

ALTERATION OF ORAL MICROBIOME IN CHILDREN AFTER USING MISWAK (*SALVADORA PERSICA* L.) MADE FROM ARAK AS A NATURAL TOOTHPASTE

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Abstract. The influence of Miswak (*Salvadora persica* L.) that is made from arak on the oral microbiome signatures of 6- to 12-year-old Saudi children was studied. Deep sequencing was done for the V3-V4 regions of bacterial 16S rRNA. Sequence tags were assigned to 291 OTUs (operational taxonomic units) across samples with $\geq 97\%$ similarity. The results indicated that factors like ethnic background and/or geographic origin can influence differences in healthy oral microbiome signatures. The results indicated some changes in the overall structure and diversity of oral microbiome. At genera level, there are five abundant phyla. At the species level, there are two opportunistic pathogens and the unassigned species as well as bacteria of the Proteobacteria family significantly decreased, the unassigned species belonging to the two genera increased due to swaking. The high abundance of *Streptococcus* and *Megasphaera* genera and low abundance of *Veillonella* genus are biomarkers of good oral hygiene as the first two genera (producer) can catabolize carbohydrates to the useful short-chain organic acids in biofilm formation, while the third (consumer) relies mainly on the fermentation of organic acids. These results shed light on the possible anti-microbial and anti-inflammatory properties of Miswak in addition to the role in removing plaque. In summary, we claim that Miswak is an excellent natural toothpaste for maintaining good oral hygiene, especially for children.

Keywords: *oral microflora, dysbiosis, Streptococcus, Megasphaera, Veillonella, organic acids*

Introduction

The human body contains ~100 trillion bacterial cells representing 1000 bacterial species or more. This bacteriome influences important parameters in human health including immune response, nutrient absorption, body weight, etc. (Gill et al., 2006; Pflughoeft and Versalovic, 2012). Although most of these bacterial species promote human health, others contribute to human illness (Greenblum et al., 2012; Ley, 2010). Other reports indicate that

the type of bacteria is not the only factor affecting human health, but the global microbiome balance was proved to have influence on human health (Huttenhower et al., 2012; Methé et al., 2012). Therefore, it is important to detect the normal symbiotic bacterial community that holds the healthy performance in human and how dysbiosis of this community encounters for diseases (Costello et al., 2009; Qin et al., 2010; Turnbaugh et al., 2009).

The mouth cavity is the major gateway to the human body, which influences the gastrointestinal microbiome and subsequent status of human health (Meurman, 2010). Dysbiosis of oral bacteriome has been linked with several life-threatening disorders including cardiovascular disease, stroke, pneumonia, etc. (Awano et al., 2008; Beck and Offenbacher, 2005; Joshipura et al., 1996, 2003; Offenbacher et al., 1998; Seymour et al., 2007). Simon-Soro et al. (2018) indicated that combined assessment of host response along with host oral microbiome can reveal clusters of health and disease. This indicates that oral microbiome can offer biomarkers of oral health (Alcaraz et al., 2012) and disease (Gomez and Nelson, 2017). Simon-Soro and colleagues also indicated that susceptibility to oral disease can be influenced by host immune factors, with emphasis to those existing in saliva. The latter contains a complex mixture of innate anti-microbial proteins and adaptive immune mediators with a significant impact on the microbial colonization of the oral cavity (Hancock et al., 2016; Mookherjee and Hancock, 2007). Previous reports indicate the high diversity among oral microbiomes of healthy individuals (inter-individual variation) (Aas et al., 2005; Nasidze et al., 2009), but little is known about the link between the structure and diversity of the oral microbiomes of healthy individuals in different ethnics/geographic regions (Mason et al., 2013). Interestingly, Nasidze and colleagues (Nasidze et al., 2009) indicated that normal saliva microbiome differs as we go further from the equator. In addition, saliva microbiome differs due to the human lifestyle and diet (Nasidze et al., 2011).

Salvadora persica L., also known as Miswak, is a tooth (or chewing) stick that is recommended by Prophet “Muhammad”. *Salvadora persica* belongs to the family Salvadoraceae and order Brassicales (Table 1). This plant is a well-branched evergreen tree, with soft yellow wood, that is capable to tolerate severe abiotic stresses (Haque and Alsareii, 2015; Khatak et al., 2010). The World Health Organization (WHO) has recommended the use of this stick as a natural toothbrush for healthy oral hygiene (WHO, 1984). The plant is known to contain important chemical constituents such as vitamin C, alkaloids, trimethylamine, tannins, saponins, organic sulphur compounds, lignan glycosides, etc. (Ohtani et al., 1992). Besides, the plant possesses a number of bioactive compounds with important pharmacological properties (Aumeeruddy et al., 2018). These properties include anti-microbial, anti-oxidant, anti-ulcer, anti-inflammatory, and anti-tumor activities besides being recently feasible in several biotechnological applications (Lebda et al., 2018).

Table 1. The taxonomic classification of *Salvadora persica*

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Brassicales
Family	Salvadoraceae
Genus	Salvadora
Species	<i>Persica oleoides</i>
Binomial name	<i>Salvadora persica</i>

The present study aims at detecting the influence of swaking with *Salvadora persica* L. (Miswak) for one month on maintaining or restoring healthy oral microbiome in Saudi children and assessing the possible protective effects against human opportunistic pathogens.

Materials and methods

Recruitment of participants and sample collection

The study was approved by the Ethics Committee of King Abdulaziz University Hospital (KAUH, Jeddah, Saudi Arabia) under number 066-16 in 2018 and written informed consent was obtained from parents of all participant children. All participant children received oral examination and those with a history of immunosuppression or systemic diseases, use of medications that reduce saliva flow, or exposure to antimicrobials in the previous three months were excluded from the study. A total of 10 Saudi children were selected based on the previous criteria with age ranging from 6 to 12 years old. Children have used Miswak once a day for one month. The saliva samples were collected before (BEF) and after (AF) swaking in Oragene-DNA (OG-500) Self-Collection kit (DNA Genotek Inc., Canada) and mixed with stabilizing reagent in the collection tubes per manufacturer's instructions and stored at 4 °C.

DNA extraction and partial 16S rRNA gene sequencing

Genomic DNA was extracted using the QIAamp® DNA Mini kit (Qiagen®51306; Hilden, North Rhine-Westphalia, Germany) according to the manufacturer's instructions. DNA purity was evaluated via A260/A280 ratio using NanoDrop 7000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and DNA integrity was checked by 1% agarose gel electrophoresis. PCR amplification of the V3-V4 regions of bacterial 16S rRNA was performed using the universal primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). PCR program was the following: initial denaturation at 95°C for 5 min; 25 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 40 s; and final extension of 72 °C for 10 min. Amplicons were run on agarose gel (1.2%), then gel-purified using DNA Gel Extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Amplicons were, then, shipped to Beijing Genome Institute (BGI) in China for library construction and deep sequencing on Illumina Miseq platform to recover ~300 bp pair-end reads of the V3 and V4 regions. The ends of each read were overlapped to generate high-quality, full-length reads. The resulted sequencing data has been deposited in the European Nucleotide Archive (ENA) (<https://www.ebi.ac.uk/ena/submit/sra/#studies>) under the project number PRJEB27276.

16S dataset processing and statistical analysis

Sample size estimation was performed to determine the probability that the samples are representative (Motulsky, 2010). The raw sequencing data were analyzed using the Quantitative Insights Into Microbial Ecology 2 (QIIME2) package v.2018.11; (<https://qiime2.org>) (Bokulich et al., 2018; Bolyen et al., 2019). V3-V4 16S rRNA sequence reads were trimmed using trimmomatic software (Version 0.33) and merged into single sequences using FLASH (Version 1.2.10). Merged sequences were filtered to remove the low-quality sequences. The latter are the reads shorter than 110

nucleotides, reads truncated at any site with an average quality score of < 20 over a 50-bp sliding window, or the truncated reads that were shorter than 50 bp. Only sequences that overlapped for more than 10 bp were assembled. The unique sequence set was linked to tags and classified into operational taxonomic units (OTUs) with a cutoff of 97% identity using the *de novo* OTU selection strategy. We retained only OTUs with at least 0.01% mean relative abundance, as predominant. OTUs were ranked by the relative abundance values as x- and y-axis, then the rank curve was drawn by software R (Version 3.1.1). Taxonomies were assigned by the RDP classifier (Version 2.2) (Cole et al., 2013) against the Human Oral Microbiome Database (Chen et al., 2010) (HOMD RefSeq, Version 13.2) and the Greengenes database (version 13.8: 16S rDNA database, http://qiime.org/home_static/dataFiles.html) with a confidence threshold of 0.7. Chimeric sequences were removed using Usearch (Version 8.0).

Alpha diversity was assessed by Shannon and Simpson indices that were calculated by Mothur (v1.31.2), and the corresponding boxplot of alpha diversity and rarefaction curve were drawn by software R (Version 3.1.1). Drawing rarefaction curve was based on calculating OTU numbers of the extracted tags (in multiples of 500) and detecting the maximum depth (no. reads) permitted to retain all samples in the dataset. Sequences were extracted randomly according to the minimum sequence number for all samples, and the extracted sequences formed a new 'OTU table biom' file. To detect the beta diversity within and between groups, the weighted and unweighted UniFrac distances were calculated (Lozupone et al., 2011) and plotted *via* principal coordinate analysis (PCoA) using package 'ade4' of software R (Version 3.1.1). UniFrac uses the system evolution information to compare the composition of community species between samples. The results can be used as a measure of beta diversity. It takes into account the distance of evolution between species, and the bigger the index, the greater the differences between samples. The UniFrac is divided into weighted UniFrac and unweighted UniFrac of which the weighted UniFrac considers the abundance of sequences, while unweighted UniFrac gives more weight on species presence/absence.

Heat maps were generated using the package 'gplots' of software R (Version 3.0.3) (<https://github.com/talgalili/gplots>). At phylum level, all species were used to draw the heat map and taxa of which abundance was less than 0.5% in all samples were classified as 'others'. To minimize the differences degree of the relative abundance value, the values were all log transformed. The representative sequences were aligned against the Silva core set. Representative OTU phylogenetic tree was constructed using the QIIME 2 (v.2018.11) built-in scripts including the fast-tree method for tree construction. The tags with the highest abundance of each genus was chosen as the corresponding genus representative sequences, and genus level phylogenetic tree was obtained by the same way as the OTU phylogenetic tree. Then, the phylogeny tree was imaged by R phylogenetics packages. Venn diagram was drawn by Venny (<http://bioinfogp.cnb.csic.es/tools/venny/>), while differences in the relative abundances of taxa at the phylum, genus and species levels were analyzed using Metastats (Paulson et al., 2011). PERMANOVA was used to test significance among values. All statistical tests were two-sided, and P value ≤ 0.05 was considered significant. Benjamini-Hochberg false discovery rate (FDR) correction was used to correct for multiple hypothesis testing where applicable. A GitHub repository link (<https://github.com/shandley/Microbiome-Analysis-Using-R.git>) providing the applied codes was added.

Results

Statistics of oral 16S rRNA sequence datasets

In the present study, the oral microbiome was detected for a group of 10 Saudi children (subjects) who used Miswak as a natural toothpaste. Illumina MiSeq was used in analyzing 20 salivary samples based on the 16S rRNA. Statistics of the raw data description and its processing is shown in *Table 2*. The average sequence length per read was 297 bp across different samples ranging from 293 to 300 bp. A total of 2,305,029 clean sequence reads were generated across subjects before and after swaking with average read numbers of 116,476 and 114,027 per subject, respectively (*Fig. 1*). A total of 2,241,369 tag-linked sequences were generated across subjects before and after swaking with average read numbers of 113,346 and 110,791 per subject, respectively. While, a total of 1,700,687 sequence tags were generated across subjects before and after swaking with average read numbers of 86,072 and 83,995 per subject, respectively (*Fig. 1*). These sequence tags were assigned to 291 OTUs (operational taxonomic units) across samples with $\geq 97\%$ similarity. A summation of 4,147 OTUs for the 20 samples were generated with an average of 207 OTUs per sample ranging from 146 to 257 OTUs and averages of 206 and 208 OTUs per subject before and after swaking (*Figs. 2* and *A1*). The overall number of OTUs before swaking (BEF) was 289, while 282 after swaking (AF). The results for the number of observed species (number of OTUs) per subject indicated increases in four subjects, while resulted in decreases in five subjects and no change in one subject (*Fig. 2*).

Table 2. Statistics of data generated from deep sequencing for 10 Saudi children before (BEF) and after (AF) swaking for one month

Sample ID	Reads length (bp)	Raw data (Mbp)	N base (%)	Low quality (%)	Clean data (Mbp)	Data utilization (%)	Raw reads	Clean reads	Read utilization (%)
BEF2	300:295	89.24	0.002	7.765	73.06	81.87	149,985	130,663	87.12
BEF6	299:295	79.81	0.001	6.864	66.83	83.74	134,352	119,170	88.70
BEF8	298:295	73.22	0.001	7.257	60.23	82.26	123,481	108,164	87.60
BEF9	296:295	84.64	0.001	7.787	69.78	82.44	143,209	125,526	87.65
BEF10	297:294	70.10	0.001	7.719	57.33	81.79	118,613	103,360	87.14
BEF12	296:294	65.60	0.000	7.847	53.49	81.55	111,179	96,635	86.92
BEF14	294:294	79.26	0.001	7.752	64.36	81.21	134,790	117,250	86.99
BEF15	293:294	70.97	0.002	7.396	57.99	81.71	120,904	105,761	87.48
BEF19	300:294	92.49	0.001	7.376	76.47	82.69	155,701	136,473	87.65
BEF20	299:294	82.00	0.001	7.163	67.93	82.84	138,284	121,761	88.05
AF2	294:296	74.17	0.002	6.002	62.50	84.27	125,711	113,133	89.99
AF6	293:296	64.83	0.001	6.418	54.24	83.67	110,064	98,130	89.16
AF8	300:296	83.51	0.001	6.229	70.41	84.32	140,110	125,524	89.59
AF9	299:296	88.14	0.003	5.953	74.70	84.76	148,127	133,382	90.05
AF10	298:296	65.37	0.001	6.203	54.80	83.82	110,056	98,229	89.25
AF12	296:296	80.26	0.001	6.397	67.79	84.46	135,575	121,608	89.70
AF14	297:295	88.56	0.002	7.310	73.27	82.74	149,588	131,711	88.05
AF15	296:295	69.51	0.003	7.725	57.17	82.25	117,610	102,860	87.46
AF19	294:295	64.55	0.001	7.524	52.85	81.87	109,588	95,807	87.42
AF20	293:295	80.93	0.001	7.712	66.05	81.62	137,635	119,882	87.10

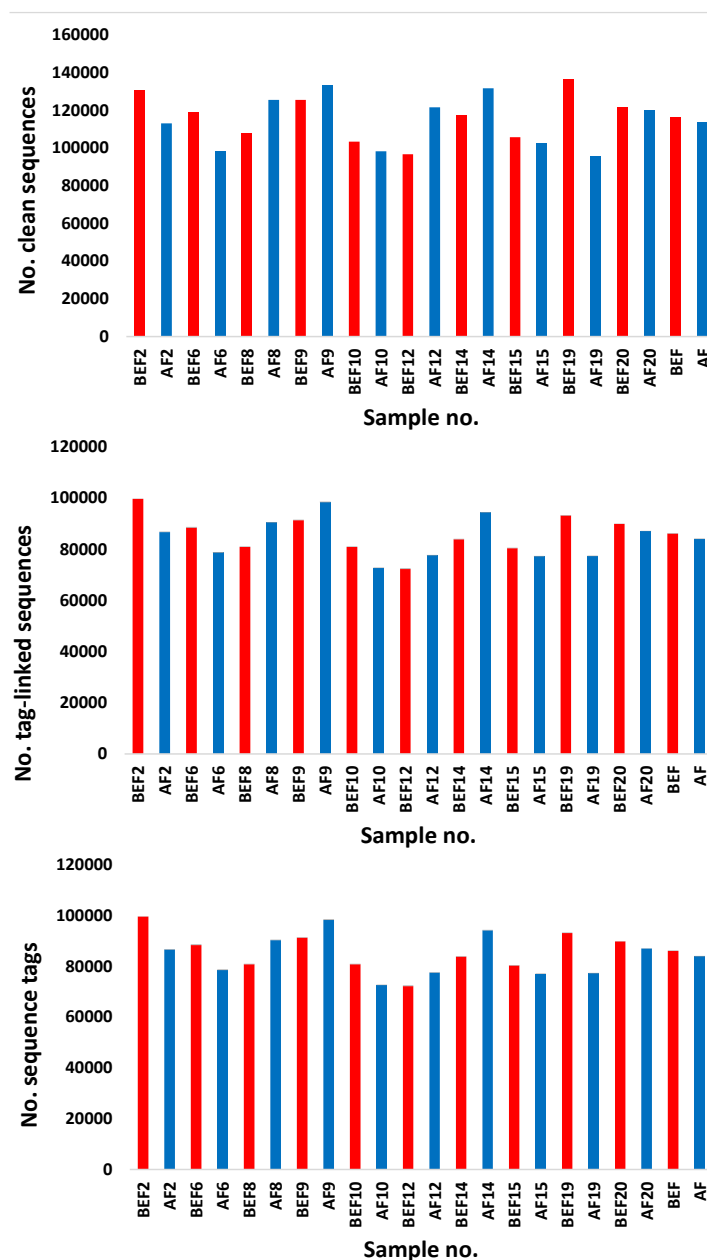


Figure 1. Comparison among numbers of clean and tagged sequences along with the recovered sequence tags at the sample and group levels before (red) and after (blue) swaking for one month to sequences statistics. BEF = before swaking, AF = after swaking

Diversity and rarefaction curve analysis

Description of observed species detected from OTU annotation is shown in *Table A1*. Alpha diversity was applied to analyze complexity of species. Shannon and Simpson indices as alpha diversity measures indicated no significance between BEF and AF groups (*Fig. 2*). Shannon and Simpson values reflect the species diversity of the community at both species' richness and evenness levels. But Shannon index comprises more weight on sequence richness, while Simpson index comprises more weight on evenness. With the same species richness, the greater the species evenness, the greater the community diversity. Alpha diversity per subject indicated increases in six subjects

due to swaking for one month in terms of Shannon measures (Fig. 2). Almost opposite results were detected in terms of Simpson measures (Fig. 2). These results indicated that the use of Miswak has resulted in increased microbial species richness and decreased species evenness. In other words, Miswak likely changed the overall structure of oral microbiome during such a short period of treatment. Alpha diversity at the group level showed much higher diversity in AF group due to swaking than BEF group (Fig. 3). However, further prolonged studies might be required to prove whether salivary bacterial diversity is related to oral hygiene status, especially after swaking, or not.

