

# Analysis of Oral Microbial Dysbiosis Associated With Early Childhood Caries

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## Research article

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# Abstract

**Background:** "Core microbes" play a key role in the development of caries and lead to microflora disorders. Our goal was to detect the core microbes associated with the microbial flora imbalance in early childhood caries (ECC).

**Methods:** Fifteen caries-free children and fifteen high-caries (DMFT $\geq$ 10) children aged 4-6 years old were recruited according to the diagnostic criteria of caries suggested by the WHO. The 16S rRNA genes from plaque samples loaded in saliva were amplified by PCR, and the PCR products were sequenced by the Illumina Miseq platform. The sequencing results were analyzed by professional software to determine the composition and structure of the saliva microorganisms.

**Results:** There were statistically significant differences between the groups regarding the relative abundance of *S. mutans* (Wilcoxon rank-sum test,  $P < 0.05$ ). No significant difference was found between the groups regarding other species or functional genes.

**Conclusion:** Neither unique microbes leading to early caries in deciduous teeth nor characteristics of a microbial community with a disordered structure were found. Some microorganisms related to dental caries in young children were found, but whether these microbes were truly related or were false positives requires further verification.

## Background

Early Childhood Caries (ECC) is defined as one or more decayed (non-cavitated or cavitated lesions), missing or filled (due to caries) surfaces presenting in any deciduous tooth of a child under six years old [1]. The high prevalence of ECC among these young children around the world has a significant impact on children's health and social costs. However, surveys have made it clear that, except for the prevalence of ECC, it is largely untreated in children under the age of three [2]. The caries rate of deciduous teeth in China is 71.9% in 5-year-old children, and the DMFT is 4.24. The significant caries index (SIC) of these children was found to be 9.61 [3]. Severe early childhood caries can cause masticatory dysfunction, malocclusion, jaw dysplasia, temporomandibular joint disorder, facial aesthetic problems and abnormal pronunciation, which will have unpredictable impacts on children's lives. Therefore, the tasks of prevention and treatment of deciduous teeth caries are still arduous, and more research on the etiology of early childhood caries is urgently required.

According to the quadruple factors theory of caries, bacteria, food, host and time are all necessary conditions for caries occurrence. Dental caries have been defined as microbial dysbiosis [4]. Bacterial factors are important factors in caries etiology and bacteria depend on biofilm to cause caries. For the microbial factors of dental caries, it is generally accepted that microorganisms ferment carbohydrates in dental plaque to produce acid, which leads to the breakdown of the balance between demineralization and remineralization of enamel [5].

At present, *Streptococcus mutans*, *Lactobacillus*, *Actinomyces* and other microorganisms are associated with caries, which is also related to the individual's behaviors and caries resistance. However, no microorganism has been found to conform to Koch's postulates of pathogenic bacteria that can prove that caries is an

infectious disease caused by a single species. Currently, based on molecular biology methods such as denaturing gel gradient electrophoresis (DGGE) [6], human oral microbe identification microarray (HOMIM)[7] and 16S rRNA gene sequencing [8], no caries-specific bacteria have been found, but different caries-related bacteria species have been observed in different studies.

Research on the microbial etiology of dental caries has not been well developed, partly due to the complexity of the etiology of dental caries itself. It is estimated that dental plaque contains approximately 700 organic combinations and interacting bacterial communities, which greatly increases the difficulty of the research on the pathogenic bacteria of dental caries. The specific plaque hypothesis argues that specific bacterial species in the plaque, such as *Streptococcus mutans*, are the key pathogenic factors of caries [9], while according to the nonspecific plaque hypothesis, the acidic secretion activity of the entire microbial plaque community is increased, leading to enamel demineralization [9].

Regarding the relationship between dental caries and microorganisms, an increasing number of scholars agree with the ecological plaque hypothesis [10, 11], which postulates that the occurrence of dental caries is the result not of the activity of a specific microorganism but rather of external factors leading to the destruction of the microecological balance in plaque biofilm and to the disorder of the overall microbial community structure, resulting in the occurrence and development of dental caries. Therefore, it is necessary to study the composition and structure of the entire microbial community and to obtain comprehensive information on the microorganisms in the plaque biofilm. With the development of metagenomic technology and DNA sequencing technology, it is now possible to directly analyze the species composition and functional genes in the community by obtaining the microbial genome of the whole community [12].

Studies on the microflora associated with early childhood caries suggest that the microecological imbalance leading to caries in children may be caused by multiple microorganisms, which differs from the suggestion that the pathogenic microorganisms, obtained through traditional culture methods, are *Streptococcus mutans*, *Lactobacillus* and *Actinomyces*. Based on the existing literature on caries disease and early childhood caries and on some clinical studies of caries-susceptible children, we proposed the hypothesis that there are some "core microbes" associated with caries in dental plaque and that these "core microbes" play a key role in the development of the occurrence of dental decay and determine the microflora disorders.

In this study, in order to analyze the salivary microbial composition and structure, we compared the plaque samples loaded in saliva from children with no caries and children with ECC using 16S rRNA gene sequencing technology. Our aim was to detect pathogenic bacteria that were related to the microbial dysbiosis of ECC, providing a theoretical basis for caries prevention and therapy.

