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ORIGINAL RESEARCH

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Oral microbiome characteristics in children with and without early childhood caries

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Abstract

Objective: Early childhood caries (ECC) negatively affects children's growth due to its close relation to an imbalance of the oral microbiota. This study aimed to evaluate the distribution of the oral microbiota in children with ECC and healthy individuals. Methods: The oral microbiota of 20 children with dental caries from both carious teeth (CC cohort) and healthy teeth (CH cohort), and the oral microbiota of 20 healthy control children (HH cohort) were subjected to 16S rDNA sequencing. Results: The results revealed significant differences between the microbial structure of the CC and CH cohorts of every child with ECC. The most common microbes were *Streptococcus*, *Neisseria*, *Leptotrichia*, *Lautropia* and *Haemophilus*. Specifically, the CC cohort contained *Lactobacillus*, *Veillonella*, and *Prevotella 7*, the CH cohort contained *Actinomyces*, *Bifidobacterium* and *Abiotrophia*, and the HH cohort mainly contained *Neisseria*, *Leptotrichia*, *Porphyromonas* and *Gemella*. Lastly, we established a random forest model consisting of 10 genera (*Prevotella 7*, *Actinobacillus*, *etc.*) which demonstrated promising clinical diagnostic ability (area under the curve (AUC) = 89.8%). These findings indicate that oral microbiota can potentially be used as therapeutic targets or diagnostic markers for the early prediction and prevention of caries in children.

Keywords

Early childhood caries; Oral microbiota; 16S rDNA sequencing; Randomforest model; Biomarker

1. Introduction

Dental caries is a chronic progressive disease mainly caused by bacteria and is the most prevalent oral disease in humans [1]. The prevalence of early childhood caries (ECC) is approximately 530 million, according to the world health organization (WHO) report [2]. ECC is defined as the presence of one or more decayed, missing or filled tooth surfaces in any primary tooth in a child under 6 years old, with irreversible damage to the teeth. Once started, the child usually suffers from a higher risk of new lesions or even tooth loss over their entire lifespan [3].

The oral microbiota is the second most complex community and is important for human health [4, 5]. Some studies revealed that oral microbiota is associated with several oral diseases [6, 7] and suggested that it could be regarded as a biomarker to predict certain diseases [8, 9]. These findings indicate that the analysis of the oral microbiota might be advantageous in future clinical diagnosis of diseases.

Although previous studies explored the oral microbiota characteristics of ECC [10–12], these studies only compared the oral microbiota between patients with ECC and healthy individuals. As a result, only the results of past disease progression could be confirmed and the microbiota distribution of healthy sites in individuals with ECC remains uninvestigated. Since there are changes in the oral microbiota during the transformation of healthy primary teeth into cavities, it is important to investigate the spatial differences in the microbiota in the same child with ECC to distinguish the differences between healthy teeth states.

In this study, to evaluate the distribution of the oral microbiota in children with ECC and healthy individuals, the oral microbiota from carious teeth (CC cohort) and healthy teeth (CH cohort) in 20 children with dental caries were subjected to 16S rDNA (16S ribosomal DNA) sequencing. The same sequencing was also performed on the oral microbiota (HH cohort) from a control group comprising 20 healthy children.

2. Materials and methods

2.1 Cohort enrollment and volunteer clinical information

Forty children aged 3–6 years (20 children with ECC and 20 healthy children) were recruited for this study after obtaining permission and informed consent from their guardians. The severity of primary caries of ECC subjects was 5–6 according

TABLE 1. Patients' characteristics.

Variable	Healthy (n = 20)	ECC (n = 20)	*p*-value
Boy	10	12	
Girl	10	8	
Age, mean ± SD (yr)	5.63 ± 1.50	5.11 ± 0.87	0.38
Height, mean ± SD (cm)	106.42 ± 12.89	110.75 ± 10.65	0.17
Weight, mean ± SD (kg)	18.68 ± 7.40	18.56 ± 7.23	0.23
BMI	15.38 ± 0.94	16.31 ± 0.86	0.38
Dmfts, mean ± SD	0	10.35 ± 2.62	
Frequency of brushing teeth			
≥2 times/day	12	18	
≤1 times/day	8	2	
Times of eating dessert			
≥2 times/week	17	17	
≤1 times/week	3	3	
The only-child			
Yes	8	9	
No	12	11	

ECC: early childhood caries; SD: standard deviation; BMI: Body Mass Index.

to the International Dental Caries Detection and Assessment System (ICDAS-II) and the numbers of decayed, missing, and filled teeth (DMFT) of the subjects were determined by an experienced dental surgeon [13]. Children with mixed dentition were excluded from the study group. The inclusion criteria were as follows: (I) no antibiotics used within 1 month; (II) no fluoride toothpaste used and fluoride treatment within 1 month; (III) the children had no other diseases except for caries; and (IV) DMFT ≥6 in children with ECC, and DMFT = 0 in the healthy controls [14]. The clinical data of the volunteers are shown in Table 1.

2.2 Sample collection

The subjects were told not to eat or drink 2 h before sample collection. The plaques were collected from the gap of the first and second primary molars of healthy controls using dental excavators (Shanghai Kangqiao Dental Instruments Factory, China) by a dental surgeon and were assigned to the HH cohort. For each ECC patient, plaques were collected mainly from two sites. One site was the gap of the first and second primary molars (without caries), and the other was from the cavitated site of carious lesions (the CH and CC cohorts, respectively). The samples were transferred into a labeled sterile centrifuge tube (Maisinuo, China) containing 1 mL sterile phosphate-buffered saline (PBS), then transported to a laboratory refrigerator at −80°C for freezing within 2 h since sampling. The samples were kept on ice during the transfer process to avoid contamination.

