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SHORT COMMUNICATION

Diversity and homogeneity of oral microbiota in healthy Korean pre-school children using pyrosequencing

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ABSTRACT

Objectives The purpose of this study was designed to identify the oral microbiota in healthy Korean pre-school children using pyrosequencing. **Materials and methods** Dental plaque samples were obtained form 10 caries-free pre-school children. The samples were analysed using pyrosequencing. **Results** The pyrosequencing analysis revealed that, at the phylum level, *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Fusobacteria* showed high abundance. Also, predominant genera were identified as core microbiome, such as *Streptococcus*, *Neisseria*, *Capnocytophaga*, *Haemophilus* and *Veilonella*. **Conclusions** The diversity and homogeneity was shown in the dental plaque microbiota in healthy Korean pre-school children.

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Introduction

Recent advances in microbial analysis, including high-throughput pyrosequencing, have enabled a better understanding of the complex oral microbiota. Compared to traditional sequencing techniques, pyrosequencing provides a larger number of readings, resulting in great depth of coverage and allowing detection not only of the most dominant microbial community members, but also of the low-abundance taxa.[1]

Most studies have focused on oral microbiota in children with early childhood caries (ECC) and suggested that dental plaque microbiota is closely correlated with ECC.[2] Current literature has revealed that *Streptococcus*, *Veillonella*, *Lactobacillus*, *Actinomyces*, *Prevotella* and *Granulicatella* are associated with progression of ECC.[1] However, oral microbiota in healthy pre-school children can have significance as a standard only a few studies have issued. The purpose of this pilot study was to identify the dental plaque microbiota in healthy pre-school children using the high-throughput pyrosequencing and define the oral microbiota in primary dentition of healthy children.

Materials and methods

Ten healthy children who were 5 year olds (five boys, five girls) were recruited as study subjects. Inclusion criteria were that children had no dental caries or had not received any treatment of dental caries and had not used systemic antibiotics within the previous 3 months. The children were instructed not to brush their teeth for 24 h before sample collection and not to eat or drink before the appointment. Dental plaque was collected from intact enamel surface. The samples were assayed using pyrosequencing, based on the V1–V3 hypervariable region of the 16S rRNA gene. Reads obtained from the samples were categorized by means of the unique barcodes of each PCR product and the taxonomic classification of each read was assigned against the EzTaxon-e database.[3] The data were analysed using the CLcommunity software (ChunLab Inc., Seoul, Korea).

Results

Pyrosequencing of the 10 samples finally yielded 52 268 reads and the average number of species-level OTUs per sample was 291.8, with a range of 91–416 species. By analysing the plaque microbiota of samples, nine phyla were found. Five phyla, including *Proteobacteria* (37.8%), *Firmicutes* (25.1%), *Bacteroidetes* (15.0%), *Actinobacteria* (13.4%) and *Fusobacteria* (8.0%), were predominant, while other phyla (SR1, *Spirochaetes*, GN02 and TM7) presented in relatively low proportions. However, the relative bacterial abundance for each predominant phylum differed between samples. At the genus level, 79 different genera were found. Ten genera were observed in all 10 samples, which comprised 77.3% of the total oral microbiota; including *Streptococcus* (14.9%), *Neisseria* (13.9%), *Capnocytophaga* (8.5%), *Lautropia* (8.2%), *Leptotrichia* (6.0%), *Corynebacterium* (6.0%), *Haemophilus* (5.8%), *Actinomyces* (5.4%), *Veillonella* (4.4%) and *Kingella* (4.2%). However, their relative abundance in each sample varied (Figure 1). The multidimensional UniFrac distance matrix was used to estimate similarity between samples.[4] The average of this index was high (0.061 ± 0.014, ranging from 0.036–0.089), suggesting the similarity between samples.

