Association of oral streptococci community dynamics with severe early childhood caries as assessed by PCR-denaturing gradient gel electrophoresis targeting the \textit{rnpB} gene

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This study sought to investigate the possible association between the dynamics of oral streptococci community profiles and severe early childhood caries (S-ECC) development, compared with caries-free (CF) controls. Supragingival plaque samples were evaluated from 8–32-month-old children who had previously been assessed for overall profiles of their oral microbial community. Twelve children were in each group. Bacterial genomic DNA was extracted and amplified using \textit{rnpB}-specific primers for streptococci; the products were then subjected to denaturing gradient gel electrophoresis (DGGE) and sequence analysis. We observed that the mean values for species richness (\(N\)) and diversity of oral streptococci (\(H\)) were significantly lower in the S-ECC group than in the CF group (\(N = 1.25 \pm 4.14\) vs \(14.92 \pm 2.84\); \(H = 1.41 \pm 0.29\) vs \(1.64 \pm 0.18\)) at 32 months of age (\(P < 0.05\)). Significantly higher detection rates of \textit{Streptococcus sanguinis} and \textit{Streptococcus gordonii} were found in the CF group compared with the S-ECC group at 32 months of age (\(P < 0.05\)). Cluster analysis of DGGE profiles showed that most of the clusters were constructed from one individual over time. These results suggested that the onset of S-ECC is accompanied by reduced diversity of oral streptococci, that the detection rates of \textit{S. sanguinis} and \textit{S. gordonii} have negative correlations with S-ECC; and that there are high levels of intra-individual similarity for the oral streptococci community over time.

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INTRODUCTION

Severe early childhood caries (S-ECC) is a form of rampant caries that severely compromises the primary dentition during the early stages of life (Drury et al., 1999). Despite its public health significance, the cause of S-ECC remains unclear. Dental plaque biofilm is known to be the main aetiological factor for carious progression (Selwitz et al., 2007). Its development is heavily influenced by oral streptococci, which are considered to be the primary component of early colonizers. Initial attachment of oral streptococci determines the composition of later colonizers in the oral biofilm and impacts the caries status (Filoche et al., 2010). Although most investigations implicate mutans streptococci as the principal aetiologic agents in early childhood caries, due to their prevalence in carious lesions (Thenisch et al., 2006), the mere presence of mutans streptococci is not sufficient to predict the formation of dental caries; other oral streptococcal species may also moderate caries outcome (Kanasi et al., 2010). To date, little is known about the changes in the diversity and composition of oral streptococci in relation to S-ECC. According to the ecological plaque hypothesis, the relative numbers of these commensal species are thought to be fairly constant in health, and shifts in their relative proportions may result in caries (Marsh, 1994). Therefore, monitoring oral streptococci in the development of S-ECC will be valuable for increasing our knowledge about the cariogenic plaque.

Previous studies by our group (Tao et al., 2013) and others (Jiang et al., 2011; Li et al., 2007; Ling et al., 2010) demonstrated the superior utility of PCR-denaturing gel gradient electrophoresis (PCR-DGGE) relative to other methods in evaluating the oral microbiota composition. With this approach, microbial profiles of plaques or saliva from various individuals can be compared, and shifts in the

Abbreviations: CF, caries-free; DGGE, denaturing gradient gel electrophoresis; S-ECC, severe early childhood caries; UPGMA, unweighted pair group method with arithmetic averages.
ecological balance of the biofilms can be evaluated. Because one of the known limitations of DGGE is that the identification is generally based on partial 16S rRNA gene sequences, some plaque samples may only be identifiable at the genus level (Watanabe et al., 2001). For species identification of oral streptococci, specific primers were needed. An alternative candidate was the \textit{rnpB} gene, which codes for the RNA subunit of RNase P. Compared with the 16S rRNA gene, which may have several copy variants in the genome and could therefore result in erroneous genotyping, the \textit{rnpB} gene exhibits single-copy expression of the gene and has a higher information value per nucleotide position. Combined with the advantage of its short length, the \textit{rnpB} gene has been suggested to have greater potential for species discrimination in streptococci than the 16S rRNA gene (Täpp et al., 2003). Recently, the \textit{rnpB} gene has been shown to be an excellent target for oral streptococci differentiation in a cross-sectional study (Jiang et al., 2011). Although previous studies have investigated the oral streptococci associated with caries using cross-sectional methods, there are few longitudinal studies reporting the succession pattern of oral streptococci in early childhood caries over time.

