



Xylitol-Containing Chewing Gum Reduces Cariogenic and Periodontopathic Bacteria in Dental Plaque – Microbiome Investigation

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Specialty section:

This article was submitted to
Nutrition and Microbes,
a section of the journal
Frontiers in Nutrition

Received: 24 February 2022

Accepted: 11 April 2022

Published: 11 May 2022

Citation:

Wu Y-F, Salamanca E, Chen I-W,
Su J-N, Chen Y-C, Wang SY,
Sun Y-S, Teng N-C and Chang W-J
(2022) Xylitol-Containing Chewing
Gum Reduces Cariogenic
and Periodontopathic Bacteria
in Dental Plaque – Microbiome
Investigation. *Front. Nutr.* 9:882636.
doi: 10.3389/fnut.2022.882636

Background: Dental caries and periodontal disease remain the most prevalent oral health problems in the world. Chewing xylitol gum may help reduce the risk of caries and periodontitis for dental health benefits. However, little evidence has shown healthy food estimation by sequencing 16S rDNA in oral microbial communities. This study investigated the clinical effect of xylitol chewing gum on dental plaque accumulation and microbiota composition using the PacBio full-length sequencing platform in 24 young adults ($N = 24$). The participants were randomly assigned to xylitol chewing gum and control (no chewing gum) groups. Participants in the chewing gum group chewed ten pieces of gum (a total of 6.2 g xylitol/day). Dental plaque from all teeth was collected for weighing, measuring the pH value, and analysis of microbial communities at the beginning (baseline, M0) and end of the 2-week (effect, M1) study period.

Results: The results suggested a 20% reduction in dental plaque accumulation ($p < 0.05$) among participants chewing xylitol gum for 2 weeks, and the relative abundance of *Firmicutes* (a type of pathogenic bacteria associated with caries) decreased by 10.26% ($p < 0.05$) and that of *Bacteroidetes* and *Actinobacteria* (two types of pathogenic bacteria associated with periodontitis) decreased by 6.32% ($p < 0.001$) and 1.66% ($p < 0.05$), respectively. Moreover, the relative abundance of *Fusobacteria* was increased by 9.24% ($p < 0.001$), which has been proven to have a higher proportion in dental plaque of healthy adults. However, the dental plaque pH value stayed in a healthy range for the two groups.

Conclusion: In conclusion, chewing xylitol gum would benefit cariogenic and periodontal bacterial reduction in the oral cavity, which could help to prevent the diseases related to these bacteria.

Keywords: oral microbiota, oral health and hygiene, dental plaque, dental caries (DMFT), 16S rDNA sequencing analysis, xylitol gum, periodontal health, periodontopathic bacteria

INTRODUCTION

Dental caries and periodontal disease are the most prevalent oral problems in humans, and each one alone can lead to tooth loss. The World Health Organization (WHO) has indicated that almost all adults and over 60% of children have dental caries (1). The occurrence of caries from clinical research is higher in patients with diets of high carbohydrate intake and lower saliva production, and cariogenic microorganisms adhere to the biofilm on the tooth surface. These factors can process hard tissue minerals over time and destroy their organic components (2). For example, *Streptococcus* is considered a highly cariogenic bacterium capable of this destruction (3, 4). Many assessments can evaluate the oral health status, such as the weight of dental plaque (5), microbial community (6), pH value of dental plaque (7), and colonization measurement of specific cariogenic bacteria (8, 9).

Xylitol is a 5-carbon sucrose substitution ($C_2H_{12}O_5$, molecular weight: 152.15 g/mol) derived from beechwood, also found naturally in small amounts inside strawberries and cauliflowers (10). This natural sugar is accepted as a safe substance to reduce dental caries risks (11). Xylitol can disrupt energy production process (EPS) of cariogenic microorganisms and decrease acid production from bacterial fermentation and bacterial adherence ability on teeth surfaces (12). Chewing gum is one of the most ubiquitous products around the globe, with a world market fabrication of more than 560,000 tons annually (13). Xylitol gum has been proven to have many advantages, such as stimulating saliva flow, increasing the pH value, and eliminating food debris and dental plaque easily (14, 15). In the last decades, the Food and Drug Administration (FDA) and European Food Safety Authority (EFSA) have approved Xylitol consumption as part of a balanced diet (16, 17). Different researchers have studied the inhibition of cariogenic bacteria levels and the reduction in dental plaque accumulation after short-term (18–20) and long-term (12) usage of xylitol gum in many clinical studies. Previous clinical findings have indicated that the effective dose of xylitol is over 5–6 g per day with a chewing time of at least 5 min to significantly decrease the risk of caries (21). Additionally, a higher dose of xylitol does not have any additional improvement; instead, it may cause side effects such as diarrhea (22). However, few studies have investigated whether chewing xylitol decreases oral inflammation and changes the oral microbiota through third-generation sequencing technology.

Oral microbiota research has been conducted for diagnosis in the last decade. Comprehensive genomic profiling in dental caries using supragingival plaque has been investigated in children and elderly patients using next-generation sequencing (NGS) technology (23, 24). *Streptococcus* was found to be significantly increased in dental caries. However, the difficulty from previous studies was short-length read assembly with lower resolution despite the lower cost (25). The PacBio platform involves third-generation sequencing (PacBio SMRT; Pacific Biosciences of California, Inc., Menlo Park, CA, United States) for accurate analysis with the longest read lengths in microbial communities

(26, 27). The error rate can be effectively reduced to 1% by single-molecule circular consensus sequencing (28).

Although several xylitol-related clinical experiments have been performed, there have not been many comprehensive investigations of the oral microbiota among young adults using a strict protocol (29, 30). In this study, we hypothesized that xylitol could influence cariogenic and periodontopathic bacterial growth in the oral cavity, which could later influence the oral microbiota. A randomized controlled trial (RCT) was designed to investigate microbiota changes using PacBio platform third-generation sequencing in young adults after chewing xylitol gum and those who did not chew gum for 2 weeks.

