

# A Three-year Follow-up Study for Levels of Salivary *Streptococcus mutans* and *Streptococcus sobrinus* from Japanese Adolescents by Quantitative Real-time PCR

ISHIHARA Yoko

Department of Operative Dentistry, Tsurumi University School of Dental Medicine  
R & D Department, GC Corporation

## Abstract

**Purpose:** The purpose of this study was to investigate changes in *Streptococcus mutans* and *Streptococcus sobrinus* levels in saliva using TaqMan real-time PCR, once when the subjects were aged 12-13 years, and again 3 years later when the subjects were aged 15-16 years, in order to gain insights into caries prevention during adolescence.

**Methods:** The subjects were 88 Japanese adolescents (46 boys and 42 girls) in their first year of Tsurumi Junior High School in 2010, and in their first year of high school in 2013. Stimulated saliva was collected, and salivary *S. mutans* and *S. sobrinus* levels were obtained by real-time PCR. DMFT scores were also examined.

**Results:** Neither *S. mutans* nor *S. sobrinus* had formed colonies in 32 students (36.4%) at age 12-13 years, and remained undetected ( $<10^3$  cells/ml) in 24 of these students (75%) 3 years later. These results suggested that individuals in whom *S. mutans* and *S. sobrinus* have not formed colonies by age 12-13 remained non-carriers ( $<10^3$ ) for 3 years. In 9.1% of students, bacteria were detected in 2013 only, possibly due to horizontal transmission. In 5.7% of students, *S. mutans* and *S. sobrinus* were only detected in 2010, suggesting that transient flora had been detected. In 57.9% of students, bacteria were detected at both time points, indicating possible colonization. DMFT scores in *S. mutans* and *S. sobrinus* carriers tended to be higher than in non-carriers.

**Conclusion:** This study demonstrated the importance of inhibiting *S. mutans* and *S. sobrinus* colonization to prevent dental caries. As two or more bacterial tests must be conducted in order to determine *S. mutans* and *S. sobrinus* colonization, it is recommended that such bacterial tests be conducted at a dental clinic or during school health check-ups.

**Key words :** real-time PCR, *Streptococcus mutans*, *Streptococcus sobrinus*

## Introduction

Dental caries is a common infectious disease caused by dental plaque. Among 500 oral bacterial species, mutans streptococci (MS) such as *Streptococcus mutans* and *Streptococcus sobrinus* are conducive to caries initiation and development in humans<sup>1-3</sup>. These two bacteria synthesize adherent water-insoluble glucans from sucrose and adhere to the tooth surface. Once adhered, they form a biofilm with other oral flora and produce acid that demineralizes the tooth surface within the biofilm<sup>4</sup>.

In addition to dental caries, *S. mutans* has also been implicated in infective endocarditis<sup>5</sup>, and recent mouse studies have suggested that it is a risk factor for hemorrhagic stroke<sup>6</sup> and colitis<sup>7</sup>. In other words, preventing the colonization of *S. mutans* in the oral environment may not only help prevent caries, but also be important for general health overall.

Previously, the culture method using a mutans group-specific selective medium, such as Mitis-Salivarius agar and/or Mitis-Salivarius-Bacitracin agar<sup>8</sup>, has been used to obtain *S. mutans* and *S. sobrinus* levels<sup>1,9-11</sup>. However, while the culture method is able to identify bioactive bacteria, it is difficult to process large numbers of samples, as the method is labor- and time-intensive.

Thus, convenient methods, such as the "Strip mutans test"<sup>12</sup>, have been used for the determination of MS levels. The disadvantage of this convenient method is lower sensitivity and accuracy in clinical samples; the method can only divide results into classes 0 to 3: class 0 for MS=0, class 1 for  $5 \times 10^5$  CFU/ml or less, class 2 for 5 to  $9 \times 10^5$  CFU/ml and class 3 for  $10^6$  CFU/ml or more.

On the other hand, recent advances in molecular biology have enabled the development of Polymerase Chain Reaction (PCR) methods using DNA<sup>13-15</sup>. In addition, real-time PCR methods have been developed for quantifying DNA from specific pathogens. This method allows easy, rapid, and accurate quantitative detection of pathogens in clinical samples.

Salivary *S. mutans* and *S. sobrinus* levels obtained using real-time PCR have been reported<sup>16-19</sup>. However, these studies quantified bacterial levels at only one time point in each subject and did not clarify the

changes over time. Furthermore, even follow-up studies reporting bacterial counts have used the culture method<sup>20</sup>. Hence, it is important to determine changes in bacterial counts of a large number of subjects using methods such as real-time PCR to obtain accurate bacterial counts.

