BMC Oral Health

RESEARCH

Open Access

The impact of *Limosilactobacillus reuteri* in combination with non-surgical periodontal therapy on periodontal clinical parameters and salivary and subgingival microbiota composition in individuals with stage III–IV periodontitis: a randomized controlled trial

Pengcheng Huo[1†], Li Deng[1†], Jiawei Lu[1], Powen Kan[1], Rui Jing[1] and Li-jun Luo[1*]

Abstract

Background The clinical and microbiological outcomes of probiotic-assisted periodontal therapy remain inconclusive in prior research. This ambiguity may stem from uncertainties regarding the duration, dosage, and administration route of probiotics. Additionally, earlier studies predominantly concentrated on the identification of individual bacterial species, thereby limiting the ability to thoroughly elucidate the intricate composition of microbial communities and the synergistic or antagonistic interactions among their constituents. The study aimed to investigate the effect of combining probiotics with non-surgical periodontal therapy on clinical and microbiota changes in patients with stage III–IV periodontitis.

Methods A total of 40 patients were randomized into two groups to receive non-surgical periodontal treatment. The test group received *Limosilactobacillus reuteri* for 21 days along with treatment. Periodontal indicators were examined at baseline and 1 and 6 months after treatment. Saliva and subgingival biofilm samples were collected for 16 S rRNA gene sequencing analysis.

Results After treatment, both groups showed significant improvements in clinical parameters. In the test group, attachment loss and the medium pocket were significantly reduced at 6 months compared to that at 1 month. The presence of *Tannerella forsythia* in subgingival biofilms decreased significantly in the test group after treatment. Among salivary microorganisms, the abundance of *Prevotella nanceiensis* significantly increased in both groups, while that of *Streptococcussp*. was significantly reduced in the control group. Linear discriminant analysis indicated that

[†]Pengcheng Huo and Li Deng contributed equally to this work and should be considered co-first authors.

*Correspondence:
Li-jun Luo
juneluo@yeah.net

Full list of author information is available at the end of the article

the most significant distinction between the groups was observed in the subgingival biofilm samples 1 month after treatment.

Conclusions Combining *L. reuteri* with non-surgical periodontal therapy may not directly improve clinical indicators. The treatment showed potential benefits by changing the microbial composition of subgingival biofilm and enhancing treatment sensitivity.

Trial registration The trial was approved by the Chinese Clinical Trial Registry (ChiCTR) on March 25, 2021, with registration number ChiCTR2100044638.

Keywords *Limosilactobacillus reuteri*, Adjunctive periodontal therapy, Microbiology, Non-surgical periodontal therapy, Clinical trial

Background

Periodontitis is a chronic inflammatory disease initiated and perpetuated by a dysbiostic biofilm, resulting in the progressive destruction of the hard and soft tissues of the periodontium. It is widely recognized as a significant contributor to edentulism globally [1]. According to a recent meta-analysis, periodontitis and severe periodontitis in dentate adults were estimated at approximately 62% and 23.6%, respectively [2]. In pathological conditions, the microbiota shifts from symbiosis to dysbiosis. Disease-associated microbial changes signify an overall alteration in the structure of the entire microbial population and the functional properties of the entire community [3].

Non-surgical periodontal therapy (NSPT) is the most reliable and fundamental treatment for periodontal inflammation. The primary objective of NSPT is to control the amount of periodontal pathogenic microbiota [4]. However, a limitation of mechanical microbial biofilm control is that the microbiome composition may shift back to pre-treatment levels in the short term [5]. In patients with moderate to severe periodontitis, NSPT may be ineffective in deep periodontal pockets, furcation involvements, and root trunk concavities [6, 7]. In this context, research interest in methods to assist NSPT in clinical periodontology has been growing.

Probiotics are living microorganisms that provide health benefits to the host when consumed in sufficient quantities. Probiotics restore the balance of the oral microbial ecosystem by increasing the proportion of beneficial bacteria through the competitive inhibition of periodontal pathogens, thereby modulating the host's subsequent response [8]. *Limosilactobacillus*, a commonly used probiotic, may prevent and cure numerous illnesses in animals and humans [9]. Several studies have suggested that adding *Limosilactobacillus reuteri* to NSPT results in greater improvements in the gingival index, bleeding on probing, and probing depth (PD) compared with a placebo 3 months post-treatment [10–14]. Other studies have found that improvements in PD and attachment loss due to probiotic treatment can last up to 6 months [15–17]. However, some clinical trials found no statistical difference in clinical parameters between groups using *L. reuteri* in addition to NSPT and those using NSPT alone [18–20]. The meta-analysis conducted by Ng et al. [21] demonstrated that the use of adjunctive probiotics did not confer any additional benefit in terms of the percentage change in the total number of deeper periodontal sites (≥ 5 mm, ≥ 6 mm, ≥ 7 mm) before and after treatment. Furthermore, no significant differences were observed in the mean reduction of probing pocket depth at 3 and 6 months post-therapy.

Studies have shown changes in oral microbial content after applying *L. reuteri*. Teughels et al. [14] showed that using *L. reuteri* auxiliary NSPT significantly reduced the incidence of *Porphyromonas gingivalis* in subgingival, supragingival, and saliva plaques, along with a notable decrease in salivary *Prevotellaintermedia*. Tekce et al. [13] found that administering *L. reuteri* with NSPT reduced the percentage of specialized anaerobic bacteria in subgingival biofilm over 3 months. Nevertheless, some studies indicate contrary findings. Laleman et al. [16] studied the additional effects of *L. reuteri* probiotics on treating persistent pockets. They found no significant changes in the abundance of pathogenic bacteria *P. gingivalis*, *P. intermedia*, *Fusobacterium nucleatum*, or *Aggregatibacteractinomycetemcomitans* in supragingival, subgingival, and salivary plaques [16]. Moreover, meta-analyses report that adjuvant probiotics did not elicit short-term effects on reducing the abundance of periodontal pathogens, including *A.actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, and *F. nucleatum*, in substrates [22, 23].

