Streptococcus mutans in saliva: intraindividual variations and relation to the number of colonized sites

Jörgen Togelius, Katarina Kristoffersson, Harald Anderson and Douglas Bratthall

Department of Cariology, Faculty of Odontology, University of Lund, Malmö, and Department of Mathematical Statistics, University of Lund, Lund, Sweden

Togelius J, Kristoffersson K, Anderson H, Bratthall D. *Streptococcus mutans* in saliva: intraindividual variations and relation to the number of colonized sites. Acta Odontol Scand 1984;42:157–163. Oslo. ISSN 0001–6357.

Analysis of the salivary levels of *Streptococcus mutans* may contribute to the evaluation of the caries risk. It is therefore important to identify factors that might influence the outcome of such analyses. This investigation consisted of three parts. The first aim was to evaluate the short-term variation of *S. mutans* concentration in saliva. Furthermore, we estimated the effect of discontinued oral hygiene on salivary *S. mutans*. We also analyzed the relation between salivary levels of *S. mutans* and the number of colonized approximal and occlusal sites. Tongue samples were also included in the comparison. Systematic short-term variation could not be demonstrated. A 95% confidence interval for an observed *S. mutans* value was obtained by multiplying and dividing the observed value by a factor of 5 (for *S. mutans* samples $<10^6$) or 2.3 (*S. mutans* $> 10^6$). One week of discontinued oral hygiene did not significantly change the level of *S. mutans* in saliva. Saliva samples correlated significantly with tongue samples and with the number of colonized approximal sites. The results of these studies confirm the stability of the *S. mutans* colonization level. □ *Caries; oral hygiene; oral ecology*

Douglas Bratthall, University of Lund, School of Dentistry, Carl Gustafs väg 34, S-214 21 Malmö, Sweden

Qualitative and quantitative analyses of the oral microflora may be a valuable tool in predicting dental caries. *Streptococcus mutans* and lactobacilli have been of special interest in this context (1, 2). For *S. mutans* both plaque and saliva samples have been used. In routine clinical work, saliva samples are often preferred owing to easier handling, and there are indications that such samples under normal conditions reflect the number of tooth surfaces colonized with *S. mutans* (3, 4). It is considered important to ascertain that results for the saliva analyses are reliable and that the factors possibly influencing the level of *S. mutans* colonization are identified.

The aim of the present study was to ascertain whether samples taken a few hours or a day apart differed significantly with regard to the level of *S. mutans*. Second, we investigated whether a temporary change in oral hygiene had any such effects. We also studied the relation between the salivary level of *S. mutans* and the tongue, proximal, and occlusal colonization levels of *S. mutans*.

Materials and methods

Short-term variation of S. mutans *level in saliva*

Saliva samples analyzed for *S. mutans* were taken 1–2 h after brushing the teeth and breakfast at about 0830 hours and compared with samples taken at 1530 the same day and with samples taken at 0830 on the following day.

Seventy-two adults participated in a study comparing morning and afternoon samples. Sixty-nine adults participated in a study comparing two consecutive morning samples. They were all recruited from the professional staff at the School of Dentistry in Malmö.

Paraffin-stimulated whole saliva was collected via sterile funnels into sterile test tubes. One milliliter of saliva was then transferred to 3 ml of a transport medium, VMG-II (5), and within 3 h processed for *S. mutans* by standard procedures (6). MSB agar (7) selective for *S. mutans* was used. In the data analyses, the participants were

divided into three *S. mutans* classes: $<10^5$, 10^5–10^6, and $>10^6$ *S. mutans* per 1 ml saliva.

Colonies that were not typical of *S. mutans* were isolated and identified (8).

The effect of temporarily discontinued oral hygiene on the number of S. mutans *in saliva*

Changes in salivary *S. mutans* were evaluated for 24 adults, students at the School of Dentistry in Malmö, who did not clean their teeth for 7 days. No dietary restrictions were issued.