The incidence of caries among young people in Japan and other advanced nations has been declining in recent years. According to the Survey of Dental Diseases<sup>21</sup> conducted every six years in Japan, DMFT score at age 12 peaked in 1981 at 5.9 and dropped to 1.7 in 2005 and 1.4 in 2011. However, the number of caries continues to increase after age 12, and DMFT scores in the Survey were 3.2 for age 15-19, 5.9 for age 20-24 and 8.9 for age 25-29. The major reasons for this age-related increase may be that oral care is no longer managed by caregivers and schools as young people get older and the transition from student to adult is accompanied by lifestyle changes<sup>22</sup>.

In order to maintain a low incidence of caries after age 12, it is essential to determine the changes in caries risk factors during adolescence.

The purpose of this study was to investigate the caries risk factors in the same subjects twice, once when they were 12-13 years of age, and again three years later when they were 15-16 years of age. In particular, the study focused on *S. mutans* and *S. sobrinus*, which are known to be cariogenic bacteria, and used real-time PCR to investigate the detailed changes in bacterial levels. The effects of *S. mutans* and *S. sobrinus* levels on caries during adolescence were examined in order to gain insights into caries prevention.

## Materials and Methods

### 1. Subjects and survey of lifestyle habits

The subjects were 88 students (46 boys and 42 girls) who were members of Tsurumi University Junior High School in Yokohama, Kanagawa, Japan, at age 12-13 years in 2010, and 3 years later, when they attended Tsurumi University High School at age 15-16 years in 2013.

This study was approved by the Tsurumi University ethics committee (No. 813), was permitted by the school principal, and complied with the Declaration of Helsinki.

Before conducting the study, the purpose of the study was explained to the subjects and informed con-

sent was obtained. They also completed a questionnaire about lifestyle habits that are predicted to have an effect on the oral environment.

## 2. Saliva sampling

Before dental examination, at least 1 h had passed since eating or tooth brushing. Stimulated saliva was obtained from subjects by chewing tasteless paraffin gum for 2 min, and was placed in a centrifuge tube. Saliva samples were immediately cooled on ice, and were transported to the laboratory within 4 h. Samples were stored at  $-30^{\circ}\text{C}$  prior to DNA extraction, then were tested one time per sample by real-time PCR.

## 3. Dental examination

Examination was carried out in accordance with the checkup methods outlined by the School Health and Safety Act<sup>(23)</sup> in Japan by a team of eight expert dentists using a standardized procedure. The same team performed the examinations with the same procedure in 2010 and 2013. A mirror and LED light were used for the examinations, which were performed in the school gymnasium. The examination criterion was the number of decayed, missing and filled teeth (DMFT).

## 4. DNA extraction and quantitative PCR

Genomic DNA was isolated from saliva samples using a QuickGene DNA tissue kit S (Kurabo Industries) in accordance with the manufacturer's instructions. 5' Nuclease-based real-time PCR assay was performed according to the previously described method<sup>(24)</sup>. For real-time PCR assay, 20  $\mu\text{L}$  of a mixture containing 1  $\mu\text{L}$  of template DNA, 1  $\times$  TaqMan Fast Universal PCR Master Mix (Life Technologies Japan), 200 nmol/l forward and reverse primers, and 100 nmol/l TaqMan probe were placed in each well of a 96-well plate. Amplification and detection were performed using the 7500 Fast Real-Time PCR System (Life Technologies Japan) with the following cycle profile: 95 $^{\circ}\text{C}$  for 20 s, and 40 cycles of 95 $^{\circ}\text{C}$  for 3 s, and 60 $^{\circ}\text{C}$  for 30 s.

Sequences of *S. mutans*-specific primers were as follows: forward primer (Smut3368-F) was 5'-GCCTA-CAGCTCAGAGATGCTATTCT-3', reverse primer (Smut3481-R) was 5'-GCCATACACCACTCAT-GAATTGA-3', and amplicon size was 114 bp. The TaqMan fluorescent probe (Smut3423T) was 5'-FAM-TGGAAATGACGGTCGCCGTTATGAA-TAMRA-3'.

Sequences of *S. sobrinus* specific primers were as follows: forward primer (Ssob287-F) was 5'-TTCAAAGC-CAAGACCAAGCTAGT-3', reverse primer (Ssob374-R)

was 5'-CCAGCCTGAGATTCAGCTTGT-3', and amplicon size was 88 bp. The TaqMan fluorescent probe (Ssob298T) was 5'-FAM-CCTGCTCCAGCGA-CAAAGGCAGC-TAMRA-3'.

Standard curves for each organism were plotted for each primer-probe set using *Ct* values obtained from the amplification of genomic DNA extracted from samples of *S. mutans* strain and *S. sobrinus* strain. Numbers of bacteria were determined by plating culture dilutions on Brain Heart Infusion agar (Difco Laboratories, USA).