## Methods

### 1. Participants recruiting

Fifteen caries-free children were recruited in control group (group C) and 15 high-caries children in disease group (group D) for this project. Their basic information (comprehending sex, age, feeding habits and oral hygiene) and their caries situation were recorded. Informed consent was obtained from the children's parents for this research. The inclusion criteria for the children with high caries (DMFT  $\geq$  10) were based on the

diagnostic criteria of caries formulated by the WHO (Oral Health Surveys: Basic Methods (fourth edition)). The inclusion criteria were as follows: 1) evidence of caries present (a lesion in a pit or fissure or on a smooth tooth surface, an unmistakable cavity, undermined enamel, detectably softened floor or wall, a tooth with a temporary filling or one that was sealed but also decayed, including white spot lesions and dental caries losses); 2) children were between 4 and 6 years old, with primary teeth that have erupted completely and permanent teeth that have not yet erupted.

The exclusion criteria for all children were as follows: 1) There was evidence of other oral diseases, family hereditary diseases, systemic diseases, long-term medication history or use of antibiotics in the past three months. 2) Those who with first permanent molars and/or permanent incisors erupted. Fifteen children classified as healthy (dmft = 0) were included in the control group according to the exclusion criteria.

The project was approved by the Ethics Committee of the Stomatological Hospital Affiliated with Fujian Medical University (committee's reference number: 2017–30).

## **1. Collection of plaque and salivary mixture specimens**

The dental plaque of the children was sampled 0.5 to 3 hours after breakfast without brushing teeth. Oral examination was performed by an experienced dentist in the form of a visual examination combined with exploratory probing. When sampling, the researchers let the children brush their teeth without gargling by an aseptic toothbrush and spit their saliva into an aseptic paper cup; the cup contents were then poured it into aseptic 50 mL centrifugal tube, transferred to the laboratory in an ice box and stored in a freezer at  $-80^{\circ}\text{C}$ .

## **3. Sample processing and DNA extraction**

The plaque and saliva mixtures were thawed in the centrifugal tube. A bacterial genomic DNA extraction kit (OMEGA) was used to extract the bacterial genomic DNA from the samples. The extraction procedure was carried out according to the instructions of the kit.

## **4. DNA library construction and sequencing**

The DNA solution of all samples was examined by 1% agarose gel electrophoresis. A Qubit fluorometer was used for DNA quantitation and quality inspections. The DNA samples were stored at  $-20^{\circ}\text{C}$  after extraction until further processing. Universal primers for the bacterial 16S rRNA gene were used to amplify the bacterial 16S rRNA gene v3-v4 variable regions (338F: 5'-ACTCCTACGGGAGGCAGCAG-3', 806R: 5'-GACTACHVGGGTWTCTAAT-3'). The primers contained a tag sequence of 8 nt (Barcode) for distinguishing each sample. Each sample underwent three PCRs, with each 25  $\mu\text{L}$  PCR containing 10  $\mu\text{L}$  Pyrobest buffer, 2  $\mu\text{L}$  dNTPs (2.5 mM), 1  $\mu\text{L}$  primer (10  $\mu\text{M}$ ), 0.4 U Pyrobest DNA polymerase, and 15 ng genomic DNA (template DNA). The PCR conditions used included denaturation at  $95^{\circ}\text{C}$  for 5 min, followed by 25 cycles of denaturation at  $95^{\circ}\text{C}$  for 30 s, annealing at  $55^{\circ}\text{C}$  for 30 s, extension at  $72^{\circ}\text{C}$  for 30 s, and a final extension at  $72^{\circ}\text{C}$  for 10 min. The PCR products were used for subsequent Miseq sequencing (Illumina Inc., CA, USA). The PCR products were electrophoresed in a 2% agarose gel, followed by use of the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, CA, USA) according to the product manuals. The 16S rDNA library with barcodes was acquired after extension. QuantiFluor™-ST (Promega, Madison, USA) was used to quantify the DNA. The DNA of each sample was mixed in equal amounts and the DNAs were sequenced on the Illumina Miseq

platform according to the standard operating procedure (2 × 300 bp paired-end). Quality control of the DNA, construction of the 16S rRNA gene fragment DNA library, and DNA sequencing were all performed by the sequencing company (Allwegene, Beijing, China).

## 5. Data analysis of the sequencing results

(1) The original data (\*.fastq file) were split according to the specific label sequence (barcode) of the sample, and the FastQC software was used to check whether the quality and quantity of the original data met the basic requirements of the analysis;

(2) A trimmingReads.PL script in ngs-qc-toolkits was used to remove the 30 bp sequence at the 3' end of read2 and to retain the high-quality 270 bp at the 5' end;

(3) An IlluQC.PL script in ngs-qc-toolkits was used to screen out sequences whose quality Q20 was less than 90% (parameter: -l 90-s 20);

(4) Paired read1 and read2 were processed in the Mothur pipeline and first merged using Mothur > make.contigs, with default parameters. Merged sequences with a length > 489 bp were discarded. Follow-up analysis was performed according to the analytical procedures of Mothur. The SILVA database was selected for alignment and taxonomic annotation. Chimera removal was not performed, and 80% for the taxonomic annotation cutoff was selected. A cluster.split method was used to cluster sequences into OTUs. This resulted ultimately in a \*. biom file;

(5) QIIME 1.8 was used to further analyze the \*. biom file. We removed the low abundance OTUs in the \*.biom file (total count 2 in all samples) and generated the relative abundance table from the phylum to the genus level;

(6) In QIIME 1.8, a certain sequence number (n = 18600) was randomly selected to calculate the alpha diversity of all samples (Chao1 index and Shannon index);

(7) The Bray-Curtis distance was calculated between samples (Qiime > beta\_diversity.Py -m bray\_curtis). At the same time, based on the Bray-Curtis distance matrix, the PCoA visualization of the distance between the samples was carried out with R language.

