

## Original Article

# Bacterial community of saliva in adults with and without periodontitis

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**Abstract:** Objective: The studies of microbial polymorphism of periodontitis were limited and mainly focused on the subgingival plaque. This study was to compare the bacterial community in the saliva of adults with and without periodontitis. Methods: 29 adults samples (15 periodontitis and 14 healthy) who lived in same place were selected. 16S rDNA cloning library technology, ClustalX2, and Mothur v.1.32.1ect. software and the vegan package of R. LDA Effect Size (LEfSe) were used to compare the bacterial community in the saliva. Results: The major phyla were similar in adults with and without periodontitis. Especially, the genus *Prevotella* was considered to be the feature in periodontitis samples, while the proportion of *Lactobacillus* and *Granulicatella* were lower in adults with periodontitis. Conclusions: These results indicate that the change of the bacterial community at the lower taxonomic levels in saliva of adults may be one pathogenic factor of the periodontitis. This would be taken into account in further treatment or prevention of the periodontitis.

**Keywords:** Periodontitis, bacterial community, 16S rDNA cloning library

## Introduction

Periodontitis is an inflammatory disease, which is not only injurious to oral health, but also is a variety of systemic disease risk factors such as: cardiovascular disease, nephritis, adverse pregnancy outcomes, diabetes, and rheumatoid arthritis [1]. Studies have demonstrated that pathogenic bacteria was an important factor of periodontitis [2] and there may be some disease-associated species of periodontal ecosystem [3]. However, the pathogenic bacteria of the periodontitis were still not entirely clear.

In the past, microbes were traditionally identified by culture-based methods, which excluded numerable uncultivated species. Therefore, it was difficult to discern the pathogenic bacteria of periodontitis wildly. With the popularity of molecular biology methods, such as 16S rDNA clone library and 454 pyrosequencing, now some new view is: the change of the microbial community structure caused the occurrence of

periodontal disease [4, 5]. When compared the subgingival bacterial communities of healthy and periodontitis subjects, the data showed that there were some certain more common species in disease [6-8]. Nevertheless, the main bacteria in patients with periodontitis in these studies were not always the same, which may due to that the methodological and individual differences. Accordingly, study on the microbiological diversity in patients with periodontitis is still needed.

Containing a variety of bacterial species from different oral sites (tongue, subgingival plaque, and supragingival plaque), saliva has been considered to be the sample which can provide more information of the bacteria for oral disease or oral health including periodontitis in the entire oral cavity [9-11]. But to the best of our knowledge, the researches of the microbiological diversity of periodontitis were more focused on the subgingival plaque, and there were little studies focused on the saliva. In this study, we

**Table 1.** The information of samples in this study

Group	Healthy	Periodontitis
Gender	7 M; 7 F	7 M; 8 F
Age	31.3±9.7	51.1±9.1
Probing depth	2.01±0.51	6.77±2.2
Attachment loss	0	3.36±1.49
Bleeding on probing (%)	0	50.3%±5.8%

M: Male; F: Female.

analyzed more than 1000 cloned 16S rDNA gene sequences from 29 individuals (including 15 periodontitis and 14 healthy subjects), and then examined the differences between microbiological diversity of saliva in adults with and without periodontitis.

## Materials and methods

### *Subjects and specimen collection*

All human host volunteers were from resident population of Yugur autonomous county, Gansu province, China, in September 2012. At last, 29 (15 periodontitis (P) subjects and 14 healthy oral (H) subjects) volunteers were selected. They were fully oriented regarding the research objectives and agreed to sign an informed consent form after study approval by the Ethics Committee of Northwest University for Nationalities. Their ages were range from 22 to 55, and there were 7 male and 8 female in periodontitis group and 7 male and 7 female in healthy group. A complete clinical examination was performed by one trained examiner, including medical and dental histories, an intra-oral examination. The subjects with periodontitis had pocket probing depths of  $\geq 5$  mm, attachment loss of  $\geq 3$  mm and gingival bleeding  $\geq 50\%$  at the CPI index teeth [12]. The pocket probing depths and attachment loss were measured at six sites per CPI index tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual). The healthy subjects had no probing depths that were  $< 3$  mm in full mouth.