2.3 DNA extraction PCR and Illumina sequencing

The genomic DNA of dental plaque samples was extracted using the Fast DNA^{TM} Spin Kit for Soil (16560200, MP, CA, USA) following the manufacturer's instructions. Subsequently, the V3–V4 region (forward primer, 5′-CCTACGGGNGGCWGCAG-3′; reverse primer, 5′-GGACTACHVGGGTATCTAAT-3′) of the 16S rDNA gene fragments were amplified [15, 16]. The 50 μL reaction system contained 25 μL Premix Taq^{TM}plus dye (TaKaRa, China), 0.5 μL forward primer, 0.5 μL reverse primer, 2 μL DNA template, and 22 μL ddH_2O. The amplification conditions were as follows: 95 ℃ for 5 min; 40 cycles of 95 ℃ for 30 s, 52 ℃ for 30 s, 72 ℃ for 30 s, and maintained at 12 ℃ for 10 min.

The PCR products were purified after amplification using the QIAquick PCR Purification Kit (28106, Qiagen, Germantown, USA), and paired-end sequencing was performed on the Illumina Miseq platform [17].

After sequencing, Quantitative Insights into Microbial Ecology 2 (QIIME2, Boulder, CO, USA) was used to analyze the high-quality sequences.

2.4 Microbial diversity assessment

Microbial diversity, including alpha-diversity and beta-diversity, was analyzed using QIIME2. Alpha diversity was evaluated using Shannon and Simpson indices [18]. Beta-diversity was determined based on Bray-Curtis dissimilarity and unweighted-UniFrac dissimilarity [19]. Microbial community clustering was arrayed using the principal coordinate analysis (PCoA), followed by visualization with the R (version 3.6.0, Ross Ihaka & Robert Gentleman,

Oakland, New Zealand) "ggplot" package.

2.5 Linear discriminant analysis effect size (LEfSe) analysis

The LEfSe analysis (https://huttenhower.sph.harvard.edu/galaxy/) was used to identify the taxa and functions of the CC, CH, and HH cohorts [20]. The Kruskal-Wallis test was applied, and the *p*-value was set at 0.05. For taxa differences, the LDA (linear discriminant analysis) score was set at 3.5. As a supervised learning method, LDA analysis can distinguish taxa and functions to the maximum extent [21]. The analysis was performed using the R "mass" package.

2.6 Classification model construction by randomforest

The R "random forest" package was used to classify the different cohorts based on the relative abundance at the genus level, and to identify the potential diagnostic biomarkers [22]. Subsequently, 10-fold cross-validation and 10-time repeats were used to assess the model's performance. Lastly, the areas under curve index (AUC) and receiver operating characteristic (ROC) curves were analyzed by the "pROC" package, and the model was visualized with the "ggplot" package.

2.7 Statistical Analysis

Clinical indicators were statistically analyzed using the SPSS software (version 26.0; International Business Machines Corporation, Amonk, NY, USA). The non-parametric Mann-Whitney test was used to test the differences in age, height, weight, and other indicators of subjects in each cohort. The R platform (http://www.r-project.org/) was used for statistical analysis of the sequencing data. The difference in the diversity of oral microbiota was compared using the function of the Kruskal-Wallis test and Permutational multivariate analysis of variance (PERMANOVA) of the R "vegan" package [23]. The data are presented as mean ± SD for each cohort. Statistical significance was set at $p < 0.05$.

3. Results

3.1 The diversity of oral microbiota in caries and healthy teeth

The V3–V4 regions of the 16S rDNA of the 60 investigated samples (20 from healthy individuals and 40 from ECC children) were sequenced. A total of 837,136 high-quality sequencing reads were obtained, ranging from 2594 to 42,458 sequences, (mean = 13,952 sequences, and median = 10,997 sequences). The Shannon and Simpson indices were used to characterize the microbial richness and uniformity of the CC, CH, and HH cohorts. The results of comparative analyses showed no significant difference among the three groups (Fig. 1A,B; Kruskal-Wallis test, $p > 0.05$). The beta-diversity values based on Bray-Curtis (Fig. 1C) and Unweighted-UniFrac (Fig. 1D) distance metrics were calculated to reflect the levels of similarities of composition among these cohorts. The results showed that the beta-diversity values of the CC and CH cohorts were similar. The lower value of the HH cohort (Fig. 1C,D; Kruskal-Wallis test, $p < 0.001$) indicated that the community similarity of the HH cohort was more stable.

To determine the similarity of the microbiota composition (Fig. 1E) and functional composition (Fig. 1F) in healthy and carious sites of each child with ECC, beta-diversity was characterized using the common PCoA based on Bray-Curtis distance metrics. The two ends of each line represent the plaque samples of different health statuses of one child with ECC. The results showed a significant difference in the taxonomical and functional composition (PERMANOVA, $p = 0.003$ and $p = 0.001$, respectively) between healthy and carious sites in patients with ECC.

3.2 Shifts of the oral microbiota in children with ECC and healthy individuals

To understand the species distribution of different healthy states, the microbiota was analyzed at the phylum and genus levels. A total of 17 phyla, 26 classes, 51 orders, 104 families, and 273 genera were detected in the oral plaque samples. At the phylum level, *Firmicutes*, *Proteobacteria*, and *Fusobacteria* were the main phyla among the three cohorts (Fig. 2A). Specifically, the relative abundance of *Firmicutes* in the CC cohort (50.6%) was higher than that in the CH and HH cohorts (34.1% and 34.5%, respectively). In the CH cohort, the relative abundance of *Proteobacteria* (34.4%) was higher than that in the CC cohort (21.2%) and was similar to that of the HH cohort (31.3%). *Fusobacteria* was mainly concentrated in the HH cohort, with a relative abundance of 15.3%. In the CC and CH cohorts, the relative abundance of *Fusobacteria* was 8.2% and 9.9%, respectively.

At the genus level, the top three genera in the three cohorts were *Streptococcus*, *Neisseria*, and *Leptotrichia* (Fig. 2B). The relative abundance of *Streptococcus* was roughly equal among the three cohorts, while the relative abundance of *Neisseria* decreased with the degree of ECC. For each cohort, the relative abundance of *Neisseria* in the CC, CH and HH cohort was 6.1%, 10.6%, and 14.9%, respectively. The relative abundance of *Leptotrichia* was 10.5% in the HH cohort, which was higher than that in the CC (6.2%) and CH cohorts (6.5%).