## 6. Specific details on the real-time PCR assays

Universal primers targeted to total bacteria and specific primers targeted to *Streptococcus mutans*, *Lactobacillus* and *S. sobrinus* were chosen to determine the bacterial load by real-time PCR (qPCR). Functional genes, including the *ldh* gene and the F-ATPase gene, were also quantified by real-time PCR (qPCR). The primers are listed in Table 1. The samples were tested in triplicate. Each PCR was performed in a total volume of 20 µl, which contained 10 µl of Fast Power SYBR green PCR Master Mix, 500 nM of each primer pair, 1 µl DNA template and PCR water. The thermocycler conditions included a denaturation step of 95 °C (2 min) and 40 cycles of 95 °C (15 s) and 60 °C (1 min). All samples were amplified in triplicate, and the mean value of the targeted molecule was used for analysis. We used the total (universal) bacterial 16S rRNA gene as the reference gene to calculate the relative abundance of the specific 16S rRNA genes of the target

bacteria and the relative expression level of the functional genes. For total bacteria determination, the DNA templates were diluted 100-fold, and for the functional gene determination, the templates were diluted 10-fold. The undiluted DNA solutions were used as the DNA templates in each PCR. The relative abundance of the specific taxa was simply defined as the relative abundance of the detected gene sequences mapped to the reference genome. The relative abundance of specific taxa or functional genes was simply defined as the ratio of DNA fragments detected to the total bacteria detected via qPCR. The Ct values of the target genes (Ct target) minus the Ct values of the total bacterial gene (Ct total-bacteria) were used to determine the  $\Delta Ct$  values. The values of  $2^{-\Delta Ct}$  were defined as the relative abundance of the specific taxa or genes.

Table 1  
Primers used in qPCR

	Forword Primer	Reverse Primer	Amplicon size
Total bacteria	5'-TCCTACGGGAGGCAGCAGT - 3'	5'-GGACTACCAGGGTATCTAATCCTGTT-3'	466 bp
S. mutans	5'-GCCTACAGCTCAGAGATGCTATTCT-3'	5'-GCCATACACCACTCATGAATTGA-3'	98 bp
Lactobacillu	5'-TGGAACAGGTGCTAATACCG-3'	5'-GTCCATTGTGGAAGATTCCC-3'	231 bp
S.sobrinus	5'-TTCAAAGCCAAGACCAAGCTAGT-3'	5'-CCAGCCTGAGATTCAGCTTGT-3'	88 bp
ldh gene	5'-CTTGATACTGCTCGTTTTCCGTC-3'	5'-GAGTCACCATGTTCACCCAT-3'	93 bp
F-ATPase gene	5'-CGGATGCGTGTTGCTCTTACTG-3'	5'-GGCTGATAACCAACGGCTGATG-3'	167 bp

## 7. Statistical analysis

Alpha diversity among the different groups was compared by an independent sample t test. The comparison of the relative abundance of the bacterial species used the Wilcoxon rank-sum test. Multiple comparisons were compared by the Bonferroni test correction. The data in this paper are expressed as the mean + standard deviation. There was statistical significance if the P Value was less than 0.05.

## Results

After quality inspection, trimming, filtering, splicing and refiltering of the raw sequencing data, 4 samples were excluded, due to the number of sequences in a single sample of less than 5000 (including 2 samples from the caries-free group and 2 samples from the high-caries group). Thirteen samples from the control group (group C) and 13 samples from the disease group (group D) were obtained. A total of 683,709 sequences were obtained, with sample C27 showing the largest number (38,055) of sequences and sample D33 showing the smallest number (18,657) of sequences, with a median sequence number of 25,816.

## Alpha diversity analysis based on OTUs

With the minimum sequences, sample D33 (18,657 sequences) was defined as the threshold for sequence re-sampling, 18,600 sequences were randomly selected from all samples (10 times for each sample) to calculate the alpha diversity. The Chao1 index (indicating flora abundance) and Shannon index (indicating flora diversity) of group C and group D were not significantly different ( $P > 0.05$ , Fig. 1).

## **Bacterial community structure characteristics based on the Bray-Curtis distance**

We converted the OTU relative abundance table into a relative abundance table of taxonomic level from phylum to genus. We calculated the Bray-Curtis distance between the samples based on the relative abundance table of all taxonomic levels, after which PCoA visualization was performed (Fig. 2). The samples from group C and group D could not be separated significantly in principal composition 1 or principal component 2. We further analyzed whether there were significant differences in the taxonomic composition between the two groups based on the Bray-Curtis distance matrix, and no significant differences were found (permutation 999 times, p value of adonis was 0.272, p value of anosim was 0.368). According to this result, we found that we cannot distinguish the children with caries from the children without caries based on the bacterial community structure above the species level.

## **Analysis of bacterial composition of the control group and caries group**

We generated the relative abundance table of taxa at each level (phylum, class, order, family and genus) from the OTU relative abundance table, and the relative abundance of taxa at the genus level is shown in Fig. 3. *Streptococcus* (23.8%), *Neisseria* (8.1%), *Prevotella* (6.2%), *Actinomyces* (5.6%) and *Leptotrichia* (5.4%) were found to be of the highest relative abundance. Based on our detection level and the current information of the 16S rRNA gene database, no specific taxa were found in the ECC group in this study. The relative abundance of the taxa at the different levels was compared. After multiple comparison corrections ( $P < 0.05$  as the boundary), no taxa with significant differences were found at any taxa levels. However, we found some genera that may be associated with caries, but those differentially present genera were all of low abundance (Table 2).