The purpose of this study was to monitor changes in the diversity and composition of the oral streptococci community in dental plaques and to test their association with the S-ECC status in children.

**METHODS**

**Subjects.** Study subjects were first recruited from our previous study (Tao et al., 2013), in which we monitored the longitudinal changes in oral microbial diversity of 8-month-old children at 6, month intervals for 2 years and evaluated their S-ECC status. According to the criteria of the American Academy of Pediatric Dentistry, any sign of smooth-surface caries was diagnosed as S-ECC in children younger than 3 years of age (American Academy on Pediatric Dentistry & American Academy of Pediatrics, 2008–2009). In our previous study, a total of 12 children attended all follow-up appointments and those found with S-ECC were formed into the S-ECC group; two children acquired S-ECC at 26 months of age and 10 acquired S-ECC at 32 months of age. The control group consisted of 12 caries-free (CF) age- and gender-matched children who showed no caries until 32 months of age. Each examination of the subjects included a dental examination and dental plaque sample collection. Dental caries examinations were scored for dmfs: decayed (d), missing due to caries (m), and filled (f) surfaces (s), using World Health Organization diagnostic criteria (WHO, 1997). The major socio-economic background and developmental characteristics of the two groups of children were not significantly different, as analysed in our previous study (Tao et al., 2013). Written informed consent by the parents was obtained for all the subjects.

In the present study, 12 children, with eight boys and four girls per group, were recruited to assess the dynamics of oral streptococci in childhood dental plaques and their relationship with S-ECC development, compared with CF controls. This study was approved by the ethics committee of Sun Yat-sen University.

**Sample collection.** Pooled supragingival plaque samples were collected from the accessible surfaces of the deciduous anterior teeth, using a sterile cotton tip, from each child at 8, 14, 20, 26 and 32 months of age. The plaque samples were collected in sterile 1.5 ml microcentrifuge tubes containing 1.0 ml saline solution and stored at 80 °C prior to analysis.

**DNA extraction.** Genomic DNA was extracted from the samples as described previously (Tao et al., 2013). Briefly, a QIAamp DNA Mini kit (Qiagen) was used for bacterial cell lysis and total DNA precipitation according to the manufacturer’s instructions with some modifications. The final quantity and quality of the DNA was evaluated using a UV spectrophotometer at \(A_{260}/A_{360}\). The DNA was dissolved in sterile \(d_2\)O and stored at 20 °C until it was analysed.

**Touchdown PCR amplification.** The following primers were used to target the \textit{rnpB} gene of streptococci: A1118BP-GC (5’-CGCCCGCCGCGGGGGCCGCGGGGGCCGCGGGGGCCGCGGGGGCCGCGGGGGCCGCGGGGGCGGGGGGAATTTTTTGATATTGCT-3’), which contains a 40 bp GC clamp that makes it suitable for DGGE, and A1121RPB (5’-TGGGTTGCTAGCT-TGAGG-3’). The PCR mixture comprised a total volume of 50 μl, which consisted of 2 μl template DNA at 10 ng μl\(^{-1}\), 50 pM each primer, 200 μM each dNTP, 5 μl 10 × PCR buffer, 1.5 mM MgCl\(_2\) and 1 U Platinum Taq polymerase (Invitrogen).

Amplification was performed using the following ‘touchdown’ cycling profile: 95 °C for 5 min for initial denaturing; one cycle of 94 °C for 40 s, 65 °C for 1 min and 72 °C for 45 s; and a subsequent nine cycles lowered the annealing temperature (65 °C) by 1 °C per cycle. A final 25 cycles were carried out of 94 °C for 40 s, 56 °C for 1 min and 72 °C for 45 s, with a final extension of 7 min at 72 °C, which finished the amplification cycle, followed by cooling to 4 °C. The presence of PCR products was confirmed by electrophoresis on 2 % (w/v) agarose gels.

