Streptococci and activities of sucrases and α-amylases in supragingival dental plaque and saliva in three caries activity groups

Nils-Erik Fiehn, Vibeke Oram and Dennis Moe
Departments of Microbiology, Cariology, and Biochemistry,
Royal Dental College, Copenhagen, Denmark

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Thirty-eight young adults participated in the study. They were divided in a caries-inactive group, a low caries activity group and a moderate to high caries activity group. Total cultivable bacteria, *Streptococcus salivarius*, and *S. mutans* in plaque and saliva were quantitated on TSA, MS, and MSB plates, respectively. Sucrase activity was determined by measuring reducing sugars in plaque and saliva after incubation with sucrose. α-Amylase activity was determined by Pharmacia Phadebas® Amylase test. The data were analyzed with the non-parametric Mann–Whitney U test. The only significant difference was observed for plaque α-amylase activity between the caries-inactive group and the moderate–high caries activity group ($P < 0.05$). The lack of differences concerning the other variables is discussed mainly on the basis of the multifactorial character of dental caries and the possible insufficiency of the applied methods. □ *Cariology; enzymes; microbiology;* Streptococcus mutans; Streptococcus salivarius

N.-E. Fiehn, Department of Microbiology, Royal Dental College, Juliane Maries vej 30, DK-2100 Copenhagen Ø, Denmark

Development of dental caries is closely associated with the formation of dental plaque, in which bacteria belonging to many different species are present. The metabolic bacterial degradation of certain carbohydrates to low molecular weight organic acids can lead to dental decay. *Streptococcus mutans* is considered an important bacterial species in relation to dental caries owing to its great potential to ferment dietary sugars and to form extracellular polysaccharides from sucrose (1). Furthermore, the ability of this bacterium to produce caries lesions in monoinfected, gnotobiotic rats fed a carbohydrate-containing diet supports a relationship to dental caries (1, 2).

The most important bacterial enzymes involved in the caries process are, besides the glycolytic enzymes, the carbohydrate-hydrolyzing enzymes. Since sucrose is the most important dietary carbohydrate, the sucrases are essential carbohydrate-splitting enzymes in dental plaque (3, 4). Several bacteria in dental plaque other than *S. mutans* have the ability to ferment dietary sugars, and among these, strains of *S. salivarius* have been observed to produce high levels of sucrases (5). *S. salivarius* is predominant on the oral mucosa and in saliva, and in spite of its low occurrence in human dental plaque the cariogenic potential of this streptococcal species has been verified in experimental rat caries studies (2, 6).

Most of the carbohydrate-degrading enzymes in plaque and saliva originate from dental plaque bacteria (7), except for the α-amylases, which are mainly of salivary origin (8). The α-amylases degrade starch, and these salivary enzymes may have an influence on the caries process (9). Despite many caries studies, it is not clear to what extent the different virulence factors contribute to the caries process, and it is therefore difficult to identify individuals at risk of developing caries by measuring single virulence factors in dental plaque and saliva.

In the present study percentages of *S. mutans* and *S. salivarius* and activities of sucrases and α-amylases were determined in supragingival dental plaque and saliva in three caries activity groups. Finally, statistical comparisons among the groups were carried out.

Materials and methods

Subjects

Thirty-eight young adults participated in the study. They were divided into three caries activity groups: a caries-inactive group (CI), a low caries activity group (LCA), and a moderate to high caries activity group (M-HCA). The subjects in CI were selected as follows: no carious lesions could be observed at the examination, at sampling time, or a year before; DMFS values were below 20. The subjects in LCA had one or two carious lesions at the sampling time and a DMFS value between 20 and 35. The subjects in M-HCA had more than two decayed surfaces and a DMFS value higher than 35. Table 1 shows further details concerning the individuals.

Caries examination was carried out with mirror, probe, and two bitewing radiograms.

All subjects used fluoride-containing toothpaste twice daily; no subjects had been treated with antibiotics during the last 3 months before examination and sampling.