All the subjects were free of caries. The patients with a history of immunosuppression or systemic diseases (e.g., diabetes and HIV), those using medications that reduce saliva flow and those under treatment with antimicrobials within the previous two months and smokers were excluded from this study.

### *DNA extraction, PCR amplification, 16S rDNA gene clone and sequencing*

One-milliliter un-stimulated saliva samples were collected using an asepsis tube containing 500  $\mu$ L of TE buffer (25 mM Tris-HCl, 10 mM EDTA, pH 8.0) from each subject, who had not brushed their teeth, gargled or eaten for 12 hours. The bacterial g DNA was extracted by Qiagen Stool Mini Kit (Qiagen, Valencia, CA) following the manufacturer's instructions. The 16S rDNA region was amplified with the primers 8F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1391R (5'-GACGGGCGGTGTGTRCA-3') [13]. Purified amplicons were ligated into plasmid vector PMD18-T (Takara, Japan) and then transformed into *Escherichia coli* using the Takara Cloning kit (Takara, Japan) according to the manufacturer's instructions. Sanger sequencing was performed on the plasmid inserts of the purified PCR products (Life Genomics Institute, Beijing), and 600-800 bp sequencing was obtained.

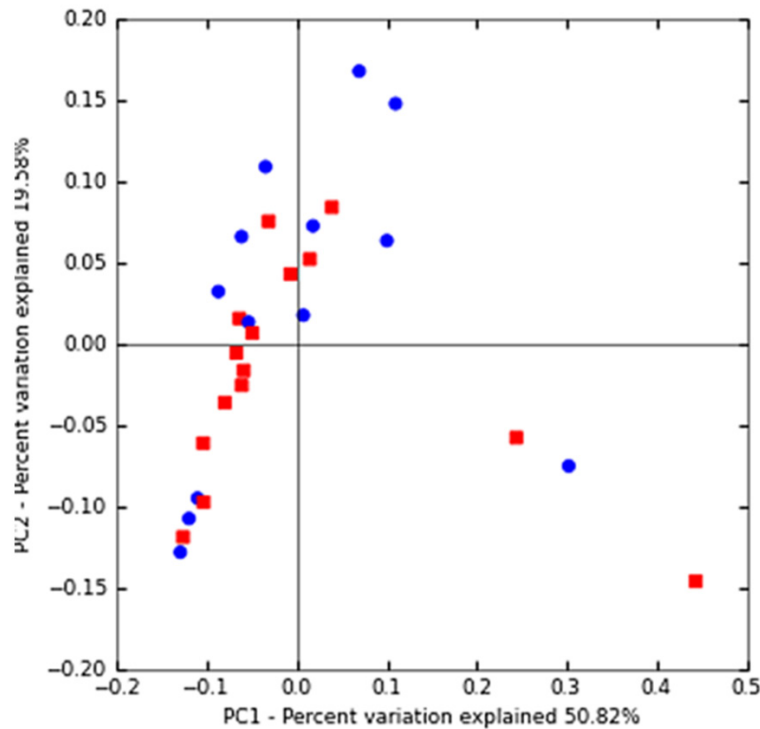
### *Data analysis*

The sequences were aligned and edited using ClustalX2 and BioEdit7.0.9.0 software, respectively. The vector sequence regions and low-quality sequences with chimeras were removed after processing with the program Vec Screen. Operational taxonomic units (OTUs; phylotypes) were defined using a 97% sequence similarity cutoff, which in this context roughly corresponds to species-level groupings. One representative for each of the OTUs identified in this study was deposited in the RDP database (<http://rdp.cme.msu.edu/>). Mothur v.1.32.1 software was used to calculate Ace and Chao 1 richness estimator at different cutoffs by T-test. Rarefaction curves were used to evaluate the sample volume. Principal Component Analysis (PCoA) and bacterial community distribution were performed using the vegan package of R. LDA Effect Size (LEfSe) was used to representation the characteristic bacteria at difference taxonomic levels [14].

