons reported that cariogenic strain 1A produced GT and that this trait was required for the development of plaquelike deposits in vitro (31). Strain 27006 is cariogenic for hamsters (15) and gnotobiotic rats (17) and has now been shown to produce a readily detectable level of GT. However, it remains to be established whether this GT is required for the observed pathogenicity in rodents. Strain 13419 has occasionally produced fissure caries (45), but is not cariogenic for smooth surfaces (24, 32, 45), and it synthesized relatively little GT (Table 4). It would be interesting to evaluate strains with high GT activity (9759, 25975, CNII, MEPI, etc.) for cariogenicity. It would also be useful to know if levan production contributes to the pathogenesis and alveolar bone loss observed in animals. Whereas Kelstrup and Gibbons suggested that the nature and amount of polysaccharides formed might be decisive factors in the type of colonization that develops (31), Guggenheim has stated that levan formation might interfere with colonization of smooth surfaces (24).

Overall, the results presented here suggest that strains of *S. salivarius* with high GT activity could contribute indirectly to sucrose-dependent plaque formation. Transfer of GT from *S. salivarius* to *Veillonella* cells could, presumably, occur locally via the tongue dorsum, where both are abundant. Transfer of GT to other loci in the mouth would require distribution through the mouth by oral fluids. If this mechanism were operative, then one might expect to find GT in whole saliva. We tested this last hypothesis and found that a majority of human volunteers had measurable GT activity in their oral fluids (manuscript in preparation). The possibility that this GT adsorbs to teeth and thus facilitates adherence also has to be considered, because GT from both *S. mutans* (25) and *S. sanguis* (6) adsorbs to hydroxyapatite in vitro. *S. salivarius* becomes established in the mouths of infants within a day of birth (5, 36). In contrast, *S. mutans* colonizes later and more sporadically (5, 19, 53)

and does not spread readily (19, 30). Consequently, it is conceivable that if strains with high GT activity have become established in the mouth, they might contribute to colonization of teeth soon after eruption.

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