Samplings

Before caries examination sampling of plaque and saliva was carried out. The subjects were instructed not to brush their teeth in the morning before the sampling, which was carried out at about 1000 h. Supragingival plaque from all smooth surfaces in the premolar–molar regions was removed with a sterile Capland 9/10 instrument and transferred to and pooled in 3 ml reduced transport fluid (RTF). To avoid contamination with saliva, cotton rolls and compressed air were used. Soon after, 3 ml of paraffin-stimulated saliva was collected in a sterile glass tube. The samples were immediately transported to the laboratory for microbial cultivation and biochemical analysis. In both plaque and saliva samples sucrase and α-amylase activity were determined and the percentages of *S. mutans* and *S. salivarius* of total cultivable bacteria were calculated.

Plaque treatments

The plaque samples were sonicated for 15 sec in an ice water bath, using an MSE 100-W ultrasonic disintegrator at an amplitude of 6 μm. One hundred microliters of the sample was 10-fold serially diluted in RTF (up to 10^{-8}). Then 100 μl inoculum were spread on the following substrates: enriched tryptic soy agar (TSA), Difco 0369-01-4 with 5% horse blood for total cultivable bacteria, mitis salivarius agar (MS), Difco 0298-01 for *S. salivarius* enumeration, and mitis salivarius agar with 20% sucrose and 0.2 U bacitracin/ml (MSB) (10) for *S. mutans* enumeration. The TSA plates were incubated in an anaerobic glove box (70% N_2, 20% H_2, 10% CO_2) 5 days at 35°C. The MS and MSB plates were incubated in the glove box for 2 days, followed by 1 day of incubation aerobically at 35°C. Typical colonies representing *S. salivarius* and *S. mutans* were counted on MS and MSB plates and calculated in percentage of total cultivable bacterial

Table 1. Distribution of the individuals in the three experimental groups

Caries activity groups	No.	Male, *n*	Female, *n*	Age, years	DMFS	No. of decayed surfaces at examination time
Caries-inactive group	13	6	7	20–30	< 20	0
Low caries-active group	14	8	6	20–30	20–35	1–2
Moderate–high caries-active group	11	5	6	20–30	> 35	3–11

counts. Material from representative colonies of the two streptococcal species was identified by the following criteria: Gram staining; catalase; fermentation of sorbitol, manitol, salicin, and inulin; acetoin production; H_2O_2 production; hydrolysis of arginine and esculin; and dextran formation from sucrose (11).

The remaining undiluted plaque sample was centrifuged at 2500 *g* for 15 min. The resulting supernatant was removed for sucrase, α-amylase, and protein determination (12). The wet weight of the pellet was determined to the nearest tenth of a milligram. The pellet was divided into two parts, one for sucrase determination and the other for α-amylase determination. Both parts were weighed as previously.

Saliva treatments

The saliva samples were Vortex-mixed for 60 sec and 10-fold serially diluted in PY (13). TSA, MS, and MSB plates were inoculated and incubated as above. Representative colonies of *S. mutans* and *S. salivarius* were identified as mentioned earlier. Likewise, the percentages of the two streptococcal species were calculated. Finally, sucrase and α-amylase activities were determined in the undiluted saliva.

Enzyme assays

Sucrase. The enzyme activities in saliva, plaque supernatant, and plaque pellet were determined, and the two latter were added to give the total activity for plaque. The incubations and determination of glucose plus fructose were carried out as described earlier (4, 5). The plaque pellet was weighed and resuspended in 250 µl 0.2 M acetate buffer, pH 5.0, before incubation with sucrose. Just before the measuring of glucose plus fructose the incubation mixture was centrifuged at 10,000 *g* for 15 min. The enzyme activity was expressed in units per milligram plaque or saliva, and 1 unit is defined as 1 µmol monosaccharide formed per 1 min. In the calculations, 1 ml saliva corresponds to 1000 mg.