## Result and discussion

### *The information of samples and the whole condition of sequencing*

The information of samples in this study was listed in **Table 1**. Human oral cavity is a complex microecosystem and mainly refers to the



**Figure 1.** Principal coordinate analysis of the 29 samples using pairwise Bray-Curtis dissimilarity matrices for the bacterial community based on OTUs at the 97% similarity level.

interactions between resident microorganisms and host responses [15]. The factors of diet, environment, or lifestyle combined affect the oral microbial homeostasis [16-18]. However, most previous studies did not avoid the influence of these factors. Therefore, in this study, we selected resident population of a remote area in Gansu province of China. The people living in this area have similar living environment (a pasturing area) and classic nomadic living and diet (tea with milk, meat, noodle and rarely vegetables). Moreover, they rarely communicate with other places due to the difficult traffic. Thus, the background of the individuals in this study was relatively consistent.

Across the 29 individuals, 1,450 clones (50 per samples) were sequenced and 1,334 qualified sequences were manually assigned to 326 OTUs using a cutoff of 97% sequence identity. Though the high-throughput sequencing is widely used today, the 16S rDNA clone technology is still a good method to investigate the oral bacterial community [13, 19-22] due to its advantages, such as saving the strains plasmids to the further study and more accurate sequencing.

#### Bacterial community of the healthy and periodontitis saliva

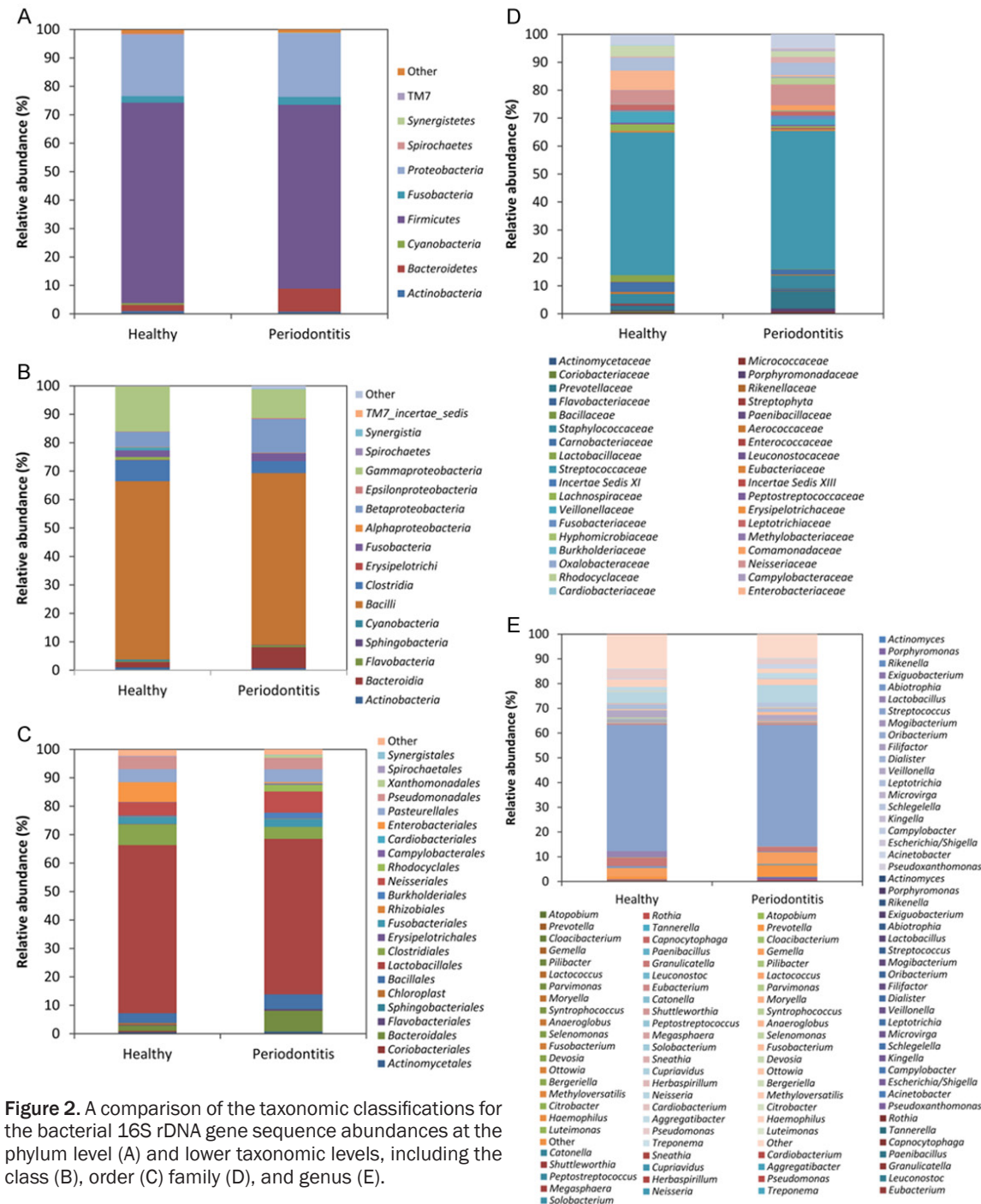
Detected between the healthy and periodontitis saliva of adults, OTU richness had not statistical differences. However, the similarity/dissimilarity of the bacterial communities across the 29 samples was measured using principal coordinate analyses (PCoA) for the pairwise Bray-Curtis dissimilarity matrices. The healthy samples clustered into the second and third quadrant, while the periodontitis samples were more scattered (**Figure 1**).

