Oral Microbiota in Healthy Thai Adults in Bangkok and Nearby Provinces

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Abstract— Oral cavity is a complex organ including teeth, tongue, gingival and cheeks, that exhibits a variety of bacteria. More than 700 species of bacteria have been reported in oral cavity. Dysbiosis in oral microbiota can increase a risk of oral disease and may lead to systematic disease. Oral microbiota diversity could be indicated oral health and there are several factors that affect to oral microbiota composition such as ages, gender and race. Next-generation sequencing is a technique that used to study all microorganisms including non-culturable microorganisms. In this study, we collected saliva and oral rinse from 20 healthy Thai adults. Metagenomic DNA were amplified V4 region of the 16s rRNA gene using universal primers. The number of OTUs and alpha-diversity index by Chao (richness) between saliva and oral rinse were significantly different, but by Shannon (diversity) were similar. Then, we separated into 5 groups according to ages. The results demonstrated that community richness only in between 20s and 30s groups which was significantly different in saliva, whereas in oral rinse the 20s group was all significantly different when compared with all other groups. However, a community diversity between age remains similar in both sample types. Abundant bacterial phyla were Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria and Fusobacteria. Yet, compared with 30s, Firmicutes increased markedly in 60s in saliva (P-value = 0.047). In contrast to oral rinse, the significantly difference was observed between 20s and 30s, 20s and 50s (P-value = 0.039 and 0.022, respectively). Moreover, Non-metric multidimensional scaling to visualize the closeness of bacterial composition between ages, the results revealed that 30s was likely separated from older groups. In conclusion, these suggested that ages might play a crucial role in bacteria diversity alteration and the results might represented as preliminary oral microbiota information of Thai individuals in Bangkok and nearby provinces.

Keywords— Oral, Microbiota, 16S rRNA.

I. INTRODUCTION

Oral cavity is a complex organ that contains several parts such as teeth, tongue, gingival and cheeks that have a variety of bacteria. More than 700 bacterial species in oral cavity were found such as *Actinomyces*, *Streptococcus*, *Neisseria*, *Veillonella*, *Porphyromonas*, and*Selenomonas*[1].They have an important role to help digestion, nutritional absorption and protection against potential pathogens. In contrast, some species can harm our health. Thus, oral health care is very important for many people because bad oral hygiene can affect a quality of life and our overall health.

Bad oral health can increase a risk of oral diseases such as periodontitis and dental caries. These oral diseases are caused by bacterial infections. An oral pathogen, Streptococcus mutans, is commonly found in oral cavity, however it cannot colonize in good oral hygiene. Furthermore, several studies reported that oral infection could lead to systematic diseases such as diabetes, cardiovascular disease and pneumonia [2]. Oral microbiome diversity can be indicated oral health and disease. In our body, the beneficial microorganisms play important role against the pathogens in several ways. However, the chance of pathological change in oral microbiome can occur, and it may lead to oral diseases. For example, the difference in microbiota composition between partial and complete denture wearer had related with progression of denture related stomatitis and complete denture wearer has more influence on progression of disease [3].

Oral microbiota composition can be altered by many factors such as ages, gender, dietary intake and climate zone. The previous study compared oral microbiome in 3 sample groups from different climate zones (Alaska, Germany and Africa) and microbiome from Africans group has a little different from Alaskans and Germans group [4]. This indicated that different climates zone and individual lifestyle can affect the oral microbiome. However, still less reported about oral microbiome in Thais. Knowing an oral microbiota in each region will lead to more understand the relationship

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between microorganisms and oral disease. Increasing of age is associated with all organs in our body. There have reported that aging process changed the proportion of Firmicutes and abundance of facultative anaerobes in human gut microbiota [5]. In oral cavity, it has evidence that aging reduced salivary flow in elderly people [6]. However, oral microbiota associated with aging still controversy.

Next-generation sequencing (NGS) technology has allow the researcher to study microbiome that associated with oral health because all DNA sequences in microorganisms including non-culturable microorganisms were obtained. The experiment was conducted using Illumina Miseq platform with universal primers that covered on 16s rRNA genes V4 regions.

In this study, we aim to characterize microbiota of Thai adults in saliva and oral rinse sample in each age and investigate whether which bacterial species are associated for used as preliminary information of Thai patients.

II. MATERIALS AND METHODS

Sample collections

40 subjects (20saliva samples and 20 oral rinse sample) were collected by Faculty of Dentistry, Chulalongkorn University. 1 mL Saliva were collected directly from each participant. Oral rinse participants were rinsed their mouth with 15 mL sterile Phosphate Buffer Saline (PBS) and expectorated to collected tube [7]. Saliva and the cell pellet from oral rinse were stored at -80^{o}C.