3.3 Difference of oral microbial taxonomical and functional composition in caries and healthy children

LEfSe analysis was performed to identify the dominant microbes and their functions. We obtained 29 taxa with LDA > 3.5 (Fig. 3A) and 34 signaling pathways with LDA >2.0 (Fig. 3B). The main microbes were different in all the three cohorts. As shown in Fig. 3A, the main genera of the HH cohort were *Neisseria*, *Leptotrichia*, *Porphyromonas*, and *Gemella*. The CC cohort included *Lactobacillus*, *Veillonella*, and *Prevotella 7*. While the dominant bacteria in the CH cohort were *Actinomyces*, *Bifidobacterium*, and *Abiotrophia*.

According to the third level of KEGG pathway analysis, the functions were mainly involved in cellular processes, environmental information processing, genetic information processing, human diseases, metabolism, and organismal systems (Fig. 3B). For example, the HH cohort was involved

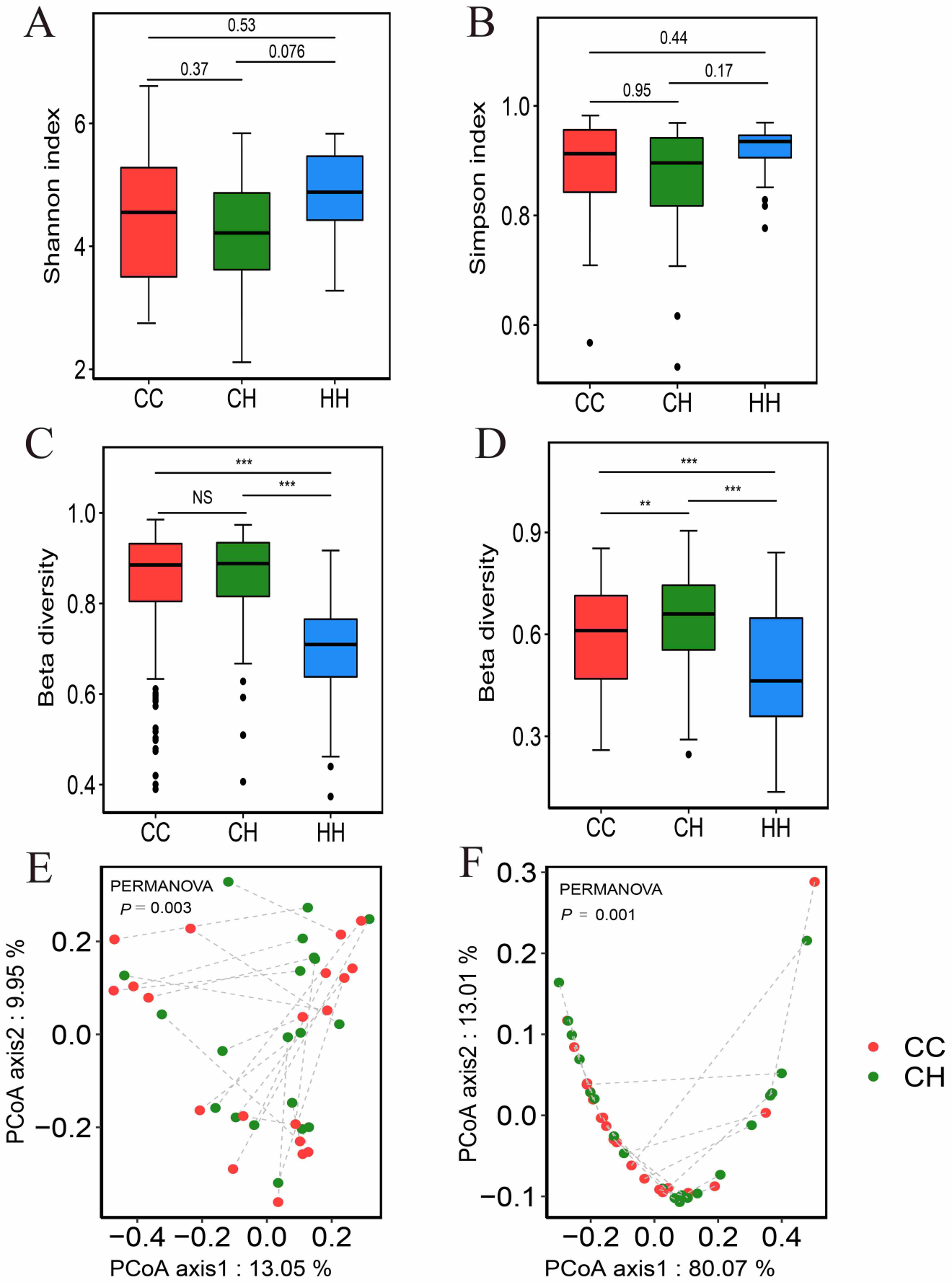

FIGURE 1. The diversity and oral microbiota composition in carious and healthy sites of ECC (CC and CH) and healthy controls (HH). (A) Shannon index and (B) Simpson index of microbial diversity revealed no significant difference among the CC, CH and HH cohorts. (C) Bray-Curtis analysis and (D) Unweighted-UniFrac analysis revealed that the beta-diversity of the HH cohorts was lower than that of other cohorts. The taxonomical composition (E) and functional composition (F) in each child (CC and CH cohorts) based on PCoA revealed significant differences between different sites in one child. The dotted line indicates that the oral samples were from the same individual (Bray-Curtis distance, PERMANOVA). (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; NS: No Significance; Kruskal-Wallis test and PERMANOVA).