Totally, nine bacterial phyla were identified and further divided into 16 classes, 23 orders, 42 families, and 59 genera (**Figure 2A-E**). Across the healthy and the periodontitis samples, *Firmicutes* was the dominant phylum,

followed by *Proteobacteria*, *Fusobacteria*, *Bacteroidetes*, and *Actinobacteria*, though with different proportion in each group (**Figure 2A**). The same dominant phyla were also found in other individual with previously [9] and the same condition was reported in populations with caries [23]. All these suggesting that the influence of in saliva of adults with periodontitis was low at the phylum level.

Differences between the healthy and periodontitis human mouth were more obvious at lower taxonomic levels (**Figure 2B-E**). In the most common genus, our data confirmed that the most common genus in saliva of hosts with periodontitis was *Streptococcus*, accounting for 49.7% (**Figure 2E**), while the proportion of the genus connected with the periodontitis was low, such as *Porphyromonas* (0.7%) and *Aggregatibacter* (1.8%). These data was same to the previous study [9]. The *Streptococcus* is connected with caries [24], which is an infectious diseases of teeth. What is the truly role of the *Streptococcus* in saliva of host with periodontitis is worth study deeply due to the higher proportion. Further, more unknown sequences were found at lower taxonomic levels

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**Figure 2.** A comparison of the taxonomic classifications for the bacterial 16S rDNA gene sequence abundances at the phylum level (A) and lower taxonomic levels, including the class (B), order (C) family (D), and genus (E).

(Figure 2B-E), extending the list of oral microbial candidate divisions that are divergent from the known taxa.