## 5. Statistical analysis

All statistical analyses were performed using SPSS Statistics Version 17.0 (IBM Japan). Wilcoxon signed-rank tests were performed in order to analyze changes in *S. mutans* and *S. sobrinus* levels from 2010 to 2013. Kruskal-Wallis tests were performed in order to compare mean DMFT at a significance level of 0.05.

## Results

Table 1 shows the number of subjects classified by *S. mutans* and *S. sobrinus* levels in 2010 and 2013.

The detected *S. mutans* level ranged from 0 to  $3.7 \times 10^5$  (cells/ml) in 2010 and from 0 to  $6.2 \times 10^5$  (cells/ml) in 2013. *S. mutans* was not detected at all in 15 students (17.0%) in 2010 and in 23 students (26.1%) in 2013. *S. mutans* was not detected at all in either 2010 or 2013 in nine of those students (10.2%).

With regard to *S. sobrinus*, this bacteria was detected in the range of 0 to  $2.9 \times 10^5$  (cells/ml) in 2010 and 0 to  $6.6 \times 10^4$  (cells/ml) in 2013. In 2010, 82 students (93.2%) were non-carriers and in 2013, 81 students (92.0%) were non-carriers of *S. sobrinus*. *S. sobrinus* was not detected at all in either 2010 or 2013 in 80 of those students (90.9%).

Ten students (11.4%) in 2010 and six students (6.8%) in 2013 (one subject had both  $9.6 \times 10^4$  cells/ml *S. mutans* and  $1.2 \times 10^4$  cells/ml *S. sobrinus*) had MS levels of  $10^5$  cells/ml or more (Table 1).

As shown in Table 2, the students were divided into four groups; A, B, C and D. Group A comprised students who were non-carriers ( $<10^3$ ) in both 2010 and 2013. Group B comprised students who were non-carriers ( $<10^3$ ) in 2010, but who were carriers in 2013. Group C comprised students who were carriers in 2010, but who were non-carriers ( $<10^3$ ) in 2013. Group D

**Table 1** Number of subjects classified by *S. mutans* and *S. sobrinus* levels in 2010 and 2013

Bacterial levels (cells/ml)	2010		2013	
	<i>S. mutans</i>	<i>S. sobrinus</i>	<i>S. mutans</i>	<i>S. sobrinus</i>
0	15	82	23	81
0 < <math>10^2</math>	0	0	1	0
<math&gt;10^2 &lt;="" 10^3&lt;="" \leq="" math&gt;<="" td=""> <td>19</td> <td>1</td> <td>7</td> <td>0</td> </math&gt;10^2>	19	1	7	0
<math&gt;10^3 &lt;="" 10^4&lt;="" \leq="" math&gt;<="" td=""> <td>15</td> <td>1</td> <td>17</td> <td>1</td> </math&gt;10^3>	15	1	17	1
<math&gt;10^4 &lt;="" 10^5&lt;="" \leq="" math&gt;<="" td=""> <td>30</td> <td>3</td> <td>35</td> <td>6</td> </math&gt;10^4>	30	3	35	6
<math&gt;10^5 &lt;="" 10^6&lt;="" \leq="" math&gt;<="" td=""> <td>9</td> <td>1</td> <td>5</td> <td>0</td> </math&gt;10^5>	9	1	5	0
Total (subjects)	88	88	88	88

**Table 2** Number and ratio (%) of mutans streptococci (*S. mutans* and *S. sobrinus*) carriers in 88 adolescents in 2010 and 2013

	2013	
	-	+
2010	-	24 (27.3%) <sup>A</sup>
	+	5 (5.7%) <sup>C</sup>
		8 (9.1%) <sup>B</sup>
		51 (57.9%) <sup>D</sup>

-: no mutans streptococci were detected ( $<10^3$ )

+: mutans streptococci were detected ( $\geq 10^3$ )

comprised students who were carriers ( $\geq 10^3$ ) in both 2010 and 2013.

Twenty-four students belonged to Group A (27.3%). Eight students belonged to Group B (9.1%), and carried *S. mutans* in 2013, including one student who also carried *S. sobrinus* in 2013. Five students belonged to Group C (5.7%). They carried *S. mutans* and did not include any *S. sobrinus* carriers. Fifty-one students belonged to Group D (57.9%). In Group D, 45 students were carriers of only *S. mutans* in both years, two students were carriers of only *S. sobrinus* in both years, three students were carriers of both *S. mutans* and *S. sobrinus* in both years and one student was a carrier of *S. mutans* in both years and *S. sobrinus* only in 2013.

Changes over 3 years in *S. mutans* levels ( $\geq 10^3$ ) in students are shown in Fig. 1.