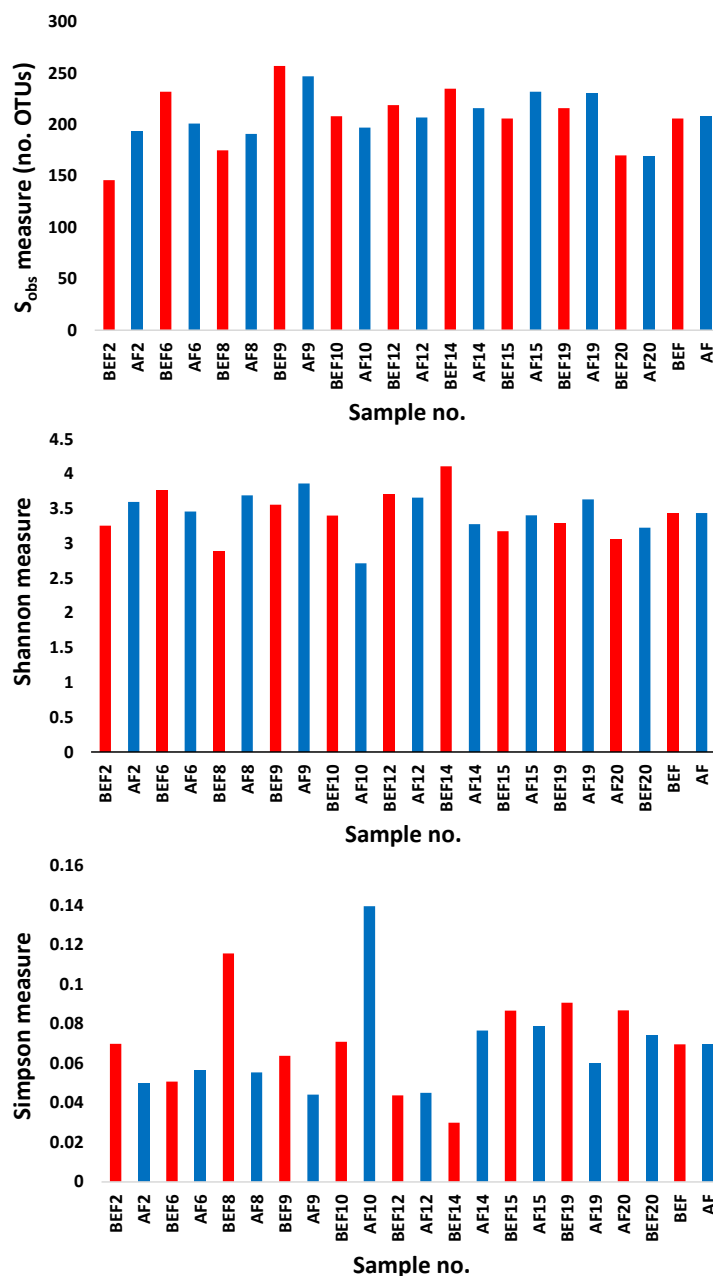


Figure 2. Alpha diversity measures at the sample and group levels before (red) and after (blue) swaking for one month to describe number of species (OTUs) per sample or group, sample or group richness (Shannon index) and evenness (Simpson index). BEF = before swaking, AF = after swaking

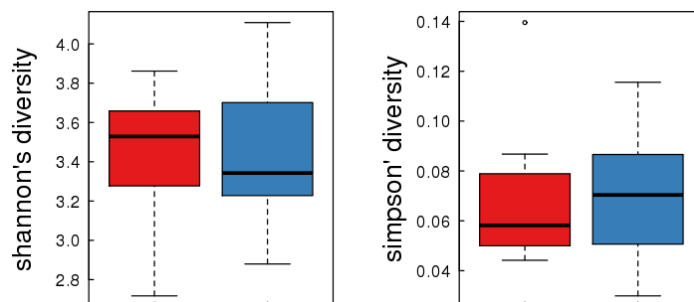


Figure 3. Alpha diversity indices as boxplots to describe richness and evenness at the group level of the samples before (red) and after (blue) swaking for one month

Rarefaction curves based on stacked number of OTUs (Fig. 4) were analyzed in order to describe the maximum depth permitted to retain all samples in the dataset for studying taxonomic relative abundance and to evaluate if produced data is enough to cover all species in the microbial community. When the curve tends to drop (Fig. 4) or no longer climbs, this indicates that the produced data is enough for further analysis. The more the curve continues to climb with increasing sequencing reads, the higher the complexity will be in samples, i.e., there will still be species uncovered by the sequencing data. The two rarefaction measures indicated that the maximum number of sequences reads to be used for further analysis of taxonomy abundance is 72,000 (Fig. 4).

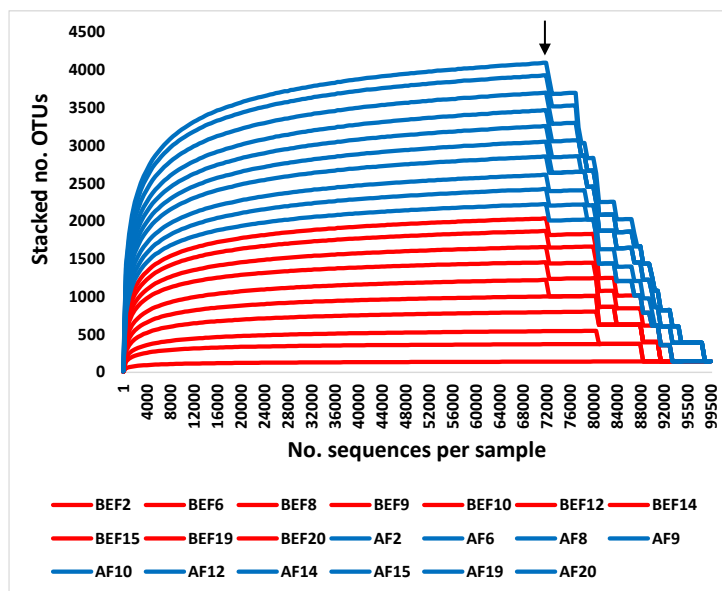


Figure 4. Stacked number of OTUs as rarefaction measures to describe the maximum depth permitted to retain all samples in the dataset for studying taxonomic relative abundance. The arrows indicate the suitable sample size for analyzing taxonomy abundance (72,000 sequence reads). BEF = before swaking, AF = after swaking

In order to display the diversity and differences of OTU composition in different samples and groups, principal coordinate analysis (PCoA) was used (Fig. 5). PCoA summarizes factors mainly responsible for this difference. When similarity is high, the two groups are closely located. Based on the OTU abundance information, the relative

abundance of each OTU in each sample and group was calculated, thus the PCoA of OTUs was plotted (Fig. 5). The PCoA plot partially showed a similar tendency in the distances within and between groups. The diversity of BEF subjects was higher towards PCoA 2 direction (PC2), while diversity of AF subjects was higher towards PCoA 1 direction (PC1). Overall, the diagram showed that mean value of BEF group was localized in the positive directions of PCoA 1 and PCoA 2 (PC1 and PC2), while that of AF group was localized in the negative directions (Fig. 5). These results indicated that the microbiome signatures of the two groups differed due to swaking, while holding relatively similar microbiome compositions as the swaking time of the experiment was short.

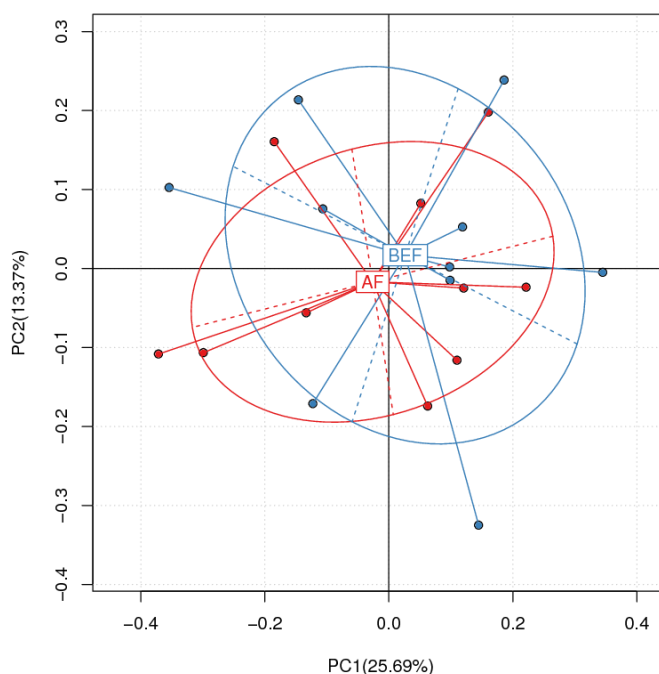


Figure 5. PCoA based on OTU abundance of different samples. Blue box indicate sample mean before swaking. Red box indicate sample mean after swaking for one month. X-axis is the first principal coordinate and Y-axis is the second. Numbers in brackets represent contributions of PCoAs to differences among samples. A dot represents each sample, and different colors represent different groups

Oral microbiomes at phylum and genus levels

Phylogenetic tree describing taxonomic groups of oral microbiomes at phylum and genus levels are shown in Figure 6. A phylogenetic tree is a branching diagram showing the inferred evolutionary relationships among various biological taxa based upon similarities and differences in their physical or genetic characteristics. The evolutionary distance between taxa is closer if the branch length is shorter. Besides the taxa composition and abundance analysis, phylogenetic tree could clarify the species evolutionary relationship further. The results indicated that the most common phyla are Actinobacteria (seven genera), Bacteroidetes (six genera), Firmicutes (28 genera), Fusobacteria (two genera), Proteobacteria (11 genera), Spirochaetes (one genus), Synergistetes (two genera) and Tenericutes (two genera) (Fig. 6).

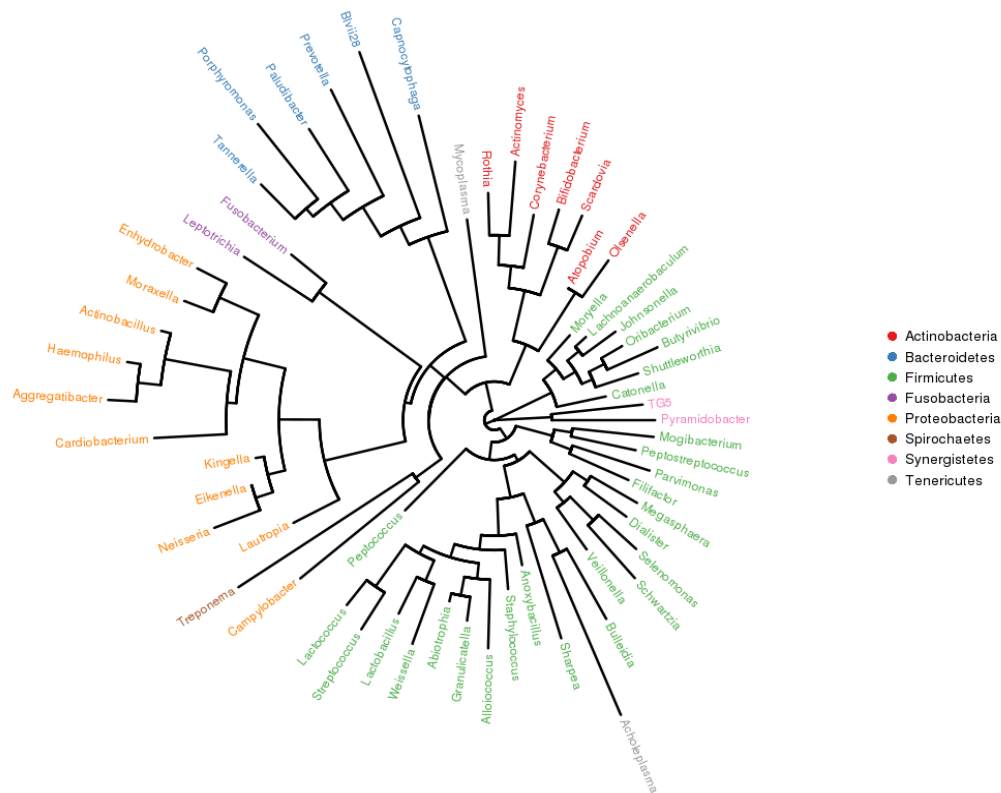


Figure 6. Genus level phylogenetic tree of oral microbiome. Genera with the same color belong to the same phylum

Abundance of individual OTUs across samples was also studied in which OTUs with number of sequences over 10,000 were considered highly abundant (Fig. 7). This criterion was met by a number of 29 out of the 219 OTUs (Table A2). These selected OTUs are OTU1-OTU12, OTU14, OTU15, OTU17, OTU20, OTU21, OTU23, OTU25-OTU29, OTU32, OTU33, OTU37, OTU40, OTU193 and OTU261. Table A2 also indicates richness of these OTUs for different samples before and after swaking. Description of these selected highly abundant OTUs in terms of taxonomy of their phyla, genera and/or species is shown in Table A3. The results of Table A3 indicate that the highly abundant OTUs belong to five of the previously mentioned phyla (e.g., Bacteroidetes, Firmicutes, Proteobacteria, Fusobacteria, Actinobacteria) in addition to the recently discovered phylum of Saccharibacteria, previously known as TM7. These results are consistent with those of log-scaled percentage heat map at the phylum level (Fig. A2). A heat map is a graphical representation of data where the individual values contained in a matrix are represented as colors.

The five highly abundant phyla included a number of 25 genera/species (Table A3). The latter include assigned and unassigned species of genera *Prevotella* and *Porphyromonas* of Bacteroidetes; *Streptococcus*, *Veillonella*, *Gemella*, *Megasphaera*, *Clostridium* and *Granulicatella* of Firmicutes; *Haemophilus*, *Campylobacter*, *Neisseria*, *Moraxella* and *Pasteurella* of Proteobacteria; *Fusobacterium* and *Leptotrichia* of Fusobacteria; while *Rothia* of Actinobacteria. However, no genus/species information is available for the new phyla Saccharibacteria TM7-3 because, up to date, these bacteria is culture-independent.

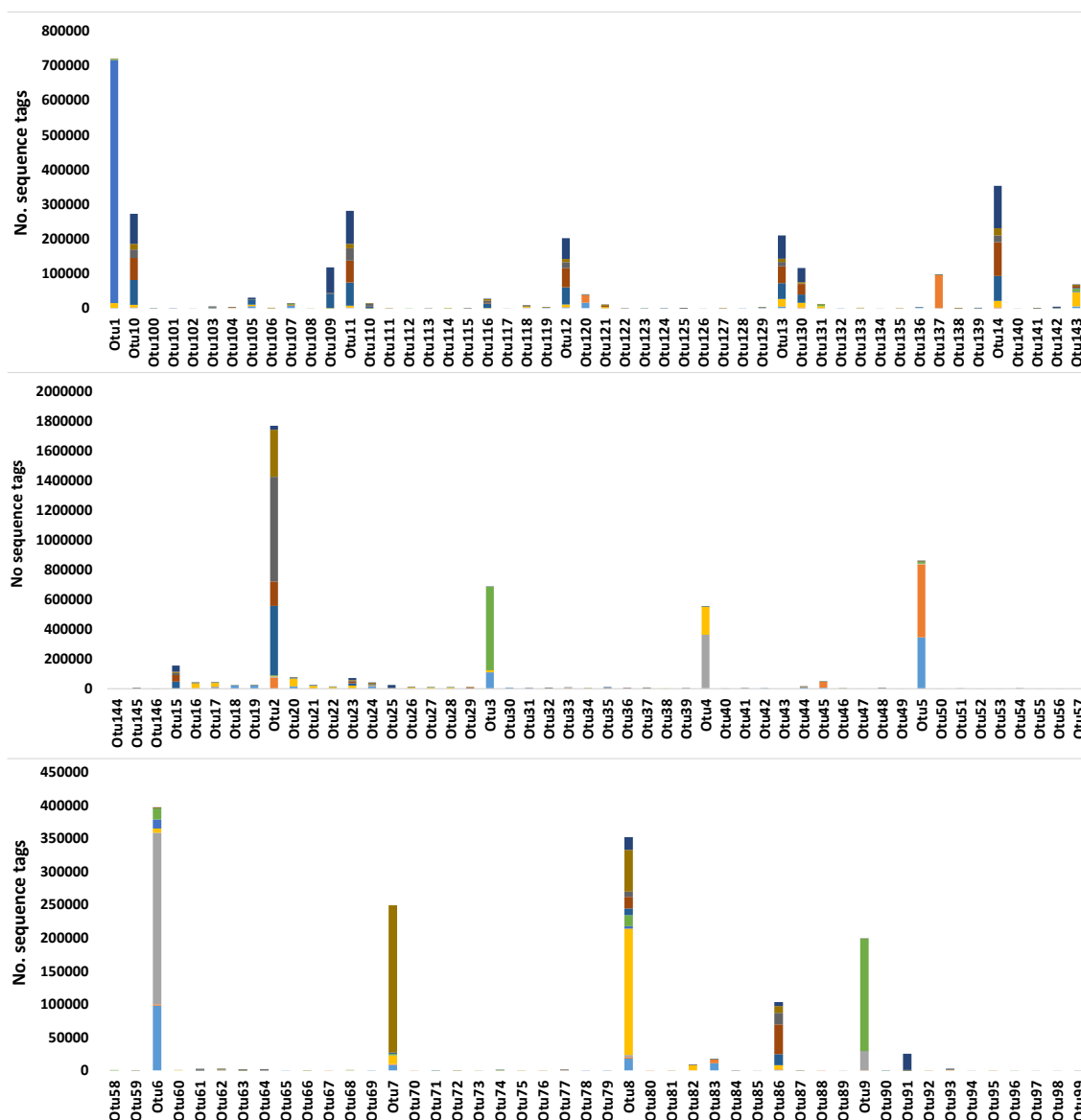


Figure 7. OTU abundances across samples

At the genus level, *Streptococcus* was the most abundant genus, with *S. infantis* as the most abundant species, followed by *Veillonella* (mainly *V. dispar*), *Prevotella* (mainly *P. melaninogenica*), *Haemophilus* (mainly *H. parainfluenzae*), *Rothia* (mainly *R. mucilaginosa*), *Neisseria* (mainly *N. subflava*), *Fusobacterium*, *Campylobacter* and the candidate phyla *Saccharibacteria* (Table A3). These results are consistent with those of log-scaled percentage heat maps at genus and species levels (Figs. A3 and A4). Many of the highly abundant genera (e.g., *Veillonella*, *Campylobacter*, *Clostridium*, *Prevotella*, etc.) and species (e.g., *V. dispar*, *H. parainfluenzae*, *R. mucilaginosa*, etc.) are opportunistic pathogens and reflect the lack or the poor hygiene that requires attention, especially at childhood period.

Venn diagram indicated the existence of 280 common OTUs in both the BEF and AF groups (Fig. 8). The number of OTUs uniquely found in BEF group was nine representing the taxonomic groups *Streptococcus* spp., *Aggregatibacter*,

Veillonellaceae, *Prevotella* spp., *Pyramidobacter piscolens*, *Bifidobacterium* spp., *Bulleidia* spp., *Prevotella* spp. and *Streptophyta* I), while two (*Actinobacillus* spp. and *Kingella* spp.) in AF group. Each of these taxonomic groups were present in only 1-2 samples, while only *Streptococcus* spp. was present in five BEF samples. However, this result was not considered affective because the number of sequences for the specific OTU of this taxa (e.g., OTU138) is as little as 72. Thus, the 11 group-specific OTUs were not considered effective in distinguishing between the two groups or in detecting the influence of swaking for one month on children oral microbiomes (Fig. 8).