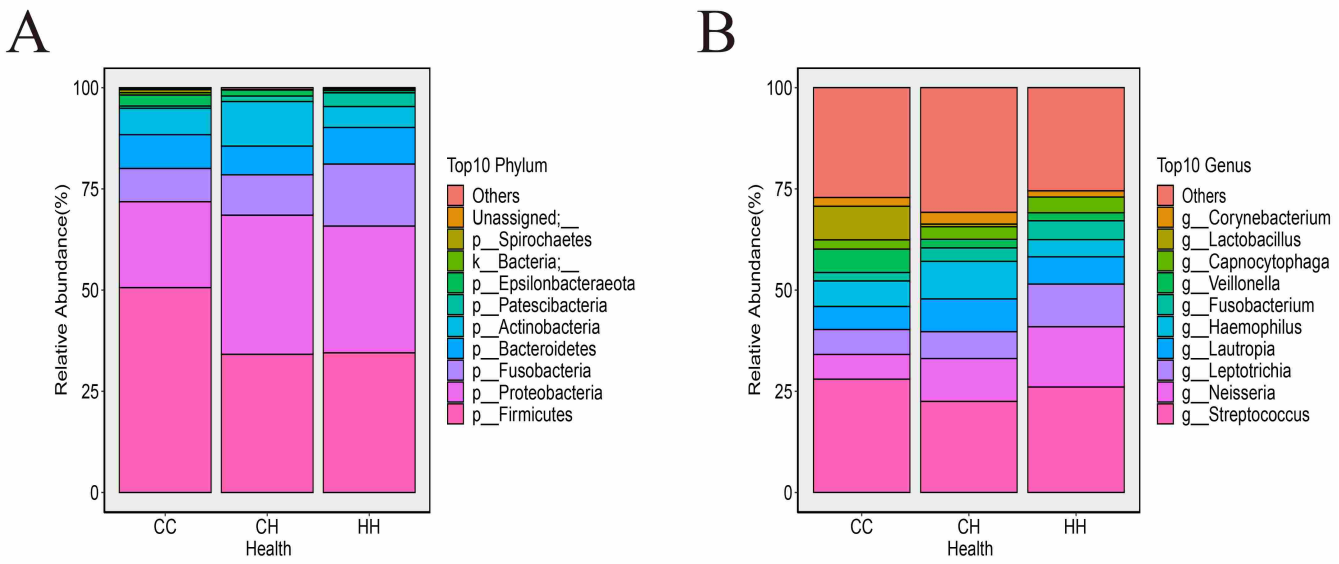

FIGURE 2. Shifts of the oral microbiota in children with ECC and healthy individuals. The relative abundance of oral microbiota at the phylum (A) and genus (B) levels showed the dominant microbiota among CC, CH and HH cohorts. The top 10 phyla and genera are presented.

in the biosynthesis of terpenoids and steroids, lipoic acid metabolism, and biotin metabolism, while the CC cohort was associated with histidine metabolism, biosynthesis of amino acids, and fructose and mannose metabolism. In the CH cohort, the main functions included biosynthesis of unsaturated fatty acids and the adipocytokine signaling pathway.

LDA, a supervised discrimination method, was used to assess the microbial composition (Fig. 3C) and functional characteristics (Fig. 3D). The results confirmed that the microbial composition and function varied due to differences in the oral health states.

3.4 Analysis of oral microbiota competition in different cohorts

Co-occurrence networks help us to better understand the relationship among the oral microbiotal components from an ecological perspective. Hence, the interactions of dominant genera based on the SparCC algorithm were studied. In the genus-genus co-occurrence network, 35 different abundant genera with positive or negative relationships were identified in the CC cohort (Fig. 4). Similarly, 30 and 39 different abundant genera were obtained in the CH and HH cohorts, respectively (Fig. 4). In terms of edges, two related genera were connected, and the number of edges was positively correlated with complexity. The edges of the CC, CH, and HH cohorts were 56, 59, and 63, respectively. Genera of the same color were grouped into the same module. The HH cohort had the largest number of modules and edges, which indicated that the relationship among genera in the healthy oral environment was more complicated.

Streptococcus showed a significant positive correlation with many other genera in the CC cohort, such as *Bergeyella*, *Abiotrophia*, and *Rosia* (Fig. 4A). While in the CH cohort, *Streptococcus* was positively correlated with *Actinomyces* and negatively correlated with the *Candidy Division SR1 bacterium MGEHA* (Fig. 4B). *Streptococcus* had the most complex network relationship in the HH cohort, but this relationship was mainly negatively correlated (*e.g.*, *Campylobacter*, *Prevotella*, and *Fusobacterium*; Fig. 4C). This demonstrated that diverse microbes dominated in different groups, which caused the diverse functional modules to be enriched in the different cohorts.

3.5 Identification of the biomarkers for ECC

Considering that each cohort had distinctive microbiota, it was possible to search for biomarkers representing different healthy states. A random forest model was constructed at the genus level. Through 10-fold cross-validation and 10 repetitions, the results showed that the error rate decreased sharply as the number of genera increased. The lowest classification error rate was observed when 10 genera were selected as biomarkers (Fig. 5A). According to the Mean Decrease Accuracy, the ranking index of the importance of characterizing genera was used to determine the top 10 genera (Fig. 5B), which were chosen as biomarkers. Their relative abundances are shown in Fig. 5C. The biomarkers included *Firmicutes* (*Gemella*, *o-Lactobacillus*, *Veillonella*, *Lactobacillus* and *Abiotrophia*), *Proteobacteria* (*f-Neisseria and Cardiobacterium*), *Actinobacteria* (*Actinobacillus*), and *Bacteroidetes* (*Prevotella 7*). The accuracy of these biomarkers for model classification was assessed (Fig. 5D), which shown an AUC of 89.8% (95% CI: 73.1%–100%). These results indicated that the classification model had a high predictive ability, and the biomarkers explored could be of great value in preventing and diagnosing caries in children.