MATERIALS AND METHODS

Participant Screening and Recruitment

For initial screening, each 2 (mL) of saliva was collected from 100 volunteers. The sample was first diluted 50-fold with phosphate-buffered saline (PBS) and further diluted 10-fold serial dilution for several times. Afterward, 50 μ l of diluted saliva was plated

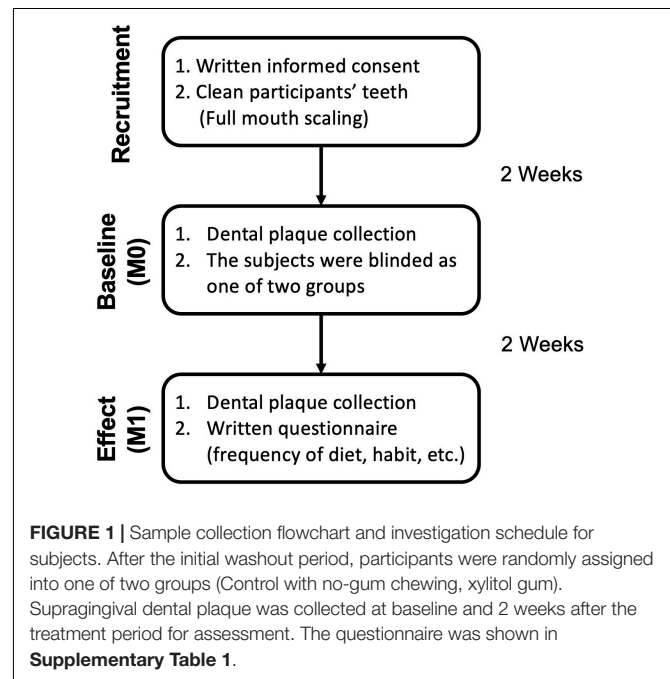


TABLE 1 | Characteristics of participants from the control and xylitol gum groups.

Basic profile	Control	Xylitol gum
Sample size, <i>N</i>	11	13
Male: Female	6:5	6:7
Age, years	24.91 ± 3.91	23.31 ± 3.12
Decayed, Missing, and Filled Teeth (DMFT) index	10 (8–13)	9 (6–13)
Number of decayed teeth (DT value)	2 (1–3)	1 (1–4)
Number of missing teeth (DM value)	3 (2–4)	4 (1–4)
Number of filled teeth (DF value)	4 (2–9)	3 (1–5)

There was no statistically significant difference between the two groups.

on Mitis Salivarius-Bacitracin (MSB) agar and incubated for 3 days in an anaerobic chamber (37°C, 5% CO₂ plus 95% N₂) following bacterial colony counts. The volunteers were required to have at least 10⁵ CFU/mL *S. Mutant* colonization in the saliva, representing a high risk for dental caries (31).

After screening, we found 24 young adults ($N = 24$) participants were qualified for the study. All participants had more than 20 teeth, provided written informed consent, and were asked not to take any medication for 3 days before the experiment. Participants were excluded if they had a systemic disease or conditions (e.g., diabetes, cancer, cardiovascular disease), localized pain (e.g., localized abscess, localized infection), pregnant women, habitual consumers of xylitol-containing products, smoking, or alcohol. The decayed, missing, and filled teeth (DMFT) index was calculated, and full mouth scaling was offered 2 weeks prior to the baseline stage for clinical examination.

Subsequently, baseline (M0) and effect (M1) dental plaque samples were collected for further examination. The flowchart is shown in **Figure 1**. The protocol was approved by the Taipei Medical University Joint Institutional Review Board (Approval No. N201910042) and followed the guidelines of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement. All participants were randomly assigned into one of two groups after baseline measurement.

Chewing Gum Selection

Xylitol gum (King Car Industrial Co., Ltd., Taipei, Taiwan) contained 0.62 g/pellet of xylitol, and the gum base included mint flavoring, soy lecithin, glycerin, titanium dioxide, and carnauba wax. The chewing gum was labeled with a letter on the package to differentiate between them.

Study Protocol

Each participant was requested to allocate 1 month for this clinical study and use the same brand of toothpaste throughout the study period. In addition, the use of mouthwash and other xylitol-related products during this period was forbidden. After the washout period, subjects were randomly classified into one of two groups: a control group (no gum chewing) and xylitol gum chewing group. All subjects received a 2-week “washout period” during which no gum was chewed after full mouth scaling, followed by a 2-week treatment period. The subjects in the chewing gum group were asked to chew two pieces per time,

five times every day, and at least 5 min for each chewing (30). The subjects in the control group were requested to brush twice a day, using the same brand of fluoride-containing toothpaste as the chewing gum group, but without chewing any gum during the whole period. The assessment of the effect on dental plaque was recorded after a 2-week trial.

Sample Collection

The total period of this study was 4 weeks. For the baseline (M0) and effect (M1) examinations, subjects were asked to refrain from the following: brushing their teeth for at least 48 h, eating at least 6 h before the examination, and drinking at least 2 h before the examination. Gracey curettes were used to collect the supragingival plaque from all teeth of each subject without activating it on the tooth's buccal and lingual/palatal surfaces. Curettes were sterilized before use, and the samples were stored in a sterile Eppendorf tube for further analysis. After the sampling was completed, the sample was labeled according to the grouping. Supragingival plaque samples were analyzed for weight, pH value, and 16S rDNA full-length sequencing.

Experimental Design

This study mainly followed the “Estimate the Health Food Effect for Dental Health” guidelines, which were released by the Ministry of Health and Welfare (MOHW) (31). Changes in dental plaque accumulation, pH value, and 16S full-length rDNA sequencing were assessed for the effects on subjects' dental health. At baseline (M0) and 2 weeks after baseline (M1), the dental plaque from all teeth of each subject was collected, weighed for plaque accumulation, and measured for pH value. The dental plaque weight was evaluated using a digital electronic microbalance, and the pH value was determined using a micro pH electrode (Orion Model 9863BN, Thermo Scientific, Chelmsford, MA, United States) to estimate the caries susceptibility.

For 16S rDNA full-length amplicon sequencing with the PacBio SMRT sequencing platform, plaque was extracted and prepared according to the manufacturer's protocol of the GenElute™ Bacterial Genomic DNA Kit (Sigma-Aldrich, St. Louis, MO, United States). The final DNA solution from this sample was collected with an elution solution (10 mM Tris-HCl, 0.5 mM EDTA, pH 9.0) for further sequencing. The full-length 16S rDNA sequence was amplified using the bacterial-specific universal PCR primers 27F and 1492R according to the manufacturer's instructions. The raw reads were processed

TABLE 2 | Average dental plaque weight, pH value from the two groups at M0 and M1, respectively.