**DGGE analysis.** PCR amplicons of the \textit{rnpB} gene from oral streptococci, originally present in supragingival plaque samples, were separated via DGGE using the D-Code system (Bio-Rad). Each PCR product (20 μl) was loaded onto 8 % (w/v) polyacrylamide gels (acrylamide/bisacrylamide = 37.5:1) in 1 × TAE buffer with an increasing linear gradient of denaturants of 30–60 % in the direction of parallel electrophoresis, where a 100 % denaturant was defined as 7 M urea and 40 % (v/v) deionized formamide. To evaluate the accuracy of comparisons within and across gels, a molecular standard marker was introduced into all gels. The synthetic standard was constructed after mixing equal volumes of the reamplified PCR products from the excised representative bands of the DGGE gels, with the above parameters. Electrophoresis was performed in 1 × TAE buffer with a constant voltage of 100 V at 60 °C for 14 h. Gels were then stained using SYBR Green I nucleic acid stain (1 : 30 000 in 1 × TAE buffer), visualized on a UV transilluminator and photographed (Alpha Innotech Corporation). The migration and intensity of the DGGE bands were analysed with GelCompare II software (version 6.5; Applied Maths), as described previously (Fromin et al., 2002). Levels of similarity among fingerprints were calculated using the Pearson product moment correlation coefficient (Smalla et al., 2001). The unweighted pair group method with arithmetic averages (UPGMA), which is advantageous in enabling the rapid assessment of the coherence of fingerprinting patterns, was applied to identify the samples that generated similar patterns in the DGGE profiling (Fromin et al., 2002). The results were displayed graphically as a dendrogram.

**Cloning and sequence analysis of PCR-DGGE fragments.** DNA fragments of visible DGGE bands to be sequenced were excised with a sterile scalpel and eluted in 50 μl sterile water. Bands in different lanes that shared the same horizontal position were considered to be the same species, and one representative of each was randomly chosen for sequencing. The DNA was diffused at 4 °C overnight. Approximately 2–4 μl of the eluate was used as a template and reamplified with the same primer without the GC clamp as described above. The fresh PCR
products were purified (QIAquick PCR Purification kit; Qiagen) and then cloned into the TOPO10 vector (TOPO TA Cloning kit; Invitrogen) following the manufacturer’s protocol. The transformed cells were screened using X-Gal (40 mg ml⁻¹) medium containing kanamycin (50 μg ml⁻¹). Inserted colonies (white) were randomly selected and used for extraction of plasmid DNA with a QIAprep Spin Miniprep kit (Qiagen). Sequencing of the purified plasmid DNA was performed at the Invitrogen Sequencing Department (Invitrogen). Similarity was tested to sequences available at the National Center for Biotechnology Information using BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST).

**Statistical analysis.** Species diversity consists of two components: species richness and evenness. Richness, defined as the number of species (N), was calculated as the total number of bands per sample. Evenness refers to the proportion of species in each sample. The Shannon–Wiener index (H') was used to determine the species diversity of oral streptococci present in all plaque samples. This index was calculated by the following equation:

\[ H' = -\sum_{i=1}^{s} p_i \ln p_i \]

where \( s \) is the number of species and \( p_i \) is the proportion of species in sample \( i \). A higher \( H' \) indicates a higher diversity. Repeated-measures ANOVA tests were used to compare the variables between the two groups at the various time points (8, 14, 20, 26 and 32 months of age). Geisser–Greenhouse correction was applied for repeated-measures analysis to correct for violations of the sphericity assumption. Multiple comparisons correction was performed with the Bonferroni test. Statistical analyses were carried out using SPSS software version 16.0 (SPSS Inc.). \( P<0.05 \) was considered statistically significant.

**RESULTS**

**DGGE profiles analysis**

Touchdown PCR-DGGE analysis with primers targeting the *rnpB* gene of streptococci was used to analyse the dominant species of oral streptococci in two groups at different months of age. As shown in Fig. 1, the DGGE profiles of the two groups at five time points revealed multiple structures and compositions of oral streptococci. The band number of DGGE profiles, which represent species richness of oral streptococci, was found to be up to 29 with various densities.

To compare the species richness and diversity of the predominant oral streptococci in each sample, the numbers of distinct bands (N) in the DGGE profiles and the Shannon–Wiener index (H’) were analysed (Fig. 2). By performing longitudinally repeated measures ANOVA of the mean N and H' values, we found a significant association with the time factor (N: F=3.985, P=0.00; H': F=2.934, P=0.025), but no significance between the two groups (N: F=0.222, P=0.642; H': F=0.039, P=0.846) over time, thus confirming the influence of time on the species richness and diversity of oral streptococci communities in dental plaques. The post-hoc Bonferroni test revealed significant differences in the mean N (11.25 ± 4.14 vs 14.92 ± 2.84, \( P=0.019 \)) and \( H' \) (1.41 ± 0.29 vs 1.64 ± 0.18, \( P=0.025 \)) values between the S-ECC group and the CF group at 32 months of age, indicating that the caries status was associated with a lower diversity of oral streptococci.