The minimum and maximum *S. mutans* levels in Group B in 2013 were  $2.2 \times 10^3$  and  $2.6 \times 10^5$  (cells/ml), respectively. The minimum and maximum *S. mutans* levels in Group C in 2010 were  $1.2 \times 10^3$  and  $4.7 \times 10^4$  (cells/ml), respectively. The minimum and maximum *S. mutans* levels in Group D were  $1.2 \times 10^3$  and  $3.7 \times 10^5$  (cells/ml), respectively, in 2010 and  $1.2 \times 10^3$  and  $6.2 \times 10^5$  (cells/ml), respectively, in 2013. The mean *S.*

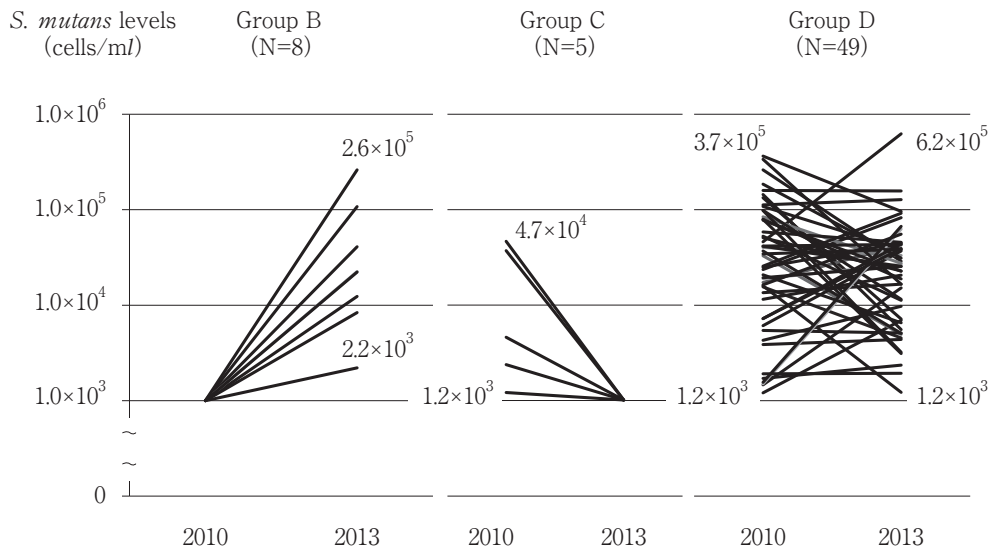
*mutans* level for Group D was  $6.2 \times 10^4$  in 2010 and  $4.4 \times 10^4$  (cells/ml) in 2013, and there were no statistically significant differences between the means for these time points.

Changes in *S. sobrinus* levels over 3 years for the seven students in whom *S. sobrinus* was detected ( $\geq 10^3$ ) are shown in Fig. 2. The *S. sobrinus* level in Group B in 2013 was  $5.7 \times 10^4$  cells/ml. The maximum *S. sobrinus* level in Group D was  $2.9 \times 10^5$  cells/ml in 2010, and the minimum and maximum *S. sobrinus* levels were  $1.6 \times 10^3$  and  $6.6 \times 10^4$  (cells/ml), respectively, in 2013. The mean *S. sobrinus* level for Group D was  $7.2 \times 10^4$  in 2010 and  $2.4 \times 10^4$  (cells/ml) in 2013, and there were no statistically significant differences between the means for these time points.

Mean DMFT per group in 2010 and 2013 and changes in the distribution of number of students in each DMFT category per group are shown in Table 3. DMFT in one student in Group A increased by 14 during the 3-year period; it is likely that factors other than *S. mutans* and *S. sobrinus* caused the increase in the number of caries in this individual. Excluding this student as an outlier, analyses gave a mean DMFT of 0.9 for Group A in 2013.

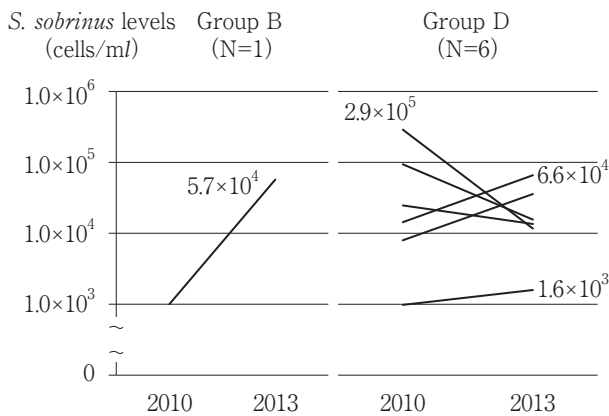
Mean DMFT differed significantly between Group A and Group D in 2010, but did not differ significantly in 2013. Many students in Group D had a high DMFT when compared with students in other groups, and this group tended to include many students with a large number of caries indicated by a DMFT of 3 or higher.