Variable	Control		Xylitol gum	
	M0	M1	M0	M1
Average dental plaque weight, mg	32.00 (19.15–66.60)	34.30 (22.10–49.50)	34.80 (30.50–57.60)	26.40* (16.17–37.80)
Average pH value	6.53 (6.35–6.79)	6.58 (6.49–6.80)	6.55 (6.28–6.79)	6.58 (6.30–6.67)

* $p < 0.05$ by Wilcoxon's test; The average dental plaque weight was significantly decreased following chewing xylitol gum treatment.

TABLE 3 | Detection selected abundance of *Abiotrophia*, *Actinomyces*, *Porphyromonas*, *Alloprevotella*, *Porphyromonas*, *Streptococcus*, *Fusobacterium*, and *Leptotrichia* species carried by control and xylitol groups.

Species level	Control group			Xylitol group				
	Significance	Trend	Relative median abundance (%)		Significance	Trend	Relative median abundance (%)	
			M0	M1			M0	M1
<i>Abiotrophia defective</i> (nutritionally variant <i>Streptococci</i>)			0.336	0.258	**	↓	0.141	0.09
<i>Actinomyces unclassified</i> (<i>Actinomyces</i> spp.)			0.018	0.016	*	↓	0.026	0.01
<i>Actinomyces gerencseriae</i>			0.032	0.009	*	↓	0.01	0
<i>Actinomyces meyeri</i>			0.001	0.001	**	↓	0.02	0.003
<i>Actinomyces odontolyticus</i>			0.003	0.001	**	↓	0.008	0.005
<i>Actinomyces</i> sp._HMT_448			0.009	0.005	*	↓	0.026	0
<i>Porphyromonas unclassified</i> (<i>Porphyromonas</i> spp.)			0.358	0.279	***	↓	0.929	0.412
<i>Porphyromonas catoniae</i>			0.528	0.314			0.466	0.390
<i>Porphyromonas</i> sp._HMT_278					**	↓	0.041	0.018
<i>Porphyromonas</i> sp._HMT_284			0.007	0.006			0.008	0.004
<i>Alloprevotella</i> sp._HMT_914			0.123	0.107	**	↓	0.344	0.083
<i>Alloprevotella rava</i>			0.081	0.087			0.065	0.099
<i>Prevotella unclassified</i> (<i>Prevotella</i> spp.)			4.239	2.747			2.330	2.920
<i>Prevotella denticola</i>			0.081	0.146			0.127	0.119
<i>Prevotella histicola</i>			0.014	0.009			0.003	0
<i>Prevotella intermedia</i>			0.006	0.0			0.043	0.004
<i>Prevotella melaninogenica</i>			0.517	0.6			0.225	0.273
<i>Prevotella nigrescens</i>			0.247	0.263			0.172	0.157
<i>Prevotella oulorum</i>			0.055	0.072			0.326	0.149
<i>Prevotella pallens</i>			0.048	0.035			0.011	0.016
<i>Prevotella veroralis</i>			0.040	0.047			0.344	0.212
<i>Streptococcus unclassified</i> (<i>Streptococcus</i> spp.)			4.335	1.642			3.270	1.621
<i>Streptococcus anginosus</i>			0	0			0.031	0.013
<i>Streptococcus cristatus</i> _clade_578			0.030	0.013			0.051	0.029
<i>Streptococcus gordonii</i>			0.160	0.114	*	↓	0.197	0.039
<i>Streptococcus intermedius</i>			0	0			0.017	0.008
<i>Streptococcus oralis</i> _subsp._dentisani_clade_058			2.338	1.906			1.744	0.858
<i>Streptococcus oralis</i> _subsp._dentisani_clade_398			0	0			0.003	0.003
<i>Streptococcus oralis</i> _subsp._tigurinus_clade_071			0.078	0.145			0.177	0.172
<i>Streptococcus salivarius</i>			0.024	0			0.003	0
<i>Streptococcus sanguinis</i>			0.041	0.032			0.038	0.030
<i>Fusobacterium unclassified</i> (<i>Fusobacterium</i> spp.)			9.807	8.878	*	↑	8.541	10.199

(Continued)

TABLE 3 | (Continued)

Species level	Control group			Xylitol group				
	Significance	Trend	Relative median abundance (%)		Significance	Trend	Relative median abundance (%)	
			M0	M1			M0	M1
<i>Fusobacterium naviforme</i>			0	0.001		↑	0	0.015
<i>Fusobacterium nucleatum_subsp._animalis</i>	*	↑	0.492	1.391	*	↑	1.774	3.697
<i>Fusobacterium nucleatum_subsp._polymorphum</i>			0.120	0.072			0.077	0.099
<i>Fusobacterium nucleatum_subsp._vincentii</i>	**	↑	0.359	1.500	**	↑	1.281	2.083
<i>Fusobacterium periodonticum</i>			0.958	0.598	*	↓	0.324	0.097
<i>Fusobacterium p._HMT_203</i>	*	↑	0.065	0.118			0.081	0.116
<i>Lautropia mirabilis</i>			0.840	0.330	***	↓	0.446	0

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ by Wilcoxon's test.

using pbccs (v.3.4.0) in SMRT Link Analysis v6.0.0, which generated consensus sequences with low error rates. Sequences for each sample were processed using the software package QIIME version 1.9.0 (32). To avoid possible sequencing errors, operational taxonomic unit (OTU) picking was performed using mothur v.1.39.5 with 97% identity (33). Chimeric sequences were identified using UCHIME v4.2 with the Gold database and made OTU table (34). The microbial diversity analyses of our samples were based on the Human Oral Microbiome Database (HOMD) v15.2 using QIIME.

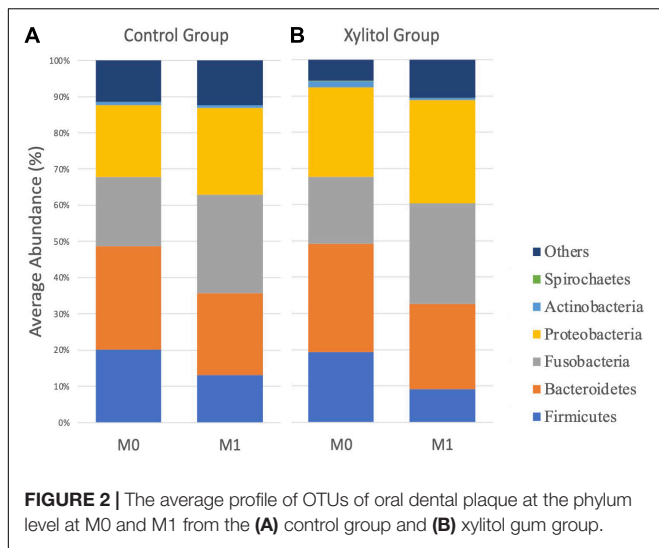
We chose IBM SPSS, version 19 (IBM Co., Armonk, NY, United States) for further statistical analysis and compared the difference between effect and baseline assessments in each group. The effect of the gum was assessed using the Student's *t*-test or Wilcoxon test. The microbial community analyses from the OTUs were performed using the Kruskal–Wallis test and Wilcoxon test, when appropriate.