α-Amylase. The activity of this enzyme was determined in the same materials as sucrase activity. Pharmacia Diagnostics' Phadebas® Amylase test (52-1395-00/1) was used, and the determination was carried out in accordance with the manufacturer's specifications. Specifically, the plaque pellet was resuspended in 250 µl phosphate buffer (Sörensen), pH 6.9, before incubation, and 200 µl material was used for both plaque components, whereas a 50-µl sample from 10-fold diluted saliva was used. The dilution was made in saline containing 0.2% bovine serum albumin and 20 mM $CaCl_2$. The enzyme activity was expressed in units per milligram plaque or saliva, and 1 unit corresponds to hydrolysis of 1 µmol glycoside bond per 1 min. In the calculations of the enzyme activity in saliva 1 ml ~ 1000 mg.

Statistical analysis

The relationships between the CI and LCA groups and between CI and M-HCA groups were examined for the percentages of *S. mutans* and *S. salivarius* and for the activities of sucrases and α-amylases in both pooled plaque and saliva. The non-parametric Mann–Whitney U-test was applied as a one-tailed test at the significance level $P \sim 0.05$.

Results

The data are presented in Tables 2–5. A significant difference ($P < 0.05$) was observed between the CI and M-HCA groups for α-amylases in pooled plaque. Among the other factors examined only the salivary α-amylases in the two above-mentioned groups approached a significant difference (calculated U-value, 48.5; critical U-value 42). With regard to the α-amylases in plaque, 95% confidence limits were calculated. For the CI group this interval, at the average value of 0.053 U/mg, was 0.017–0.089 U/mg; for the M-HCA group the average value was 0.143 U/mg, and the corresponding confidence interval was 0.059–0.227 U/mg. Five observations in the CI group are higher than the lower limit of the 95% confidence interval of the M-HCA

eptococcus mutans in pooled supragingival plaque and saliva samples in the three caries activity groups*

Caries-inactive group			Low caries activity group				Moderate–high caries activity group			
	Saliva				Saliva				Saliva	
Plaque, %	%	CFU/ml	Subject no.	Plaque, %	%	CFU/ml	Subject no.	Plaque, %	%	CFU
0	0	6.2×10^5	102	0.02	0.05	2.9×10^6	106	0	0	0
0	0.60	2.5×10^5	107	0	0	0	115	0	0	0
0	0	0	109	0	0	0	119	1.63	1.22	$2.2 \times$
0	0.01	1.0×10^4	110	0.15	0.07	3.7×10^5	120	0.03	0.39	$2.7 \times$
0.50	0.22	2.2×10^6	114	0.05	0.16	6.0×10^4	124	0.05	0	$2.0 \times$
0.41	0.04	8.3×10^5	117	1.00	0.18	8.6×10^5	125	0	0	0
0.06	0.29	2.0×10^5	121	0	0	5.5×10^4	127	0	0	0
2.64	0.02	7.0×10^4	122	0	0.15	1.2×10^5	128	0	0	0
0	0.11	1.7×10^6	126	0	0	0	133	0	0	0
0	0	0	129	0	0	0	134	0.05	0	0
0	0	0	132	9.70	0.04	1.9×10^7	138	0.95	0.11	$4.8 \times$
0	0	0	135	0	0.10	5.1×10^7				
0	0	0	136	0	0	0				
			137	0.28	0.03	3.0×10^4				
0.28	0.10	4.5×10^5		0.80	0.06	5.3×10^6		0.25	0.16	$4.6 \times$
0	0.01	7.0×10^4		0	0.04	5.8×10^4		0	0	0
0–	0–	0–		0–	0–	0–		0–	0–	0-
2.64	0.60	2.2×10^6		9.70	0.18	5.1×10^7		1.63	1.22	$4.8 \times$

rcentage (with two decimals) of total cultivable bacteria; CFU = colony-forming units.