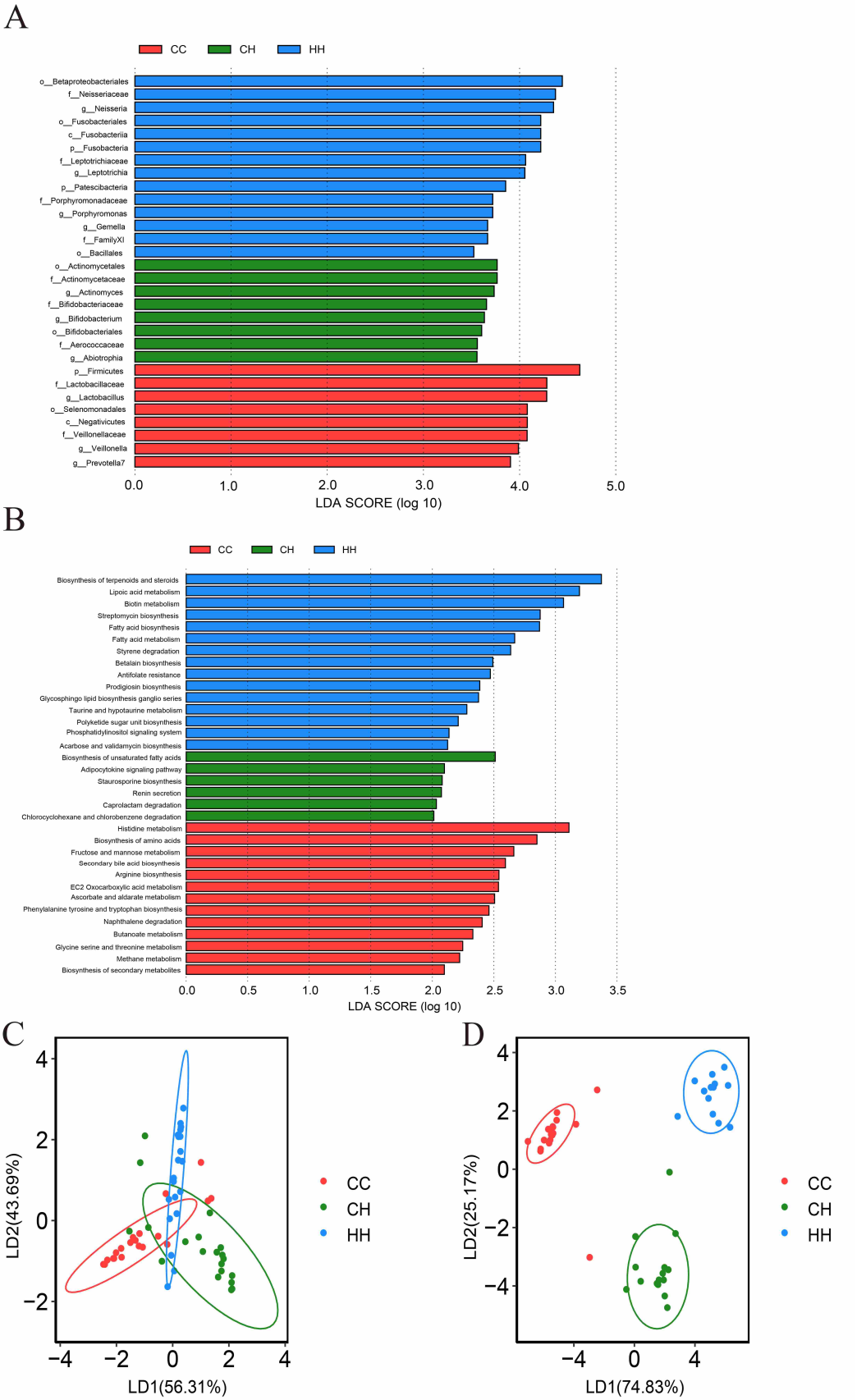

FIGURE 3. Difference of oral microbial taxonomical and functional composition in caries and healthy children. Taxonomical composition (A) and functional composition (B) for identification of the CC, CH and HH cohorts presented the oral microbiota taxa and functions with significant differences. Linear discriminant analysis was performed to maximize the separation of the CC, CH and HH cohorts based on (C) taxonomical composition and (D) functional composition.