RESULTS

The background and dental information from all participants are shown in **Table 1**. There were 13 subjects in the xylitol gum group and 11 subjects in the control group. The average participants' age range was 23–25 years, and the DMFT index ranged from 9 to 10. Age and DMFT index were not significantly different between the two groups because the present study is a randomized clinical trial. The demographics and specific information of participants were listed in **Supplementary Table 2**.

The average dental plaque weight, pH value, unique tags, Good's coverage, and alpha diversity are shown in **Table 2**. At least 10,000 effective unique tags were classified into OTUs, and 99.0% of Good's coverage remained in all groups (**Supplementary Table 3**). Thus, the sequencing depth was sufficient to recover the oral bacterial communities. Interestingly, the present data showed that chewing xylitol gum for 2 weeks (at M1) resulted in a 19% significant reduction in dental plaque weight ($p < 0.05$), while controls had an increase of 7%. In addition, the Shannon diversity index of the microbiota in the xylitol gum group significantly decreased after the trial due to the reduction in pathological microorganisms ($p < 0.05$), but no significant difference was observed in the effects in the control group. The effect on plaque samples in the xylitol gum group was reduced weight. A smaller amount of dental plaque was presented with a small taxonomically diversity than the control group. However, the average pH value in both groups remained within the healthy range with no significant change before and after the study period.

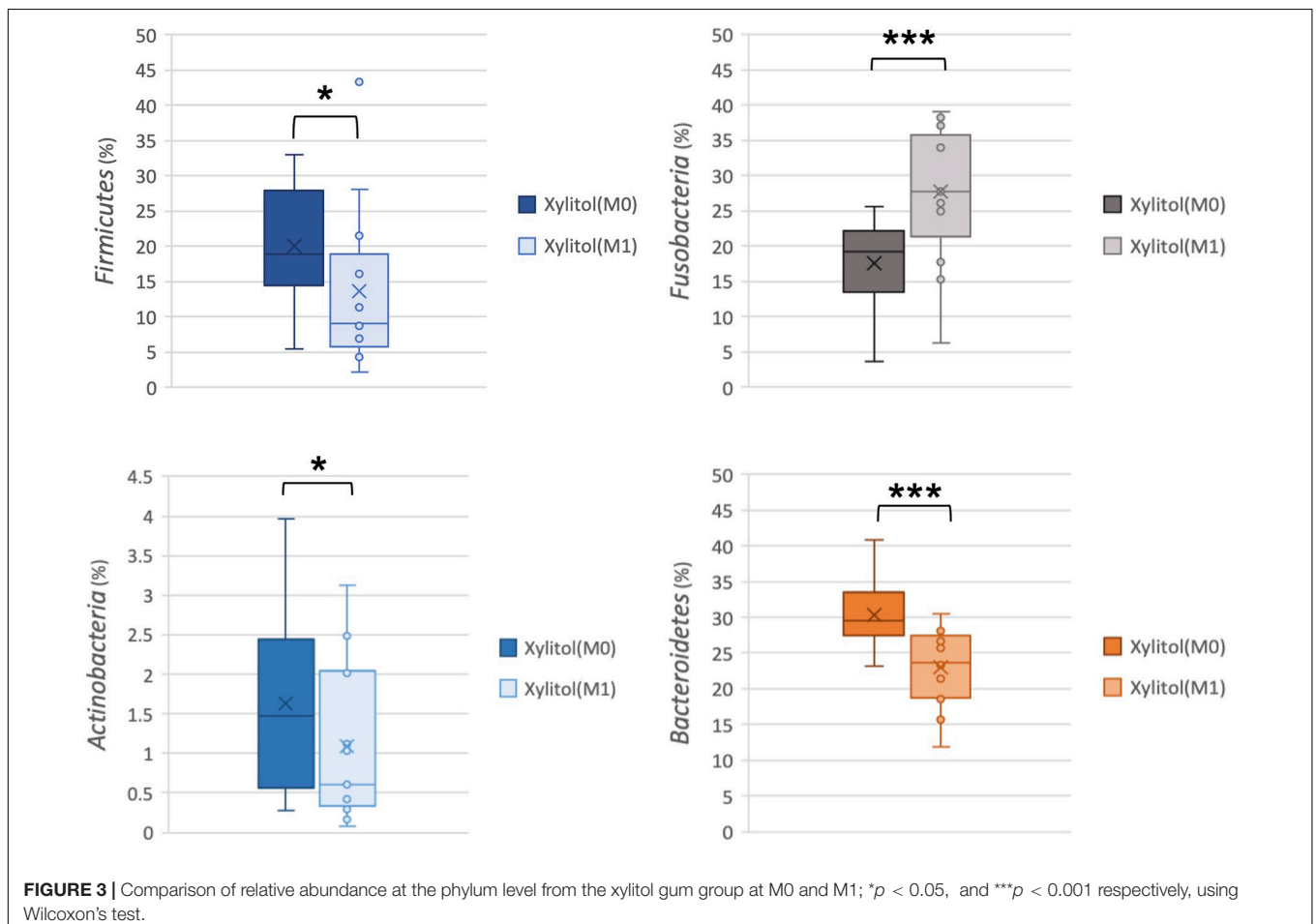
Figure 2A shows the histogram of the relative abundance of oral bacterial phyla at M0 and M1 for the two groups. In the control group, the fluoride-containing toothpaste effect decreased the average abundance of *Firmicutes* and *Bacteroidetes* from 20.14 and 28.49% at baseline to 13.02 and 22.62%, respectively. The average abundance of *Proteobacteria* slightly increased from 19.80 to 23.89%, and that of *Fusobacteria* significantly increased from 19.12% at baseline to 27.28% after 2 weeks ($p < 0.05$). The results from the control group indicated