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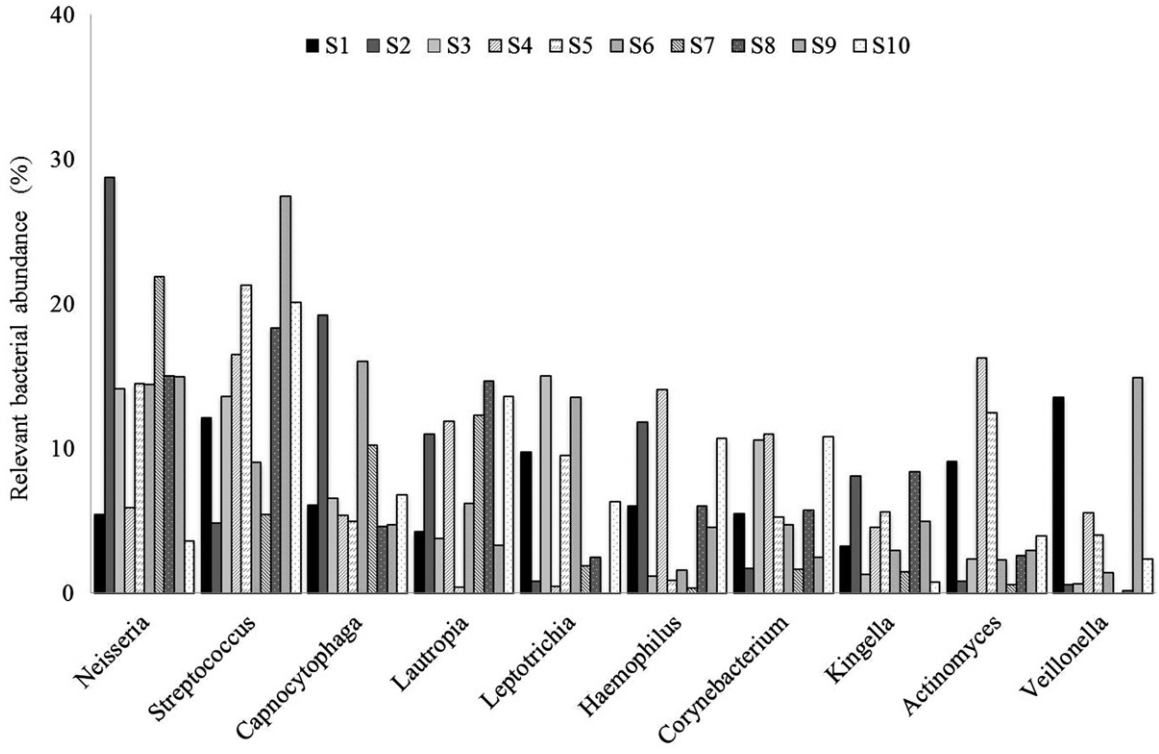


Figure 1. Relative bacterial abundance in each sample for each major genus.

Discussion

Comprehensive investigation of the composition of the oral microbiota is essential for a better understanding of the aetiology of dental caries.[5] Although there were some trials that investigated the oral bacterial communities, previous culture-dependent methods of microbial identification were insufficient for defining oral microbiota communities.[6] In the present study, a total of 52, 268 high-quality sequences were produced, demonstrating that deep coverage of pyrosequencing was enough to facilitate access to low abundance bacterial phylum. Relative bacterial abundance differed with individual variations both at the phylum and genus level. The obtained results indicate the diversity of oral microbiota in healthy Korean pre-school children.

At the phylum level, five phyla showed high abundance. These results are in agreement with those of previous studies investigating oral microbiome of healthy children.[7] Interestingly, similar patterns in distribution of phyla level were observed, in spite of differences in geography, race and age.

The composition of the microbiota is variable in different individuals. However, recent research revealed that there are similarities between oral microbiomes of individuals at the genus level and species level.[8] In this study, 10 predominant genera were present. These findings were consistent with the core microbiome of previous research that reported the spectrum of bacterial genus of healthy children.[8] It is supporting that there may be homogeneity of oral microbiome in healthy children.

In conclusion, diversity of the dental plaque microbiota was investigated by pyrosequencing and analysis of metagenomics. Within the limits of the present study, there may be a core microbiome in the dental plaque microbiota in healthy Korean pre-school children. We believe that understanding the oral microbiome of healthy pre-school children could lead to better prevention and treatment of ECC.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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