Table 3. *Streptococcus salivarius* in pooled supragingival plaque and saliva samples in the three caries activity groups*

Caries-inactive group				Low caries activity group				Moderate–high caries activity group			
		Saliva				Saliva				Saliva	
Subject no.	Plaque, %	%	CFU/ml	Subject no.	Plaque, %	%	CFU/ml	Subject no.	Plaque, %	%	CFU/ml
103	0	7.51	6.2×10^5	102	0.07	10.17	6.0×10^8	106	0	0.34	5.9×10^5
104	0	5.11	2.3×10^7	107	0	0.35	7.0×10^5	115	0	2.21	2.1×10^6
105	0	0.11	5.5×10^8	109	0	1.40	2.8×10^5	119	2.00	11.11	2.0×10^7
108	0.06	0.20	4.0×10^5	110	1.17	2.40	1.2×10^7	120	0	3.04	2.1×10^6
111	0	4.80	4.8×10^7	114	0	20.53	7.8×10^6	124	0.01	1.92	1.5×10^7
112	0.01	4.78	1.1×10^8	117	0	1.39	6.8×10^6	125	0	0.23	2.7×10^5
113	0.06	7.68	5.3×10^6	121	0	0.65	1.5×10^6	127	0	0	1.5×10^5
116	0.02	3.33	1.3×10^7	122	0.03	3.59	2.8×10^6	128	0	0	4.7×10^5
118	0	2.81	4.5×10^7	126	0	0.49	2.0×10^5	133	0	4.67	9.8×10^6
123	0	0.04	9.5×10^4	129	0	0	5.0×10^4	134	0.09	99.11	2.9×10^9
130	1.47	0.46	1.1×10^7	132	0	2.24	1.1×10^9	138	0	0	1.6×10^6
131	0.10	51.00	5.1×10^7	135	0.01	2.16	1.1×10^9				
139	0	1.49	1.3×10^6	136	0.01	3.56	3.9×10^8				
				137	1.33	4.89	4.6×10^6				
Mean	0.13	6.87	6.6×10^7		0.19	3.84	2.3×10^8		0.28	11.15	2.6×10^8
Median	0	3.33	1.3×10^7		0	2.20	5.7×10^6		0	1.92	2.1×10^6
Range	0–	0.04–	9.5×10^4–		0–	0–	5.0×10^4–		0–	0–	1.5×10^5–
	1.47	51.00	5.5×10^8		1.33	20.53	1.1×10^9		2.00	99.11	2.9×10^9

* % = percentage (with two decimals) of total cultivable bacteria; CFU = colony-forming units.

Table 4. Sucrase activity in pooled supragingival plaque and saliva samples in the three caries activity groups*

Caries-inactive group			Low caries activity group			Moderate–high caries activity group		
Subject no.	Plaque, U/mg	Saliva, U/mg	Subject no.	Plaque, U/mg	Saliva, U/mg	Subject no.	Plaque, U/mg	Saliva, U/mg
103	3.01	0.08	102	3.51	0.35	106	1.35	0.10
104	4.76	0.13	107	9.01	0.08	115	6.86	0.08
105	4.07	0.07	109	5.47	0.08	119	4.02	0.12
108	10.63	0.31	110	7.03	0.20	120	4.94	0.07
111	9.31	0.41	114	10.71	0.03	124	3.27	0.18
112	9.93	0.10	117	2.97	0.02	125	10.89	0.25
113	4.17	0.08	121	11.51	0.10	127	3.74	0.24
116	1.13	0.08	122	14.83	0.04	128	7.56	0.19
118	2.17	0.44	126	3.27	0.23	133	15.77	0.24
123	5.54	0.04	129	4.22	0.06	134	3.76	0.35
130	11.33	0.15	132	14.61	0.44	138	11.97	0.10
131	2.03	0.46	135	3.88	0.04			
139	4.11	0.06	136	5.07	0.12			
			137	3.15	0.06			
Mean	7.01	0.19		7.09	0.13		6.74	0.17
Median	4.76	0.13		5.27	0.08		4.94	0.18
Range	1.13–21.03	0.04–0.46		2.97–14.83	0.02–0.44		1.35–15.77	0.07–0.35

* U/mg = units per milligram (see Materials and methods).

group, and five observations in the M-HCA group were below the upper limit of the 95% confidence interval of the CI group (Table 5).