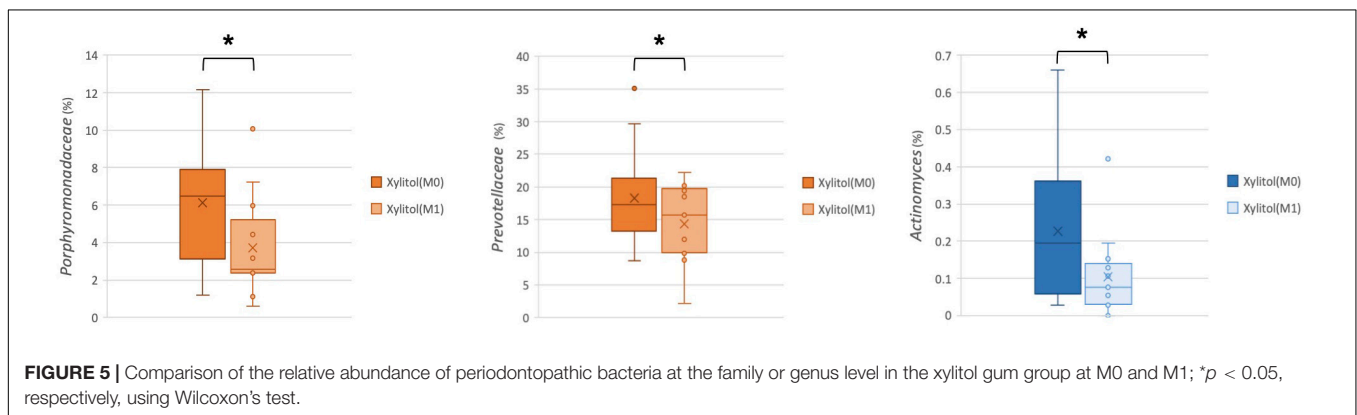
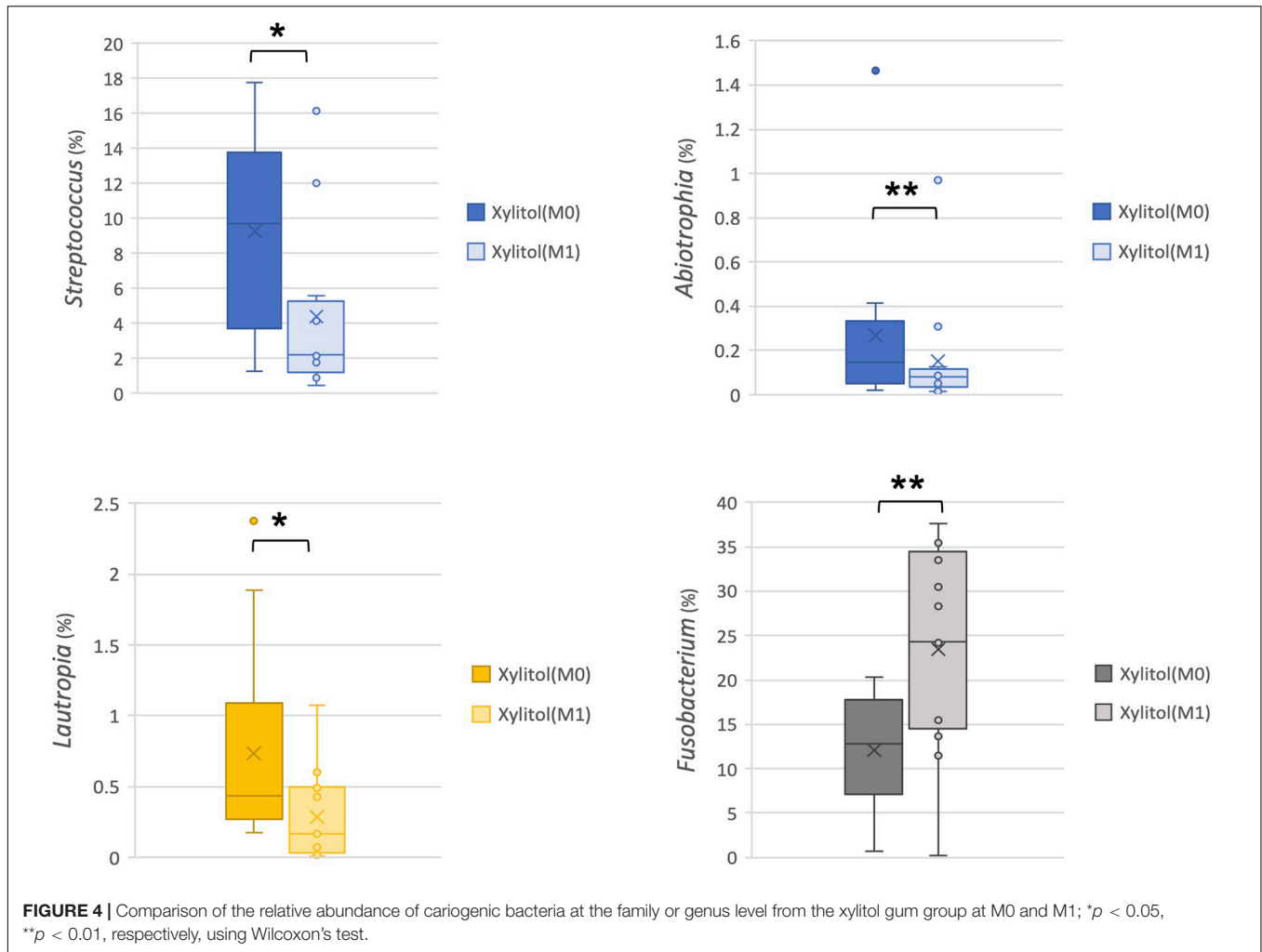


In **Figures 2B, 3**, the xylitol gum group shows a decrease in oral *Firmicutes*, and *Bacteroidetes* from 19.28 and 29.90% to 9.02 ($p < 0.05$) and 23.58% ($p < 0.001$), respectively. Notably, the average abundance of *Actinobacteria* was significantly decreased by 1.66% after 2 weeks of chewing xylitol gum ($p < 0.05$). The average abundance of *Fusobacteria* was increased by 6% after 2 weeks in the xylitol gum group ($p < 0.001$). The xylitol group had a significant increase ($p < 0.001$) in the relative abundance of *Fusobacteria* compared to that in the control group ($p < 0.05$).