Metagenomic DNA extraction and 16s rRNA gene library preparations

Metagenomic DNA were extracted using DNeasy Powe rSoil kits (Qiagen, Germany) according to the manufacturer's instructions with minor modifications. Each sample was amplified using 515F and 806R primers with appended barcode sequences following conditions: 1) initial denaturing at 94^{o}C for 3 min, 2) denaturing at 94^{o}C for 45 sec 3) annealing at 50^{o}C for 60 sec 4) extension at 72^{o}C for 90 sec, repeat steps 2) to 4) for 28 cycles and 5) further extension at 72^{o}C for 10 min [8]. 381bp amplicon was checked by agarose gel electrophoresis. Gels were extracted using PCR clean-up & Gel extraction kit (GeneDireX, Inc., USA) and DNA quantity were measured using Qubit® 3.0 Fluorometer and Qubit®dsDNA HS Assay kit (Invitrogen). Sequencing was performed by Illumina Miseq platform (Faculty of Medicine, Chulalongkorn University).

Bioinformatics and statistical analysis

Sequences were analyzed using Mothur software[9]. Sequences which less than 100 bp, more than 8 ambiguous and chimera sequences were removed. Silva (V132 release) database was used for alignment and Green genes was used for taxonomy assignment. Alpha-diversity were estimated by Shannon index, Chao, observed OTUs and good's coverage. Non-metric Multidimensional scaling (NMDS) was continually analyzed to determine dissimilarity between pairs microbial community using Morisita-Horn distance method.

For statistical analysis, a student's t-test was used to compare microbial diversity and relative abundances of each bacterial taxon.

III. RESULTS

Participants information

The participants in this study were Thai adults ages between 20-65 years old, without history of smoking or any systematic diseases as showed in Table 1.

Table 1 Participants information.

Sample ID	Age	Systemic disease	Medication
Subject 1	22	no	no
Subject 2	27	no	no
Subject 3	33	no	no
Subject 4	33	no	no
Subject 5	34	no	no
Subject 6	35	no	no
Subject 7	36	no	no
Subject 8	40	no	no
Subject 9	41	no	no
Subject 10	45	no	no
Subject 11	48	no	no
Subject 12	51	no	no
Subject 13	52	no	no
Subject 14	52	no	no
Subject 15	53	no	no
Subject 16	57	no	no
Subject 17	59	no	no
Subject 18	60	no	no
Subject 19	62	no	no
Subject 20	65	no	no

Alpha-diversity and taxonomy assignment

Average 110,444 and 56,818 sequences reads per sample of saliva and oral rinse respectively, were analyzed alpha-diversity. Good's coverage indicated that our data likely represented the most bacterial sequences from saliva and oral rinse samples. We found significantly difference in number of OTUs and Alpha-diversity index by Chao (richness) between saliva and oral rinse sample (*P*-value = 0.0012 and 0.0018, respectively), whereas by Shannon (diversity) had similar (*P*-value = 0.8871) (Figure 1A). Then, we separated sample into 5 groups according ages (20-29, 20s; 30-39, 30s; 40-49, 40s; 50-59, 50s and 60-69, 60s) as showed in Table 2. Diversity within groups weren't significantly difference in both sample type, but Chao richness estimate had

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significantly difference between 20s and 50s in saliva and all groups in oral rinse when compared with 20s (Figure 1B).

Table 2 Alpha diversity index in each ages of each sample types.

Sample	No. of Sequences	Good's coverage	OTUs	Chao	Shannon
Saliva					
20s	77,535	99.97%	113	136.45	2.66
30s	59,172	99.92%	200	236.48	2.81
40s	106,724	99.98%	140	159.58	2.68
50s	148,526	99.96%	153	186.44	2.73
60s	146,636	99.97%	174	206.35	3.02
OR					
20s	23,024	99.97%	78	82.00	2.75
30s	41,377	99.96%	99	116.90	2.50
40s	43,352	99.95%	102	121.66	2.76
50s	79,518	99.96%	125	157.87	2.90
60s	77,635	99.97%	147	175.12	3.15