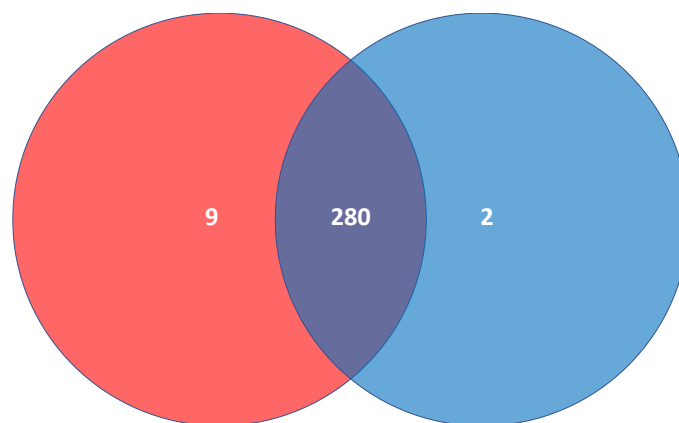


Figure 8. Venn diagram describing the unique (nine for BEF and two for AF) and shared OTUs (280) between the two groups of samples before (red) and after (blue) swaking for one month. BEF = before swaking, AF = after swaking. The nine BEF OTUs involve OTU138, OTU181, OTU224, OTU232, OTU243, OTU257, OTU260, OTU266 and OTU 267. The two AF OTUs involve OTU127 and OTU269. The other OTUs (280) are shared between the two groups with different relative abundances

Differential abundance of microbes due to swaking for one month

Differential abundance of microbes of different subjects before and after swaking for one month was studied at phylum, genus and species levels (Figs. 9, 10 and 11, respectively). Weighted Unifrac diversity distances within subjects indicated major changes in microbiome signature due to swaking in six out of the ten subjects, while unweighted Unifrac diversity distances within subjects indicated major changes in only two subjects. We did not consider the unweighted Unifrac results as the number of diverged subjects is less than four. As indicated earlier, prolonged swaking time might result in higher distances at the weighted and unweighted Unifrac levels. A number of 12 phyla, 27 genera and 21 species showed considerable changes within each subject's microbiome due to swaking (Figs. 9, 10 and 11, respectively). Overall, phyla Actinobacteria and Bacteroidetes decreased, while Firmicutes increased due to swaking for one month.

Statistical analysis for the highly abundant OTUs recovered from oral microbiomes with number of sequences over 10,000 indicated significant increases due to swaking across subjects in two OTUs referring to unassigned species of the genera *Streptococcus* and *Megasphaera* (Fig. 12; Table A4), while significant decreases in five OTUs referring to opportunistic pathogens *Veillonella dispar* and *Rothia mucilaginosa*; unassigned species of genera *Campylobacter* and *Prevotella* as well as bacteria of the family Pasteurellaceae (Fig. 13; Table A4).

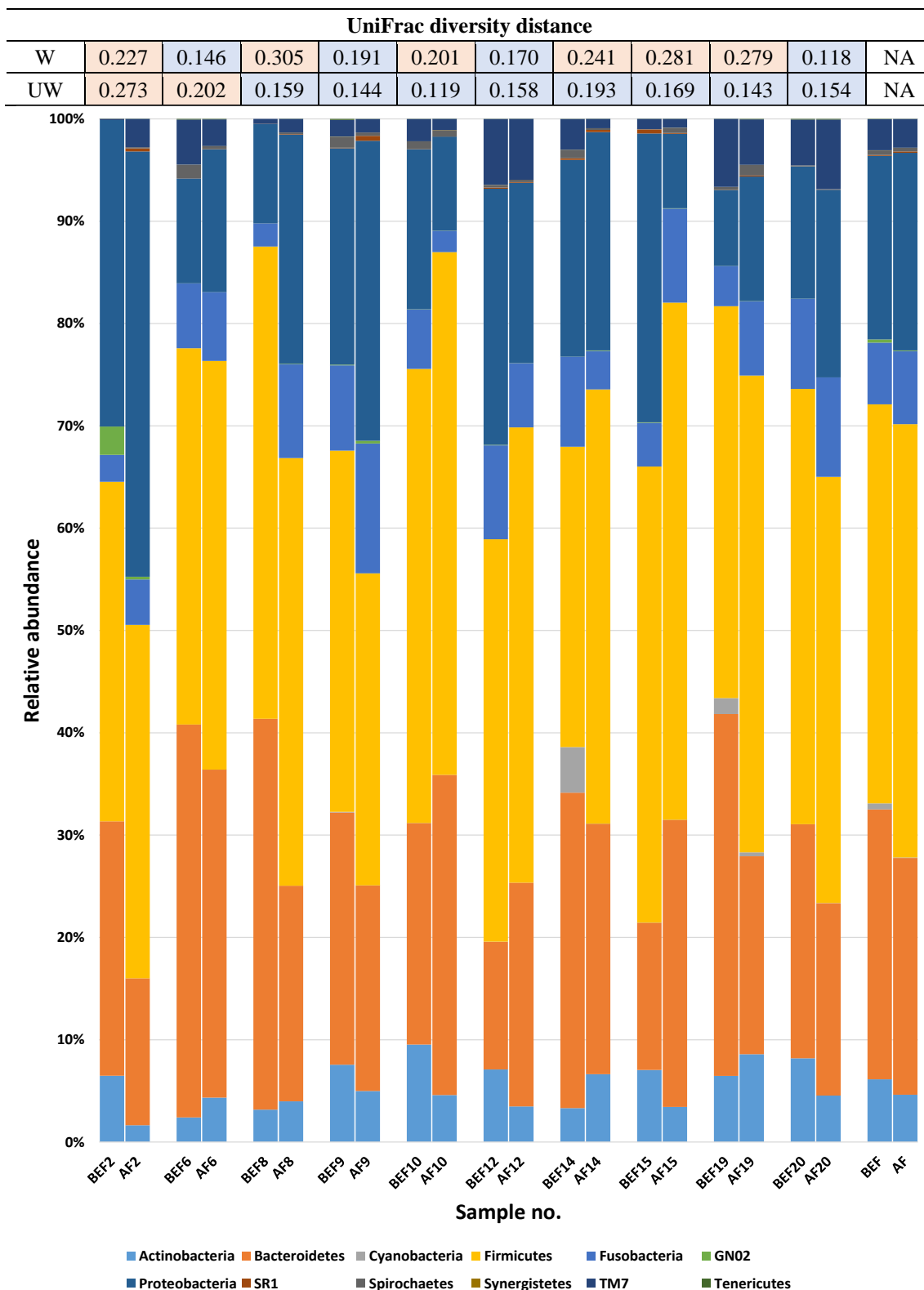


Figure 9. Relative abundance at phylum level as measured by Metastats at sample and group levels before (red) and after (blue) swaking for one month. BEF = before swaking, AF = after swaking. On top of the figure: pink box = distance of ≥ 2 , while blue box = distance of < 2 to describe both weighted_Unifrac (W) and unweighted_Unifrac (UW) diversity distances between sample pairs of each subject (before/after)

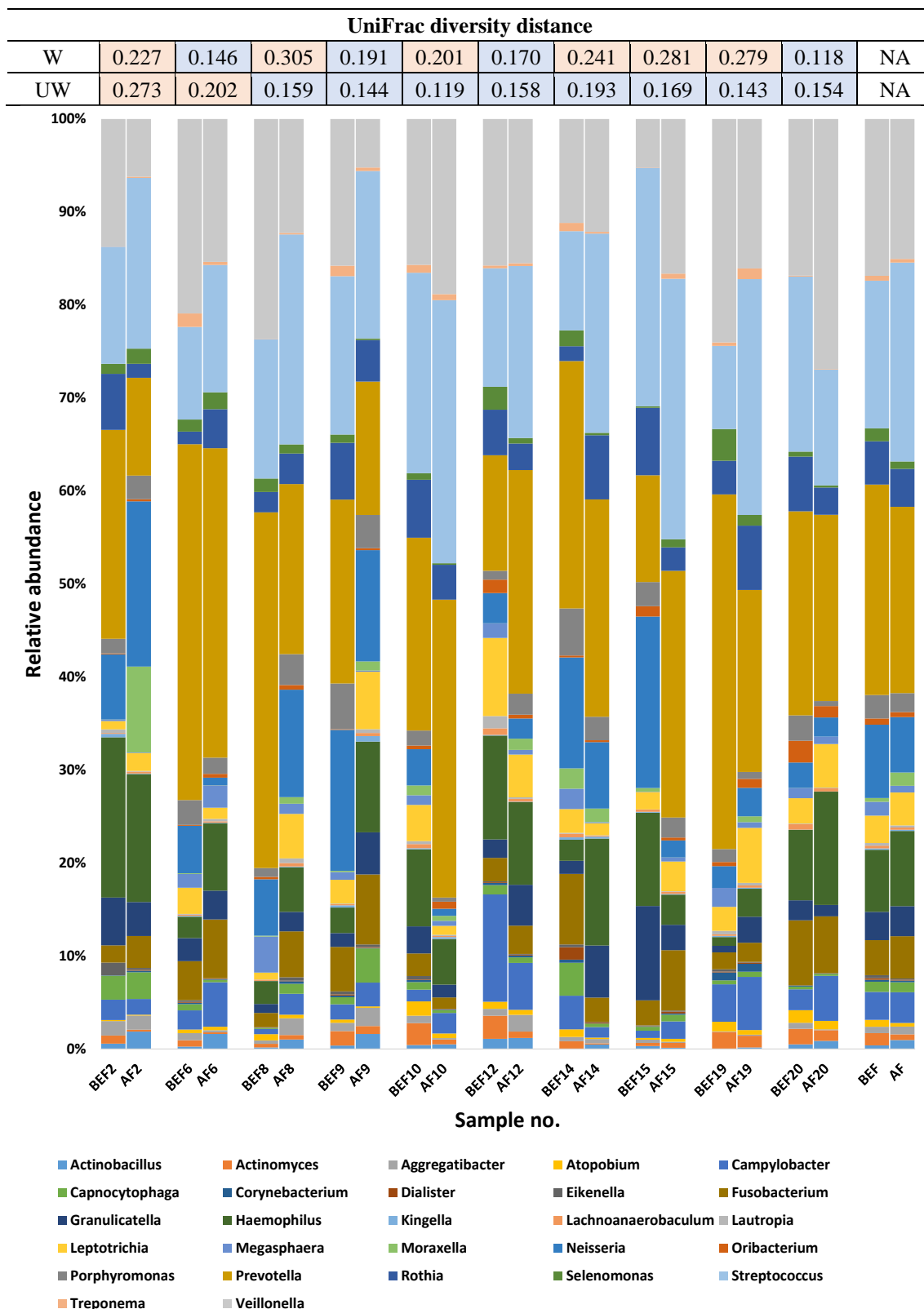


Figure 10. Relative abundance at genus level as measured by Metastats at sample and group levels before (red) and after (blue) swaking for one month. BEF = before swaking, AF = after swaking. On top of the figure: pink box = distance of ≥ 2 , while blue box = distance of < 2 to describe both weighted_Unifrac (W) and unweighted_Unifrac (UW) diversity distances between sample pairs of each subject (before/after)

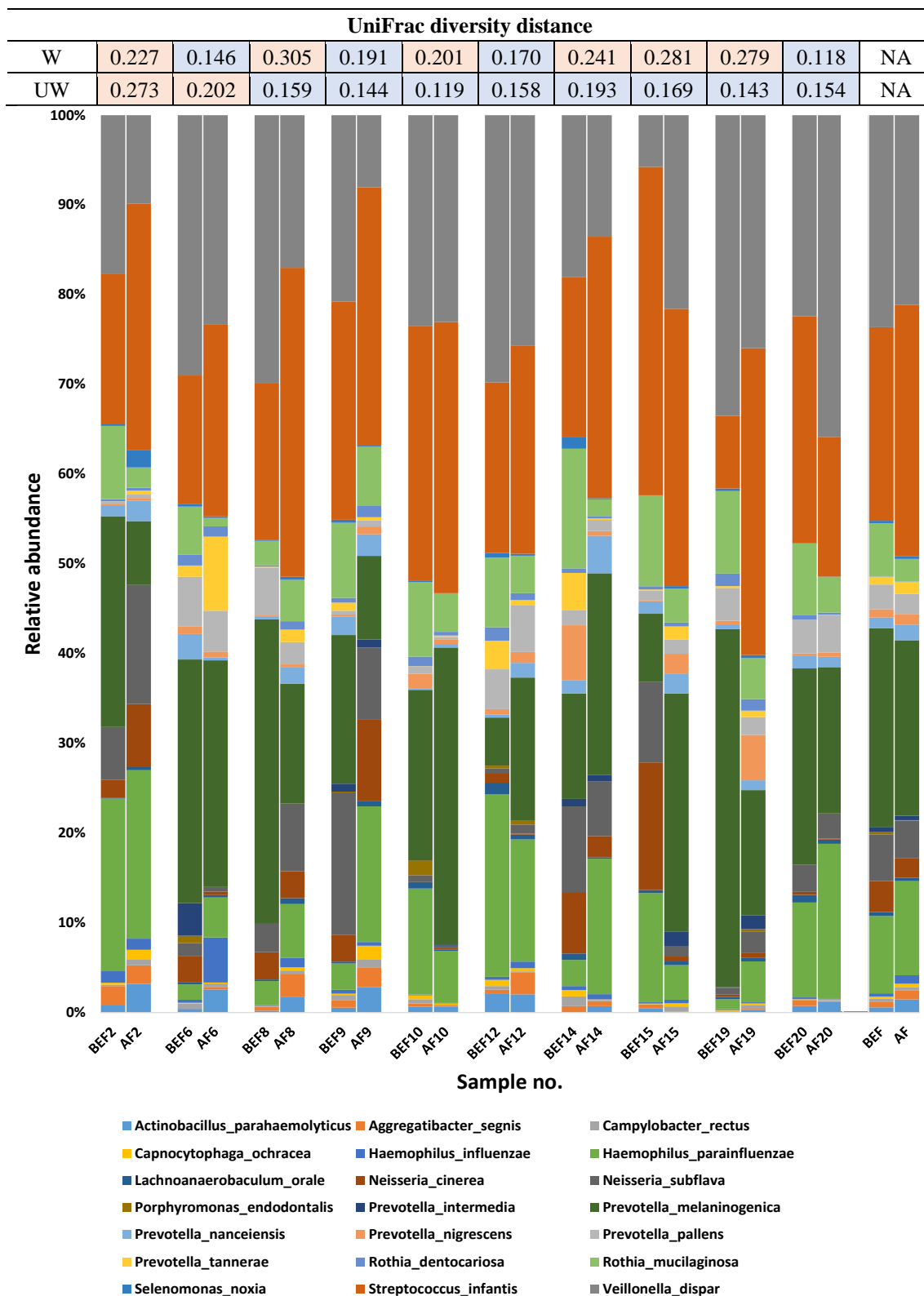


Figure 11. Relative abundance at species level as measured by Metastats at sample and group levels before (red) and after (blue) swaking for one month. BEF = before swaking, AF = after swaking. On top of the figure: pink box = distance of ≥ 2 , while blue box = distance of < 2 to describe both weighted_Unifrac (W) and unweighted_Unifrac (UW) diversity distances between sample pairs of each subject (before/after)

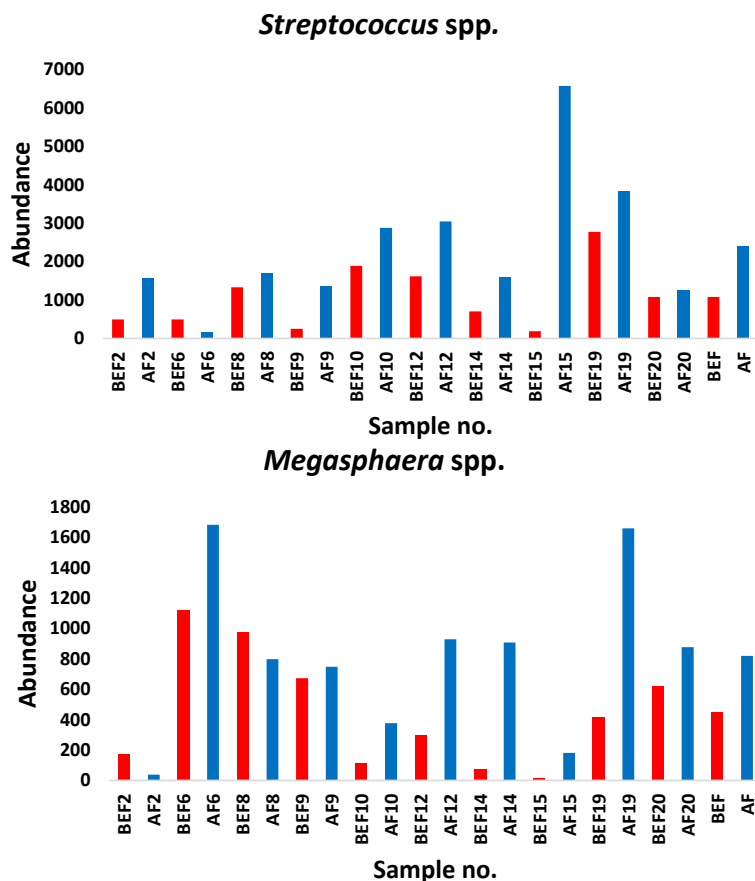


Figure 12. High abundance of the unassigned species of the genera *Streptococcus* and *Megasphaera* due to swaking for one month. BEF = before swaking, AF = after swaking

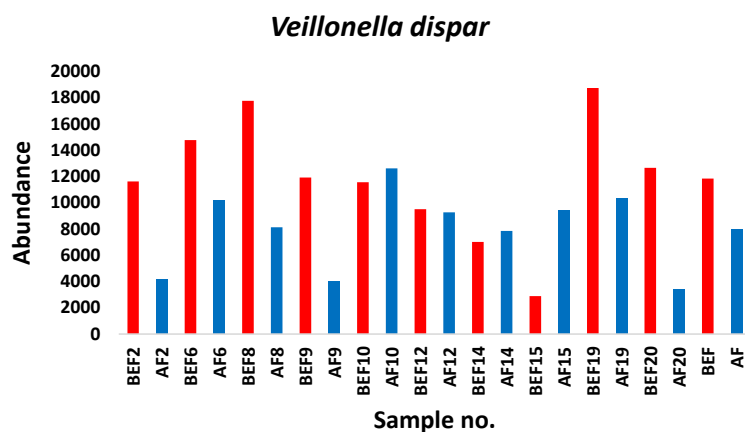


Figure 13. Low abundance of *Veillonella dispar*, *Rothia mucilaginosa*, unassigned species of the genera *Campylobacter* and *Prevotella* as well as bacteria of the family *Pasteurellaceae* due to swaking for one month. BEF = before swaking, AF = after swaking

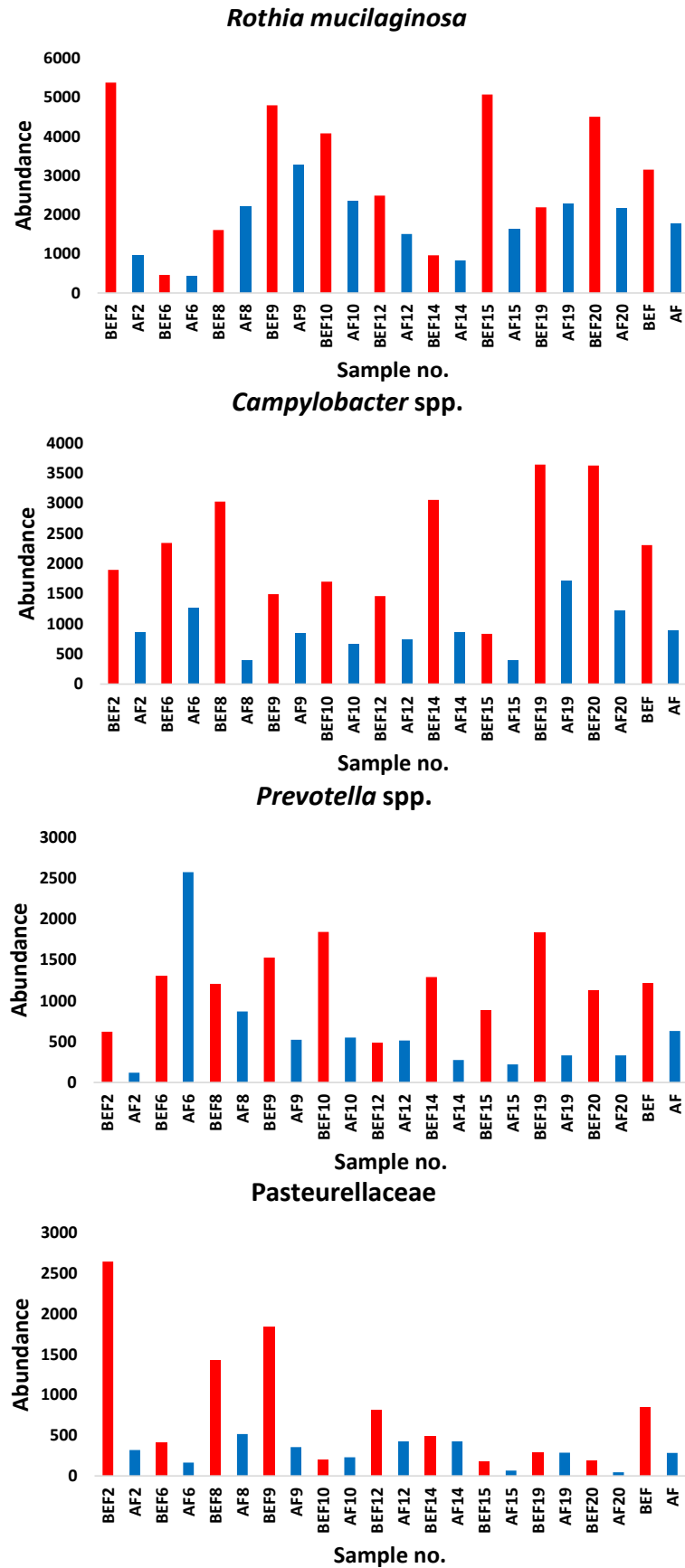


Figure 13. Continued

Discussion

Oral microbiome significantly affects overall human health. It is organized as biofilms adapted to every niche within the mouth. This biofilm has fundamental role in host homeostasis and the protection against oral as well as gastric diseases (Ismail et al., 2009; Kau et al., 2011).