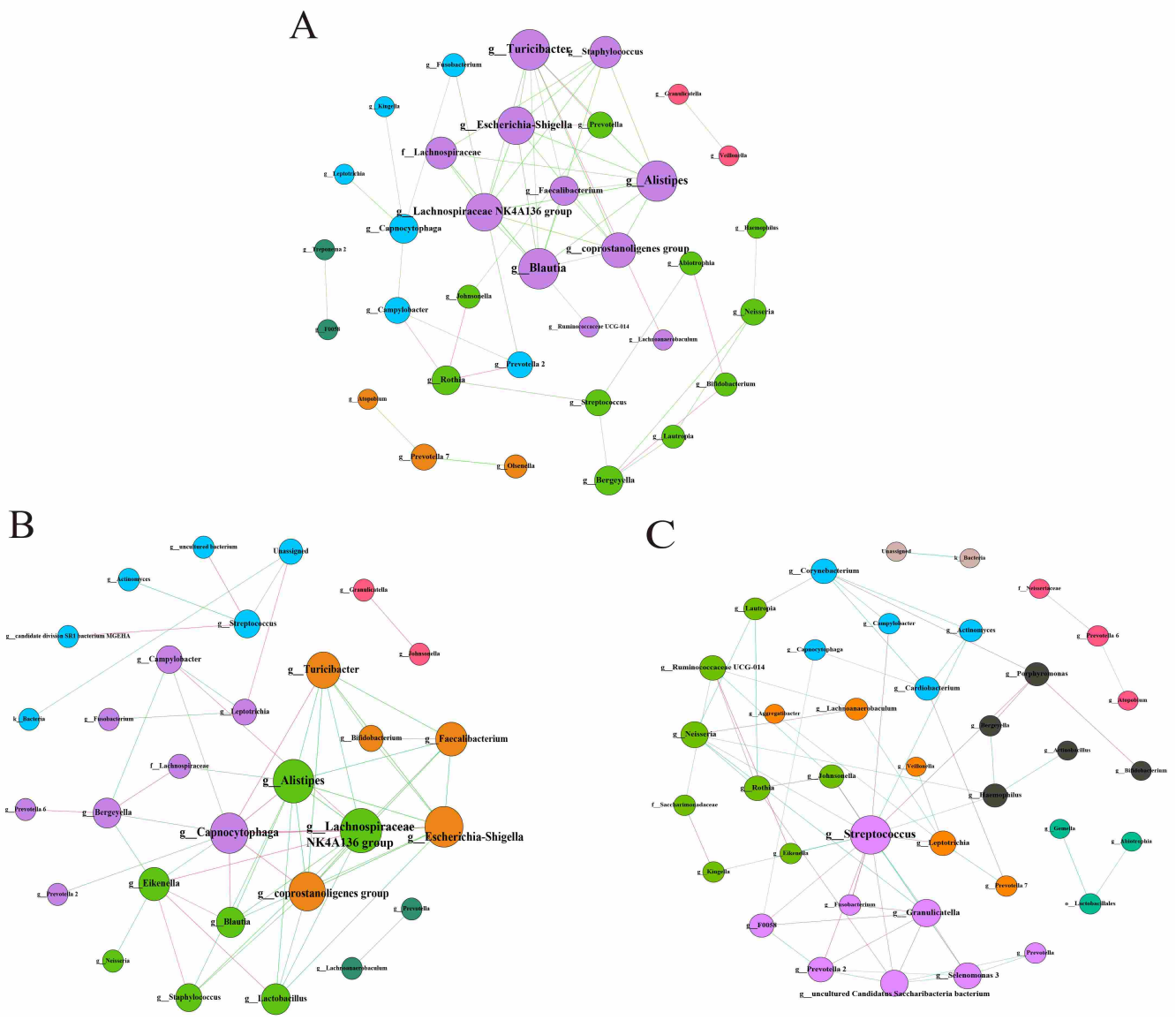

FIGURE 4. Correlations between enriched microbiota of the CC (A), CH (B) and HH (C) cohorts. The nodes represent different genera, and their sizes represent the relative abundance; same color represents a module. The edges among them indicate correlations; red indicates a negative correlation, and green indicates a positive correlation.

4. Discussion

As one of the most common chronic oral diseases, ECC could delay children's growth, if not treated in time [24]. In this study, we found differences in the oral microbiota between children with ECC and healthy individuals. The genera of the healthy cohort included *Neisseria*, *Leptotrichia*, *Porphyromonas* and *Gemella*. Comparatively, the genera of the CC cohort were *Lactobacillus*, *Veillonella* and *Prevotella 7*. More importantly, the oral microbiota in healthy sites of children with ECC was investigated, and the genera *Actinomyces*, *Bifidobacterium*, and *Abiotrophia* were found to play important roles in the transition from healthy to ECC states. Additionally, our constructed diagnostic model based on the random forest of 10 genera demonstrated a prediction ability of 89.8%.

The diversity in an ecosystem is often termed alpha diversity, which is a comprehensive indicator reflecting the richness and evenness of a species in a community [25]. Community diversity is usually represented by the Shannon and Simpson indices, which assign more weight to rare species, while the Simpson index emphasizes the importance of common species. This study demonstrated no significant difference in the alpha diversity among the CC, CH and HH cohorts, which showed that the occurrence of ECC did not change the biodiversity of the oral microbiota. The results were consistent with the findings reported by Chen *et al.* [26], who found that the richness and diversity of the bacterial communities were similar between children with caries and those who were caries-free [26]. Other studies found that the dental plaque of healthy individuals showed higher diversity than patients with caries [27]. The controversial conclusions could be attributed to individual variations, different sample sizes or analytic procedures used in these studies. Hence, more rigorously designed experiments are required to shed more light on this issue.