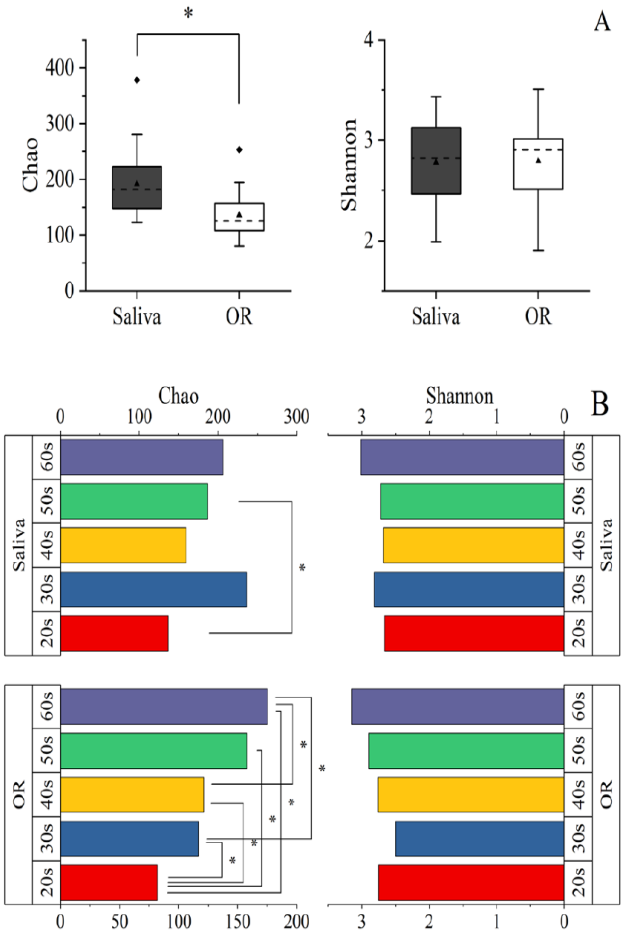

Figure 1 showed Alpha diversity estimated chao richness and Shannon diversity index. A) represented between sample types (saliva and oral rinse). B) represented between ages groups of each sample types. Comparison between groups was by Student's *t* test (*, p < 0.05).

Then we tried to assigned bacteria taxonomy at phylum level. The major phylum wasProteobacteria, Firmicutes, Bacteroidetes, Actinobacteria and Fusobacteria but we found a significantly increased of phylum Firmicutes in 60s when compared with 30s of saliva sample (*P*-value = 0.039) but decreased in 30s and 50s when compared with 20s (Figure 2).

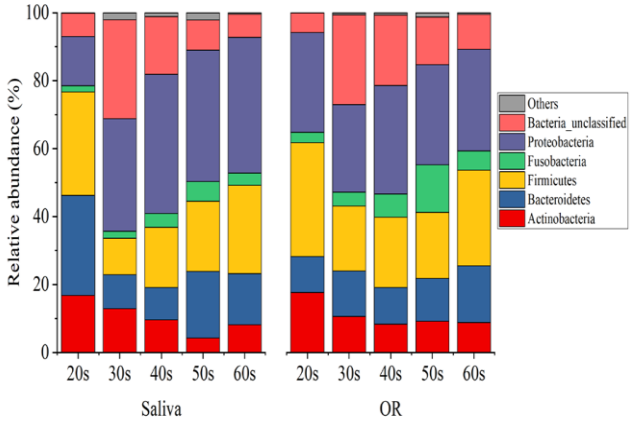

Figure 2 Bacteria relative abundance among ages at phylum level.

Comparison of bacterial composition between ages groups

We used Non-metric Multidimensional scaling (NMDS) to compared Beta-diversity between ages groups using Morisita-Horn distance method. The results showed all groups were clustered together excepted 30s, it was likely separated from another groups. This may indicate that in ages between 30-40 years have a difference on salivary microbiota structure that may affected by lifestyle of individual.

IV. DISCUSSIONS

First of all, we used two sample types to study oral microbiota. Saliva was commonly used for studied oral microbiota but saliva wasn't covered all oral microbiota community, so we decided to used oral rinse along with saliva. Because oral rinse able to collect genomic of the organisms from the dorsum of the tongue and the oral mucosal [7]. Our results showed oral rinse have lower number of OTUs than saliva,this may because participants used phosphate buffer saline (PBS) to rinse their mouth, PBS may diluted concentration of sample. In contrast, participants expectorated their saliva directly without using any buffer.

Lifestyle of each ages may affected on oral microbiota. Our result showed no statistically significant difference on bacteria composition. This related with Belstrøm et al. study, they reported that aging didn't influences on bacteria composition [10]. However, there have reported that ages are significant alterations in the proportion and composition of the different microbial taxa and leading to reduced microbiota diversity [11].

Although our results didn't showed significant difference on bacterial composition, we found pattern that likely difference between ages on salivary bacteria composition. It maycaused by another factor such as dietary intake. In Bangkok, there have a variety of foods so this may affect on oral bacteria composition. Older people can digest and absorb nutritions

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less than younger people but kind of foods and nutritions may help shape bacteria composition. However, this study have a limitation to defines factors that may associated with oral microbiota.

V. CONCLUSION

In this study, our results suggested that ages may change bacteria diversity, and represented a preliminary oral microbiota information of Thai individuals in Bangkok and nearby provinces.

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