Normally, the predominant bacteria in saliva are commensal and produce enzymes that degrade biofilm matrix polymers (Kaplan et al., 2004; Pereira et al., 2012). Poor oral hygiene can lead to changes in microbial communities and cause dental caries and periodontal disease that seriously affect children's overall life-time health status (Marsh, 2003; Prabhu and John, 2015). Therefore, it is important to detect oral microbe biofilm signatures in healthy and infected individuals in order to develop preventive strategies, especially in childhood.

Earlier studies indicated that changes in the microbiome balance might have a more important influence on human health than a distinct microbial species being either beneficial or harmful (Huttenhower et al., 2012; Methé et al., 2012). Although a distinct microbial species might not be harmful in its primary habitat, oral bacteria were linked with systemic, life-threatening disorders including cardiovascular disease, pneumonia and stroke (Awano et al., 2008; Beck and Offenbacher, 2005; Joshipura et al., 2003; Joshipura et al., 1996; Seymour et al., 2007). Oral microbiome is highly divergent among healthy individuals as several ecological factors, such as life style, external environment and oral hygiene, can contribute to this divergence (Aas et al., 2005; Crielaard et al., 2011; Nasidze et al., 2009; Sheiham and Watt, 2000).

Other factors include ethnic background and/or geographic origin (Aas et al., 2005; Ledder et al., 2007). Several bacterial genera/phyla fundamentally influence human oral health/disease status. They include *Actinomyces* spp., *Gemella* spp., *Granulicatella* spp., *Veillonella* spp., *Haemophilus* spp., *Capnocytophaga* spp. and TM7. For example, early colonization of *Veillonella* spp. and existence of phylum TM7 guide the development of microbial biofilm communities (Liu et al., 2012; Periasamy and Kolenbrander, 2010). As indicated earlier, *Streptococcus* was the most abundant genus in oral microbiomes of Saudi children regardless of swabbing, followed by *Veillonella*, *Prevotella*, *Haemophilus*, *Fusobacterium*, *Rothia*, *Neisseria*, *Campylobacter* and the candidate phyla *Saccharibacteria* (Table A3). These results are consistent with previous studies of the human oral microbiota (Hoffman et al., 2018; Li et al., 2014; Takeshita et al., 2014).

High-throughput technologies are employed in detecting microbiome signatures in different human organs and their relation to disease risk/status (Gao et al., 2017; Jo et al., 2017). The approach of the cost-efficient, high-throughput characterization of the human microbiome relies on the use of microbial 16S ribosomal RNA (rRNA) gene sequence that is considered as a barcode of microbes, either culturable or unculturable (Ahn et al., 2011). Such an approach can provide new insights into the diversity, normal microbial signatures and health/disease status (Ahn et al., 2011; Belda-Ferre et al., 2012; Johansson et al., 2016; Zhou et al., 2016). Many problems raised when isolating oral bacterial DNA from clinical samples due to the lack of bacterial lysis uniformity, particularly for gram-positive bacteria (Lazarevic et al., 2013). Although Vesty et al. (2017) recommended the use of enzymatic lysis method for bacterial DNA extractions, Garbieri et al. (2017) recommended the use of commercial DNA extraction kits. We have chosen the QIAamp® DNA Mini kit (Qiagen®51306; Hilden, North Rhine-Westphalia, Germany) for extracting high-quality oral bacterial DNA. Another problem lies in the choice of the hypervariable regions (V1-V9) of 16S rRNA gene that

adequately and reliably detect oral microbiome signatures. Besides, it is important to know the proper database for OTU annotation. Based on the most recent studies, the 16S V3-V4 regions of oral microbiome were successful in detecting accurate microbial signatures and in providing adequate phylogenetic assignment (Furquim et al., 2017; Jiang et al., 2016; Yu et al., 2017). Accordingly, we have chosen the V3-V4 regions for analyzing the oral microbiome. We have also chosen the Greengenes (version 13_8: 16S rDNA database, http://qiime.org/home_static/dataFiles.html) (DeSantis et al., 2006) and Human Oral Microbiome (HOMD, <http://www.homd.org/>) (Chen et al., 2010) databases for annotating the oral microbiome sequences.

The influence of swaking on human oral microbiome was statistically studied on the 29 most abundant families, genera and species (Table A4). The overall results indicated that swaking for one month resulted in the significant increases of the unassigned species of the genera *Streptococcus* and *Megasphaera* (Table A4; Fig. 12) and the significant decreases of the bacteria *Veillonella dispar* and *Rothia mucilaginosa*; unassigned species of genera *Campylobacter* and *Prevotella* as well as bacteria of the family Pasteurellaceae (Table A4; Fig. 13). Although extremely abundant, *Streptococcus anginosus* (previously known as *Streptococcus milleri*) and *S. infantis* showed insignificant increases due to swaking (Table A4). Otherwise, the influence of Miswak was arbitrary for the other highly abundant taxa with no specific pattern across subjects. Although it resides in normal flora of the oral cavity, *Streptococcus anginosus* is considered as a strong virulent pathogen (Liu et al., 2018). It is speculated that the significant increase of oral *Streptococcus anginosus* associates with periodontal diseases (Drucker and Green, 1977; Liu et al., 2018; Rawlinson et al., 1993). *Streptococcus infantis* is a microbe that lives mainly in the human upper respiratory tract and usually found in healthy mouths, e.g., free of or are at low risk of tooth decay (<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2444020/>, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3472979/>).

Miller et al. (2017) showed an increased evidence that the commensal acetaldehydogenic microorganism *Rothia mucilaginosa* can be an opportunistic pathogen in immune-compromised hosts, while Amer et al. (2017) indicated that the increased abundance of *R. mucilaginosa* is linked to oral leukoplakias from lingual sites. Elevated levels of *R. mucilaginosa* was also shown to be associated with human endocarditis, meningitis and peritonitis (<https://en.wikipedia.org/wiki/Rothia>). Amer et al. (2017) also indicated that the increased levels of *Campylobacter concisus* is associated with severe dysplasia. *Campylobacter* spp. is the main cause of food poisoning (campylobacteriosis) affecting up to 1% of the world (<https://en.wikipedia.org/wiki/Campylobacter>). Recently, Liu et al. (2018) detected high abundance of *Prevotella intermedia* in saliva of gout and hyperuricemia (HUA) patients and suggested that abundance of this bacterial species is linked with gum inflammation and the progression of periodontitis. This conclusion complements a previous one made by Kamma et al. (1994). As for the bacteria of the family Pasteurellaceae, it was recently reported to be associated with caries in young children living in a rural province in China (Xu et al., 2018). The significant decreases of *Rothia mucilaginosa*, *Campylobacter* spp., *Prevotella* and Pasteurellaceae due to swaking in the present study (Fig. 13) can be considered as biomarkers of good hygiene in children.

Most of the normal commensal oral bacteria benefit the host through the production of various metabolites. They mostly include short chain fatty acids, vitamins, co-factors and other metabolites (Nallabelli et al., 2016). However, there is a strong reverse action

of the two colonizers, e.g., *Streptococcus* and *Veillonella* in oral cavity bacteria (Hoffman et al., 2018). Mashima et al. (2017) indicated that unassigned species of *Streptococcus* spp. decreased, while *Veillonella dispar*, *V. parvula* and unassigned species of *Veillonella* spp. increased with poor oral hygiene status. *Streptococcus*, a facultative anaerobe and an initial colonizer, is able to catabolize (and transport) carbohydrates to short-chain organic acids, e.g., lactic acid and pyruvic acid (Cotter and Hill, 2003). On the other hand, *Veillonella*, an obligate anaerobe and subsequent colonizer, is unable to catabolize sugars, thus, it relies mainly on the fermentation of organic acids to propionic and acetic acids, carbon dioxide, and hydrogen (Delwiche et al., 1985). It is reported that oral *Veillonella* depends on organic acids produced by oral *Streptococcus* (Mashima and Nakazawa, 2014; Palmer et al., 2006). The proximity of the producer/consumer shuttle is important in such metabolite transfers (Mashima and Nakazawa, 2014; Palmer et al., 2006). Organic acids participate mainly in biofilm formation due to the action of *Streptococcus* (Periasamy and Kolenbrander, 2010). Anaerobic environment promotes growth of *Veillonella* due to the prior growth of aerobic and facultative organisms that results in an increased plaque thickness to yield conditions suitable for anaerobic growth (Ritz, 1967). The growth of *Streptococcus* may be inhibited by anaerobic conditions that favor the growth of *Veillonella*. Therefore, the high abundance of *Veillonella* in salivary microbiome is considered as a biomarker of poor oral hygiene and occurrence of caries in children (Fig. 14). Pharmacological studies indicated that Miswak possesses anti-microbial, anti-plaque and anti-inflammatory activities that makes it useful in defeating plaque (Lebda et al., 2018). The latter potential activities align with our results in terms of the decrease of *Veillonella* spp., the bacteria that favors the accumulation of plaque. *Veillonella* was also found in higher proportion in caries or periodontal subjects (Zhang et al., 2015; Zhou et al., 2016).

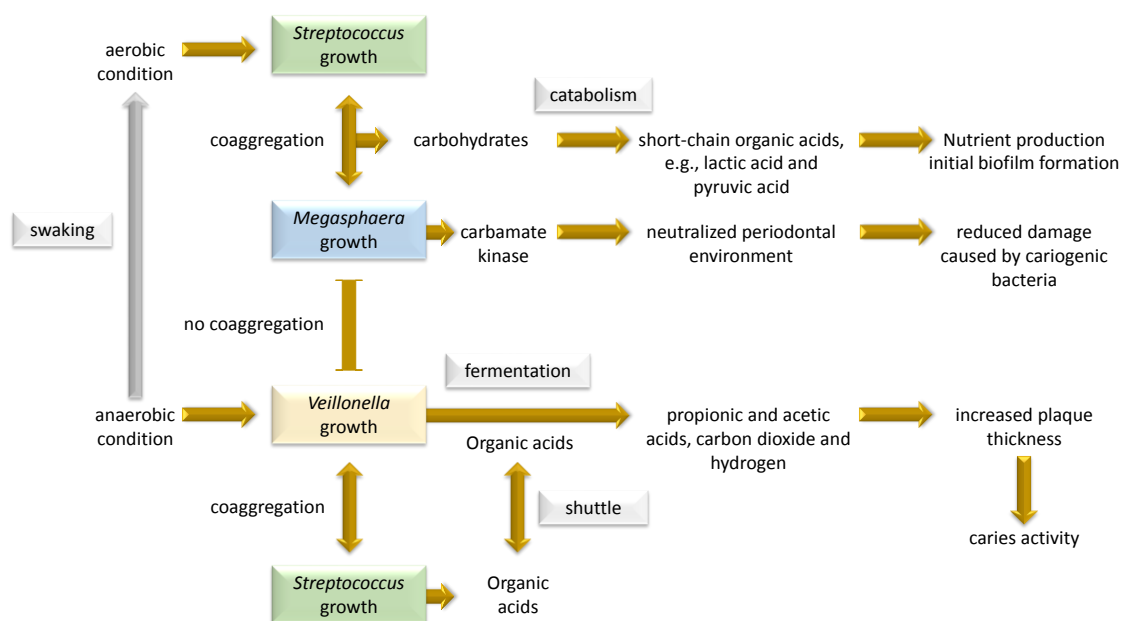


Figure 14. Roles of the three genera *Streptococcus*, *Veillonella* and *Megasphaera* and possible influence of swaking on oral hygiene

Conclusion

In general, we claim that swaking hinders the anaerobic condition required for *Veillonella* growth, thus favors the aerobic condition that promote *Streptococcus* growth. Our results also indicate parallel significant increases of the unassigned species of the genera *Streptococcus* and *Megasphaera* (Fig. 12; Table A4). Interestingly, *Megasphaera* spp. strain DISK18, a nonpathogenic species, was recently reported to coaggregate with *Streptococcus* during oral early colonization but not with *Veillonella* (Nallabelli et al., 2016). It is speculated that genera *Megasphaera* and *Veillonella* compete for coaggregating with *Streptococcus* due to homology of surface receptors of these two genera (Kreth et al., 2009; Nallabelli et al., 2016). Whole genome sequencing of the *Megasphaera* strain showed a lack of virulence genes, that are associated with oral pathogenesis, or genes encoding collagenase or gelatinase. The latter indicates that *Megasphaera* unlikely participates in periodontal disease. Instead, this bacteria harbors carbamate kinase that participate in neutralizing the periodontal environment, thus, reduce the damage caused by cariogenic bacteria (Fig. 14). In summary, we consider Miswak as an excellent natural toothpaste with differential influence on oral microbiome that makes it feasibly useful for maintaining good oral hygiene, especially for children. Future studies on the new selected species are still required in order to support the results of the present research.

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APPENDIX

OTU Rank Curve

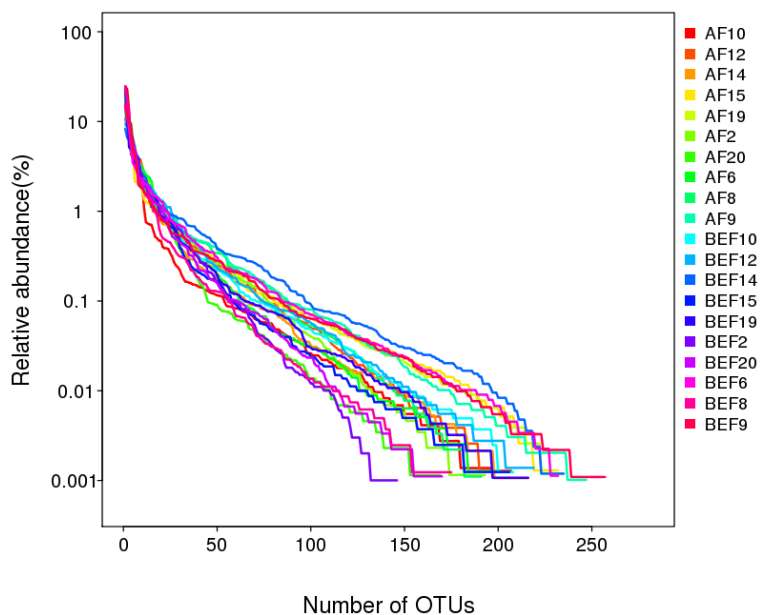


Figure A1. Number and relative abundance of OTUs of different samples. BEF = before swaking, AF = after swaking

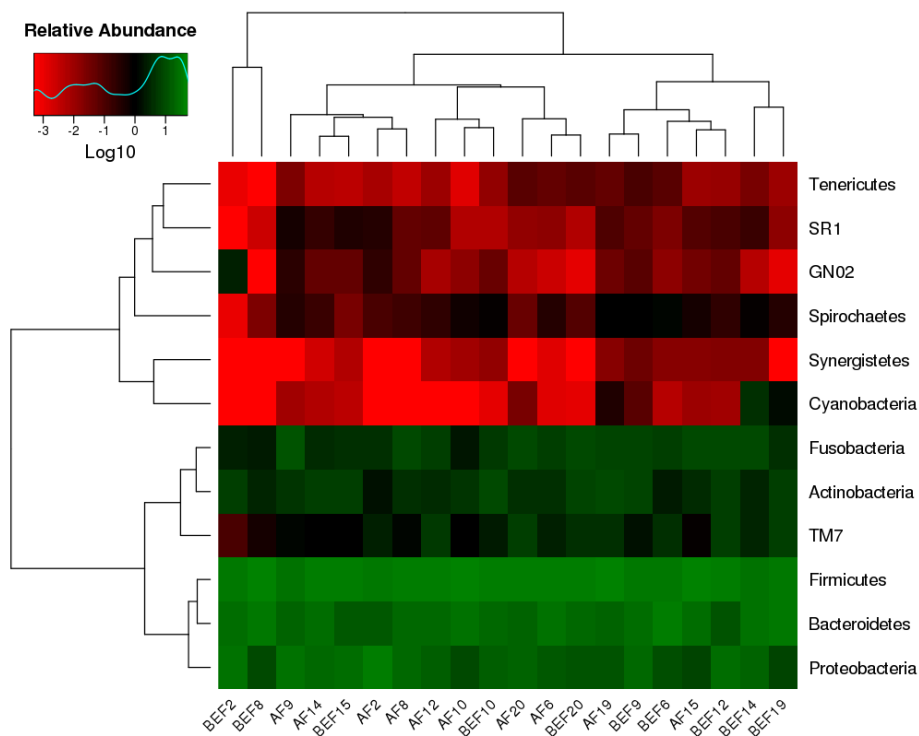


Figure A2. Log-scaled percentage heat map at phylum level. BEF = before swaking, AF = after swaking