**Sequence analysis of selected DGGE bands**

Dominant bands from multiple positions in the DGGE profiles were excised and sequenced. A total of 201 dominant bands were identified as belonging to specific species of oral streptococci except for two pairs of species – *Streptococcus mitis/pneumoniae* and *Streptococcus infantis/parasanguinis*. The data in Table 1 show the distribution of species at five time points. In both groups, *S. mitis/pneumoniae* was detected in virtually all the dental plaque samples. *Streptococcus parasanguinis*, *Streptococcus oralis*, *Streptococcus salivarius*, *Streptococcus parasanguinis* and *Streptococcus gordonii* were detected from over 50 % of all samples. To analyse the association between the distribution of species and caries status, Fisher’s exact test was conducted, and the detection rates of *S. sanguinis* and *S. gordonii* in the S-ECC group were significantly lower compared with the CF group at 32 months of age (\( P<0.05 \)).

**Clustering of DGGE profiles**

The DGGE profiles of supragingival plaque samples from the two groups at different ages were generated using the Pearson product moment correlation coefficient and dendrograms, which were constructed based on the UPGMA method. Fig. 3 shows the results of hierarchical clustering analyses of the DGGE profiles for both groups at 8, 14, 20, 26 and 32 months of age. As shown in Fig. 3, we found that samples from the S-ECC and CF group were not significantly different, regardless of the time point. Similarly, Figs 4 and 5 depict dendrograms of samples at 8–32 months of age from the S-ECC and CF groups, respectively. In Fig. 4 (S-ECC group), there were eight of...
DISCUSSION

Disruption of microbial ecological balance in dental biofilms is an important cause of early childhood caries (March, 1994). Oral streptococci are major constituents of dental plaques which have specific temporal and spatial distributions that are crucial for the ecology of dental plaques. The 12 clusters (66%, clusters 1, 2, 4–6 and 9–11) with more than 50% of the samples from the same person in each cluster. From these figures, we found that the samples from one individual in each time point assorted into different clusters, whereas intra-individual high similarity in both groups was observed. Image analysis did not reveal obvious motifs associated with the onset of S-ECC.

Table 1. Distribution of oral streptococci according to rnpB gene sequence analysis detected from the study subjects (8-32 months)

<table>
<thead>
<tr>
<th>Species</th>
<th>CF group (n=12)</th>
<th>S-ECC group (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8 months</td>
<td>14 months</td>
</tr>
<tr>
<td>Streptococcus mutans</td>
<td>0 (0)</td>
<td>1 (8.3)</td>
</tr>
<tr>
<td>Streptococcus sobrinus</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Streptococcus mitis/pneumoniae</td>
<td>11 (91.7)</td>
<td>11 (91.7)</td>
</tr>
<tr>
<td>Streptococcus oralis</td>
<td>5 (41.7)</td>
<td>8 (66.7)</td>
</tr>
<tr>
<td>Streptococcus sanguinis</td>
<td>8 (66.7)</td>
<td>9 (75.0)</td>
</tr>
<tr>
<td>Streptococcus gordonii</td>
<td>6 (50.0)</td>
<td>7 (58.3)</td>
</tr>
<tr>
<td>Streptococcus parasanguinis</td>
<td>5 (41.7)</td>
<td>3 (25.0)</td>
</tr>
<tr>
<td>Streptococcus cristatus</td>
<td>4 (33.3)</td>
<td>1 (8.3)</td>
</tr>
<tr>
<td>Streptococcus infantis/peroris</td>
<td>1 (8.3)</td>
<td>3 (25.0)</td>
</tr>
<tr>
<td>Streptococcus anginosus</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Streptococcus intermedius</td>
<td>2 (16.7)</td>
<td>1 (8.3)</td>
</tr>
<tr>
<td>Streptococcus salivarius</td>
<td>3 (25.0)</td>
<td>2 (16.7)</td>
</tr>
</tbody>
</table>

*P<0.05, by Fisher’s exact test.