The same sampling technique as described above was used. Three base-line samples were taken on 3 consecutive days before the week of experimental withdrawal from oral hygiene practices, to establish the *S. mutans* values under normal conditions. The mean value of the base-line samples was calculated for each individual. During the week of the experiment saliva samples were obtained between 1000 and 1500 hours on days 4 and 7.

The amount of plaque was measured on days 1, 4, and 7 in accordance with Silness & Löe (9). The mesial, distal, buccal, and lingual surfaces of all teeth except the third molars were scored, and the mean value was calculated for each participant. Plaque was recorded after the bacterial sampling and by one dentist.

Comparison of saliva samples of S. mutans *with tongue, proximal, and occlusal samples*

Saliva samples of *S. mutans* were taken and compared with tongue samples, proximal samples, and samples from occlusal pits and fissures. *Streptococcus mutans* estimates were derived as described above.

Thirty-four adult patients, previously treated at the School of Dentistry in Malmö, participated in this experiment. They had no open carious lesions. Sampling took place on one occasion in the following order:

1. Saliva samples were taken with the spatula technique reported by Köhler & Bratthall (10). Each individual was placed in one of four groups: no detectable *S. mutans* colony-forming units (CFU), 1–20 CFU, 21–100 CFU, and >100 CFU.

2. Tongue sampling was done with a wooden spatula pressed against the dorsum of the tongue, which had been dried with compressed air. Tongue sampling was also done after the tongue had been scraped with a tongue cleaner (Sakool®, Cuyahoga Falls, Ohio, USA). The tongue cleaner was bent lightly to an arc, pressed to the tongue, and pulled forward three times. The cultivation and enumeration procedures were identical to those for saliva samples.

3. The occlusal samples were obtained with a sharpened instrument (no. 611–116, Dentaurum). The instrument was pulled along the entire fissure or margin of a restoration and the collected material was then spread on MSB-agar plates. The total number of colonized occlusal surfaces for each individual was calculated.

4. Proximal bacterial sampling was performed with wooden triangular toothpicks as described by Kristoffersson & Bratthall (11). The total number of colonized interproximal spaces for each individual was calculated. The number of sites colonized with >75 CFU was also determined.

Statistical analyses

The difference between morning and afternoon samples was tested with Student's *t* test for paired comparisons, using the logarithms of the concentrations. To study the random variation between samples taken within 24-h intervals, it was assumed that the logarithm of an observed value was normally distributed with an expected value depending on the individual subject and a standard deviation depending only on which of the *S. mutans* classes the value belonged to. These standard deviations were calculated from the two samples.

Changes in bacterial concentrations from base line to 4-day and 7-day samples were tested with Student's *t* test for paired comparisons, using the logarithms of the concentrations.

To study the relation between the number of CFU in saliva and the number of sites colonized with *S. mutans*, the data were

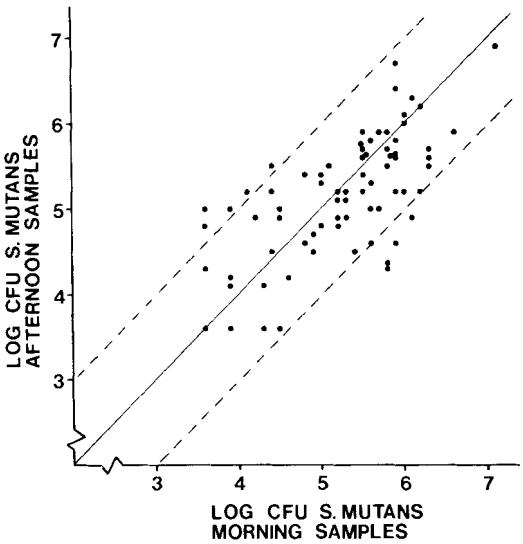

Fig. 1. Comparison between morning and afternoon samples of *S. mutans* (logarithms). The broken lines represent a divergence of ± a factor of 10.