Table 2  
The difference of relative abundance of genera between control group and caries group

Genus	Wilcoxon rank-sum Test P Value	C group (Health)		D group (ECC)	
		Mean value	s.d.	Mean value	s.d.
Bifidobacterium	0.001235	0.000834	0.002135	0.005777	0.015335
Proteiniphilum	0.010454	0.000321	0.000407	9.35E-05	0.000267
Shuttleworthia	0.025254	5.17E-05	6.01E-05	0.00027	0.000528
Legionella	0.037349	0	0	2.04E-05	4.11E-05
Hespellia	0.037349	2.15E-05	4.97E-05	0	0
Anaerospobacter	0.037481	0.000307	0.000305	0.000119	0.000164
Dysgonomonas	0.041659	0.000135	0.000179	2.42E-05	5.95E-05
Caldicoprobacter	0.043438	8.91E-05	9.75E-05	2.86E-05	7.6E-05
<b>s.d.: Standard deviation</b>					

## Description of qPCR results

The relative abundance of bacteria and expression of genes were calculated with the total number of bacteria as the internal parameter. There were statistically significant differences between the groups regarding the relative abundance of *S. mutans* (Wilcoxon rank-sum test,  $P < 0.05$ ). No significant difference was found between the groups regarding other species (Fig. 4). No significant differences were found in functional genes (Fig. 5). The *S. mutans* of group C was maintained at a low level, with an average value of  $3.78 \times 10^{-6}$  (with a range from  $1.46 \times 10^{-10}$  to  $2.24 \times 10^{-5}$ ), while the relative abundance of *S. mutans* in group D varied, with a mean value of  $8.89 \times 10^{-3}$  (with a range from  $2.72 \times 10^{-10}$  to  $4.30 \times 10^{-2}$ ).

## Discussion

For decades, acid-producing bacteria, represented by *Streptococcus mutans*, have been considered to be the main pathogenic bacteria of caries. The characteristics of extracellular polysaccharide production, acid production and acid tolerance support the role of *S. mutans* as a potential cariogenic bacteria, and most preventive measures and risk assessment methods for caries have been targeted at *S. mutans* [13]. Recently, a metagenomic method was used to study caries disease-related microbes, which found that *S. mutans* in plaque biofilm was not the dominant bacteria and that its proportion in the whole flora was very low. In some studies, *S. mutans* often cannot be detected, even by using metagenomic detection methods [14, 15] We sequenced saliva samples from two groups of children, and there was no differential presence between the two groups in *S. mutans* found in the saliva. This does not mean that *S. mutans* is unimportant. The possible explanation is that *S. mutans* plays a role in the initial stage of caries formation (demineralization period) and, at a low abundance, also may play a role as a key pathogenic bacteria in the bacterial community. After caries formation, the local niche may be dominated by other bacteria, so that it is difficult to detect the *S. mutans* in the caries cavity. The keystone pathogen theory of periodontists holds that those bacteria that

play a large role in the community but are of low abundance are considered to be the "keystone" members in the microbial ecology. Members of the microbiome with a low abundance may still be key species for complex community behavior [16]. *S. mutans* may be the "keystone" member of caries pathogens.

Some scholars have found that *Streptococcus mutans* can be detected in the caries, such as in the results of the study by Aas *et al.* using 16S rRNA sequencing to detect dental plaque. Aas *et al.* [17] found that *S. mutans* could not be detected in the plaque from healthy enamel and white plaque (demineralized surface of enamel) but could be found in most of the deep dentin plaque in caries cavity lesions. Research sample sources, sampling sites, sampling methods and research methods vary greatly, resulting in different abundances and detection rates of *Streptococcus mutans* in different studies. In this study, through qPCR detection, we found that the relative abundance of *S. mutans* in the saliva of children with early caries was significantly higher than that of children without caries, which was consistent with the previous results based on qPCR detection [18].

However, an increasing amount of evidence supports the idea that *S. mutans* is not the single causative factor of caries [19, 20]. In addition to *S. mutans*, bacteria of other genera, including *Lactobacillus*, *Actinomyces*, *Bifidobacterium*, *Veronococcus*, *Propionibacterium*, etc., have members that are believed to be involved in the occurrence and development of caries [17, 21]. Some bacteria, such as *Veronococcus*, have been found to be maintained in a high relative abundance at all stages of caries sites [17], and *Veronococcus* can participate in acid production at high glucose levels [22]. Interestingly, *Veronococcus* bacteria can synergize with *S. mutans*, and the coculture of *Veillonella alcalescens* and *S. mutans* can produce more acid than any single bacteria [23] suggesting that both may play a key role in cariogenic disorders in the microbial community. Although this study did not find other microorganisms that might play a synergistic role with *S. mutans*, we still believe that dental caries is a multipathogen disease, and some core pathogens, including *Streptococcus mutans*, play a prominent role in the occurrence and development of dental caries.

Our hypothesis is based on the "core-pathogen complex" hypothesis. The oral cavity is home to hundreds of bacterial species, most of which are symbiotic and need to maintain balance with the oral ecosystem. Dental caries are caused or maintained by resident or temporary microorganisms, which are usually small in number and do not cause disease, but infection can occur in certain circumstances [24]. Some of the bacteria in the microbiome are the core pathogenic bacteria, which play key roles, while the rest are ordinary members or transient bacteria that do not play a key role in the microbiome. When all or some of the core pathogenic bacteria in the microbiome act together, the whole microbiome structure can be disturbed and progress towards the pathogenic state. Takahashi *et al.* [25] reported that the cariogenic microecosystem is a microenvironment that changes from a dynamic stability stage (maintaining dynamic stability on the tooth surface) to an acidogenic stage (increased proportion of acid-producing bacteria) and finally to an aciduric stage (promoting caries formation). We hypothesized that *Streptococcus mutans*, together with other core pathogens, may be the key bacteria for regulating the microecological balance of caries. Yang *et al.* [26] analyzed the saliva samples of caries-free and carious participants and found that it was difficult to generalize the caries-related "core microbiome" from the taxa level, but that the caries group had "core functional genes" at the gene level. Josh *et al.* [27] supported the idea that caries is a metabolic disorder of the microbial community rather than a change in species abundance. Due to the presence of homologous

genes and horizontal gene transfer, different species of microorganisms may play similar functions in the host, so it is necessary to simultaneously analyze the species and genetic composition of caries-related microorganisms. We used a qPCR method to quantitatively identify the genes related to bacterial metabolism related to caries, such as the *ldh* gene and the F-ATPase gene. However, no significant differences in the functional genes were found between the two groups, which may be because the expression level of the bacterial genes was more important than the presence of the functional genes, and thus the detection of the metatranscriptome may be more important.