Furthermore, no connection was observed between percentages of *S. mutans* and sucrase activity in plaque or saliva. Likewise, no relationship between *S. salivarius* counts in saliva and sucrase activity in saliva or plaque was observed.

Table 5. α-Amylase activity in pooled supragingival plaque and saliva samples in the three caries activity groups*

Caries-inactive group			Low caries activity group			Moderate–high caries activity group		
Subject no.	Plaque, U/mg	Saliva, U/mg	Subject no.	Plaque, U/mg	Saliva, U/mg	Subject no.	Plaque, U/mg	Saliva, U/mg
103	0.005	0.034	102	0.012	0.120	106	0.108	0.113
104	0.003	0.058	107	0.029	0.056	115	0.024	0.160
105	0.003	0.066	109	0.080	0.067	119	0.071	0.124
108	0.186	0.078	110	0.034	0.134	120	0.015	0.032
111	0.058	0.042	114	0.057	0.124	124	0.140	0.180
112	0.101	0.152	117	0.026	0.056	125	0.068	0.128
113	0.021	0.063	121	0.045	0.054	127	0.212	0.090
116	0.083	0.168	122	0.063	0.118	128	0.317	0.076
118	0	0.096	126	0.089	0.100	133	0.222	0.059
123	0.013	0.076	129	0.033	0.018	134	0.012	0.032
130	0.130	0.017	132	0.048	0.116	138	0.382	0.108
131	0.078	0.031	135	0.020	0.024			
139	0.007	0.098	136	0.028	0.076			
			137	0.114	0.064			
Mean	0.053	0.075		0.048	0.081		0.143	0.099
Median	0.021	0.076		0.040	0.072		0.108	0.108
Range	0–0.186	0.017–0.168		0.012–0.114	0.018–0.134		0.012–0.382	0.032–0.180

* U/mg = units per milligram (see Materials and methods).

Discussion

In the past decade many investigators have studied several factors in plaque and saliva in groups of caries-free, caries-inactive, and caries-active individuals, to use such factors to predict caries risk and to select individuals exposed to dental caries for a particular therapy. The factors most frequently examined are *S. mutans* and lactobacilli in plaque and saliva, the buffer capacity of saliva, the acidogenic potential of dental plaque, and the sucrose metabolism in dental plaque and of certain bacterial species in plaque (14–21). The present study was originally planned as a combined cross-sectional and longitudinal study comprising the CI and LCA groups with a simultaneous registration of the caries increment. The two groups were selected so that the caries-active group a priori did not differ much from the inactive group. The intention was to establish a basis for identifying individuals at risk of developing caries by clinically usable laboratory tests before the disease becomes clinically visible. Since no difference was found between the two groups, a third—caries active, M-HCA—group with higher caries experience was included in the study. Since practically no difference between this group and the caries-inactive group was found either, the longitudinal part of the study was dropped.

It may seem surprising that almost none of the virulence factors examined showed significantly increased values in the caries-active groups compared with the caries-inactive group, but these observations support the general concept that dental caries is a multifactorial disease. The methods used are the simplest methods existing today for quantitation of *S. mutans*, *S. salivarius*, sucrase, and α-amylase activities. It is possible that these methods, or some of them, are not sensitive and specific enough to measure the selected variables (22), but it is very important that the methods are relatively simple if they are to be included in routine clinical practice.