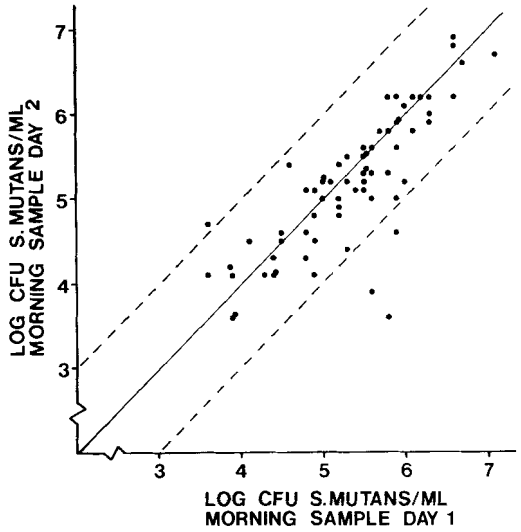

Fig. 2. Comparison between two morning samples of *S. mutans* taken with a 24-h interval (logarithms). The broken lines represent a divergence of ± a factor of 10.

reduced to 2×2 contingency tables, and exact tests of independence were performed.

Results

Short-term variation of S. mutans *level in saliva*

The results of the comparisons between morning and afternoon samples (logarithms) are presented in terms of log CFU in Fig. 1. Out of 72 individuals, 63 (87.5%) lay within a band formed by the lines drawn respectively one unit above and below the diagonal. In this region the number of *S. mutans* in the morning and afternoon samples differed by a factor of less than 10. No significant systematic difference could be found between morning and afternoon samples ($0.10 < p < 0.20$).

The comparison of samples taken with 24-h intervals is illustrated in Fig. 2. Only 4 of the 69 pairs (5.8%) differed by a factor of more than 10. The standard deviation of the logarithm of an observed value was estimated to be 0.40 in the class $<10^5$ (24 observations), 0.30 in the class 10^5–10^6 (33 observations), and 0.18 in the class $>10^6$ (12 observations). The estimates for the two lowest classes did not differ significantly (F-test), and they have been pooled to a common estimate ($s = 0.35$), whereas the estimate for the highest class is significantly lower (F-test, $p < 0.01$) than the other two. These results can be translated to random variation of an *S. mutans* number in the following manner. Assume that the variation of the logarithm of an individual is approximately normally distributed. A 95% confidence interval for the true value is then obtained by dividing and multiplying the observed value by a factor 10^{2xs}, which is 5.0 when $s = 0.35$ (*S. mutans* $<10^6$) and 2.3 when $s = 0.18$ (*S. mutans* $>10^6$). For example, an observed number of 375,000 *S. mutans* per 1 ml saliva yields the interval 75,000–1,875,000 for the true value. For an observed value of 2,000,000, the interval would be 870,000–4,600,000.

The effect of temporarily discontinued oral hygiene on the number of S. mutans *in saliva*

Fig. 3 shows that there was a significant increase of plaque scores during the week of discontinued oral hygiene. Fig. 4 illus-

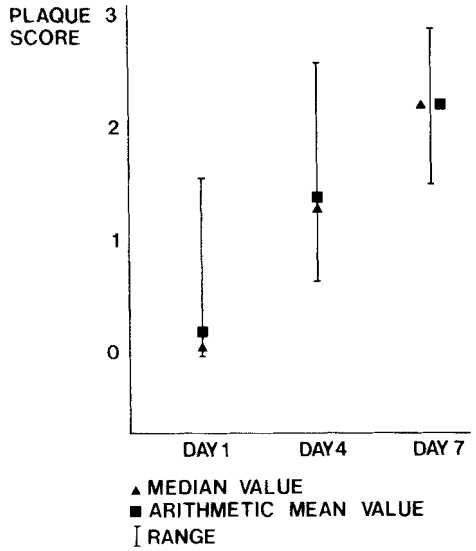

Fig. 3. Increase of plaque scores during 1 week of discontinued oral hygiene (Silness & Löe (9)).