The qPCR quantitative method can only be used to quantify a limited number of microbial species of interest in the community. Research on a single or limited species of bacteria can only be carried out selectively, which often fails to give a more comprehensive description of the overall community structure. High-throughput sequencing of the bacterial 16S rRNA gene fragment amplified by PCR (16S rRNA gene sequencing) based on a nonculture method is a popular method for the study of the bacterial community, providing a simple and easy method for the species composition and structure of the entire bacterial community. However, at present, this approach is faced with several problems, such as the short lengths of the second-generation sequencing of the DNA fragment (which has insufficient analytic power for species), PCR amplification bias and chimera formation, too many variables leading to false positive statistical results, false negatives after multiple test corrections, and unknown sequences that do not match any known database. We did not find significant differences between the two groups with the results of 16S rRNA gene sequencing, which may be caused by the limited accuracy of fragment sequencing analysis of the 16S rRNA gene. The advantage of the "shotgun" metagenomic research method is that it can determine not only the species composition and relative abundance of the microbial community but also the microbial gene content and functional gene diversity. Moreover, the "shotgun" metagenomic method can obtain information at the bacterial strain level with a sufficient sequencing depth [28]. In this study, the *ldh* gene and the F-ATPase gene of bacteria that were related to caries formation were chosen for qPCR, and no differences were found. The differences in the genes need to be further studied through a shotgun metagenomic study with a large number of samples. However, from a single cross-sectional analysis and limited amount of samples, this study cannot determine whether variation across individuals represents a pattern of microbe interacts with human host. Further research should be undertaken to clarify the mechanisms that the inter-ration between microbial members and the association between microbial dysbiosis and human diseases.

## Conclusion

1. No unique microorganism of early caries of deciduous teeth was found.
2. no characteristic of disorder of microbial community structure was found (using PCoA), based on the relative abundance of species composition.
3. There are some microorganisms found in our study related to early childhood caries. Whether these microbes are truly related or false-positive requires further verification.
4. The abundance of *ldh* gene and F-ATPase gene were found to be no differences between the two groups. The differences of functional genes needs to be further study by shotgun metagenomic study.

# Abbreviations

ECC

Early childhood caries; DMFT:Decayed, missing, and filled teeth; WHO:World Health Organization; SIC:Significant caries index; DGGE:Denaturing gel gradient electrophoresis; HOMIM:human oral microbe identification microarray; OTUs:Operational taxonomical units.

# Declarations

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## Availability of data and materials

The dataset used and/or analyzed during the present study available from the corresponding author on reasonable request.

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### **Contribution**

Hui Zheng contributed to collecting the clinical data, drafting and writing the manuscript. Tengfei Xie, Shaokai Li and Xiaotong Qiao contributed to the sample processing and data analysis. Yan Feng and Youguang Lu made substantial contributions to the conception, design, data analysis and interpretation and revised the manuscript. All authors approved the final manuscript.

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### **Ethics approval and consent to participate**

Written informed consent was obtained from the children's parents for this research. The project was approved by the Ethics Committee of the Stomatological Hospital Affiliated with Fujian Medical University (committee's reference number: 2017–30).

### **Consent for publication**

Not applicable.

### **Competing interests**

The authors declare that they have no competing interests.

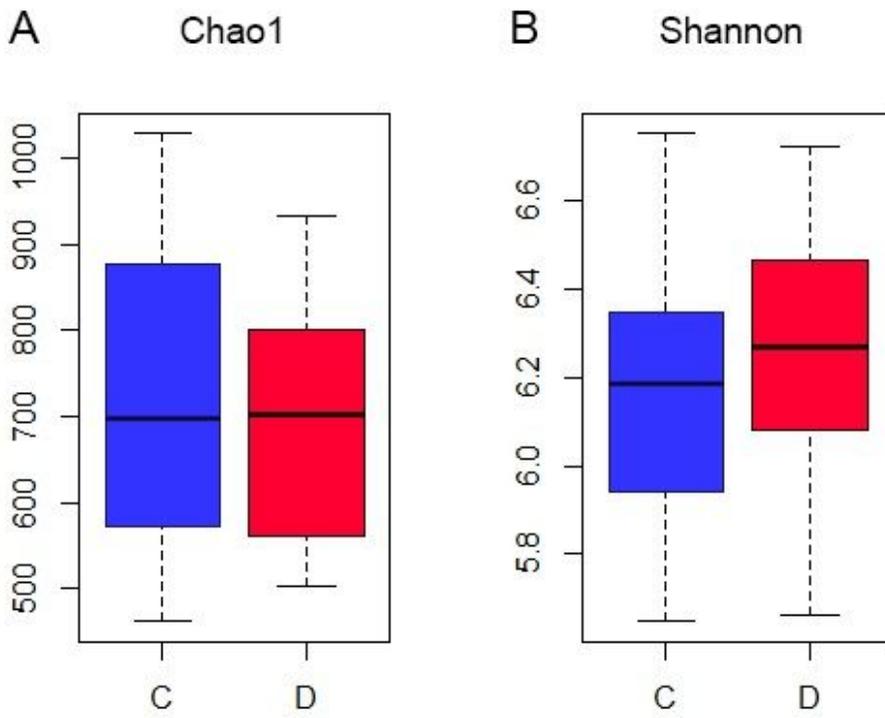
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## Figures