In order to examine the effects on changes in *S. mutans* and *S. sobrinus* levels in saliva, questionnaires were administered to the subjects with questions on lifestyle habits that are predicted to have an effect on



**Fig. 1** Changes in *S. mutans* levels for 62 subjects in groups B, C and D in whom *S. mutans* was detected in saliva. See Table 2 for groups B, C and D.

In group B, two subjects were included the point at  $2.2 \times 10^4$  cells/ml in 2013.



**Fig. 2** Changes in *S. sobrinus* levels for 7 subjects in groups B, C and D in whom *S. sobrinus* was detected in saliva. See Table 2 for groups B and D.

the oral environment (snacking, typically consumed drinks, tooth brushing, time taken for tooth brushing, use of dental floss and fluoride use). Logistic analysis did not reveal any clear associations with increases or decreases in *S. mutans* or *S. sobrinus* levels or their presence (data not shown).

### Discussion

#### 1. Cut-off levels of *S. mutans* and *S. sobrinus* for non-carriers

In this study, students with MS levels of less than

$1.0 \times 10^3$  cells/ml were deemed to be non-carriers. This was selected as a cut-off (Figs. 1, 2) because there are about  $10^8$  to  $10^9$  cells/ml of bacteria in the oral cavity in total, compared to which  $10^3$  cells/ml is minuscule. In addition, MS levels of  $10^5$  cells/ml or less have been reported to be a low caries risk using the conventional ‘Strip mutans’ test<sup>25</sup>. Even if changes in bacterial levels were examined in more detail, the effects of MS levels of  $10^3$  cells/ml or less on caries incidence can be considered very small.

#### 2. MS non-carriers group

The results in Groups A and B (Table 2) showed that neither *S. mutans* nor *S. sobrinus* were present in 32 students (36.4%) at age 12-13 years in 2010, and remained undetected ( $<10^3$ ) in 24 of these students (75% ; Group A) 3 years later. In this study, a real-time PCR method was used and this enabled very precise measurement. As oral bacterial count fluctuates, the absence of bacteria in one test does not necessarily mean that there is no colonization. In addition, as *S. mutans* is reported to have an infection rate of 82% or higher<sup>1,9-11</sup>, the colonization rate of MS was believed to be high. However, as bacteria were undetected ( $<10^3$ ) in the students in Group A not only once but again 3 years later, this strongly suggests that *S. mutans* and *S. sobrinus* did not colonize the oral environment in these students during those 3 years.

Loesche<sup>4</sup>) suggested that preventing colonization of

**Table 3** Mean DMFT and number of subjects in each DMFT category (0, 1 or 2, 3≤) in 2010 and 2013

Group* <sup>1</sup>	Subjects	mean* <sup>2</sup>	DMFT						
			2010			2013			
			0	1, 2	3≤	mean	0	1, 2	3≤
A	23	0.1	21	2	0	0.9	14	6	3
B	8	0	8	0	0	0.4	6	1	1
C	5	0.2	4	1	0	0.6	3	2	0
D	51	0.9	34	10	7	2.0	25	10	16

In group A, one subject whose DMFT score increased from 1 to 14 was excluded for the statistical reasons.

\*<sup>1</sup>: Group A: mutans streptococci were detected in neither 2010 nor 2013

Group B: mutans streptococci were detected in 2013 only

Group C: mutans streptococci were detected in 2010 only

Group D: mutans streptococci were detected in both 2010 and 2013

\*<sup>2</sup>:  $p < 0.05$ , Kruskal-Wallis test.

*S. mutans* and *S. sobrinus* in the pits and fissures in the first molar occlusal surface that erupt under conditions without MS is a major step in preventing caries in permanent teeth. As per Loesche's suggestion, this follow-up study demonstrated that it is possible to maintain no oral colonization of MS for at least 3 years from the age of 12–13 years without any special intervention.

The “window of infectivity” when *S. mutans* and *S. sobrinus* first begin colonizing the oral cavity in humans is reportedly between 19–33 months of age<sup>26</sup>. According to another study, the source of infection is the child's caregiver<sup>27</sup>. It is highly likely that students in Groups A and B in whom *S. mutans* and *S. sobrinus* were not detected at age 12–13 years, had never been carriers from childhood.

A number of host factors are thought to play a role in whether bacteria form a colony. The conditions for colonization of *S. mutans* and *S. sobrinus* in the oral cavity were examined by questionnaire, but no clear associations were found. The lack of any associations may be the result of having surveyed conditions at age 12–13 years rather than in infancy.

Kanagawa Prefecture, where the study took place, had the second smallest caries rate out of the 47 prefectures in Japan in 2010<sup>28</sup>. It has also been reported that preventive intervention in mothers was able to suppress caries development in their children<sup>29</sup>. It has therefore been suggested that there were low levels of MS in caregivers in this region, and it is also possible that MS was not transmitted to the subjects.

### 3. Carriers of MS

In Group D, as MS was detected both in 2010 and 2013, MS colonization was strongly suspected (Table 2).