In addition, the cariogenic and periodontopathic bacteria at the family or genus level from the xylitol gum group are shown in **Figures 4, 5**. This result indicated a significantly decreased tendency of *Streptococcus* ($p < 0.05$), *Lautropia* ($p < 0.01$), and *Abiotrophia* ($p < 0.05$) (nutritionally variant streptococci), all of which are related to dental caries. Additionally, a lower abundance ($p < 0.05$) of *Prevotellaceae*, *Porphyromonas*, and *Actinomyces* after 2 weeks of chewing xylitol gum was observed, these bacteria are associated with periodontal disease. In more detail, the result of the major species-level bacterial community from control and xylitol groups were listed in **Table 3**. Taxa detected periodontopathic bacteria of *Actinomyces* spp., *Actinomyces gerencseriae*, *Actinomyces meyeri*, *Actinomyces odontolyticus*, *Porphyromonas* sp., *Alloprevotella* sp._HMT_914, *Porphyromonas* spp., *Porphyromonas* sp._HMT_278,

that regular tooth brushing with fluoride-containing toothpaste and improved oral hygiene behaviors would have a slightly beneficial effect on dental health.

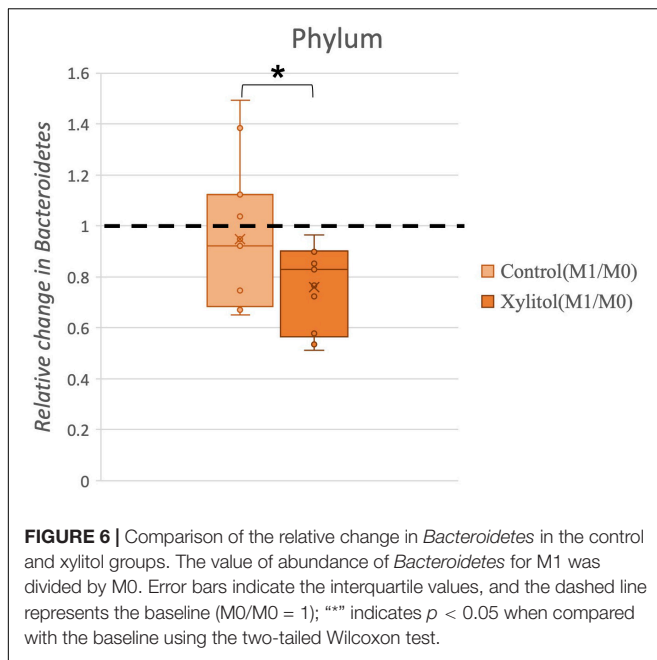




Fusobacterium periodonticum were significantly decreased in xylitol gum group than control group. Similarly, cariogenic microorganisms of *Abiotrophia defectiva*, *Streptococcus anginosus*, *Streptococcus gordonii*, *Streptococcus intermedius*, *Streptococcus oralis*, *Streptococcus salivarius*, *Lautropia mirabilis* were also decreased in xylitol gum group. These results indicated that only chewing xylitol gum exerts a suppressing effect on both

cariogenic and periodontopathic microorganisms in the oral cavity after 2 weeks of use.

To account for individual physiological differences, the relative change index (% effectiveness/baseline) from oral bacteria was calculated for the xylitol gum and control groups. **Figure 6** shows a significant relative change ($P < 0.05$) for these two groups, which indicates that chewing xylitol gum



significantly suppressed the growth of *Bacteroidetes* compared to that of the control group.

In **Figure 7A**, a linear discriminant analysis effect size-based taxonomic cladogram depicts successive circles that represent a phylogenetic level and a linear discriminant analysis (LDA) bar graph. The brightness of each dot is proportional to its effect size, and each LDA bar length was represented by a \log_{10} transformed LDA. The taxa that met a significant LDA threshold value of $>|\pm 3|$ are shown. Only the species in the family *Fusobacteriaceae* were enriched in the control group (**Figure 7A**). The species in the phylum *Firmicutes*, phylum *Bacteroidetes*, phylum *Fusobacteria*, class *Burkholderiaceae*, class *Fusobacteriia*, and genus *Lautropia* were enriched in the xylitol gum group (**Figure 7B**). Similarly, **Figure 8** shows a region of oral health distribution by confidence ellipses plotting the total cariogenic, periodontopathic bacteria and dental plaque accumulation. After 2 weeks of chewing xylitol gum (**Figure 8A**), most of the dots are found in the lower left area representing the effectiveness of xylitol inducing less dental plaque, and less total bacteria count. On the other hand, a slight change after 2 weeks in control group (**Figure 8B**).