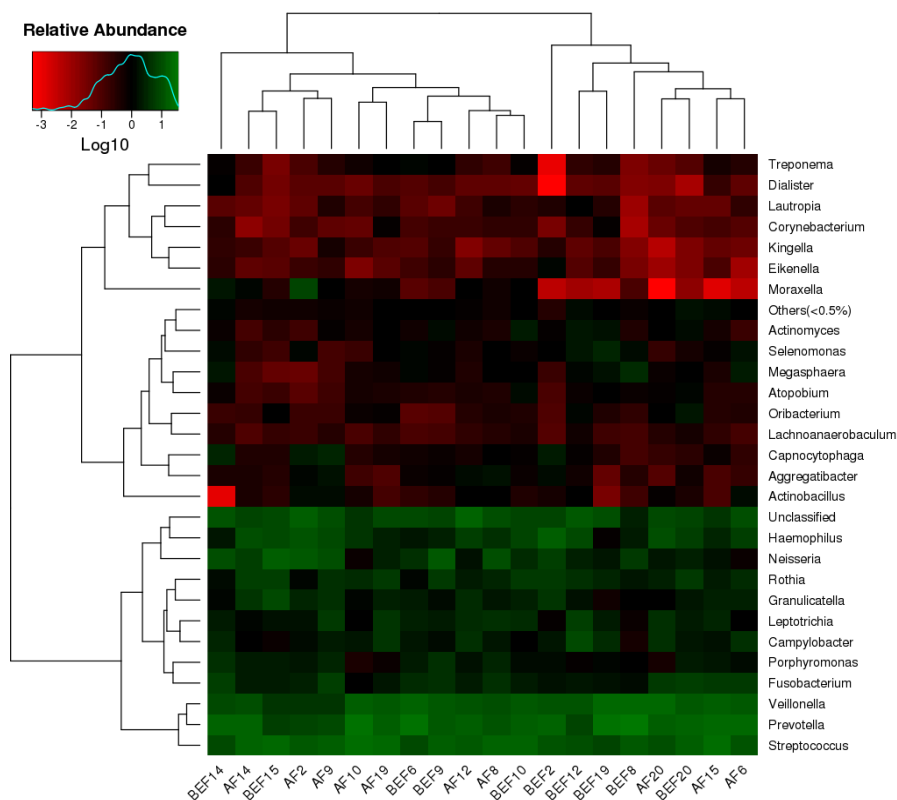


Figure A3. Log-scaled percentage heat map at genus level. BEF = before swaking, AF = after swaking

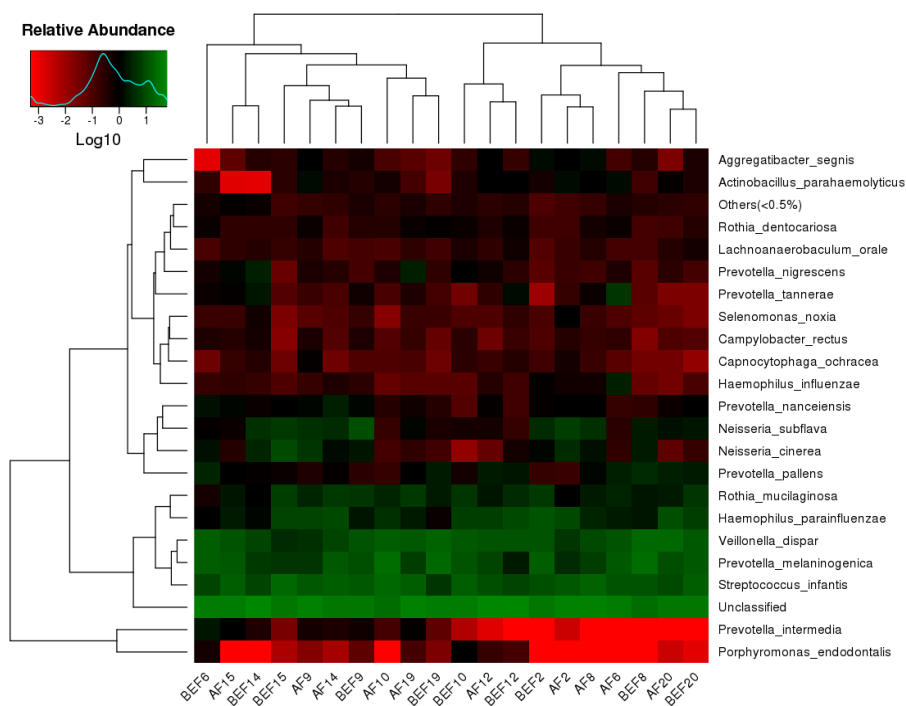


Figure A4. Log-scaled percentage heat map at species level. BEF = before swaking, AF = after swaking

Table A1. Description of observed species detected from OTU annotation across subjects and swaking

OTU no.	OTU abundance	Taxonomy
Otu2	235993	Bacteria; Firmicutes; Bacilli; Lactobacillales; Streptococcaceae; Streptococcus; Streptococcus_infantis
Otu1	195394	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella; Prevotella_melaninogenica
Otu3	163677	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Veillonella; Veillonella_dispar
Otu4	92051	Bacteria; Proteobacteria; Gammaproteobacteria; Pasteurellales; Pasteurellaceae; Haemophilus; Haemophilus_parainfluenzae
Otu11	58531	Bacteria; Fusobacteria; Fusobacteriia; Fusobacteriales; Fusobacteriaceae; Fusobacterium
Otu8	58093	Bacteria; Actinobacteria; Actinobacteria; Actinomycetales; Micrococcaceae; Rothia; Rothia_mucilaginoso
Otu32	50183	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Veillonella; Veillonella_dispar
Otu14	46624	Bacteria; Firmicutes; Bacilli; Gemellales; Gemellaceae
Otu6	40872	Bacteria; Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae; Neisseria; Neisseria_subflava
Otu5	39815	Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacteriales; Campylobacteraceae; Campylobacter
Otu12	34029	Bacteria; Firmicutes; Bacilli; Lactobacillales; Streptococcaceae; Streptococcus
Otu10	33068	Bacteria; TM7; TM7-3
Otu7	32641	Bacteria; Firmicutes; Bacilli; Lactobacillales; Carnobacteriaceae; Granulicatella
Otu29	29782	Bacteria; Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae; Neisseria
Otu261	27911	Bacteria; Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae; Neisseria; Neisseria_cinerea
Otu17	23783	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella; Prevotella_pallens
Otu15	22697	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu25	20994	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Porphyromonadaceae; Porphyromonas
Otu21	18426	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Paraprevotellaceae; Prevotella
Otu20	17155	Bacteria; Fusobacteria; Fusobacteriia; Fusobacteriales; Leptotrichiaceae; Leptotrichia
Otu37	14672	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Megasphaera
Otu27	13910	Bacteria; Firmicutes; Bacilli; Lactobacillales; Carnobacteriaceae; Granulicatella
Otu26	13468	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella; Prevotella_nanceiensis
Otu9	13242	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Moraxellaceae; Moraxella
Otu40	13051	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Veillonella
Otu33	12906	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Paraprevotellaceae; Prevotella
Otu193	12432	Bacteria; Proteobacteria; Gammaproteobacteria; Pasteurellales; Pasteurellaceae
Otu23	11707	Bacteria; Firmicutes; Clostridia; Clostridiales
Otu28	10024	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Paraprevotellaceae; Prevotella; Prevotella_tanneriae
Otu109	9878	Bacteria; Proteobacteria; Gammaproteobacteria; Pasteurellales; Pasteurellaceae; Haemophilus
Otu49	9750	Bacteria; Proteobacteria; Gammaproteobacteria; Pasteurellales; Pasteurellaceae; Actinobacillus; Actinobacillus_parahaeolyticus
Otu18	9704	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella; Prevotella_nigrescens

OTU no.	OTU abundance	Taxonomy
Otu55	8814	Bacteria; Fusobacteria; Fusobacteriia; Fusobacteriales; Leptotrichiaceae; Leptotrichia
Otu24	8592	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Oribacterium
Otu38	8380	Bacteria; Actinobacteria; Coriobacteriia; Coriobacteriales; Coriobacteriaceae; Atopobium
Otu42	8302	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Selenomonas
Otu68	8159	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu53	8127	Bacteria; Proteobacteria; Gammaproteobacteria; Pasteurellales; Pasteurellaceae; Aggregatibacter; Aggregatibacter_segnsi
Otu245	7056	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Veillonella
Otu51	6869	Bacteria; Actinobacteria; Actinobacteria; Actinomycetales; Micrococcaceae; Rothia; Rothia_dentocariosa
Otu41	6741	Bacteria; Firmicutes; Clostridia; Clostridiales; Mogibacteriaceae
Otu50	6255	Bacteria; Actinobacteria; Actinobacteria; Actinomycetales; Actinomycetaceae; Actinomyces
Otu19	6183	Bacteria; Proteobacteria; Gammaproteobacteria; Pasteurellales; Pasteurellaceae; Haemophilus; Haemophilus_influenzae
Otu39	6124	Bacteria; TM7; TM7-3; CW040
Otu277	5959	Bacteria; Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae; Neisseria; Neisseria_subflava
Otu65	5820	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Porphyromonadaceae; Porphyromonas
Otu31	5140	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu35	5103	Bacteria; Fusobacteria; Fusobacteriia; Fusobacteriales; Leptotrichiaceae; Leptotrichia
Otu22	5023	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella; Prevotella_intermedia
Otu13	4905	Bacteria; Cyanobacteria; Chloroplast; Streptophyta
Otu88	4889	Bacteria; Fusobacteria; Fusobacteriia; Fusobacteriales; Leptotrichiaceae; Leptotrichia
Otu155	4814	Bacteria; Fusobacteria; Fusobacteriia; Fusobacteriales; Leptotrichiaceae; Leptotrichia
Otu61	4797	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu70	4788	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Porphyromonadaceae; Porphyromonas
Otu34	4397	Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Capnocytophaga
Otu52	4247	Bacteria; Firmicutes; Clostridia; Clostridiales
Otu66	4201	Bacteria; TM7; TM7-3; CW040; F16
Otu76	4044	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Lachnoanaerobaculum; Lachnoanaerobaculum_orale
Otu69	3903	Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Capnocytophaga
Otu57	3763	Bacteria; TM7; TM7-3
Otu36	3476	Bacteria; Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae; Eikenella
Otu48	3440	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Burkholderiaceae; Lautropia
Otu16	3440	Bacteria; GN02; BD1-5
Otu64	3415	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Paraprevotellaceae; Prevotella
Otu84	3406	Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacteriales; Campylobacteraceae; Campylobacter; Campylobacter_rectus
Otu47	3381	Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; Weeksellaceae

OTU no.	OTU abundance	Taxonomy
Otu93	3367	Bacteria; Proteobacteria; Gammaproteobacteria; Pasteurellales; Pasteurellaceae; Aggregatibacter
Otu126	3331	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu87	3074	Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Capnocytophaga
Otu77	2993	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Selenomonas; Selenomonas_noxia
Otu271	2986	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella; Prevotella_melaninogenica
Otu75	2939	Bacteria; Actinobacteria; Actinobacteria; Actinomycetales; Corynebacteriaceae; Corynebacterium
Otu98	2827	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu59	2750	Bacteria; Actinobacteria; Actinobacteria; Actinomycetales; Actinomycetaceae; Actinomyces
Otu80	2727	Bacteria; Fusobacteria; Fusobacteriia; Fusobacteriales; Leptotrichiaceae; Leptotrichia
Otu86	2462	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu81	2433	Bacteria; Actinobacteria; Actinobacteria; Actinomycetales; Actinomycetaceae; Actinomyces
Otu206	2425	Bacteria; Actinobacteria; Actinobacteria; Actinomycetales; Actinomycetaceae; Actinomyces
Otu137	2241	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Selenomonas
Otu62	2126	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Catonella
Otu67	2056	Bacteria; Fusobacteria; Fusobacteriia; Fusobacteriales; Leptotrichiaceae; Leptotrichia
Otu71	2001	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu45	1972	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella; Prevotella_melaninogenica
Otu280	1908	Bacteria; Proteobacteria; Gammaproteobacteria; Pasteurellales; Pasteurellaceae; Haemophilus
Otu274	1904	Bacteria; Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae
Otu108	1902	Bacteria; Fusobacteria; Fusobacteriia; Fusobacteriales; Fusobacteriaceae; Fusobacterium
Otu117	1867	Bacteria; Spirochaetes; Spirochaetes; Spirochaetales; Spirochaetaceae; Treponema
Otu90	1864	Bacteria; Firmicutes; Erysipelotrichi; Erysipelotrichales; Erysipelotrichaceae; Bulleidia; Bulleidia_moorei
Otu72	1834	Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; Weeksellaceae
Otu100	1827	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu78	1823	Bacteria; SR1
Otu146	1794	Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacteriales; Campylobacteraceae; Campylobacter
Otu44	1788	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
Otu46	1702	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Porphyromonadaceae; Porphyromonas; Porphyromonas_endodontalis
Otu105	1665	Bacteria; Fusobacteria; Fusobacteriia; Fusobacteriales; Leptotrichiaceae; Leptotrichia
Otu58	1645	Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Capnocytophaga; Capnocytophaga_ochracea
Otu30	1638	Bacteria; Fusobacteria; Fusobacteriia; Fusobacteriales; Leptotrichiaceae
Otu79	1577	Bacteria; Fusobacteria; Fusobacteriia; Fusobacteriales; Leptotrichiaceae
Otu56	1534	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Dialister
Otu124	1505	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Selenomonas

OTU no.	OTU abundance	Taxonomy
Otu140	1498	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Paraprevotellaceae; Prevotella
Otu115	1295	Bacteria; Firmicutes; Bacilli; Lactobacillales; Aerococcaceae; Abiotrophia
Otu43	1184	Bacteria; Firmicutes; Clostridia; Clostridiales
Otu125	1134	Bacteria; Fusobacteria; Fusobacteriia; Fusobacteriales; Fusobacteriaceae; Fusobacterium
Otu114	1133	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu103	1108	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella; Prevotella_nanceiensis
Otu82	1104	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Butyrivibrio
Otu144	1087	Bacteria; Spirochaetes; Spirochaetes; Spirochaetales; Spirochaetaceae; Treponema
Otu134	1077	Bacteria; Firmicutes; Bacilli; Lactobacillales; Streptococcaceae; Streptococcus; Streptococcus_anginosus
Otu106	1067	Bacteria; Firmicutes; Clostridia; Clostridiales; Peptostreptococcaceae; Peptostreptococcus
Otu151	964	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Moryella
Otu85	953	Bacteria; Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae
Otu92	952	Bacteria; Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae; Kingella
Otu54	939	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Megasphaera
Otu97	904	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu63	884	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Porphyromonadaceae; Porphyromonas
Otu239	826	Bacteria; Proteobacteria; Gammaproteobacteria; Pasteurellales; Pasteurellaceae; Haemophilus; Haemophilus_parainfluenzae
Otu91	824	Bacteria; Firmicutes; Clostridia; Clostridiales; Mogibacteriaceae; Mogibacterium
Otu219	807	Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Capnocytophaga; Capnocytophaga_ochracea
Otu94	803	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales
Otu276	776	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu73	773	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu122	739	Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; Weeksellaceae
Otu129	711	Bacteria; Firmicutes; Bacilli; Lactobacillales; Streptococcaceae; Streptococcus
Otu169	705	Bacteria; Spirochaetes; Spirochaetes; Spirochaetales; Spirochaetaceae; Treponema
Otu145	698	Bacteria; TM7; TM7-3
Otu131	688	Bacteria; Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae; Kingella
Otu99	619	Bacteria; Cyanobacteria; Chloroplast; Streptophyta
Otu120	611	Bacteria; Firmicutes; Clostridia; Clostridiales; Tissierellaceae; Parvimonas
Otu287	608	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella; Prevotella_melaninogenica
Otu154	607	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Porphyromonadaceae; Tannerella
Otu143	604	Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Capnocytophaga; Capnocytophaga_ochracea
Otu217	556	Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Capnocytophaga
Otu268	556	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Selenomonas

OTU no.	OTU abundance	Taxonomy
Otu102	551	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu107	548	Bacteria; Spirochaetes; Spirochaetes; Spirochaetales; Spirochaetaceae; Treponema; Treponema_amylovorum
Otu139	546	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Porphyromonadaceae; Paludibacter
Otu192	540	Bacteria; Spirochaetes; Spirochaetes; Spirochaetales; Spirochaetaceae; Treponema; Treponema_socranskii
Otu60	539	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Moraxellaceae; Moraxella
Otu133	502	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu74	491	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales
Otu111	474	Bacteria; TM7; TM7-3; I025; Rs-045
Otu89	474	Bacteria; Spirochaetes; Spirochaetes; Spirochaetales; Spirochaetaceae; Treponema
Otu136	467	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu194	452	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Paraprevotellaceae; Prevotella
Otu186	435	Bacteria; Spirochaetes; Spirochaetes; Spirochaetales; Spirochaetaceae; Treponema
Otu119	433	Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; Weeksellaceae
Otu104	419	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales
Otu159	412	Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Capnocytophaga
Otu285	406	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu189	398	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae
Otu96	387	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales
Otu156	380	Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Capnocytophaga
Otu101	373	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Dialister
Otu168	368	Bacteria; Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae; Neisseria; Neisseria_oralis
Otu113	348	Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Capnocytophaga
Otu123	347	Bacteria; Firmicutes; Clostridia; Clostridiales; Peptostreptococcaceae; Filifactor
Otu118	323	Bacteria; Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae; Kingella
Otu110	321	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Moraxellaceae; Enhydrobacter
Otu121	309	Bacteria; Spirochaetes; Spirochaetes; Spirochaetales; Spirochaetaceae; Treponema
Otu83	307	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Moryella
Otu184	305	Bacteria; Proteobacteria; Gammaproteobacteria; Cardiobacteriales; Cardiobacteriaceae; Cardiobacterium
Otu132	288	Bacteria; Firmicutes; Clostridia; Clostridiales; Peptococcaceae; Peptococcus
Otu112	275	Bacteria; Firmicutes; Clostridia
Otu177	264	Bacteria; TM7; TM7-3
Otu95	261	Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Capnocytophaga
Otu164	241	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Schwartzia
Otu176	233	Bacteria; Firmicutes; Clostridia; Clostridiales; Peptostreptococcaceae

OTU no.	OTU abundance	Taxonomy
Otu183	231	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Megasphaera
Otu153	225	Bacteria; Firmicutes; Clostridia; Clostridiales; Mogibacteriaceae
Otu221	224	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Selenomonas
Otu170	218	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu165	215	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Selenomonas
Otu172	213	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Porphyromonadaceae; Tannerella
Otu162	211	Bacteria; Actinobacteria; Actinobacteria; Actinomycetales; Propionibacteriaceae
Otu147	207	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae
Otu202	207	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Veillonella
Otu180	202	Bacteria; Actinobacteria; Actinobacteria; Actinomycetales; Actinomycetaceae; Actinomyces
Otu148	198	Bacteria; Spirochaetes; Spirochaetes; Spirochaetales; Spirochaetaceae; Treponema; Treponema_amylovorum
Otu187	189	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
Otu182	188	Bacteria; Spirochaetes; Spirochaetes; Spirochaetales; Spirochaetaceae; Treponema; Treponema_amylovorum
Otu163	187	Bacteria; Actinobacteria; Actinobacteria; Actinomycetales; Actinomycetaceae; Actinomyces
Otu141	184	Bacteria; Actinobacteria; Actinobacteria; Bifidobacteriales; Bifidobacteriaceae; Bifidobacterium
Otu130	183	Bacteria; TM7; TM7-3
Otu149	178	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Shuttleworthia; Shuttleworthia_satelles
Otu281	174	Bacteria; Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae; Kingella
Otu142	169	Bacteria; Spirochaetes; Spirochaetes; Spirochaetales; Spirochaetaceae; Treponema
Otu116	155	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Porphyromonadaceae; Porphyromonas
Otu174	148	Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Capnocytophaga
Otu191	144	Bacteria; Spirochaetes; Spirochaetes; Spirochaetales; Spirochaetaceae; Treponema
Otu152	143	Bacteria; Fusobacteria; Fusobacteriia; Fusobacteriales; Leptotrichiaceae; Leptotrichia
Otu150	143	Bacteria; Tenericutes; Mollicutes; Mycoplasmatales; Mycoplasmataceae; Mycoplasma
Otu179	140	Bacteria; Fusobacteria; Fusobacteriia; Fusobacteriales; Leptotrichiaceae; Leptotrichia
Otu171	139	Bacteria; Tenericutes; Mollicutes; Mycoplasmatales; Mycoplasmataceae; Mycoplasma
Otu185	133	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Selenomonas
Otu160	131	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu196	128	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu216	126	Bacteria; Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae; Neisseria; Neisseria_bacilliformis
Otu251	125	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales
Otu128	122	Bacteria; Tenericutes; Mollicutes; RF39
Otu228	122	Bacteria; GN02; BD1-5
Otu238	111	Bacteria; TM7; TM7-3; CW040; F16