An association between *S. mutans* and dental caries has been observed in many studies (14, 16, 23). The present investigation is in conflict with these studies but in agreement with others, which have failed to demonstrate a positive relationship between *S. mutans* and caries (24, 25). The lack of a relationship can be explained as above, but specific factors of importance for the present study are that young adults participated and that the plaque samples were pooled from many smooth surfaces in the posterior regions of the mouths. Most studies showing a positive relationship are carried out on children, using saliva samples and plaque samples from specific sites. The purpose of applying pooled plaque samples was to obtain a measure for the individual as a whole, rather than for a single site. In the longitudinal study by Swenson et al. (22) an association between *S. mutans* in plaque and caries development in children was observed. They used pooled smooth surface plaque samples from first molars. The samples were cultured on MS plates instead of the more selective MSB plates, which were used in the present study. A lower recovery of *S. mutans* on the MSB plates (11) could explain the different findings in the two studies. Nevertheless, the recovery of *S. mutans* on the two substrates has been claimed to be identical (10). Although Swenson et al. (22) found a significant relationship between the percentage of *S. mutans* in plaque and caries development, they also found that the detection of *S. mutans* on MS agar plates is not sufficiently sensitive and specific to select individuals destined to develop caries or remain caries-free.

S. salivarius was included in this study because it had already been observed that strains of this species could show a rather high sucrase activity (5). Furthermore, *S. salivarius* has been shown to be cariogenic in rat experiments (2, 6). It was expected that no difference in *S. salivarius* in plaque between the groups could be observed, since the percentage of *S. salivarius* in plaque is normally very low, bit it was disappointing that no difference in the *S. salivarius* counts in saliva was found either. Furthermore, the salivary sucrase activity was very low in all three groups. A hypothesis that higher sucrase activity in saliva in caries-active individuals could originate from *S. salivarius* and serve as an indirect source of glucose plus

fructose to dental plaque was therfore rejected.

The activities of the two enzymes measured, sucrase and α-amylase, were expressed in units (U) per milligram plaque or saliva. In addition to determination of the wet weight of the plaque pellet, the protein content in the plaque supernatant was determined to ensure that plaque material was not lost during sonication. Only minute quantities (<200 μg/ml) at the same level from the different samples were liberated by the sonication. Consequently, only the pellet weight was used in the enzyme activity calculations.

The sucrase activity was higher in all plaque samples than in the corresponding saliva samples, which is in agreement with previous observations (26). Equal sucrase activity was found in the plaque pellet and plaque supernatant (data not shown), confirming earlier studies demonstrating both extracellular and cell-bound sucrases (27, 28). No difference in sucrase activities in the three caries groups was found, even though sucrase seems to be an important virulence factor, since the splitting of sucrose is a prerequisite for the further degradation of glucose and fructose to low-molecular weight acids, resulting in a pH drop in dental plaque. The lack of relationship between the sucrase activity and *S. mutans* or *S. salivarius* confirms that other bacteria in dental plaque are involved in the synthesis of these enzymes (5). Furthermore, there are considerable strain differences in the sucrase activity of the two examined streptococcal species (5). In *S. mutans* this difference is mainly between different biotypes/serotypes of the organism (5).

The α-amylase activity was in most individuals higher in the saliva samples than in the corresponding plaque samples, showing that the normal source of this enzyme is saliva. In a few cases, however, the α-amylase activity was highest in plaque in the present study, which indicates that a bacterial synthesis of this enzyme can occur, although most plaque α-amylases originate from saliva. This point has been under discussion several times, and several authors seem to agree that certain bacteria in dental plaque can produce α-amylases but that this is normally of minor importance for starch degradation in the oral cavity (8, 29–31). Interestingly, individuals in the M-HCA group seem to harbor plaque bacteria producing α-amylases, resulting in a significant difference in plaque α-amylases between this group and the CI group. The clinical value of this variable in predicting dental caries is, however, limited, which is illustrated by the calculated 95% confidence intervals.

The results of this study show that biochemical and microbiological laboratory tests are still unreliable as indicators of caries risk in young adults. This conclusion is in agreement with the observations in the recent study by Honkale et al. (17). They examined various biological factors in children and concluded that the caries experience seems to be the most reliable factor in predicting dental caries. Because of the multifactorial character of dental caries it may be utopic to predict caries risk from a single virulence factor or from a combination of a few biological variables. Initiation of the disease presumably depends on several local factors in dental plaque at the specific site and in the immediate surroundings.

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