The *S. mutans* (Fig. 1) and *S. sobrinus* (Fig. 2) levels in saliva of the students in Group D did not differ greatly between 2010 and 2013, and there were no significant differences in average levels. However, the levels varied greatly among individuals. Further studies are necessary to determine the factors that contribute to colonization of MS in the oral cavity and to the increase and decrease in bacterial level.

### 4. Hypothesis for transient and resident flora

When bacteria are transmitted from another individual, they may become “transient flora” that pass to the infected individual only temporarily, or “resident flora” that form a colony. The same applies to MS. Most of the bacteria transmitted from other individuals are only transient flora that are later expelled from the body, and only a small portion remains in the oral cavity, becoming resident flora that form micro-colonies on tooth surfaces.

In students in Groups B and C, *S. mutans* was only detected in either 2010 or 2013 (Figs. 1, 2). In Group B, *S. mutans* colonization was not observed at age 12–13 years. The detection of this type of bacteria 3 years later may have been due to horizontal transmission. The bacteria detected in Group C students may have been transient flora.

### 5. Level of MS and DMFT

As shown in Table 3, compared to subjects in other



groups, a large number of those in Group D had high DMFT scores at the start of the study in 2010. There was also an increase in the number of subjects with high DMFT scores after 3 years, indicating a high predisposition for dental caries. These results showed that oral colonization of MS may lead to increases in dental caries and reaffirmed the importance of preventing colonization of MS in order to prevent dental caries.

As noted earlier, this study was not able to identify the conditions needed to inhibit MS colonization nor was it able to determine the conditions needed to decrease MS. Therefore, further studies are necessary.

### 6. Two-point assessment of cariogenic bacteria

As in this study, two or more tests at two or more time points must be conducted in order to study bacterial colonization. It is therefore recommended to perform bacterial tests on a regular basis at a dental clinic or during school dental check-ups to enable comparison with previous values. The ideal time interval for testing bacterial colonization will be investigated in future studies.

Those suspected of MS colonization after repeated testing are considered to be at high risk of caries, so implementing an individualized program consisting of professional care (such as PMTC and fluoride application), self-care (such as the use of dental floss and fluoride toothpaste) and education on lifestyle modification (such as limiting the frequency of sugar intake) is crucial.

## Conclusions

The present study showed that in 27.3% of subjects, *S. mutans* and *S. sobrinus* had not formed colonies by the age of 12-13 years and these subjects remained non-carriers for 3 years. Conversely, MS colonization was strongly suspected in 57.9% of the subjects. DMFT scores tended to be higher in MS carriers than in non-carriers. To prevent dental caries, prevention of MS colonization was found to be crucial. To determine such bacterial colonization, two or more tests are required; thus, the incorporation of bacterial testing into regular school health check-ups is recommended.

### Acknowledgements

I sincerely thank Prof. MOMOI Yasuko (Tsurumi University School of Dental Medicine) whose enormous support and

insightful comments were invaluable during this study. I also thank Prof. HANADA Nobuhiro, Dr. NOMURA Yoshiaki and Dr. OKAMOTO Masaaki (Tsurumi University School of Dental Medicine) for their useful advice.

I would also like to thank Mr. TANAKA Hideo, Mr. NAKADA Yoshinori and Mr. ABE Shunichi of the Yamate Information Processing Center for their assistance and advice in statistical analysis. Special thanks go to Dr. FUKAYA Meri (Tsurumi University School of Dental Medicine) and Mr. TAKAYAMA Kazuto, Mr. NAITO Hiroki and Mr. KUMAGAI Tomohiro (GC Corporation).