OTU no.	OTU abundance	Taxonomy
Otu250	111	Bacteria; Spirochaetes; Spirochaetes; Spirochaetales; Spirochaetaceae; Treponema
Otu188	103	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu209	101	Bacteria; Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae
Otu190	100	Bacteria; Actinobacteria; Actinobacteria; Actinomycetales; Corynebacteriaceae; Corynebacterium
Otu201	100	Bacteria; Fusobacteria; Fusobacteriia; Fusobacteriales; Leptotrichiaceae
Otu207	99	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu204	96	Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus; Lactobacillus_helveticus
Otu205	96	Bacteria; Actinobacteria; Actinobacteria; Actinomycetales; Propionibacteriaceae
Otu135	95	Bacteria; TM7; TM7-3; CW040
Otu227	95	Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus; Lactobacillus_salivarius
Otu279	93	Bacteria; Proteobacteria; Gammaproteobacteria; Pasteurellales; Pasteurellaceae; Haemophilus; Haemophilus_parainfluenzae
Otu167	93	Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; mitochondria
Otu225	93	Bacteria; Firmicutes; Erysipelotrichi; Erysipelotrichales; Erysipelotrichaceae; Bulleidia
Otu197	92	Bacteria; Firmicutes; Clostridia; Clostridiales; Peptostreptococcaceae
Otu214	89	Bacteria; Actinobacteria; Actinobacteria; Bifidobacteriales; Bifidobacteriaceae; Scardovia
Otu240	86	Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus; Lactobacillus_vaginalis
Otu158	85	Bacteria; Actinobacteria; Coriobacteriia; Coriobacteriales; Coriobacteriaceae
Otu237	85	Bacteria; Actinobacteria; Coriobacteriia; Coriobacteriales; Coriobacteriaceae
Otu291	85	Bacteria; Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae; Neisseria; Neisseria_subflava
Otu223	84	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Schwartzia
Otu127	82	Bacteria; Proteobacteria; Gammaproteobacteria; Pasteurellales; Pasteurellaceae; Actinobacillus
Otu203	81	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu226	80	Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus
Otu233	75	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu215	75	Bacteria; Cyanobacteria; Chloroplast; Streptophyta
Otu157	73	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella; Prevotella_nanceiensis
Otu138	72	Bacteria; Firmicutes; Bacilli; Lactobacillales; Streptococcaceae; Streptococcus
Otu242	71	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu210	69	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu270	67	Bacteria; Synergistetes; Synergistia; Synergistales; Dethiosulfovibrionaceae; TG5
Otu222	67	Bacteria; Actinobacteria; Coriobacteriia; Coriobacteriales; Coriobacteriaceae; Olsenella; Olsenella_profusa
Otu256	66	Bacteria; Firmicutes; Bacilli; Bacillales; Staphylococcaceae; Staphylococcus
Otu272	66	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Dialister
Otu289	65	Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus; Lactobacillus_delbrueckii

OTU no.	OTU abundance	Taxonomy
Otu195	63	Bacteria; Firmicutes; Erysipelotrichi; Erysipelotrichales; Erysipelotrichaceae; Sharpea
Otu235	61	Bacteria; Proteobacteria; Gammaproteobacteria; Cardiobacteriales; Cardiobacteriaceae; Cardiobacterium
Otu263	61	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Oribacterium
Otu255	60	Bacteria; Synergistetes; Synergistia; Synergistales; Dethiosulfovibrionaceae; TG5
Otu234	59	Bacteria; Firmicutes; Clostridia; Clostridiales
Otu166	59	Bacteria; Firmicutes; Clostridia; Clostridiales; Mogibacteriaceae
Otu246	58	Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Capnocytophaga
Otu175	55	Bacteria; Firmicutes; Bacilli; Lactobacillales; Aerococcaceae; Alloiooccus
Otu231	53	Bacteria; Actinobacteria; Actinobacteria; Actinomycetales; Actinomycetaceae; Actinomyces
Otu252	53	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu253	52	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae
Otu218	50	Bacteria; Firmicutes; Bacilli; Lactobacillales; Streptococcaceae; Lactococcus
Otu181	50	Bacteria; Proteobacteria; Gammaproteobacteria; Pasteurellales; Pasteurellaceae; Aggregatibacter
Otu244	49	Bacteria; Actinobacteria; Actinobacteria; Actinomycetales; Actinomycetaceae
Otu229	48	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu248	46	Bacteria; Actinobacteria; Actinobacteria; Actinomycetales; Actinomycetaceae
Otu273	46	Bacteria; Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae; Kingella
Otu200	46	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; BS11
Otu224	44	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae
Otu264	42	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Dialister
Otu208	42	Bacteria; Firmicutes; Clostridia; Clostridiales; Peptostreptococcaceae
Otu247	41	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu236	40	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Johnsonella; Johnsonella_ignava
Otu290	38	Bacteria; Firmicutes; Clostridia; Clostridiales; Mogibacteriaceae
Otu211	38	Bacteria; Firmicutes; Bacilli; Lactobacillales; Streptococcaceae; Streptococcus; Streptococcus_sobrinus
Otu220	35	Bacteria; Firmicutes; Clostridia; Clostridiales; Acidaminobacteraceae
Otu265	35	Bacteria; Firmicutes; Clostridia; Clostridiales; Mogibacteriaceae
Otu178	35	Bacteria; Proteobacteria; Gammaproteobacteria; Cardiobacteriales; Cardiobacteriaceae
Otu212	35	Bacteria; Tenericutes; Mollicutes; RF39
Otu230	34	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales
Otu199	32	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales
Otu198	31	Bacteria; Proteobacteria; Gammaproteobacteria; Cardiobacteriales; Cardiobacteriaceae
Otu241	31	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
Otu288	30	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Paraprevotellaceae; Prevotella

OTU no.	OTU abundance	Taxonomy
Otu249	29	Bacteria; GN02; BD1-5
Otu232	28	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Rikenellaceae; Blvii28
Otu173	28	Bacteria; Tenericutes; Mollicutes; Acholeplasmatales; Acholeplasmataceae; Acholeplasma
Otu213	26	Bacteria; TM7; TM7-3
Otu286	25	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Catonella
Otu258	21	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu161	21	Bacteria; Spirochaetes; Spirochaetes; Spirochaetales; Spirochaetaceae; Treponema
Otu269	20	Bacteria; Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae; Kingella
Otu262	17	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Johnsonella; Johnsonella_ignava
Otu284	15	Bacteria; Actinobacteria; Actinobacteria; Actinomycetales; Corynebacteriaceae; Corynebacterium
Otu278	11	Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Anoxybacillus; Anoxybacillus_kestanbolensis
Otu259	11	Bacteria; Firmicutes; Bacilli; Lactobacillales; Leuconostocaceae; Weissella; Weissella_cibaria
Otu266	10	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella
Otu243	9	Bacteria; Synergistetes; Synergistia; Synergistales; Dethiosulfovibrionaceae; Pyramidobacter; Pyramidobacter_piscolens
Otu283	9	Bacteria; Fusobacteria; Fusobacteriia; Fusobacteriales; Leptotrichiaceae; Leptotrichia
Otu282	9	Bacteria; Fusobacteria; Fusobacteriia; Fusobacteriales; Leptotrichiaceae; Leptotrichia
Otu254	8	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales
Otu275	7	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae
Otu260	5	Bacteria; Firmicutes; Erysipelotrichi; Erysipelotrichales; Erysipelotrichaceae; Bulleidia
Otu267	5	Bacteria; Cyanobacteria; Chloroplast; Streptophyta
Otu257	4	Bacteria; Actinobacteria; Actinobacteria; Bifidobacteriales; Bifidobacteriaceae; Bifidobacterium

Table A2. Richness of OTUs of different samples before and after swaking

	BEF2	AF2	BEF6	AF6	BEF8	AF8	BEF9	AF9	BEF10	AF10	BEF12	AF12	BEF14	AF14	BEF15	AF15	BEF19	AF19	BEF20	AF20	Total
Otu2	11062	11570	7278	9371	10372	16437	13938	14394	14000	16547	6064	8382	6956	16884	18405	13464	4544	13569	14256	8500	235993
Otu1	15251	2972	12398	10906	19902	6250	8512	4404	9177	17991	1640	5479	4465	12883	3700	11478	21756	5495	12085	8650	195394
Otu3	8044	3356	9825	5142	13444	5971	10524	3200	9441	9744	5498	6325	5589	6824	2186	8438	13926	7560	10655	1798	147490
Otu4	12642	7845	842	1941	1587	2831	1634	7295	5771	3147	6416	4897	1116	8691	6067	1646	619	1804	5901	9359	92051
Otu11	1690	2370	3060	4357	1089	3688	3934	6327	1752	793	1498	1760	5147	1874	1641	4427	1395	1329	5658	4742	58531
Otu32	3563	802	4934	5040	4315	2150	1399	816	2116	2866	3997	2932	1420	1023	704	965	4818	2740	1990	1593	50183
Otu8	5378	948	458	419	1608	2198	4793	3265	4079	2346	2484	1503	959	830	5074	1636	2190	2268	4500	2157	49093
Otu14	2425	5232	2153	2774	803	3783	951	1463	2915	2086	2246	5832	1170	4094	3432	1546	236	1448	1352	683	46624
Otu6	3818	4766	708	208	1158	3386	8793	2683	371	110	27	362	3432	2877	3156	501	392	946	1669	1509	40872

	BEF2	AF2	BEF6	AF6	BEF8	AF8	BEF9	AF9	BEF10	AF10	BEF12	AF12	BEF14	AF14	BEF15	AF15	BEF19	AF19	BEF20	AF20	Total
Otu12	487	1567	468	130	1323	1673	194	1306	1862	2852	1573	2972	570	1585	179	6526	2685	3759	1072	1246	34029
Otu10	1	640	2043	969	284	488	1059	495	1233	517	3846	3684	679	222	515	397	4118	2307	3827	5744	33068
Otu7	3968	2096	1733	1851	393	1201	548	2721	284	438	385	2072	769	4233	6953	1337	133	844	534	148	32641
Otu5	1896	864	2344	1265	3027	405	1494	853	1702	662	1462	739	3057	873	835	396	3641	1719	3626	1232	32092
Otu261	1318	2960	1512	173	1817	1428	1695	4513	10	94	348	40	2661	1374	7146	241	141	193	196	51	27911
Otu29	1204	4196	1753	151	291	1799	1931	2459	276	1394	938	2197	1258	1718	495	1368	933	351	27	154	24893
Otu17	209	158	2811	1991	3170	1185	253	344	390	143	1413	1889	642	727	553	699	2042	790	2090	2284	23783
Otu15	792	455	2528	1294	3798	773	483	272	1072	1604	1043	2485	695	355	198	523	2114	674	255	1284	22697
Otu25	785	1237	597	596	331	1719	3075	1734	61	116	386	878	2679	1383	1222	760	952	311	1780	392	20994
Otu21	622	119	1301	2576	1203	870	1530	523	1843	548	485	513	1291	273	882	220	1835	330	1130	332	18426
Otu20	91	128	1132	460	105	1377	454	2226	941	247	2634	1290	584	277	166	373	1213	2217	595	645	17155
Otu27	745	527	229	314	364	487	694	1237	1869	513	809	661	251	619	413	638	442	1081	1215	802	13910
Otu26	800	947	1297	155	177	898	1172	1185	54	173	104	544	457	2199	556	751	212	338	822	627	13468
Otu9	1	6634	0	60	79	562	106	849	778	362	5	750	1575	1182	263	1	0	21	14	0	13242
Otu40	797	78	1525	386	445	1210	745	462	27	281	17	326	793	2069	718	1551	747	723	108	43	13051
Otu33	825	25	2041	579	244	1491	699	2294	0	43	63	283	390	574	1564	594	403	566	176	52	12906
Otu37	171	37	1119	1683	977	798	672	748	111	376	299	929	73	907	16	180	417	1659	623	877	12672
Otu23	201	2117	328	924	24	606	214	2345	232	49	538	1131	744	209	241	83	798	673	149	302	11908
Otu193	2650	318	416	163	1426	515	1844	354	197	230	811	427	482	428	178	65	292	285	189	45	11315
Otu28	10	185	639	3606	53	663	515	174	27	84	1015	193	1624	97	70	627	115	286	21	20	10024
Otu109	1870	1259	696	845	244	442	294	639	215	162	132	276	317	678	899	456	92	207	102	53	9878
Otu49	523	1345	204	1129	125	813	306	1411	314	340	660	731	1	413	227	1	26	105	406	670	9750
Otu18	65	140	436	277	55	136	120	437	820	281	198	440	2378	302	37	981	229	2004	120	248	9704
Otu55	264	153	309	101	178	476	1056	707	461	60	243	119	300	277	304	916	91	378	1065	1356	8814
Otu24	92	149	64	288	201	387	69	169	282	530	883	249	149	180	801	225	368	648	1902	956	8592
Otu38	106	63	321	261	527	315	297	140	1134	341	460	334	580	118	132	230	845	349	1110	717	8380
Otu42	266	35	419	953	808	287	184	29	242	71	1156	198	259	54	50	132	2355	396	288	120	8302
Otu68	738	501	886	618	89	369	408	351	131	103	556	876	377	191	96	197	700	414	194	364	8159
Otu53	1419	871	1	96	265	1246	487	1075	183	80	147	892	277	325	226	47	36	57	375	22	8127
Otu245	195	212	240	200	252	515	492	79	3	61	17	61	248	583	157	1055	225	60	986	1415	7056
Otu51	159	137	619	509	101	450	305	656	526	227	480	279	199	146	178	188	782	505	292	131	6869
Otu41	97	16	186	744	368	220	63	91	230	12	1292	545	506	532	134	421	255	108	452	469	6741
Otu50	41	39	194	45	149	265	905	423	386	119	938	161	261	2	65	150	574	252	779	507	6255
Otu19	836	520	181	2196	44	510	240	209	55	35	107	259	164	339	79	170	64	52	93	30	6183

	BEF2	AF2	BEF6	AF6	BEF8	AF8	BEF9	AF9	BEF10	AF10	BEF12	AF12	BEF14	AF14	BEF15	AF15	BEF19	AF19	BEF20	AF20	Total
Otu39	62	1011	892	320	21	178	97	525	14	64	268	477	26	200	32	63	1343	448	60	23	6124
Otu277	37	801	10	16	713	208	319	1298	8	41	130	9	276	652	1339	15	7	22	34	24	5959
Otu65	629	276	158	392	311	187	324	890	92	130	21	122	283	754	587	531	7	42	79	5	5820
Otu31	18	287	163	176	131	164	173	442	76	83	30	49	2064	678	65	337	85	86	24	9	5140
Otu35	0	4	8	69	138	854	9	395	0	0	1190	401	14	11	25	16	78	213	199	1479	5103
Otu22	0	2	1854	0	0	0	508	462	4	89	0	1	317	411	21	680	56	618	0	0	5023
Otu13	0	0	2	1	0	0	11	4	0	0	0	0	3543	4	3	2	1329	3	1	2	4905
Otu88	322	351	375	61	33	400	174	441	683	181	83	228	265	110	65	302	251	472	64	28	4889
Otu155	5	440	87	38	75	416	272	467	585	96	330	310	461	113	45	440	110	251	188	85	4814
Otu61	208	226	349	333	103	310	94	172	218	115	36	156	935	274	94	440	144	456	68	66	4797
Otu70	26	320	564	236	102	774	254	398	221	57	67	243	572	39	64	247	158	66	363	17	4788
Otu34	1344	485	86	12	9	178	112	548	35	8	53	72	737	163	139	56	180	57	87	36	4397
Otu52	57	174	85	323	105	404	48	239	25	13	326	1007	208	21	32	71	800	186	97	26	4247
Otu66	15	321	626	587	45	141	122	105	138	103	298	196	696	92	149	64	227	141	56	79	4201
Otu76	79	144	101	109	100	310	117	305	336	83	423	185	281	83	167	176	140	173	471	261	4044
Otu69	595	668	192	34	18	152	155	403	54	19	143	44	867	78	56	161	61	71	69	63	3903
Otu57	10	219	178	92	25	336	129	111	299	55	110	155	980	86	42	94	412	322	66	42	3763
Otu36	1262	142	142	7	23	301	269	247	257	18	56	49	217	58	55	88	199	55	22	9	3476
Otu16	2754	203	9	0	0	46	60	216	27	7	25	0	0	33	36	16	0	7	1	0	3440
Otu48	359	54	62	187	8	395	37	351	228	91	798	108	61	51	22	38	300	180	47	63	3440
Otu64	4	33	355	3	0	7	71	64	156	8	185	774	232	20	15	94	107	271	26	990	3415
Otu84	98	261	309	189	18	178	330	456	235	60	131	30	426	75	15	247	46	153	68	81	3406
Otu47	78	301	645	224	105	415	203	124	0	0	35	51	110	120	728	100	65	50	21	6	3381
Otu93	20	233	597	53	13	210	194	640	409	24	306	226	64	75	37	41	12	16	145	52	3367
Otu126	263	432	277	210	63	308	62	90	77	46	58	167	520	84	33	241	88	226	31	55	3331
Otu87	260	296	126	31	33	222	189	851	67	53	151	74	307	37	56	95	47	82	61	36	3074
Otu77	112	798	163	76	47	156	192	66	72	13	159	75	485	84	19	131	153	134	21	37	2993
Otu271	205	13	191	134	254	120	271	145	151	119	49	115	80	121	93	83	515	19	241	67	2986
Otu75	29	125	112	64	6	216	153	61	163	32	136	129	234	15	15	94	678	555	82	40	2939
Otu98	82	33	179	26	381	302	15	32	185	86	103	96	20	13	2	33	375	245	2	17	2227
Otu59	722	43	178	36	9	44	70	93	311	63	176	128	179	30	64	44	342	140	66	12	2750
Otu80	0	3	188	72	10	135	80	233	80	42	427	336	58	62	73	120	332	335	72	69	2727
Otu86	18	228	112	33	10	123	53	130	68	27	112	71	441	103	17	200	71	127	436	82	2462
Otu81	10	6	33	8	113	32	173	135	627	105	277	45	47	15	21	72	205	258	119	132	2433