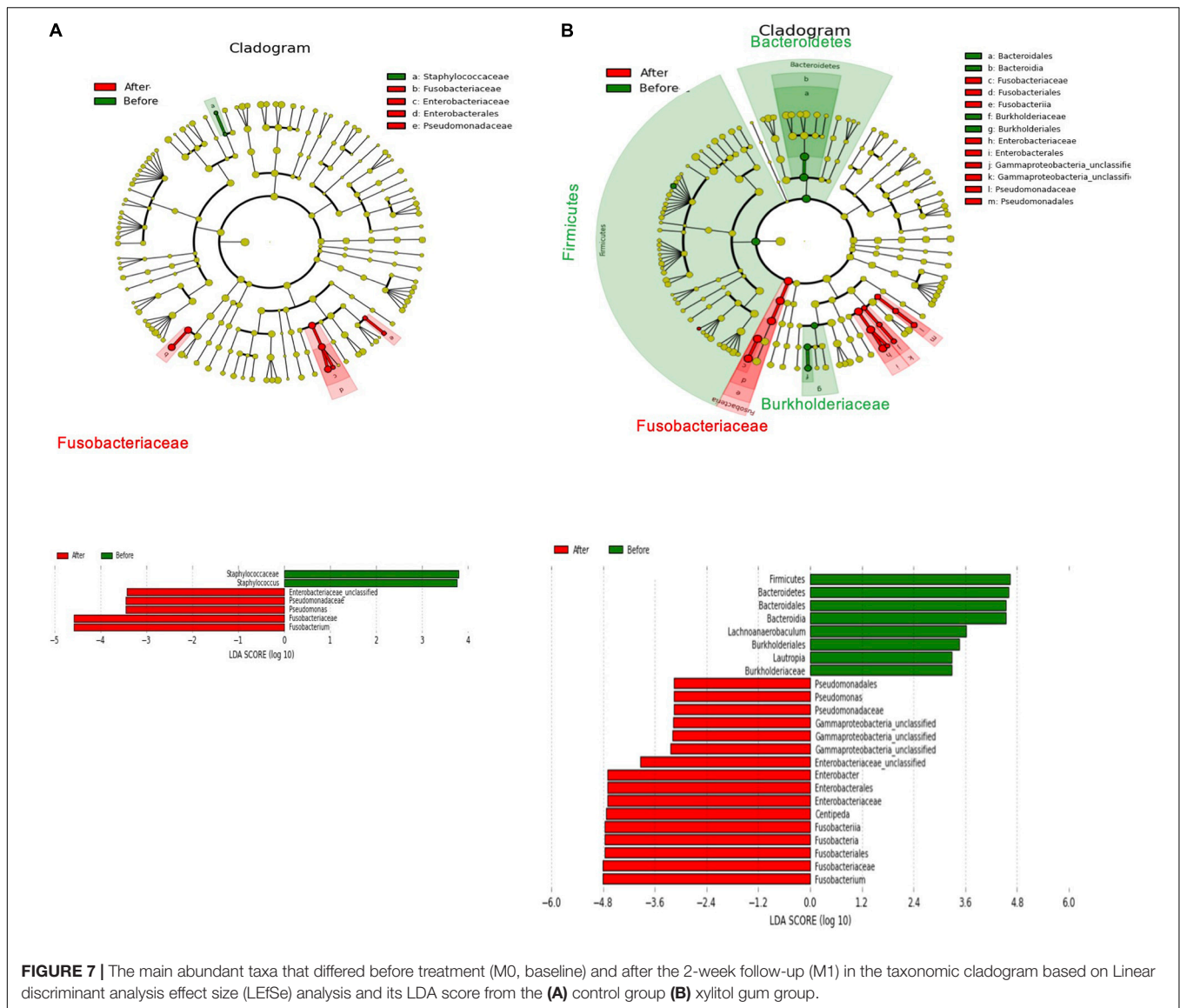
DISCUSSION

Over the years, humans have consumed various products for pleasure and health, including chewing gum (35). For decades, dentists discouraged the use of chewing gum due to sucrose additives. However, evidence showed that chewing gum would promote enamel remineralization by stimulating saliva flow and increasing plaque pH (36, 37). Chewing gum can be easily found with non-sugar sweeteners such as xylitol. The present study investigated the effect of xylitol chewing gum on dental plaque accumulation and its microbiota composition

using the PacBio sequencing platform in 34 young adults. All subjects recruited in the study had a similar taxonomic composition when comparing the number of caries, missing teeth, and the DMFT index (**Tables 1, 2**). Notably, chewing xylitol gum was significantly reduced the weight of dental plaque and the abundance of pathogenic bacteria compared to the control group (**Table 2** and **Supplementary Table 2**). Our results revealed a statistically significant difference in the plaque weight in the xylitol gum group (19% reduction) compared to the control group ($p < 0.05$). Moreover, subjects in the control group had 7% higher dental plaque weight than their baseline (**Table 2**). The increased dental plaque weight indicated a higher number of bacteria adhere to the tooth surface and the posterior biofilm formation that led to dental caries and periodontal disease.

The caries process comprises acidogenic and aciduric bacteria that are responsible for lowering the pH value and subsequent destruction of the hydroxyapatite matrix in enamel and dentine (38). In the present study, chewing xylitol gum decreased the abundance of *Streptococcus*, *Lautropia*, and *Abiotrophia* (nutritionally variant *Streptococci*) (**Figure 4**). These cariogenic microorganisms are associated with the cause or progression of the dental lesion (39). In the present study, *Streptococcus* levels had a statistically significant decrease ($p < 0.05$) between baseline and 2 weeks after the xylitol gum trial, which could prevent caries initiation or progression. This seemed to be related to the reduction of sucrose, increase in salivary flow and tooth surface self-cleaning during mastication. Xylitol's benefits can lead to a significantly lower total bacterial percentage can be seen as early as a 2-days experimental duration according to a study published in 2018 (40). In their study, researchers have also found an effect of xylitol-containing chewing gum on the oral microbiota, but no significant difference was observed in the overall composition of the salivary microbiota between the baseline and follow-up samples of the two groups, including participants aged 19–52 years with a DMFT index from 0 to 24. In addition, the pH value of the dental plaque from our results was still considered healthy after 2 weeks, which showed 6.50 and 6.21 pH levels, respectively.

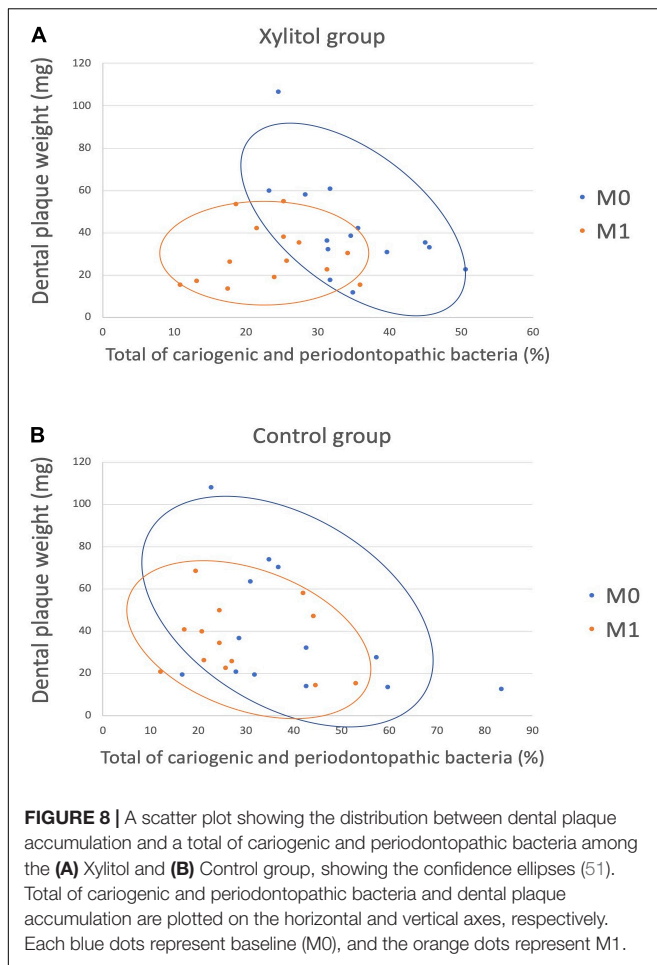
To the best of our knowledge, not only caries (41) but also patients with periodontitis (42, 43) have higher abundance levels of *Bacteroidetes* than healthy individuals. In the present study, only the xylitol gum group reduced the abundance of *Actinobacteria* (*Actinomyces* spp., *A. gerencseriae*, *A. meyeri*, *A. odontolyticus*), *Prevotellaceae*, and *Porphyromonas* (*Porphyromonas* spp., *P. sp._HMT_278*), but this reduction was not observed in the control group (**Figure 3**). The same tendency was previously described by He et al. (41) in their taxonomic and functional analyses of the supragingival microbiome from caries-affected and caries-free hosts. Their study also found that the family *Prevotellaceae* had a higher relative abundance in the supragingival dental plaque of caries patients, although the difference was not statistically significant (41). Importantly, the average abundance of *Prevotellaceae* (*P. intermedia*, *P. nigrescens*, *P. oulorum*, *P. veroralis*) in our study decreased at the family and species levels after