Fig. 2. Oral streptococci community diversity calculated using the number of DGGE bands (a) and the Shannon–Wiener index (b). The repeated-measures ANOVA and post-hoc Bonferroni test were used for statistical analysis.
Fig. 3. UPGMA cluster analysis was performed to assess differences in the compositions of the oral streptococci in the S-ECC group before (yellow squares) and after (red squares) the onset of S-ECC, and CF group samples (green squares) at various ages (months). Percentage similarity is shown on the scale above the dendrogram. The right-side legends refer to the group, subject and month of age (e.g. S9-8 is subject S-ECC9 at 8 months; C12-8 is subject CF 12 at 8 months).
Fig. 4. UPGMA cluster analysis was performed to assess differences in the compositions of the oral streptococci in the S-ECC group samples before (yellow squares) and after (red squares) the onset of S-ECC at various ages. Samples with a similar microbial community composition are clustered together on the dendrogram. Percentage similarity is shown on the scale above the dendrogram. The right-side legends refer to the group, subject and month of age (e.g. S12-26 is subject S-ECC12 at 26 months).
Fig. 5. UPGMA cluster analysis was performed to assess differences in the compositions of oral streptococci in CF group samples (green squares) at various ages. Samples with a similar microbial community composition are clustered together on the dendrogram. Percentage similarity is shown on the scale above the dendrogram. The right-side legends refer to the group, subject and month of age (e.g. C12-8 is subject CF12 at 8 months).
biofilm (Thenisch et al., 2006). Oral streptococci are divided into four different groups: the mutans group, salivarius group, anginosus group and mitis group, comprising a total of 18 species with various characteristics and functions (Whiteley & Beighton, 1998). Among these, mutans streptococci are considered to be the primary pathogens of dental caries (Thenisch et al., 2006). However, due to the complex interspecies interactions, there is also evidence to suggest that other species of oral streptococci may have different roles in the caries process (Kanasi et al., 2010). Most previous studies on the microbial risk indicators for dental caries have focused mainly on one or several possible pathogens (Caufield et al., 2007; Ge et al., 2008; Okada et al., 2005). Studies that have investigated dental plaque from a microbial ecological perspective are rare. As such, the succession of oral streptococci in dental plaque of children with or without caries is still unclear.

S-ECC can be particularly virulent forms of caries and typically develop on anterior tooth surfaces. Considering the poor cooperation and low amount of plaque in the young children that were included in this longitudinal study, supragingival plaque samples were collected from the accessible surfaces of the deciduous anterior teeth with a sterile cotton tip at each time point to maintain consistency of the plaque collection method. Such data could reflect the general microbial composition in dental plaque of the exposed tooth surface, compared with site-specific plaque sampling, for which data reflect only the situation of specific colonized sites (Tanzer et al., 2001). Previously, we and others demonstrated that PCR-DGGE is a valuable tool for intuitively observing the dynamics of microbial communities composing the dental plaque of children (Jiang et al., 2011; Li et al., 2007; Ling et al., 2010; Tao et al., 2013). In the present study, we sequenced the rnpB gene combined with DGGE to evaluate the diversity and dynamics of oral streptococci associated with S-ECC. All sequences of the dominant bands could be identified to the species level except for the species pairs of S. mitis/pneumoniae and S. infantis/peroris. The inability of our assay to distinguish these two pairs of species may due to a high level of similarity between them, making identification of some genotypes of these species difficult solely on the basis of the rnpB gene (Innings et al., 2005; Täpp et al., 2003).

The results of this study showed that the species richness and diversity of oral streptococci were significantly lower in the S-ECC group than in the CF group at 32 months of age. These findings imply that the onset of dental caries has an association with a lower diversity of oral streptococci, which is consistent with a cross-section study of preschool children in the various stages of dental caries (Jiang et al., 2011). According to these findings and the previous studies, we infer that the decline in streptococcal diversity in S-ECC plaques is possible because of shifts in the balance of the resident plaque microbiota (Jiang et al., 2011; Li et al., 2007; Ling et al., 2010). Bacterial species present in the dental biofilm communities have cooperative or competitive interactions with other members (Guo et al., 2014). Because of their genetic similarity, the oral streptococci have similar metabolic activities, and multiple species can compete for the ecological niche on the saliva-coated tooth surface. The early colonists to the niche can then produce antimicrobial compounds, such as bacteriocins and hydrogen peroxide, to affect other related species (Kreth et al., 2005; Nes et al., 2007). Moreover, in the cariogenic plaque, other caries-associated species such as Bifidobacteria and Lactobacillus might also be retained by physical or metabolic interactions with streptococci (Hojo et al., 2009). The interaction of micro-organisms, including oral streptococci, results in the deterioration of normal conditions, including acidification of plaque (pH <5), which fosters the emergence of aciduric species at the expense of diminutions in the populations of less acid-tolerant micro-organisms. These changes eventually decrease the complexity of the plaque community, as well as of oral streptococci.