	BEF2	AF2	BEF6	AF6	BEF8	AF8	BEF9	AF9	BEF10	AF10	BEF12	AF12	BEF14	AF14	BEF15	AF15	BEF19	AF19	BEF20	AF20	Total
Otu206	15	6	51	30	37	38	150	83	397	59	92	70	64	62	77	66	314	186	387	241	2425
Otu137	397	224	248	53	78	166	312	25	101	18	43	38	141	33	32	131	96	86	17	2	2241
Otu62	41	50	150	70	52	76	38	67	102	88	172	74	98	53	32	103	116	142	438	164	2126
Otu67	1	0	16	0	9	48	71	800	0	0	8	44	80	234	638	63	23	21	0	0	2056
Otu71	0	9	203	0	6	0	34	35	440	290	162	125	207	146	38	195	4	107	0	0	2001
Otu45	0	1	1189	1	0	0	710	71	0	0	0	0	0	0	0	0	0	0	0	0	1972
Otu280	475	213	72	97	30	69	64	148	34	35	41	89	28	201	188	42	10	14	28	30	1908
Otu274	459	125	33	3	43	184	252	194	93	5	66	4	269	29	19	29	52	25	15	5	1904
Otu108	1	94	204	65	28	82	58	184	51	41	27	80	181	241	158	188	83	78	33	25	1902
Otu117	0	40	308	111	11	70	88	106	174	109	63	46	233	29	12	140	111	140	57	19	1867
Otu90	28	7	30	213	217	53	98	41	84	45	68	86	66	71	65	127	88	65	199	213	1864
Otu72	145	386	30	59	15	144	49	227	71	62	49	75	62	69	142	55	9	55	92	38	1834
Otu100	18	223	81	28	19	99	14	79	53	26	31	24	701	164	22	131	28	75	5	6	1827
Otu78	0	260	21	12	2	48	49	475	4	4	80	48	144	200	315	60	14	71	5	11	1823
Otu146	13	84	55	137	7	134	192	78	29	6	105	59	228	33	19	283	67	140	46	79	1794
Otu44	0	6	9	23	54	169	5	80	19	14	806	249	15	4	22	48	74	112	12	67	1788
Otu46	0	0	421	0	0	0	52	19	815	0	97	166	0	7	5	0	23	94	1	2	1702
Otu105	135	311	82	27	25	114	12	56	121	27	155	117	98	28	16	77	25	186	41	12	1665
Otu58	38	328	22	18	15	70	58	709	160	53	57	26	24	11	9	26	1	19	0	1	1645
Otu30	0	1	0	0	0	0	1416	221	0	0	0	0	0	0	0	0	0	0	0	0	1638
Otu79	104	1	52	23	27	451	31	235	1	2	18	122	40	136	120	64	60	80	6	4	1577
Otu56	0	63	32	21	14	52	88	66	35	29	30	38	770	75	23	72	60	55	1	10	1534
Otu124	98	53	157	123	125	123	35	9	83	12	52	23	223	35	6	148	70	111	18	1	1505
Otu140	301	115	205	5	17	108	78	144	32	5	45	13	263	14	3	100	24	20	5	1	1498
Otu115	131	187	62	38	4	60	21	209	79	45	32	113	61	35	104	21	20	31	36	6	1295
Otu43	0	156	0	0	1	0	1	0	0	0	3	117	5	88	727	1	33	0	38	14	1184
Otu125	17	1	65	20	96	201	9	91	1	3	6	47	154	143	142	87	17	19	9	6	1134
Otu114	77	75	131	15	26	163	59	46	34	18	47	38	44	180	49	31	71	19	10	0	1133
Otu103	0	3	144	0	23	2	9	3	1	9	0	46	123	222	141	192	83	102	1	4	1108
Otu82	0	16	22	53	150	15	1	13	9	11	71	36	10	12	39	47	288	84	155	72	1104
Otu144	0	0	273	23	0	0	151	53	149	111	18	28	64	7	0	76	26	94	9	5	1087
Otu134	27	52	75	57	27	44	57	24	45	27	32	72	48	74	25	253	25	91	15	7	1077
Otu106	3	13	57	53	7	19	35	58	28	25	56	62	77	66	36	116	77	44	134	101	1067
Otu151	11	9	10	33	24	35	34	48	56	18	195	31	42	4	9	56	71	66	159	53	964

	BEF2	AF2	BEF6	AF6	BEF8	AF8	BEF9	AF9	BEF10	AF10	BEF12	AF12	BEF14	AF14	BEF15	AF15	BEF19	AF19	BEF20	AF20	Total
Otu85	2	3	0	1	0	0	474	311	0	2	57	37	10	41	12	2	0	0	0	1	953
Otu92	326	33	58	4	11	36	17	14	54	15	36	11	138	3	8	29	83	51	21	4	952
Otu54	0	1	35	6	55	94	22	7	5	0	17	5	568	20	15	89	0	0	0	0	939
Otu97	41	10	15	3	0	14	59	55	0	0	8	20	66	105	44	59	10	9	94	292	904
Otu63	0	0	381	0	0	0	407	96	0	0	0	0	0	0	0	0	0	0	0	0	884
Otu239	3	43	24	5	16	26	57	261	40	4	55	20	18	49	45	38	0	0	56	66	826
Otu91	6	1	46	76	13	21	15	13	72	33	193	108	2	13	8	22	14	28	84	56	824
Otu219	10	81	7	22	5	48	15	30	6	12	119	55	184	10	12	96	22	36	10	27	807
Otu94	0	0	14	19	0	0	24	24	174	55	76	31	44	18	6	51	26	200	26	15	803
Otu276	31	85	80	6	16	112	25	25	60	41	4	22	95	6	5	32	22	68	23	18	776
Otu73	0	0	109	0	0	0	8	195	11	19	0	0	368	0	0	63	0	0	0	0	773
Otu122	39	223	13	15	6	90	22	163	24	5	37	25	4	15	9	26	2	12	6	3	739
Otu129	5	15	26	36	9	31	50	64	24	30	40	72	68	15	8	50	92	67	4	5	711
Otu169	0	3	112	18	0	0	331	24	31	31	10	13	0	26	0	0	17	89	0	0	705
Otu145	11	125	71	31	1	0	6	6	40	42	36	52	61	77	32	10	26	60	2	9	698
Otu131	0	0	0	20	0	0	138	393	19	100	2	0	3	8	5	0	0	0	0	0	688
Otu99	0	0	2	0	0	0	33	4	1	0	6	0	153	1	0	6	98	293	0	22	619
Otu120	0	1	108	18	7	12	81	13	1	4	15	11	60	22	6	123	2	3	41	83	611
Otu287	2	1	47	4	0	8	8	31	21	0	20	146	25	10	9	19	50	12	6	189	608
Otu154	16	21	57	30	6	47	100	40	25	2	59	12	71	10	4	33	27	13	27	7	607
Otu143	103	56	6	11	8	49	13	40	40	6	48	52	68	17	11	32	14	28	2	0	604
Otu217	12	20	3	6	2	50	10	93	39	13	3	0	256	0	3	7	10	22	6	1	556
Otu268	11	6	9	66	53	26	3	3	17	6	65	16	23	4	7	30	69	33	92	17	556
Otu102	0	0	0	0	0	0	371	89	83	0	8	0	0	0	0	0	0	0	0	0	551
Otu107	0	0	86	0	1	11	98	28	32	16	2	18	145	21	4	34	18	32	2	0	548
Otu139	0	62	9	47	10	40	46	49	49	13	39	39	51	5	0	18	5	23	22	19	546
Otu192	1	34	34	17	7	49	44	10	35	16	31	15	108	27	2	27	27	49	3	4	540
Otu60	3	1	0	3	4	0	0	2	4	0	1	2	0	85	12	0	6	416	0	0	539
Otu133	0	115	37	14	8	27	48	26	20	12	10	15	30	33	5	42	10	37	7	6	502
Otu74	0	0	9	0	0	0	260	41	0	0	11	7	0	0	0	2	161	0	0	0	491
Otu111	0	74	39	25	10	43	7	3	29	7	89	20	13	14	1	5	44	51	0	0	474
Otu89	0	0	0	0	0	0	10	29	13	63	0	0	36	0	0	47	42	234	0	0	474
Otu136	12	93	6	7	8	116	21	28	7	4	43	15	45	9	3	13	11	17	4	5	467
Otu194	16	1	50	0	18	20	54	110	16	1	3	0	83	15	15	25	10	11	4	0	452

	BEF2	AF2	BEF6	AF6	BEF8	AF8	BEF9	AF9	BEF10	AF10	BEF12	AF12	BEF14	AF14	BEF15	AF15	BEF19	AF19	BEF20	AF20	Total
Otu186	0	3	91	8	0	10	63	18	42	12	14	3	27	9	3	28	26	67	6	5	435
Otu119	26	9	33	26	1	19	27	35	10	10	7	12	4	25	130	13	11	22	11	2	433
Otu104	0	0	162	0	0	0	186	61	0	0	0	0	0	3	7	0	0	0	0	0	419
Otu159	15	30	10	25	2	31	24	83	92	2	0	8	48	7	1	14	9	9	1	1	412
Otu285	18	6	19	11	106	25	1	1	17	19	1	22	8	16	0	4	112	8	1	11	406
Otu189	0	25	51	25	9	25	2	10	65	17	74	8	8	0	0	18	24	37	0	0	398
Otu96	0	0	52	7	0	0	199	57	0	0	0	0	21	13	6	31	0	1	0	0	387
Otu156	0	62	40	5	4	15	16	57	101	11	13	11	9	0	0	9	1	26	0	0	380
Otu101	0	0	30	26	1	0	24	2	0	0	15	10	143	10	0	62	2	33	5	10	373
Otu168	5	17	50	11	0	0	51	34	20	2	58	2	19	13	6	51	12	16	0	1	368
Otu113	0	31	26	0	0	2	7	229	0	24	0	3	0	0	0	11	0	11	1	3	348
Otu123	0	0	78	7	0	0	25	14	56	26	15	5	25	9	2	33	0	52	0	0	347
Otu118	11	7	3	1	5	1	0	5	0	4	4	5	47	154	44	13	8	10	1	0	323
Otu110	35	41	0	0	31	27	4	2	0	0	4	2	1	58	114	2	0	0	0	0	321
Otu121	0	8	28	35	0	0	9	5	78	42	18	32	0	19	3	0	5	27	0	0	309
Otu83	2	2	0	5	4	3	22	13	0	0	10	4	5	0	2	19	10	3	152	51	307
Otu184	9	13	34	8	0	15	44	41	4	3	14	2	46	2	0	15	29	18	2	6	305
Otu132	0	1	3	5	3	10	6	5	23	5	19	4	32	4	1	14	3	23	89	38	288
Otu112	0	0	49	0	0	0	13	3	124	38	8	0	0	0	0	0	2	38	0	0	275
Otu177	14	5	14	1	0	1	26	15	2	0	4	11	7	135	11	5	4	5	2	2	264
Otu95	0	0	0	0	1	34	20	165	0	0	0	4	7	4	26	0	0	0	0	0	261
Otu164	0	39	9	22	5	28	5	0	3	0	7	4	54	16	3	19	9	17	0	1	241
Otu176	0	2	0	6	5	2	6	10	23	7	3	12	14	29	8	41	4	14	27	20	233
Otu183	0	0	22	0	0	0	11	0	15	1	0	0	107	11	8	44	3	9	0	0	231
Otu153	0	0	65	1	1	9	1	6	13	11	18	19	10	21	5	24	1	15	4	1	225
Otu221	54	14	14	1	11	2	13	0	0	0	4	4	54	2	0	29	20	2	0	0	224
Otu170	1	14	17	3	2	15	26	53	0	0	2	1	42	11	1	16	9	5	0	0	218
Otu165	47	36	3	3	2	22	2	0	3	0	7	9	21	9	7	29	7	8	0	0	215
Otu172	0	0	29	24	0	0	21	2	37	15	16	5	22	6	1	19	2	11	3	0	213
Otu162	1	2	11	8	0	4	32	20	3	0	48	18	8	1	1	32	9	6	1	6	211
Otu147	0	0	23	7	5	7	2	7	8	1	1	0	107	14	0	12	6	7	0	0	207
Otu202	6	2	15	2	6	21	30	6	0	8	0	3	26	17	4	38	15	3	2	3	207
Otu180	1	10	29	5	0	3	6	4	4	4	9	7	22	1	3	41	12	31	6	4	202
Otu148	0	0	0	0	0	0	13	22	42	52	11	12	0	0	0	0	16	30	0	0	198

	BEF2	AF2	BEF6	AF6	BEF8	AF8	BEF9	AF9	BEF10	AF10	BEF12	AF12	BEF14	AF14	BEF15	AF15	BEF19	AF19	BEF20	AF20	Total
Otu187	1	7	2	1	3	9	26	63	11	0	3	3	14	1	0	9	4	4	17	11	189
Otu182	0	5	111	28	1	2	0	1	2	3	3	3	0	14	0	3	1	11	0	0	188
Otu163	0	27	36	5	1	4	2	6	12	4	6	12	17	3	4	5	23	11	5	4	187
Otu141	0	1	3	5	0	12	3	2	15	16	0	1	64	0	6	49	2	5	0	0	184
Otu130	0	0	0	16	0	0	1	0	0	0	0	0	25	25	15	10	10	81	0	0	183
Otu149	0	6	3	0	5	5	0	7	6	0	3	6	97	4	5	27	0	4	0	0	178
Otu281	0	1	3	0	2	7	43	80	4	12	0	0	1	6	5	1	1	7	1	0	174
Otu142	0	0	71	0	0	0	33	7	0	0	0	0	14	4	0	15	6	19	0	0	169
Otu116	0	0	0	0	0	0	0	0	0	0	0	0	126	4	4	21	0	0	0	0	155
Otu174	0	8	4	11	1	7	1	1	8	14	15	17	33	0	1	7	3	17	0	0	148
Otu191	0	0	5	0	0	0	97	9	28	0	2	3	0	0	0	0	0	0	0	0	144
Otu150	0	0	34	0	0	0	40	4	0	0	6	5	1	1	2	0	1	39	10	0	143
Otu152	0	0	22	2	0	0	14	73	0	0	4	1	0	0	0	14	2	9	0	2	143
Otu179	0	4	2	14	2	37	3	11	11	5	6	14	0	1	18	3	1	7	0	1	140
Otu171	1	1	26	6	0	2	29	5	5	1	1	2	15	2	1	6	7	2	5	22	139
Otu185	8	0	18	0	1	6	2	0	3	1	2	0	26	2	0	10	29	24	0	1	133
Otu160	0	0	37	0	0	0	0	0	47	22	0	0	0	1	1	0	4	19	0	0	131
Otu196	0	22	3	6	3	38	1	1	5	4	0	0	22	2	0	6	3	9	0	3	128
Otu216	33	8	9	2	0	2	7	10	9	0	1	3	28	0	2	9	1	2	0	0	126
Otu251	14	0	12	1	11	7	11	6	0	1	0	1	0	18	8	18	13	0	2	2	125
Otu128	0	5	0	4	0	1	2	11	5	0	0	0	6	0	0	1	0	0	45	42	122
Otu228	0	16	4	0	0	1	3	25	4	3	14	6	4	15	3	8	1	13	0	2	122
Otu238	0	4	5	4	1	6	5	6	0	0	3	15	28	0	1	5	1	5	10	12	111
Otu250	0	2	12	1	0	0	7	12	3	0	5	5	25	1	0	24	2	8	0	4	111
Otu188	0	0	20	0	5	23	1	0	0	0	10	11	1	5	0	0	3	24	0	0	103
Otu209	1	4	22	0	0	12	26	12	0	0	2	7	1	0	2	1	1	9	1	0	101
Otu190	0	0	2	0	0	5	2	0	10	1	9	1	0	0	11	0	27	32	0	0	100
Otu201	0	1	0	0	0	0	6	10	0	1	0	0	14	20	22	11	0	3	5	7	100
Otu207	5	14	8	4	0	0	7	2	0	0	12	1	17	0	0	22	2	5	0	0	99
Otu204	0	0	10	5	1	8	0	0	5	3	0	0	15	3	0	29	17	0	0	0	96
Otu205	0	2	9	1	4	3	5	6	7	2	1	2	24	8	2	4	11	4	0	1	96
Otu135	0	0	36	0	0	0	41	5	0	0	0	0	12	0	0	1	0	0	0	0	95
Otu227	0	0	15	3	0	2	20	3	6	13	4	0	1	1	0	23	0	0	4	0	95
Otu167	0	0	0	0	0	0	3	0	0	0	0	1	58	0	0	0	20	9	0	2	93

	BEF2	AF2	BEF6	AF6	BEF8	AF8	BEF9	AF9	BEF10	AF10	BEF12	AF12	BEF14	AF14	BEF15	AF15	BEF19	AF19	BEF20	AF20	Total
Otu225	0	2	8	1	0	0	6	1	0	0	5	3	47	1	0	19	0	0	0	0	93
Otu279	11	14	1	4	1	2	1	7	4	5	9	1	0	8	4	1	1	3	4	12	93
Otu197	0	0	4	0	5	4	17	7	5	3	1	0	6	0	0	7	3	30	0	0	92
Otu214	7	6	0	2	2	5	2	4	8	3	0	5	18	8	4	5	0	8	2	0	89
Otu240	0	0	21	5	7	10	4	5	6	3	2	0	1	8	1	13	0	0	0	0	86
Otu158	0	2	3	0	1	1	0	2	1	1	1	0	59	0	2	11	0	1	0	0	85
Otu237	0	0	43	3	9	11	0	1	7	7	0	0	1	2	0	1	0	0	0	0	85
Otu291	0	26	0	0	4	4	2	15	0	1	0	0	10	13	10	0	0	0	0	0	85
Otu223	2	4	8	0	2	8	2	0	3	0	15	4	17	2	1	2	2	12	0	0	84
Otu127	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	82	0	0	0	0	82
Otu203	0	0	0	0	0	0	51	24	0	0	0	0	0	0	0	6	0	0	0	0	81
Otu226	2	0	4	6	1	2	16	8	8	6	2	1	2	1	0	5	0	0	1	0	65
Otu215	0	0	0	0	0	0	23	0	0	0	0	0	31	0	0	0	16	5	0	0	75
Otu233	0	0	1	0	0	0	3	2	0	0	2	3	41	0	0	23	0	0	0	0	75
Otu157	0	0	0	0	0	0	3	17	12	35	3	3	0	0	0	0	0	0	0	0	73
Otu138	1	0	0	0	1	0	2	0	1	0	0	0	67	0	0	0	0	0	0	0	72
Otu242	0	13	7	2	0	0	0	0	0	0	4	0	20	3	1	15	0	6	0	0	71
Otu210	0	0	15	4	1	2	3	3	4	0	2	1	14	1	1	4	0	7	2	5	69
Otu222	0	0	2	1	0	1	1	2	1	2	0	0	34	1	1	13	0	7	1	0	67
Otu270	0	0	7	0	0	0	13	0	5	4	12	1	1	2	2	9	0	11	0	0	67
Otu256	9	11	0	2	0	1	1	0	7	1	2	2	1	8	1	2	2	11	3	2	66
Otu272	0	0	10	0	2	0	0	2	3	1	0	1	31	4	0	12	0	0	0	0	66
Otu289	0	0	6	0	0	0	3	10	0	3	0	0	19	0	0	0	24	0	0	0	65
Otu195	0	0	0	0	0	0	1	0	0	0	2	4	21	0	2	33	0	0	0	0	63
Otu235	1	0	0	2	0	8	5	1	4	1	9	1	12	0	0	11	3	3	0	0	61
Otu263	3	4	0	1	1	5	1	2	5	1	1	1	7	2	1	11	3	8	2	2	61
Otu255	0	0	8	1	0	0	11	0	5	2	4	3	17	0	2	4	0	3	0	0	60
Otu166	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	36	19	59
Otu234	0	0	5	2	0	0	11	2	10	7	0	0	0	2	1	0	0	15	2	2	59
Otu246	0	5	0	7	0	4	3	1	0	2	1	0	21	2	2	1	4	4	1	0	58
Otu175	3	1	0	0	0	0	2	0	4	0	0	0	0	0	1	0	0	43	1	0	55
Otu231	1	5	13	3	0	1	1	2	1	1	2	0	7	3	2	4	4	2	0	1	53
Otu252	0	0	8	1	0	3	1	2	12	1	5	0	17	0	1	0	0	2	0	0	53
Otu253	0	1	28	0	0	1	9	11	0	0	0	0	2	0	0	0	0	0	0	0	52