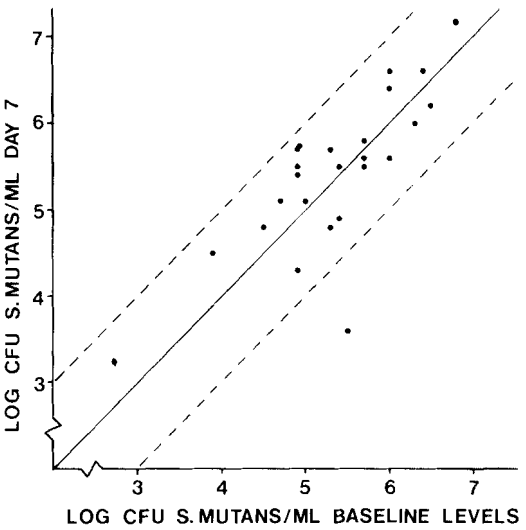

Fig. 4. Comparison of salivary *S. mutans* at base line and after 1 week of discontinued oral hygiene. The broken lines represent a divergence of ± a factor of 10.

trates the comparison between the base-line level of *S. mutans* CFU and the level after 1 week. Out of 24 participants 15 showed increased levels, whereas 9 showed decreased levels. The changes were not statistically significant (*t* test). Similarly, the *S. mutans* levels on the 4th day did not differ significantly from base line.

Comparison of saliva samples of S. mutans *with tongue, proximal, and occlusal samples*

Table 1 shows the comparison between salivary *S. mutans* and the number of interproximal sites colonized with *S. mutans*. The results indicate a significant correlation ($p < 0.001$). The figures demonstrate that

Table 1. Comparison between number of CFU in saliva and the number of interproximal sites colonized with *S. mutans*

No. of CFU in saliva samples	No. of subjects	No. of colonized interproximal sites				
		0	1–6	7–12	13–18	19–
0	6	—	4	1	1	—
1–20	11	—	2	4	4	1
21–100	11	—	—	—	3	8
>100	6	—	—	—	—	6
		No. of interproximal sites colonized with >75 CFU				
		0	1–6	7–12	13–	
0	6	6	—	—	—	
1–20	11	4	7	—	—	
21–100	11	—	8	1	2	
>100	6	—	1	2	3	

Table 2. Comparison between number of CFU in saliva and the number of occlusal sites colonized with *S. mutans*

No. of CFU in saliva samples	No. of subjects	No. of colonized occlusal sites			
		0	1–3	4–6	7–
0	6	—	3	—	3
1–20	11	—	4	5	2
21–100	11	1	4	3	3
>100	6	—	—	1	5

individuals with no detectable *S. mutans* in saliva may have colonized interproximal sites, although no interproximal site showed >75 CFU. Individuals with very high salivary levels of *S. mutans* harbored *S. mutans* in almost all interproximal spaces.

A significant positive correlation ($p < 0.01$) was also obtained when the salivary level of *S. mutans* was compared with the number of interproximal sites yielding more than 75 CFU by our sampling method (Table 1). Seven or more such interproximal sites could only be found in the two groups with the highest saliva counts.

Table 2 illustrates the comparison between salivary *S. mutans* and the number of colonized occlusal surfaces. No significant correlations could be demonstrated.

Tables 3 and 4 show the comparisons between saliva samples and tongue samples before and after tongue scraping with a tongue cleaner. There was a statistically significant positive correlation ($p < 0.001$) between *S. mutans* counts of saliva and tongue samples.

Discussion

The results did not indicate any systematic short-term changes of *S. mutans* in saliva which reached statistical significance. Even large temporary plaque accumulations over a week did not seem to influence the *S. mutans* level systematically.