Beta diversity refers to the community structure of ecosys-

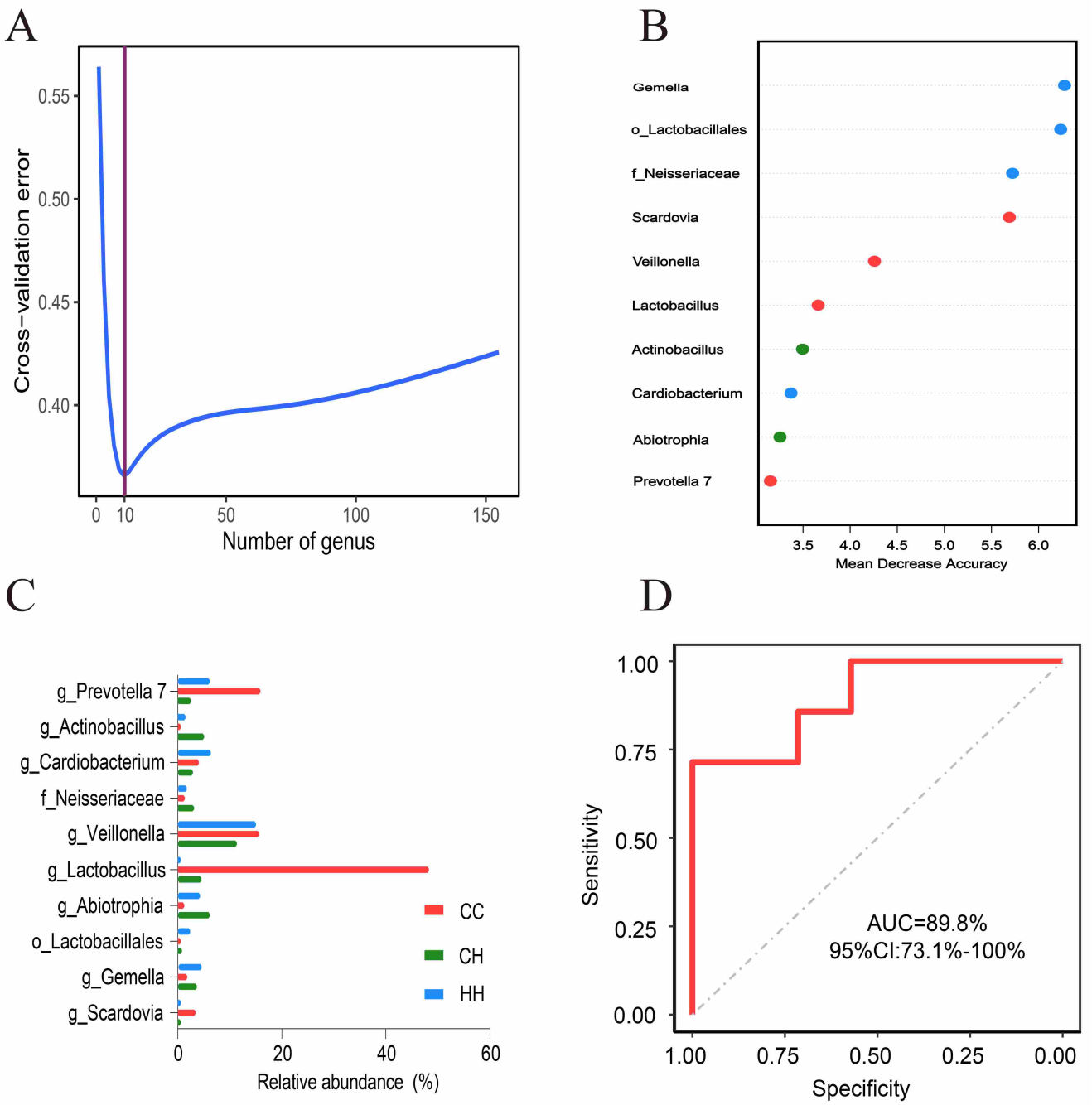

FIGURE 5. Identification of biomarkers for children with caries and healthy. (A) Correlation between the number of genera and the cross-validation error. The error rate was lowest when the number of genera was 10. (B) Mean Decrease Accuracy indices of important genera presented the contribution to the accuracy of the randomForest model. The lower the value, the smaller the effect on accuracy. (C) Specific relative abundance of biomarkers of each cohort. (D) The ROC of randomForest model constructed by 10 genera. The diagonal line in the graph markers an AUC of 0.5.

tems [28], which reflects the diversity distance relationship among samples and the degree of differentiation among biological communities. The three distance matrices, Bray-Curtis distance, weighted-UniFrac distance, and unweighted-UniFrac distance, can be used to characterize beta diversity. The Bray-Curtis distance matrix is based on the counting statistics of OTUs, which contains an abundance of information on OTUs, while the unweighted-UniFrac distance only considers the existence of OTUs in the samples. The values of the matrix distance range from 0 to 1, whereby a larger value indicates a larger difference between the samples. The results also significantly differed among the CC, CH and HH cohorts.

LEfSe analysis further screened for species with significant abundance in each cohort (Fig. 3). Among these significantly different genera, *Lactobacillus*, *Veillonella*, and *Prevotella 7* were more abundant in the CC cohort than in the other two cohorts. *Lactobacillus* is considered a cariogenic bacteria with acid-producing properties. *Veillonella*, an early oral colonization genus, was once considered helpful in preventing dental caries due to its ability to decompose lactic acid [29]. How-

ever, our study and previous studies suggested that *Veillonella* plays a more important role in caries development because it is involved in the co-aggregation and adhesion of *S. mutans* and promotes the formation of their biofilms [30, 31]. The results also showed that *Neisseria* was more abundant in the healthy cohort, which was consistent with previous findings reporting that *Neisseria* is beneficial to host health [32, 33].

The relationship between human symbiotic microbiota and the diagnosis and treatment of diseases has become a global hot topic. The random forest model was applied as a machine learning model to identify the microbiota and diagnose the diseases [34]. We aimed to identify genera that could distinguish different disease states using the random forest model to provide a theoretical basis for future disease prevention from an ecological perspective (Fig. 5). The classification effect of random forest is usually represented by AUC-ROC, whereby a larger AUC indicates better classification effects [35]. Here, the combination of 10 associated genera (*Prevotella 7*, *Actinobacillus*, *etc.*) was shown to effectively discriminate ECC patients from healthy children with a high accuracy of 89.8%. Thus, identifying microbial signatures could be a powerful tool for epidemiological studies and investigating risks of developing caries.

There were some deficiencies in this work. First, due to the highly conservative sequence of the 16S rDNA gene, the reported results can only be accurate to the level of genus and family, indicating an issue due to limited resolution. Metagenomics, as an accurate method to detect the microbiota at the level of species or strains, can obtain more information about the distribution of microbiota related to caries. Second, due to environmental, dietary and other factors, there were great differences among the investigated dataset; hence, the sample size is also a potential factor affecting the accuracy of the analysis. Thus, larger and prospective cohort studies are required to validate our proposed predictive model.

5. Conclusions

Oral microbiota can be used for the early prediction and prevention of caries in children and provide references for dietary intervention, probiotics or antibiotic target therapy. We examined the oral microbiota of the CC and CH areas of ECC compared with healthy children and explored the microbial characteristics from various aspects. Our random forest model based on 10 genera accurately predicted the individual disease status, indicating that these 10 genera could be used as biomarkers for clinical diagnosis.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

XYX, BKS and QXZ—designed the research study. XYX and BKS—performed the research. WWL and JXZ—analyzed the data. XYX and BKS—wrote the manuscript. QXZ, HZ and WC—reviewed and edited the paper.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted at Wuxi Children's Hospital, Jiangsu Province, China, and was approved by the Ethics Committee of Wuxi Children's Hospital (WXCH2020-11-001). The registration number in the Chinese Clinical Registration Center was ChiCTR2100047849. All participants agreed to participate the study.

ACKNOWLEDGMENT

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Lin T, Lin C, Pan T. The implication of probiotics in the prevention of dental caries. Applied Microbiology and Biotechnology. 2018; 102: 577–586.

[2] Organization WH. World Health Assembly Resolution paves the way for better oral health care. 2021. Available at: https://www.who.int/news/item/27-05-2021-world-health-assembly-resolution-paves-the-way-for-better-oral-health-care (Accessed: 27 May 2021).

[3] Tinanoff N, Baez RJ, Diaz Guillory C, Donly KJ, Feldens CA, McGrath C, *et al*. Early childhood caries epidemiology, aetiology, risk assessment, societal burden, management, education, and policy: global perspective. International Journal of Paediatric Dentistry. 2019; 29: 238–248.

[4] Pitts NB, Zero DT, Marsh PD, Ekstrand K, Weintraub JA, Ramos-Gomez F, *et al*. Dental caries. Nature Reviews Disease Primers. 2017; 3: 17030.

[5] Xiao J, Fiscella KA, Gill SR. Oral microbiome: possible harbinger for children's health. International Journal of Oral Science. 2020; 12: 12.

[6] Li X, Zheng J, Ma X, Zhang B, Zhang J, Wang W, *et al*. The oral microbiome of pregnant women facilitates gestational diabetes discrimination. Journal of Genetics and Genomics. 2021; 48: 32–39.