## References

- 1) Köhler B, Bjarnason S. Mutans streptococci, lactobacilli and caries prevalence in 11- and 12-year-old Icelandic children. *Community Dent Oral Epidemiol* 1987; 15: 332-335.
- 2) de Soet JJ, van Loveren C, Lammens AJ, Pavicic MJ, Homburg CH, ten Cate JM, de Graaff J. Differences in cariogenicity between fresh isolates of *Streptococcus sobrinus* and *Streptococcus mutans*. *Caries Res* 1991; 25: 116-122.
- 3) Hirose H, Hirose K, Isogai E, Miura H, Ueda I. Close association between *Streptococcus sobrinus* in the saliva of young children and smooth-surface caries increment. *Caries Res* 1993; 27: 292-297.
- 4) Loesche WJ. Role of *Streptococcus mutans* in human dental decay. *Microbiol Rev* 1986; 50: 353-380.
- 5) Miyatake K, Akaishi M, Ishizuka N, Eishi K, Kawazoe K, Nakazawa M. Guidelines for the prevention and treatment of infective endocarditis (JCS 2008). The Japanese Circulation Society. [http://www.j-circ.or.jp/guideline/JCS2008\\_miyatake\\_h.pdf](http://www.j-circ.or.jp/guideline/JCS2008_miyatake_h.pdf) (cited 2014. 01. 12)
- 6) Nakano K, Hokamura K, Taniguchi N, Wada K, Kudo C, Nomura R, Kojima A, Naka S, Muranaka Y, Thura M, Nakajima A, Masuda K, Nakagawa I, Speziale P, Shimada N, Amamo A, Kamisaki Y, Tanaka T, Umemura K, Ooshima T. The collagen-binding protein of *Streptococcus mutans* is involved in hemorrhagic stroke. *Nat Commun*; doi: 10.1038/ncomms1491
- 7) Kojima A, Nakano K, Wada K, Takahashi H, Katayama K, Yoneda M, Higurashi T, Nomura R, Hokamura K, Muranaka Y, Matsushashi N, Umemura K, Kamisaki Y, Nakajima A, Ooshima T. Infection of specific strains of *Streptococcus mutans*, oral bacteria, confers a risk of ulcerative colitis. *Sci Rep*; doi: 10.1038/srep00332
- 8) Gold OG, Jordan HV, Van Houte J. A selective medium for *Streptococcus mutans*. *Arch Oral Biol* 1973; 18: 1357-1364.
- 9) Masuda N, Tsutsumi N, Sobue S, Hamada S. Longitudinal survey of the distribution of various serotypes

- Streptococcus mutans* in infants. J Clin Microbiol 1979; 10: 497-502.
- 10) Hamada S, Masuda N, Kotani S. Isolation and serotyping of *Streptococcus mutans* from teeth and feces of children. J Clin Microbiol 1980; 11: 314-318.
  - 11) Bratthall D, Serinirach R, Carlsson P, Lekuangufu S. *Streptococcus mutans* and dental caries in urban and rural schoolchildren in Thailand. Community Dent Oral Epidemiol 1986; 14: 274-276.
  - 12) Jensen B, Bratthall D. A new method for the estimation of mutans streptococci in human saliva. J Dent Res 1989; 68: 468-471.
  - 13) Okada M, Soda Y, Hayashi F, Doi T, Suzuki J, Miura K, Kozai K. Longitudinal study of dental caries incidence associated with *Streptococcus mutans* and *Streptococcus sobrinus* in pre-school children. J Medical Microbiol 2005; 54: 661-665.
  - 14) Loyola-Rodriguez JP, Martinez-Martinez RE, Flores-Ferreira BI, Patiño-Marin N, Alpuche-Solis AG, Reyes-Macias JF. Distribution of *Streptococcus mutans* and *Streptococcus sobrinus* in saliva of Mexican preschool caries-free and caries-active children by microbial and molecular (PCR) assays. J Clin Pediatr Dent 2007; 32: 121-126.
  - 15) Okada M, Kawamura M, Oda Y, Yasuda R, Kojima T, Kurihara H. Caries prevalence associated with *Streptococcus mutans* and *Streptococcus sobrinus* in Japanese schoolchildren. Int J Paediatr Dent. 2012; 22: 342-348.
  - 16) Kishi M, Abe A, Kishi K, Ohara-Nemoto Y, Kimura S, Yonemitsu M. Relationship of quantitative salivary levels of *Streptococcus mutans* and *S. sobrinus* in mothers to caries status and colonization of mutans streptococci in plaque in their 2.5-year-old children. Community Dent Oral Epidemiol 2009; 37: 241-249.
  - 17) Choi EJ, Lee SH, Kim YJ. Quantitative real-time polymerase chain reaction for *Streptococcus mutans* and *Streptococcus sobrinus* in dental plaque samples and its association with early childhood caries. Int J Pediatr Dent 2009; 19: 141-147.
  - 18) Nurelhuda NM, Al-Haroni M, Trovik TA, Bakken V. Caries experience and quantification of *Streptococcus mutans* and *Streptococcus sobrinus* in saliva of Sudanese schoolchildren. Caries Res 2010; 44: 402-407.
  - 19) Sánchez-Acedo M, Montiel-Company JM, Dasí-Fernández F, Almerich-Silla JM. *Streptococcus mutans* and *Streptococcus sobrinus* detection by polymerase chain reaction and their relation to dental caries in 12 and 15 year-old schoolchildren in Valencia (Spain). Med Oral Patol Oral Cir Bucal 2013; 18: 839-845.
  - 20) Köhler B, Andréeen I. Mutans streptococci and caries prevalence in children after early maternal caries prevention: a follow-up at 19 years of age. Caries Res 2012; 46: 474-480.
  - 21) Ministry of Health, Labor and Welfare in Japan: The Survey of Dental Diseases, 2011. <http://www.mhlw.go.jp/toukei/list/62-23.html> (cited 2013. 12. 30)
  - 22) Holmén A, Strömberg U, Maqnusson K, Twetman S. Tobacco use and caries risk among adolescents—a longitudinal study in Sweden. BMC Oral Health; doi: 10.1186/1472-6831-13-31
  - 23) Ministry of Internal Affairs and Communications in Japan: School Health and Safety Act. <http://law.e-gov.go.jp/htmldata/S33/S33F03501000018.html> (cited 2013. 12. 30)
  - 24) Yoshida A, Suzuki N, Nakano Y, Kawada M, Oho T, Koga T. Development of a 5' nuclease-based real-time PCR assay for quantitative detection of cariogenic dental pathogens *Streptococcus mutans* and *Streptococcus sobrinus*. J Clin Microbiol 2003; 41: 4438-4441.
  - 25) Axelsson P. Diagnosis and Risk Prediction of Dental Caries, Vol. 2. Quintessence Publishing: Karlstad, Sweden; 2000. 33-40.
  - 26) Caufield PW. Dental caries—a transmissible and infectious disease revisited: a position paper. Pediatr Dent 1997; 19: 491-498.
  - 27) Kozai K, Nakayama R, Tedjosongko U, Kuwahara S, Suzuki J, Okada M, Nagasaka N. Intrafamilial distribution of mutans streptococci in Japanese families and possibility of father-to-child transmission. Microbiol Immunol 1999; 43: 99-106.
  - 28) Ministry of Education, Culture, Sports, Science & Technology in Japan: School Health Survey, 2011. [http://www.mext.go.jp/b\\_menu/toukei/chousa05/hoken/kekka/kdetail/1303380.html](http://www.mext.go.jp/b_menu/toukei/chousa05/hoken/kekka/kdetail/1303380.html) (cited 2013. 12. 30)
  - 29) Köhler B, Andréeen I. Influence of caries-preventive measures in mothers on cariogenic bacteria and caries experience in their children. Arch Oral Biol 1994; 39: 907-911.