Carlsson (12) has shown that early morning saliva samples analyzed for oral streptococci contain higher levels of bacteria than day samples. Crossner & Hagberg (13) have studied the short-term variation of salivary lactobacilli during 3 consecutive days, using the Dentocult method. Their results indicated a reduction in the lactobacillus counts when comparing morning samples (before eating and brushing of teeth) with samples obtained in the course of the day. Birkhed et al. (14) demonstrated that sampling of lactobacilli immediately on awakening yielded about four times higher values than daytime sampling. In their study different daytime samples showed only minor variations.

The results in the above studies indicate that early morning samples of oral streptococci and lactobacilli give higher values of bacteria than daytime samples. An explanation for the contrasting findings of insignificant differences between morning and afternoon samples in our study may be that

Table 3. Comparison between number of CFU *S. mutans* in saliva and on the tongue

No. of CFU in saliva samples	No. of subjects	No. of CFU tongue samples			
		0	1–20	21–100	>100
0	6	3	3	—	—
1–20	11	—	11	—	—
21–100	11	—	1	8	2
>100	6	—	—	1	5

Table 4. Comparison between number of CFU *S. mutans* in saliva and on the tongue (after scraping with a tongue cleaner)

No. of CFU in saliva samples	No. of subjects	No. of CFU tongue samples			
		0	1–20	21–100	>100
0	6	3	3	—	—
1–20	11	1	9	1	—
21–100	11	—	3	6	2
>100	6	—	—	2	4

the morning samples were obtained after breakfast and brushing the teeth.

Repeated sampling over a 24-h interval showed few strikingly deviating values. The standard deviation of a logarithmized *S. mutans* value was determined to be 0.40, 0.30, and 0.18, respectively, for the classes $<10^5$, $10^5 - 10^6$, and $>10^6$ CFU, and the confidence intervals were calculated as in Results. The variation between *S. mutans* samples may be due in part to methodological errors, such as variation in chewing intensity on the sampling occasion. Inexactness in the diluting and plating routines partly caused by agglutination of bacteria may also make a correct interpretation difficult. A contributory cause to the shown differences may also be true short-term variation of the *S. mutans* colonization level.

The random variation of an *S. mutans* determination can be reduced by taking two or more samples: if two samples, X1 and X2, are taken 24 h apart, the true mean level may be estimated by the geometrical mean $\sqrt{X1 \cdot X2}$. (The same estimate is obtained by averaging the logarithms of X1 and X2.) A 95% confidence interval for the true mean level is obtained by dividing and multiplying this estimate by $10^{2s/\sqrt{2}}$, which is 3.13 when s = 0.35 (*S. mutans* $<10^6$) and 1.80 when s = 0.18 (*S. mutans* $>10^6$). For instance, if the two values X1 = 69,700 and X2 = 152,000 are obtained, the mean level is estimated by $\sqrt{69{,}700 \times 152{,}000} = 102{,}900$, and a 95% confidence interval is (102,900: 3.13, 3.13 × 102,900) or (32,900, 322,000). Note that if the two samples are taken at the same time, the reduction of the random component will be less, since only the methodological errors are reduced and not the true short-term variation.

Discontinuing oral hygiene for a week did not markedly influence the number of *S. mutans* in saliva. This may seem strange, since all participants accumulated large amounts of plaque and since the sampling technique included chewing of paraffin to increase the shedding of bacteria from the tooth surfaces. However, *S. mutans* grows with a localized pattern and is especially found in occlusal fissures and on interdental surfaces (15–17). These sites are often difficult to clean properly and might have been covered by plaque already at the base-line registrations. The new plaque accumulated mainly on buccal and lingual surfaces. It is possible that the percentage of *S. mutans* in these deposits was low. The difficulties of *S. mutans* in spreading intraorally (18, 19) may further have contributed to the observed saliva values.