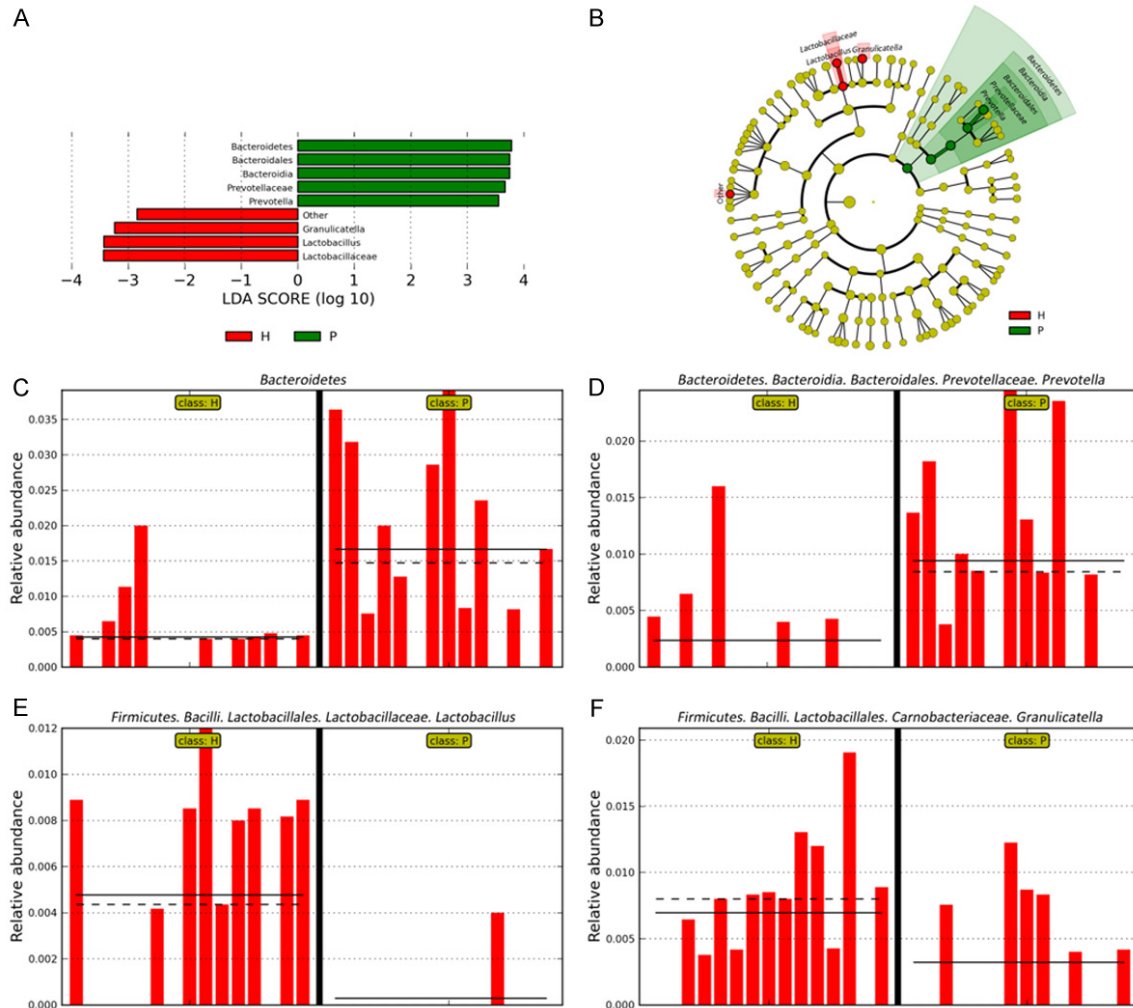
*The variance of bacterial compositions between the healthy and periodontitis saliva*

As **Figure 3** shown, the features existed at several taxonomic levels in both healthy and peri-

odontitis human mouth. The relative abundance of *Bacteroidetes* (**Figure 3A**), especially the genus *Prevotella* (belonging to class *Bacteroidales*, order *Bacteroidia*, family *Prevotellaceae*), was higher across the periodontitis samples (**Figure 3A, 3B, 3D**). *Prevotella* spp. was considered to be associated with periodontitis using cultured dependent or independent methods [25, 26]. In addition, the relative



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**Figure 3.** Cladogram representing the LefSe results on the hierarchy. “H” and “P” represent the healthy and periodontitis human mouth, respectively.

abundance of the genus *Prevotella* was also reported to be higher in subgingival plaque of adults with periodontitis [27]. However, the expected traditional periodontal pathogens, such as *Porphyromonas*, *Aggregatibacter*, *Porphyromonas* and *Treponema* etc. were not detected in this studies, suggesting that there were other pathogenic bacterium of periodontitis including the traditional periodontal pathogens, such as *Prevotella* spp.

Moreover, the genus *Lactobacillus* and *Granulicatella* were little from the periodontitis samples, but accounting for 2.46% and 3.47% from the healthy samples, respectively (Figure 3B, 3E, 3F). The two genera were normal oral bacteria and belonging to the lactic acid bacteria, a group which can metabolize sugars into lactic acid, and lower the pH of oral [28]. They

are usually with tooth hard tissue infection, and *Granulicatella* was once reported to be associated with dental plaques and endodontic infections [29]. However, why they were lower in periodontitis is worth further study, but all these data may be noticed in further diagnostic or treatment of periodontitis. In our further study, we will answer these questions by adding more samples and combining methods of high-throughput sequencing and real-time quantitative PCR. The plasmids in this study may be used in diagnosing chip for periodontitis.

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## Disclosure of conflict of interest

None.

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