subjects chewed xylitol for 2 weeks (Figure 5 and Table 3). This evidence suggests that the effectiveness of xylitol can reduce dental plaque and relieve gingival inflammation. Xylitol has only been reported to inhibit the gene expression of the inflammatory cytokines TNF- α and IL-1 β in *in vitro* studies (44). Therefore, our result of xylitol's clinical effect in microbiota suggests that chewing xylitol gum may have not only a potentially positive anti-inflammatory effect in the oral cavity but also bacteria inhibition growth, which was not previously investigated in human studies through high-throughput 16S rDNA full-length amplicon sequencing (45, 46). These data provide valuable insights into preventing oral infections such as caries and periodontal diseases by chewing xylitol gum.

The PacBio sequencing platform was used to estimate the mean percentage of supragingival plaque microbiota from each group. Our study found that only had *Fusobacterium* significantly

increased in the control group. The average abundance of *Firmicutes*, *Bacteroides*, and *Actinobacteria* significantly decreased, while that of *Fusobacterium* significantly increased in the xylitol gum group. Similar studies have also suggested that *Fusobacterium* and *Pasteurellaceae* constituted a higher proportion of dental plaque in healthy adults (47, 48). According to our present findings, only xylitol gum had a beneficial effect on dental health. The xylitol effectiveness was evaluated to reveal its clinical advantages in the preventive oral health approach. Whereas the control group did not experience any significantly higher tendency of caries and periodontal disease. These findings can be related to the longer study period, and our study participants were recruited between ages 20–30 years with high DMFT levels. Referencing the different findings among previous studies (49) and our study, the level of the DMFT index might be related to the detectability of the benefits from chewing xylitol-containing gum. The higher the DMFT level,



the more significant the clinical effect of xylitol in decreasing cariogenic and periodontopathic bacteria in dental plaque. In subjects with good oral hygiene, xylitol gum will have the same benefits for reducing the risk of caries as conventional chewing gum, such as medical reasons including halitosis and xerostomia reduction, aid to digestion, and psychological help like nervous tension relief (35). Last, the *Firmicutes/Bacteroidetes* (F/B ratio) value from all groups was less than one, which indicates no intense dysbiosis or inflammatory reaction during the study (50).

This study employed the full-length of a 16S rDNA PacBio SMRT sequence to evaluate the effectiveness of chewing gum for short-term use. This result indicated that xylitol gum had an excellent anti-cariogenic and anti-periodontopathic clinical effect based on oral microbiota modulation. However, there are still some limitations that could be improved in the future. First, a longer-term treatment over 1 month of observation was not performed in this study. Second, this method preserves the significance of differences in abundance from microbial communities. However, the increased sensitivity for specific bacteria should still be used in the traditional approach (i.e., laboratory culture-based colonization of selective media). Finally, adverse effects on the TMJ, jaw muscle pain, and even though

none of the participants had diarrhea, it may occur with excessive chewing of xylitol gum (22).

CONCLUSION

In conclusion, our study showed statistically significant differences in dental plaque accumulation and associated microbial communities after chewing xylitol gum, as determined by the PacBio SMRT sequencing platform. The results showed that dental plaque accumulation decreased due to bacterial adherence, the relative abundance of cariogenic bacteria such as *Streptococcus*, *Lautropia*, and *Abiotrophia* and that of periodontopathic bacteria such as *Porphyromonas*, *Actinomyces*, and *Prevotellaceae* bacteria were significantly decreased by daily chewing of xylitol gum (intake of 10 pellets for a total of 6.2 g xylitol/day) for 2 weeks. Xylitol gum appeared to reduce the likelihood of caries and the development of periodontal disease by limiting bacterial growth. Based on our findings, no similar studies have employed third-generation sequencing technology, which could be used in future studies for more precise results.

DATA AVAILABILITY STATEMENT

The 16S rDNA data sets generated in this study were deposited in the EBI European Nucleotide Archive database (European Bioinformatics Institute, Cambridge, United Kingdom) under the accession number: PRJEB51987.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Taipei Medical University Joint Institutional Review Board (Approval No. N201910042). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

N-CT and W-JC conceived and designed the experiments. Y-FW, ES, I-WC, J-NS, Y-CC, and SW performed the experiments. Y-FW, Y-CC, and SW analyzed the data. Y-FW, ES, I-WC, J-NS, SW, and W-JC wrote the manuscript. Y-SS, N-CT, and W-JC developed this study. All authors contributed to the article and approved the submitted version.

FUNDING

This study received funding from King Car Industrial Co., Ltd., University-Industry Collaboration, and EX-03-06-08-111 of the Southern Taiwan Science Park Bureau, Ministry of Science and Technology (MOST). The funder was not involved in the study

design, collection, analysis, interpretation of data, the writing of this article, or the decision to submit it for publication.

ACKNOWLEDGMENTS

We thank American Journal Experts (AJE) for assisting in the language usage, spelling, and grammar of our manuscript. We also appreciate Genomics BioSci & Tech. CO., LTD. (New Taipei

City, Taiwan) and Blossom Biotechnologies, Inc. (Taipei City, Taiwan) for their technical assistance.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnut.2022.882636/full#supplementary-material>

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