**Figure 1**

Comparison of microbial richness and diversity C: Control group/no caries group; D: Disease group/high caries group

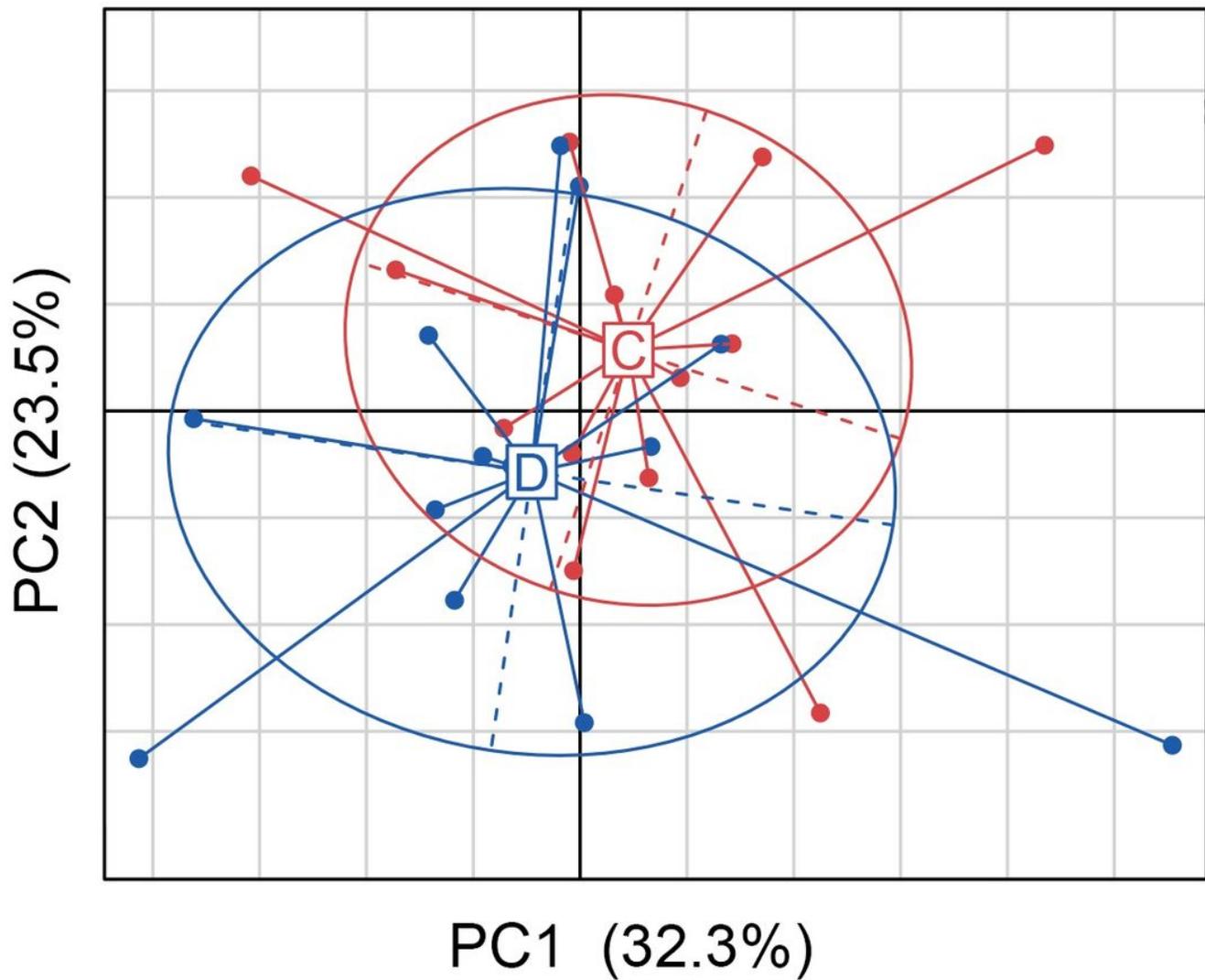
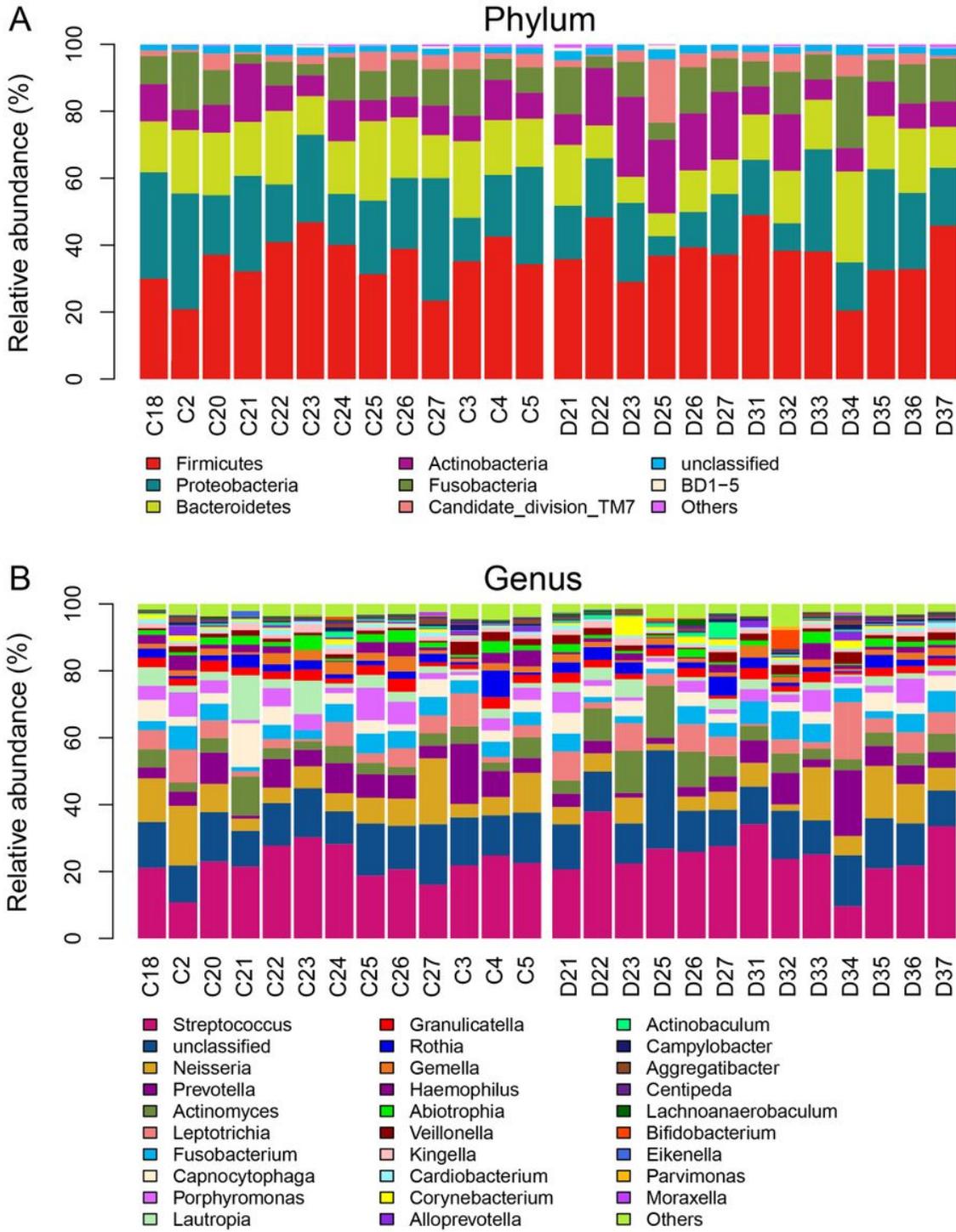