# リアルタイム PCR 法を用いて測定した唾液中の *Streptococcus mutans* と *Streptococcus sobrinus* 菌数について日本人の青年期における 3 年間のフォローアップ研究

石原容子

鶴見大学歯学部保存修復学講座  
株式会社ジーシー 研究所

## 抄録

目的：本研究の目的は、12～13歳時とその3年後の15～16歳時の2度、同一対象者に対してう蝕のリスク要因の調査、比較を試みることである。特に、リアルタイム PCR 法を用いてう蝕原因菌の *Streptococcus mutans* と *Streptococcus sobrinus* の詳細な細菌数の変化を調査することにより、この時期のう蝕に与える *S. mutans* と *S. sobrinus* 菌数の影響を検討し、青年期のう蝕予防の手がかりを得ようと試みた。

方法：2010年に中学1年生（12～13歳）で、2013年に高校1年生（15～16歳）に進学した合計88名を対象とした。口腔内検査に先立ち刺激唾液を採取して、リアルタイム PCR 法によって唾液中の *S. mutans* と *S. sobrinus* の菌数を測定した。事前に手技を統一した歯科医師によって、DMFT が調査された。

結果：12～13歳の段階で *S. mutans* と *S. sobrinus* が非検出（ $10^3$  cells/ml 未満）だった者が32名（36.4%）で、その内の75%にあたる24名は、3年後でもこれらの細菌は非検出だった。また、15～16歳時のみこれらの細菌が検出（ $10^3$  cells/ml 以上）された者は9.1%、12～13歳時のみに検出された者は5.7%だった。12～13歳時とその3年後の15～16歳時ともにこれらの細菌が検出された者は、57.9%だった。両年ともに検出された者は、その他の者たちに比べて DMFT 値が高い傾向にあった。

結論：本研究から、27.3%の者において、12～13歳時に *S. mutans* と *S. sobrinus* が定着しておらず、その後3年間非定着のまま経過することが確認された。また両年ともに *S. mutans* や *S. sobrinus* が検出された者においては、両菌の定着が強く疑われた。*S. mutans* や *S. sobrinus* が定着した者たちは、非定着の者に比べより重度なう蝕が多い傾向にあり、う蝕予防やう蝕を重症化させないためには、両菌を定着させないことが重要であることが示された。また本研究から、細菌の定着を確認するには2回以上の細菌検査が必要であり、これが学校健診などの場で実施されることが望ましいことが示唆された。

キーワード：リアルタイム PCR, *Streptococcus mutans*, *Streptococcus sobrinus*

---

責任著者連絡先：石原容子

〒174-8585 東京都板橋区蓮沼町76-1 株式会社ジーシー 研究所

TEL：03-3965-1233, FAX：03-3965-1655, E-mail：yoko\_ishihara@mb.gcdental.co.jp

受付：平成26年7月15日/受理：平成26年8月29日