[7] Wang T, Yu L, Xu C, Pan K, Mo M, Duan M, *et al*. Chronic fatigue syndrome patients have alterations in their oral microbiome composition and function. PLoS One. 2018; 13: e0203503.

[8] Wang J, Jia Z, Zhang B, Peng L, Zhao F. Tracing the accumulation of *in vivo* human oral microbiota elucidates microbial community dynamics at the gateway to the GI tract. Gut. 2020; 69: 1355–1356.

[9] Huang S, Li R, Zeng X, He T, Zhao H, Chang A, *et al*. Predictive modeling of gingivitis severity and susceptibility via oral microbiota. The ISME Journal. 2014; 8: 1768–1780.

[10] Wang Y, Wang S, Wu C, Chen X, Duan Z, Xu Q, *et al*. Oral microbiome alterations associated with early childhood caries highlight the importance of carbohydrate metabolic activities. MSystems. 2019; 4: e00450–19.

[11] Dashper SG, Mitchell HL, Lê Cao KA, Carpenter L, Gussy MG, Calache

H, *et al*. Temporal development of the oral microbiome and prediction of early childhood caries. Scientific Reports. 2019; 9: 19732.

[12] Teng F, Yang F, Huang S, Bo C, Xu Z, Amir A, *et al*. Prediction of early childhood caries via spatial-temporal variations of oral microbiota. Cell Host & Microbe. 2015; 18: 296–306.

[13] Dikmen B. Icdas II criteria (international caries detection and assessment system). Journal of Istanbul University Faculty of Dentistry. 2015; 49: 63–72.

[14] Ling Z, Kong J, Jia P, Wei C, Wang Y, Pan Z, *et al*. Analysis of oral microbiota in children with dental caries by PCR-DGGE and barcoded pyrosequencing. Microbial Ecology. 2010; 60: 677–690.

[15] Wang L, Pan M, Li D, Yin Y, Jiang T, Fang S, *et al*. Metagenomic insights into the effects of oligosaccharides on the microbial composition of cecal contents in constipated mice. Journal of Functional Foods. 2017; 38: 486–496.

[16] Wang L, Hu L, Xu Q, Yin B, Fang D, Wang G, *et al*. Bifidobacterium adolescentis exerts strain-specific effects on constipation induced by loperamide in BALB/c mice. International Journal of Molecular Sciences. 2017; 18: 318.

[17] Han M, Yang K, Yang P, Zhong C, Chen C, Wang S, *et al*. Stratification of athletes' gut microbiota: the multifaceted hubs associated with dietary factors, physical characteristics and performance. Gut Microbes. 2020; 12: 1842991.

[18] Paul D, Kumbhare SV, Mhatre SS, Chowdhury SP, Shetty SA, Marathe NP, *et al*. Exploration of microbial diversity and community structure of lonar lake: the only hypersaline meteorite crater lake within basalt rock. Frontiers in Microbiology. 2015; 6: 1553.

[19] Lozupone C, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. Applied and Environmental Microbiology. 2005; 71: 8228–8235.

[20] Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, *et al*. Metagenomic biomarker discovery and explanation. Genome Biology. 2011; 12: R60.

[21] Howsmon DP, Hahn J. Regularization techniques to overcome overparameterization of complex biochemical reaction networks. IEEE Life Sciences Letters. 2016; 2: 31–34.

[22] Svetnik V, Liaw A, Tong C, Culberson JC, Sheridan RP, Feuston BP. Random forest: a classification and regression tool for compound classification and QSAR modeling. Journal of Chemical Information and Computer Sciences. 2003; 43: 1947–1958.

[23] McGraw R, Zhang R. Multivariate analysis of homogeneous nucleation rate measurements. Nucleation in the p-toluic acid/sulfuric acid/water system. The Journal of Chemical Physics. 2008; 128: 064508.

[24] Seow WK. Early childhood caries. Pediatric Clinics of North America. 2018; 65: 941–954.

[25] Xia Y, Sun J, Chen D. Community diversity measures and calculations. Statistical Analysis of Microbiome Data with R. 2018; 14: 167–190.

[26] Chen W, Jiang Q, Yan G, Yang D. The oral microbiome and salivary proteins influence caries in children aged 6 to 8 years. BMC Oral Health. 2020; 20: 295.

[27] Xiao C, Ran S, Huang Z, Liang J. Bacterial diversity and community structure of supragingival plaques in adults with dental health or caries revealed by 16S pyrosequencing. Frontiers in Microbiology. 2016; 7: 1145.

[28] Whittaker RH. Vegetation of the siskiyou mountains, oregon and california. Ecological Monographs. 1960; 30: 279–338.

[29] Costalonga M, Herzberg MC. The oral microbiome and the immunobiology of periodontal disease and caries. Immunology Letters. 2014; 162: 22–38.

[30] Luo YX, Sun ML, Shi PL, Liu P, Chen YY, Peng X. Research progress in the relationship between Veillonella and oral diseases. Hua Xi Kou Qiang Yi Xue Za Zhi. 2020; 38: 576–582. (In Chinese)

[31] Kanasi E, Dewhirst FE, Chalmers NI, Kent, Jr. R, Moore A, Hughes CV, *et al*. Clonal analysis of the microbiota of severe early childhood caries. Caries Research. 2010; 44: 485–497.

[32] Yamashita Y, Takeshita T. The oral microbiome and human health. Journal of Oral Science. 2017; 59: 201–206.

[33] Fakhruddin KS, Ngo HC, Samaranayake LP. Cariogenic microbiome and microbiota of the early primary dentition: a contemporary overview. Oral Diseases. 2019; 25: 982–995.

[34] Blanchet L, Vitale R, van Vorstenbosch R, Stavropoulos G, Pender J, Jonkers D, *et al*. Constructing bi-plots for random forest: Tutorial. Analytica Chimica Acta. 2020; 1131: 146–155.

[35] Muschelli J. ROC and AUC with a binary predictor: a potentially misleading metric. Journal of Classification. 2020; 37: 696–708.

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