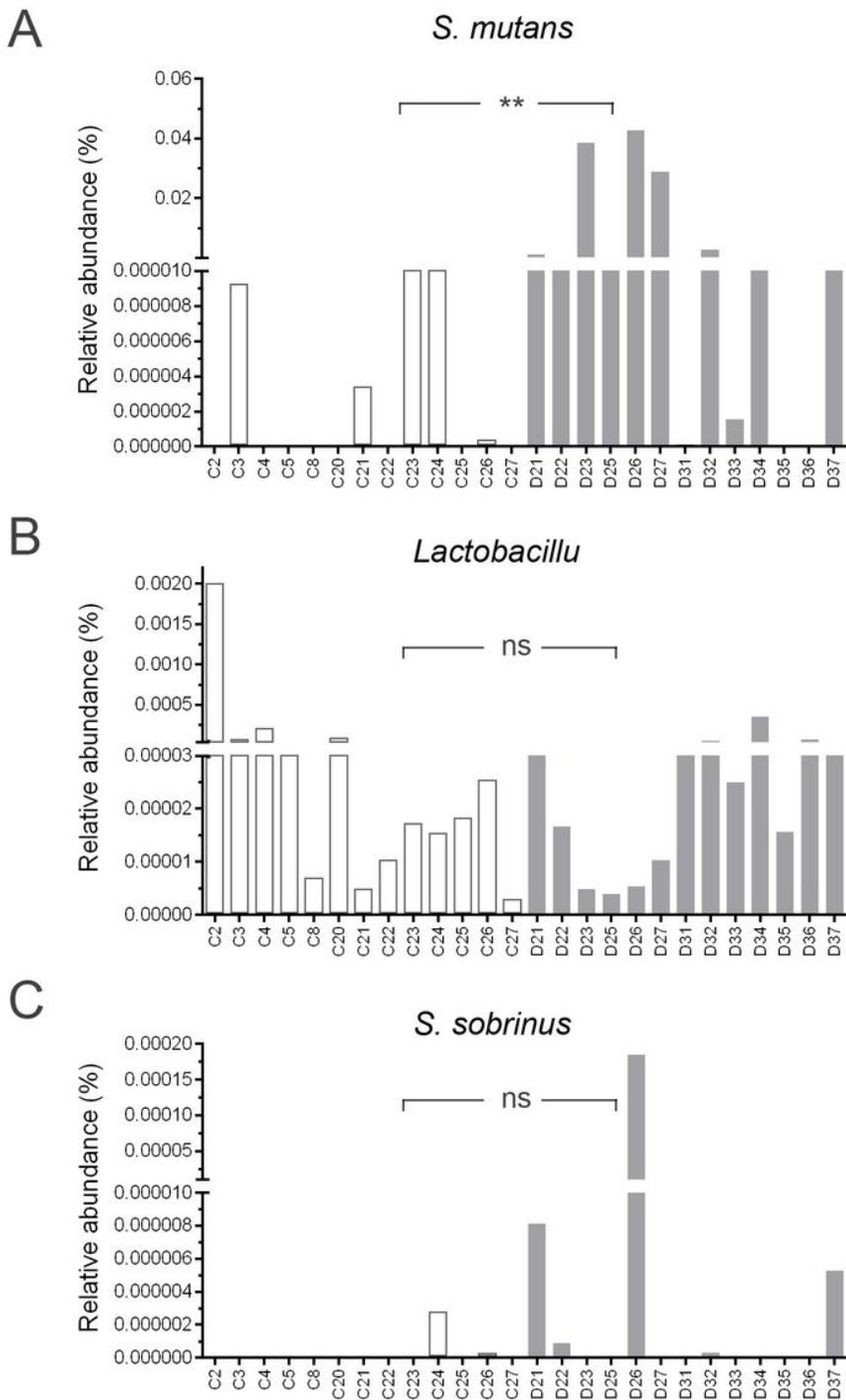
Figure 2

Comparison of microbial community structure between no caries group (C) and high caries group (D). PCoA visualization was performed according to bray-Curtis distance matrix between samples based on the relative abundance table of all taxonomic levels.



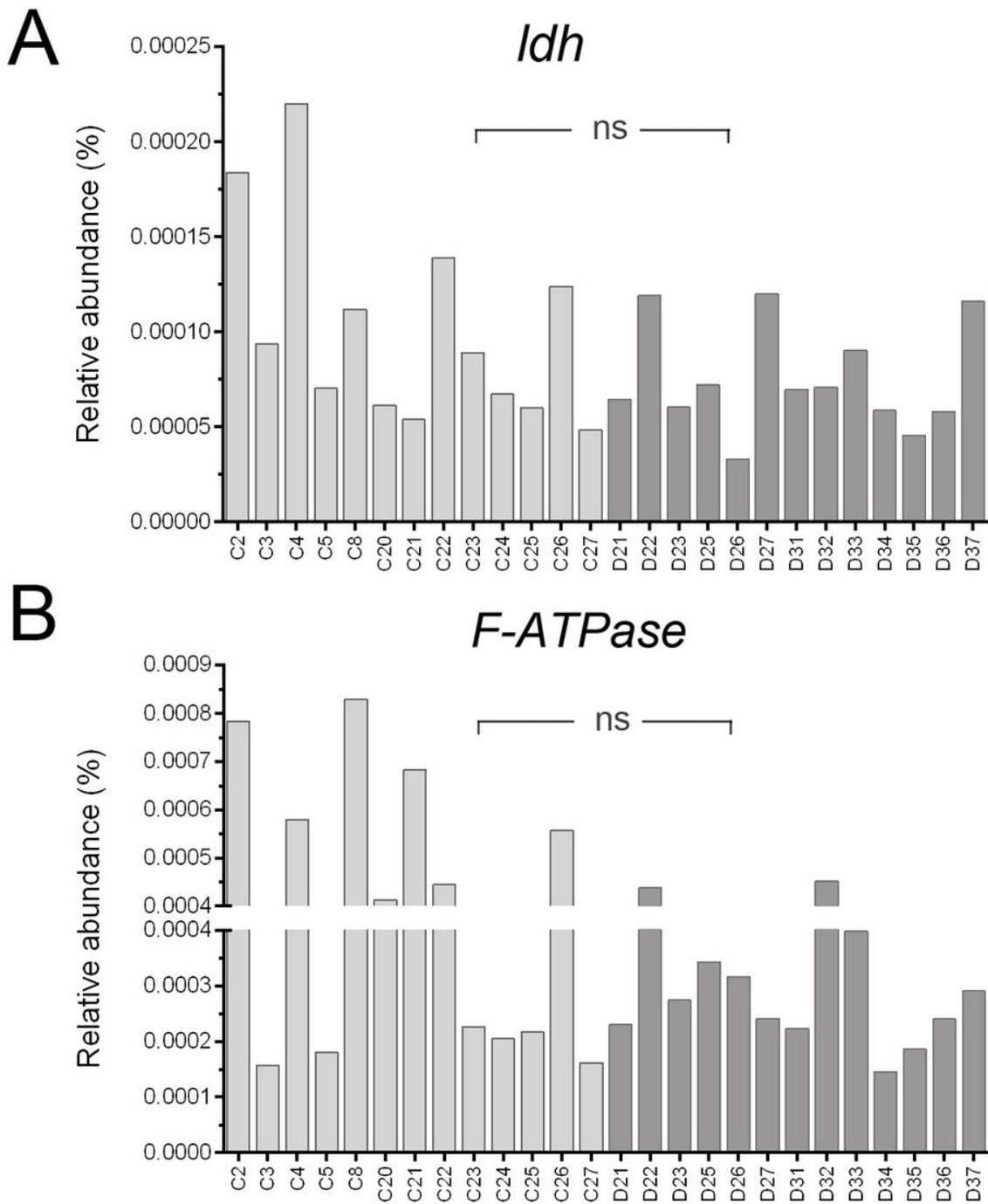
**Figure 3**

The predominant phylum and genus of the all samples.



**Figure 4**

Comparison of the relative abundance of *S. mutans*, *Lactobacillus* and *S. sobrinus*. The relative abundance were calculated based on qPCR results. *S. mutans* differing in terms of relative abundance between no caries group (C) and high caries group (D) (Wilcoxon rank-sum test,  $P < 0.05$ ). \*\*:  $P < 0.05$ , ns: not significant.



**Figure 5**

Comparison of the relative abundance of functional genes. The relative abundance were calculated based on qPCR results. ns: not significant.