	BEF2	AF2	BEF6	AF6	BEF8	AF8	BEF9	AF9	BEF10	AF10	BEF12	AF12	BEF14	AF14	BEF15	AF15	BEF19	AF19	BEF20	AF20	Total
Otu181	0	0	0	0	0	0	50	0	0	0	0	0	0	0	0	0	0	0	0	0	50
Otu218	0	2	0	0	1	0	0	2	0	16	2	0	1	1	0	2	21	1	0	1	50
Otu244	0	1	2	6	1	0	0	0	7	2	4	5	7	0	0	2	2	5	3	2	49
Otu229	0	0	0	2	0	0	0	0	0	2	0	1	39	1	0	2	0	1	0	0	48
Otu200	0	0	0	0	0	0	44	2	0	0	0	0	0	0	0	0	0	0	0	0	46
Otu248	0	2	15	1	0	0	3	2	5	0	5	3	0	0	1	1	1	7	0	0	46
Otu273	0	0	9	0	0	5	0	0	5	0	1	0	0	5	5	0	11	5	0	0	46
Otu224	0	0	44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	44
Otu208	1	0	0	0	0	0	0	0	0	0	0	0	13	0	0	1	4	23	0	0	42
Otu264	0	0	2	0	0	0	3	0	2	0	0	0	21	4	2	5	1	2	0	0	42
Otu247	0	0	0	0	0	0	2	2	0	0	0	0	16	0	0	17	0	0	4	0	41
Otu236	0	1	0	5	0	0	2	3	0	0	1	1	2	1	2	9	1	8	3	1	40
Otu211	0	0	22	3	0	0	0	2	0	9	1	0	0	1	0	0	0	0	0	0	38
Otu290	0	0	19	0	0	0	3	3	0	0	0	0	3	0	5	5	0	0	0	0	38
Otu178	0	0	0	0	0	0	5	30	0	0	0	0	0	0	0	0	0	0	0	0	35
Otu212	0	0	0	30	0	0	0	0	0	0	1	0	0	1	0	0	1	2	0	0	35
Otu220	0	0	11	0	0	0	1	0	3	0	2	4	6	1	0	6	0	1	0	0	35
Otu265	0	0	0	0	0	0	3	1	7	5	5	7	0	0	0	0	3	4	0	0	35
Otu230	0	0	0	0	0	0	14	18	0	0	0	0	0	2	0	0	0	0	0	0	34
Otu199	0	0	0	0	0	0	30	1	0	0	0	0	0	0	1	0	0	0	0	0	32
Otu198	0	0	0	0	0	0	5	26	0	0	0	0	0	0	0	0	0	0	0	0	31
Otu241	0	2	1	0	0	0	3	7	8	0	0	0	0	0	0	1	0	1	8	0	31
Otu288	0	0	0	1	0	0	4	2	0	0	0	0	17	0	1	5	0	0	0	0	30
Otu249	0	0	0	2	0	0	0	10	3	1	0	0	0	0	0	0	0	11	0	2	29
Otu173	0	0	0	0	0	0	24	4	0	0	0	0	0	0	0	0	0	0	0	0	28
Otu232	0	0	0	0	0	0	0	0	0	0	7	0	13	0	0	0	8	0	0	0	28
Otu213	0	1	0	0	1	0	3	15	0	0	0	0	0	0	0	1	1	4	0	0	26
Otu286	0	0	17	0	0	0	0	0	0	0	2	3	0	2	1	0	0	0	0	0	25
Otu161	0	0	16	0	0	0	2	3	0	0	0	0	0	0	0	0	0	0	0	0	21
Otu258	0	0	0	0	0	0	1	1	0	0	0	0	0	1	4	14	0	0	0	0	21
Otu269	0	0	0	6	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0	20
Otu262	0	0	0	0	0	0	3	0	0	0	0	0	3	0	0	5	1	5	0	0	17
Otu284	0	0	0	0	0	0	0	0	11	3	0	0	0	0	0	0	1	0	0	0	15
Otu259	0	0	0	0	0	0	0	2	0	0	0	0	6	0	0	0	3	0	0	0	11

	BEF2	AF2	BEF6	AF6	BEF8	AF8	BEF9	AF9	BEF10	AF10	BEF12	AF12	BEF14	AF14	BEF15	AF15	BEF19	AF19	BEF20	AF20	Total
Otu278	0	0	0	0	0	0	0	7	0	1	0	0	0	0	0	0	1	2	0	0	11
Otu266	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10
Otu243	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	9
Otu282	0	0	4	2	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	9
Otu283	0	0	0	0	2	0	0	3	0	0	2	1	0	0	0	0	0	0	0	1	9
Otu254	0	0	0	0	0	0	0	0	0	0	1	0	3	0	0	0	2	1	0	1	8
Otu275	0	0	6	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
Otu260	0	0	4	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	5
Otu267	0	0	0	0	0	0	1	0	0	0	0	0	4	0	0	0	0	0	0	0	5
Otu257	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	4

Table A3. Taxonomy and description of the highly abundant OTUs recovered from oral microbiomes of children regardless of swaking

Phylum	Genus/Species	Description	OTUs
Bacteroidetes	<i>Prevotella</i> spp.	A genus of gram negative anaerobic bacteria. Some strains are present in humans, including <i>Prevotella denticola</i> . They are predominantly oral and can be opportunistic pathogens [1].	15,21,33
	<i>Prevotella melaninogenica</i>	A species of bacterium in the normal flora of the upper respiratory tract. It is formerly called <i>Bacteroides melaninogenicus</i> , is a gram-negative, anaerobic, rod-shaped bacterium that inhabits the oral cavity. It is an important human pathogen in various anaerobic infections [2].	1
	<i>Prevotella pallens</i>	A genus of gram-negative bacteria. <i>Prevotella</i> spp. are members of the oral, vaginal, and gut microbiota and are often recovered from anaerobic infections of the respiratory tract [3].	17
	<i>Prevotella tanneriae</i>	An obligately anaerobic, non-spore-forming, nonmotile, gram-negative, rod-shaped bacterium that was isolated from the human gingival crevice [4].	28
	<i>Prevotella nanceiensis</i>	A novel species isolated from human clinical samples, e.g., blood cultures, lung abscess pus, broncho-alveolar lavage fluid, obligately anaerobic, non-spore-forming, non-motile, gram-negative coccoid and short rods [5].	26
	<i>Porphyromonas</i> spp.	Associated with severe and chronic periodontal (tissues surrounding and supporting the tooth) diseases. Progression of the disease is caused by colonization by this organism in an anaerobic environment in host tissues and severe progression results in loss of the tissues supporting the tooth and eventually loss of the tooth itself [6].	25
Firmicutes	<i>Streptococcus</i> spp.	First isolated in 1924 from human carious (cavities) lesions and is the main cause of tooth decay. This organism thrives in a bacterial community known as a dental plaque which forms on the surface of teeth. This organism has also been implicated in cases of infective endocarditis [7].	12
	<i>Streptococcus infantis</i>	A species of alpha-haemolytic streptococci. It has been isolated from human tooth surfaces and pharynx [8].	2
	<i>Veillonella</i> spp.	Well known for its lactate fermenting abilities. They are a normal bacterium in the intestines and oral mucosa of mammals. In humans they have been rarely implicated in cases of osteomyelitis and endocarditis [9].	40

Phylum	Genus/Species	Description	OTUs
	<i>Veillonella dispar</i>	A member of the normal human oral microbial community [10].	3,32
	<i>Gemella morbillorum</i>	Previously known as <i>Streptococcus morbillorum</i> , is an anaerobic, gram-positive coccus which is a component of the normal flora of the human gastrointestinal tract and the human oral microflora [11].	14
	<i>Megasphaera</i> spp.	A commensal genus of Firmicutes classified within the class Negativicutes as a member of the Clostridia. <i>Megasphaera</i> spp. reside in the human genitourinary tract. It has not yet been characterized using traditional methods, or the species name has not yet been validly published [12].	37
	<i>Clostridium</i> spp.	Distinguished from the Bacilli by lacking aerobic respiration. They are obligate anaerobes and oxygen is toxic to them [13].	23
	<i>Granulicatella</i> spp.	A facultatively anaerobic gram-positive, non-motile, non-sporulating bacterium. It is part of normal human oral flora and is thought to be a cause of endocarditis [14].	7
	<i>Granulicatella adjacens</i>	Formerly called <i>Streptococcus adjacens</i> or <i>Abiotrophia adiacens</i> , a species of gram-positive, non-motile, non-sporulating cocci isolated from the throat flora, urine and blood of patients with endocarditis [15].	27
Proteobacteria	<i>Haemophilus parainfluenzae</i>	A slow-growing gram negative bacteria that is a normal part of the human oropharyngeal flora. It is a cause of endocarditis in children [16].	4
	<i>Campylobacter</i> spp.	The leading cause of bacterial food poisoning (campylobacteriosis) in the world, and is more prevalent than <i>Salmonella enteritis</i> (salmonellosis). Severe health and economic problems are a result of widespread infections that affect up to 1% of the population [17].	5
	<i>Neisseria</i> spp.	A nonpathogenic, commensal bacterium closely related to the pathogenic <i>Neisseria meningitidis</i> . Of the 11 species that colonize humans, only two are pathogens, <i>N. meningitidis</i> and <i>N. gonorrhoeae</i> [18].	29
	<i>Neisseria subflava</i>	Commonly isolated from the oral and respiratory tract of humans. This bacterium can be an opportunistic pathogen and has occasionally been isolated from cases of endocarditis or bacteremia [19].	6
	<i>Neisseria cinerea</i>	Isolated in Germany from the nasopharyngeal mucosa of a healthy human [20].	261
	<i>Moraxella</i> spp.	Part of the commensal flora of the upper respiratory tract. It is also recognized as the cause of a variety of human infectious diseases including acute otitis media and sinusitis, which occur primarily in infants and young children [21].	9
	<i>Pasteurella</i> spp.	One of the first pathogens ever studied, and is named after Louis Pasteur, who used it in his vaccination studies in the 1880s. This organism usually resides in the mucous membranes of the intestinal, genital, and respiratory tissues and is an opportunistic pathogen [22].	193
Fusobacteria	<i>Fusobacterium</i> spp.	Belongs to the normal microflora of the human oral and gastrointestinal tracts. It is a very long and slender spindle-shaped bacillus with sharply pointed ends that is characterized by the ability to invade soft tissues. Although not considered a major dental pathogen on its own, this anaerobe facilitates the aggregation and establishment of several other species including the dental pathogens <i>Porphyromonas gingivalis</i> and <i>Bacteroides forsythus</i> [23].	11
	<i>Leptotrichia</i> spp.	An anaerobic, gram-negative rod bacteria. It is a constituent of normal oral flora. <i>Leptotrichiabuccalis</i> can be clearly identified using live blood analysis in dark field. They have a distinct form, which separates them from other rod forms [24].	20
Actinobacteria	<i>Rothia mucilaginosa</i>	A gram-positive, cocci-shaped bacterium that inhabits the oral cavity. The bacterium is considered an opportunistic pathogen and has been associated with endocarditis, meningitis, and peritonitis [25].	8
Saccharibacteria (TM7-3)	N/A	A major lineage of Bacteria, or a candidate phylum known solely through environmental 16S rRNA sequences as no species had been grown in the lab [26].	10

Table A4. Statistical analysis across subjects of the highly abundant OTUs recovered from oral microbiomes of children due to swaking

OTU no.	OTU abundance	Taxonomy	BEF2	AF2	BEF6	AF6	BEF8	AF8	BEF9	AF9	BEF10	AF10	BEF12	AF12	BEF14	AF14	BEF15	AF15	BEF19	AF19	BEF20	AF20	Mean BEF	Mean AF	P value
Otu2	235993	Bacteria; Firmicutes; Bacilli; Lactobacillales; Streptococcaceae; Streptococcus; Streptococcus_infantis	11062	11570	7278	9371	10372	16437	13938	14394	14000	16547	6064	8382	6956	16884	18405	13464	4544	13569	14256	8500	10687.5	12911.8	0.208
Otu1	195394	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella; Prevotella_melaninogenica	15251	2972	12398	10906	19902	6250	8512	4404	9177	17991	1640	5479	4465	12883	3700	11478	21756	5495	12085	8650	10888.6	8659.8	0.474
Otu3	147490	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Veillonella; Veillonella_dispar	8044	3356	9825	5142	13444	5971	10524	3200	9441	9744	5498	6325	5589	6824	2186	8438	13926	7560	10655	1798	8913.2	5835.8	0.08
Otu4	92051	Bacteria; Proteobacteria; Gammaproteobacteria; Pasteurellales; Pasteurellaceae; Haemophilus; Haemophilus_parainfluenzae	12642	7845	842	1941	1587	2831	1634	7295	5771	3147	6416	4897	1116	8691	6067	1646	619	1804	5901	9359	4259.5	4945.6	0.611
Otu11	58531	Bacteria; Fusobacteria; Fusobacteriia; Fusobacteriales; Fusobacteriaceae; Fusobacterium	1690	2370	3060	4357	1089	3688	3934	6327	1752	793	1498	1760	5147	1874	1641	4427	1395	1329	5658	4742	2686.4	3166.7	0.446
Otu32	50183	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Veillonella; Veillonella_dispar	3563	802	4934	5040	4315	2150	1399	816	2116	2866	3997	2932	1420	1023	704	965	4818	2740	1990	1593	2925.6	2092.7	0.049
Otu8	49093	Bacteria; Actinobacteria; Actinobacteria; Actinomycetales; Micrococcaceae; Rothia; Rothia_mucilaginos	5378	948	458	419	1608	2198	4793	3265	4079	2346	2484	1503	959	830	5074	1636	2190	2268	4500	2157	3152.3	1757	0.025
Otu14	46624	Bacteria; Firmicutes; Bacilli; Gemellales; Gemellaceae	2425	5232	2153	2774	803	3783	951	1463	2915	2086	2246	5832	1170	4094	3432	1546	236	1448	1352	683	1768.3	2894.1	0.093
Otu6	40872	Bacteria; Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae; Neisseria; Neisseria_subflava	3818	4766	708	208	1158	3386	8793	2683	371	110	27	362	3432	2877	3156	501	392	946	1669	1509	2352.4	1734.8	0.417
Otu12	34029	Bacteria; Firmicutes; Bacilli; Lactobacillales; Streptococcaceae; Streptococcus	487	1567	468	130	1323	1673	194	1306	1862	2852	1573	2972	570	1585	179	6526	2685	3759	1072	1246	1041.3	2361.6	0.05
Otu10	33068	Bacteria; TM7; TM7-3	1	640	2043	969	284	488	1059	495	1233	517	3846	3684	679	222	515	397	4118	2307	3827	5744	1760.5	1546.3	0.519
Otu7	32641	Bacteria; Firmicutes; Bacilli; Lactobacillales; Carnobacteriaceae; Granulicatella	3968	2096	1733	1851	393	1201	548	2721	284	438	385	2072	769	4233	6953	1337	133	844	534	148	1570	1694.1	0.878
Otu5	32092	Bacteria; Proteobacteria;	1896	864	2344	1265	3027	405	1494	853	1702	662	1462	739	3057	873	835	396	3641	1719	3626	1232	2308.4	900.8	0

OTU no.	OTU abundance	Taxonomy	BEF2	AF2	BEF6	AF6	BEF8	AF8	BEF9	AF9	BEF10	AF10	BEF12	AF12	BEF14	AF14	BEF15	AF15	BEF19	AF19	BEF20	AF20	Mean BEF	Mean AF	P value
		Epsilonproteobacteria; Campylobacteriales; Campylobacteraceae; Campylobacter																							
Otu261	27911	Bacteria; Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae; Neisseria; Neisseria_cinerea	1318	2960	1512	173	1817	1428	1695	4513	10	94	348	40	2661	1374	7146	241	141	193	196	51	1684.4	1106.7	0.493
Otu29	24893	Bacteria; Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae; Neisseria	1204	4196	1753	151	291	1799	1931	2459	276	1394	938	2197	1258	1718	495	1368	933	351	27	154	910.6	1578.7	0.122
Otu17	23783	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella; Prevotella_pallens	209	158	2811	1991	3170	1185	253	344	390	143	1413	1889	642	727	553	699	2042	790	2090	2284	1357.3	1021	0.204
Otu15	22697	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella	792	455	2528	1294	3798	773	483	272	1072	1604	1043	2485	695	355	198	523	2114	674	255	1284	1297.8	971.9	0.452
Otu25	20994	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Porphyromonadaceae; Porphyromonas	785	1237	597	596	331	1719	3075	1734	61	116	386	878	2679	1383	1222	760	952	311	1780	392	1186.8	912.9	0.372
Otu21	18426	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Paraprevotellaceae; Prevotella	622	119	1301	2576	1203	870	1530	523	1843	548	485	513	1291	273	882	220	1835	330	1130	332	1212.2	630.4	0.046
Otu20	17155	Bacteria; Fusobacteria; Fusobacteriia; Fusobacteriales; Leptotrichiaceae; Leptotrichia	91	128	1132	460	105	1377	454	2226	941	247	2634	1290	584	277	166	373	1213	2217	595	645	791.5	924	0.676
Otu27	13910	Bacteria; Firmicutes; Bacilli; Lactobacillales; Carnobacteriaceae; Granulicatella	745	527	229	314	364	487	694	1237	1869	513	809	661	251	619	413	638	442	1081	1215	802	703.1	687.9	0.935
Otu26	13468	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella; Prevotella_nanceiensis	800	947	1297	155	177	898	1172	1185	54	173	104	544	457	2199	556	751	212	338	822	627	565.1	781.7	0.368
Otu9	13242	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Moraxellaceae; Moraxella	1	6634	0	60	79	562	106	849	778	362	5	750	1575	1182	263	1	0	21	14	0	282.1	1042.1	0.283
Otu40	13051	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Veillonella	797	78	1525	386	445	1210	745	462	27	281	17	326	793	2069	718	1551	747	723	108	43	592.2	712.9	0.615
Otu33	12906	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales;	825	25	2041	579	244	1491	699	2294	0	43	63	283	390	574	1564	594	403	566	176	52	640.5	650.1	0.975

OTU no.	OTU abundance	Taxonomy	BEF2	AF2	BEF6	AF6	BEF8	AF8	BEF9	AF9	BEF10	AF10	BEF12	AF12	BEF14	AF14	BEF15	AF15	BEF19	AF19	BEF20	AF20	Mean BEF	Mean AF	P value
		Paraprevotellaceae; Prevotella																							
Otu37	12672	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Megasphaera	171	37	1119	1683	977	798	672	748	111	376	299	929	73	907	16	180	417	1659	623	877	447.8	819.4	0.027
Otu23	11908	Bacteria; Firmicutes; Clostridia; Clostridiales	201	2117	328	924	24	606	214	2345	232	49	538	1131	744	209	241	83	798	673	149	302	346.9	843.9	0.112
Otu193	11315	Bacteria; Proteobacteria; Gammaproteobacteria; Pasteurellales; Pasteurellaceae	2650	318	416	163	1426	515	1844	354	197	230	811	427	482	428	178	65	292	285	189	45	848.5	283	0.049
Otu28	10024	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Paraprevotellaceae; Prevotella; Prevotella_tannerae	10	185	639	3606	53	663	515	174	27	84	1015	193	1624	97	70	627	115	286	21	20	408.9	593.5	0.63