The saliva samples reflected the number of colonized interproximal sites. The number of *S. mutans* in pits and fissures on occlusal surfaces did not correlate with the saliva levels. This may be because the anatomy of occlusal surfaces admit optimal growth conditions for *S. mutans* on comparatively small and inaccessible areas. Tongue samples may be regarded as a kind of saliva sample, since it is almost impossible to remove all saliva from the dorsum of the tongue before sampling. This could be the explanation of the good correlation between tongue and saliva samples.

Summarizing the results of these studies, we have found the colonization level of *S. mutans* as observed in saliva samples to be

fairly stable from short-term periods. The samples also reflect the number of colonized tooth surfaces. Standardization of sampling time in a clinical study is certainly always preferable, but if this cannot be done, the variation of *S. mutans* during daytime seems to be fairly insignificant.

References

1. Crossner C-G. Salivary lactobacillus counts in the prediction of caries activity. Community Dent Oral Epidemiol 1981;9:182–190.
2. Klock B, Krasse B. A comparison between different methods for prediction of caries activity. Scand J Dent Res 1979;87:129–139.
3. Emilson C-G. Prevalence of *Streptococcus mutans* with different colonial morphologies in human plaque and saliva. Scand J Dent Res 1983;91:26–32.
4. Köhler B, Pettersson B-M, Bratthall D. *Streptococcus mutans* in plaque and saliva and the development of caries. Scand J Dent Res 1981;89:19–25.
5. Möller ÅJR. Microbiological examination of root canals and periapical tissues of human teeth. Methodological studies. Thesis. Odontol Tidskr 1966;74 (no. 5–6. Special article).
6. Klock B, Krasse B. Microbial and salivary conditions in 9- to 12-years old children. Scand J Dent Res 1977;85:55–63.
7. Gold OG, Jordan HV, Houte Jv. A selective medium for *Streptococcus mutans*. Arch Oral Biol 1973;18:1357–1364.
8. Carlsson J. Presence of various types of non-haemolytic streptococci in dental plaque and in other sites of the oral cavity in man. Odontol Rev 1967;18:55–74.
9. Silness J, Löe H. Periodontal disease in pregnancy. Acta Odontol Scand 1964;22:121–135.
10. Köhler B, Bratthall D. A practical method to facilitate the estimation of *Streptococcus mutans* levels in saliva. J Clin Microbiol 1979;9:584–588.
11. Kristoffersson K, Bratthall D. Transient reduction of *Streptococcus mutans* interdentally by chlorhexidine gel. Scand J Dent Res 1982;90:417–422.
12. Carlsson J. Effect of diet on presence of *Streptococcus salivarius* in dental plaque and saliva. Odontol Rev 1965;16:336–347.
13. Crossner C-G, Hagberg C. A clinical and microbiological evaluation of the Dentocult dip-slide test. Swed Dent J 1977;1:85–94.
14. Birkhed D, Edwardsson S, Anderson H. Comparison among a dip-slide test (Dentocult), plate count, and Snyder test for estimating number of lactobacilli in human saliva. J Dent Res 1981;60:1832–1841.
15. Ikeda T, Sandham HJ. Prevalence of *Streptococcus mutans* on various tooth surfaces in Negro children. Arch Oral Biol 1971;16:1237–1240.
16. Littleton NW, Kakehashi S, Fitzgerald RJ. Recovery of specific 'cariesinducing streptococci' from carious lesions in the teeth of children. Arch Oral Biol 1970;15:461–463.
17. Shklair IL, Keene HJ, Cullen P. The distribution of *Streptococcus mutans* on the teeth of two groups of naval recruits. Arch Oral Biol 1974;19:199–202.
18. Edman DC, Keene HJ, Shklair IL, Hoerman KC. Dental floss for implantation and sampling of *Streptococcus mutans* from approximal surfaces of human teeth. Arch Oral Biol 1975;20:145–148.
19. Gibbons RJ, de Paola PF, Spinell DM, Skobe Z. Interdental localization of *Streptococcus mutans* as related to caries experience. Infect Immun 1974;9:481–488.

Received for publication 2 May 1983