Subsequent cloning and sequencing of the excised bands revealed that the detection rates of S. sanguinis and S. gordonii were significantly higher in the CF group than in the S-ECC group at 32 months of age. S. sanguinis and S. gordonii are pioneering bacteria that colonize tooth surfaces and form dental plaques on tooth surfaces that are free of caries (Becker et al., 2002; Gross et al., 2010). S. sanguinis competes with other bacteria by producing a bacteriocin or excreting hydrogen peroxide to trigger the lysis of competitors during initial colonization (Kreth et al., 2008). Several investigators have shown that S. sanguinis can play an antagonistic role against Streptococcus mutans colonization, and early colonization of S. sanguinis is associated with delayed colonization of S. mutans in children (Kreth et al., 2005). Similarly, S. gordonii can also excrete hydrogen peroxide, but the present studies showed no inhibition of S. mutans by S. gordonii (Kreth et al., 2008; Tanzer et al., 2012). Furthermore, S. mutans can outcompete S. gordonii on the tooth surface (Kreth et al., 2008). Gross et al. (2010) reported that S. gordonii numbers diminish greatly in caries-associated plaque biofilms, whereas S. mutans persists. In our study, S. sanguinis and S. gordonii were maintained at relatively higher proportions in both groups, relative to S. mutans, at 8–26 months of age, but significantly decreased at 32 months of age in the S-ECC group as the teeth decayed. These results demonstrated that S. sanguinis and S. gordonii play important roles in balancing the human oral microbial ecosystem. We observed that the detection rate of S. mutans in the CF group was maintained at approximately 8 % at 14 months of age, whereas sharp increases appeared at 26 months (16.7 %) and 32 months (41.7 %) in the children in the S-ECC group. A considerable amount of epidemiological evidence has established a positive correlation between S. mutans and early childhood caries (Selwitz et al., 2007). No significant correlation was found in the analysis between S-ECC and S. mutans in this study, which might be due to the small sample size and related low detection rates of S. mutans in the young children who were examined.
As the children’s age increased, the proportion of children positive for *S. mutans* might also increase (Seki et al., 2006). Longer follow-up studies with larger sample sizes of children are needed in the future.

The results of cluster analyses of the DGGE profiles showed that the microbiota of oral streptococci within an individual had high similarity over time (Figs 4 and 5). These results implied that intra-individual variability of oral streptococci is relatively low in the early stages of a child’s life. One potential reason for this phenomenon is that dental plaque development is person specific. Diaz et al. (2006) reported that oral streptococci in the initial dental plaque community of a given subject were unique in terms of diversity and composition. As the major predominant early colonizers of dental biofilm, we conclude that oral streptococci may adapt to each other and to the host, and interact among the community participants that maintain the stability of the microbial community composition. It has been observed that children harbour more than one distinct genotype of bacteria at different ages (Klein et al., 2004). Once established, bacterial species tend to persist in children’s oral cavities. For example, it has been reported that *mutans* streptococci have a fairly high degree of consistency in children aged 3–8 years, indicating persistence of the strains (Emanuelsson & Thornqvist, 2000). Theoretically, PCR-DGGE permits the detection of even single base-pair substitutions, and different genotypes within one species could produce distinct bands (Muyzer et al., 1993). Because no correlation between these clusters and the presence of S-ECC was observed, it is possible that these clusters relate to variables beyond the scope of this investigation; the inter-individual variability of the fingerprints may come from both the presence of multiple species and multiple genotypes within a single species.

In summary, our results showed that the development of S-ECC was accompanied by a complicated shift in multiple groups of oral streptococci. *S. sanguinis* and *S. gordonii* were significant factors associated with the caries status in children. High intra-individual similarities in the oral streptococci community were found at 8–32 months of age, regardless of caries status. The results of our study also demonstrated that the *rnpB* gene is an adequate target for *Streptococcus* discrimination. Future research focusing on the interactions among oral streptococci may provide valuable insights into the prevention of dental caries.

**CONCLUSION**

We concluded that the onset of S-ECC is accompanied by a reduced diversity of oral streptococci. The detection rates of *S. sanguinis* and *S. gordonii* had a negative correlation with S-ECC. There were high intra-individual similarities in the oral streptococci community over time.

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