Previous research findings on the impact of *L. reuteri*-assisted periodontal therapy on oral cavity microorganisms are conflicting. This phenomenon can be attributed to several prevalent methodological limitations, including the absence of a standardized treatment protocol, insufficient knowledge regarding the most effective probiotic combinations, and the most suitable probiotic delivery vehicle. Additionally, there is a tendency to focus solely on detecting specific pathogenic bacteria, while neglecting a comprehensive assessment of the entire microbiota, particularly the anaerobic flora [8].

Herein, 16 S rRNA gene sequencing was performed to investigate the microbial effects of probiotics, specifically modified *L. reuteri*, after periodontal treatment. This study aimed to conduct comprehensive microbiome analyses of *L. reuteri* in the context of supporting NSPT for severe periodontitis, and to elucidate the mechanisms by which these probiotics exert their effects. Enhanced understanding of these processes may subsequently clarify the variability in clinical efficacy and inform more effective utilization strategies.

Methods

Study characteristics

This research was conducted as a single-blind, randomized clinical trial. All participants provided consent by signing an informed consent form. Recruitment was conducted by the same trained examiner at the Department of Periodontitis, Stomatology Hospital, University of Tongji. The inclusion criteria for the study were as follows: participants aged 18–60 years, individuals with 20 or more remaining teeth in the oral cavity, and those with a confirmed diagnosis of stage III–IV periodontitis based on the 2018 Periodontal Disease Classification Criteria [1, 24]. The exclusion criteria included pregnant or lactating women, individuals who had used antibiotics in the previous 3 months, those who had undergone periodontal treatment in the last 6 months, and smokers (current or with a history of smoking over 100 cigarettes).

Forty patients were enrolled in the study and randomly assigned to either the control or test groups using the Fisher and Yates random number Table [25]. Each group included 20 patients, all of whom followed the treatment regimen during the study. Six participants were excluded from the study: four and two from the control and test groups, respectively, due to antibiotic use during treatment or missed follow-up appointments. Ten patients from each group were willing to provide saliva and subgingival biofilm samples and were enrolled for further analysis. The study flow diagram is shown in Fig. 1.

The trial was approved by the Chinese Clinical Trial Registry (ChiCTR) on March 25, 2021, with registration number ChiCTR2100044638. The study protocol was approved by the ethics committee of the Stomatological Hospital of Tongji University (Approval Number: [2019]-R-011) and followed the ethical principles of the Helsinki Declaration for research involving human participants.

Microbiological sampling

After rinsing with clean water, 1–2 mL of unstimulated saliva was collected and transferred into a 1.5-mL EP tube. The tube was centrifuged at 2,000 × *g* for 20 min at 4 °C. After centrifugation, the supernatant was discarded, and the precipitate was retained for further analysis. The Ramfjord index teeth were selected for subgingival biofilm collection [26]. Teeth were selected for sampling based on preliminary probing and radiographic analyses, necessitating a probing pocket depth of ≥ 5 mm and a minimum bone resorption level of one-third of the root length. In cases where the teeth did not meet these criteria, were absent, or were pending extraction, the distal adjacent tooth was selected. Once identified, sampling was performed on the same teeth at each review. Next, the subgingival biofilm samples were collected and combined into a composite sample. Briefly, the supragingival dental biofilm was removed using a probe and then air-dried. The subgingival biofilm was collected using a 5/6 Gracey curette (HuFriedyGroup, Chicago, IL, USA) and transferred to an EP tube. After adding 200 µL deionized water, the tube was centrifuged at 2,000 × *g* for 20 min at 4 °C. Finally, the precipitate was sealed and stored in a refrigerator at -80 °C for future examination and analysis.

Periodontal measurements and therapy

Periodontal measurements per patient, including PD and clinical attachment loss (CAL), were recorded at six sites on each tooth. The bleeding index (BI) [27] was measured on the buccal and lingual sides. The examiners who conducted all measurements were masked to minimize bias. To ensure consistent results, the examiner performed calibration before the study (Kappa value = 0.87). Patients received mechanical therapy in one to two sessions after baseline measurements. Mechanical therapy included the use of sonic/ultrasonic scalers, hand instruments, and air powder devices for supragingival and subgingival debridement. This procedure was performed under local anesthesia. The patients were given study probiotics (*L. reuteri* DSM 17938 and ATCC PTA 5289; BioGaia AB, Lund, Sweden) at a dosage of 1×10^8 colony-forming units for each strain to be taken twice daily for 21 days. Subsequently, patients were seen at 1, 3, and 6 months for oral hygiene instructions and full-mouth supragingival debridement. Sites with a probing pocket depth of 4 mm or more underwent retreatment or periodontal surgery after 6 months.

Library Preparation

Library preparation included extracting microbial DNA, amplifying the 16 S rRNA gene by PCR, and recovering and purifying the PCR products. The PCR products were then measured in preparation for future online sequencing analysis.

Bioinformatics

The double-terminal data underwent overlap splicing, followed by quality control and chimera filtering processes to obtain high-quality CleanData. The Divisive Amplicon Denoising Algorithm (DADA2) was introduced as a new approach that eliminates the need for

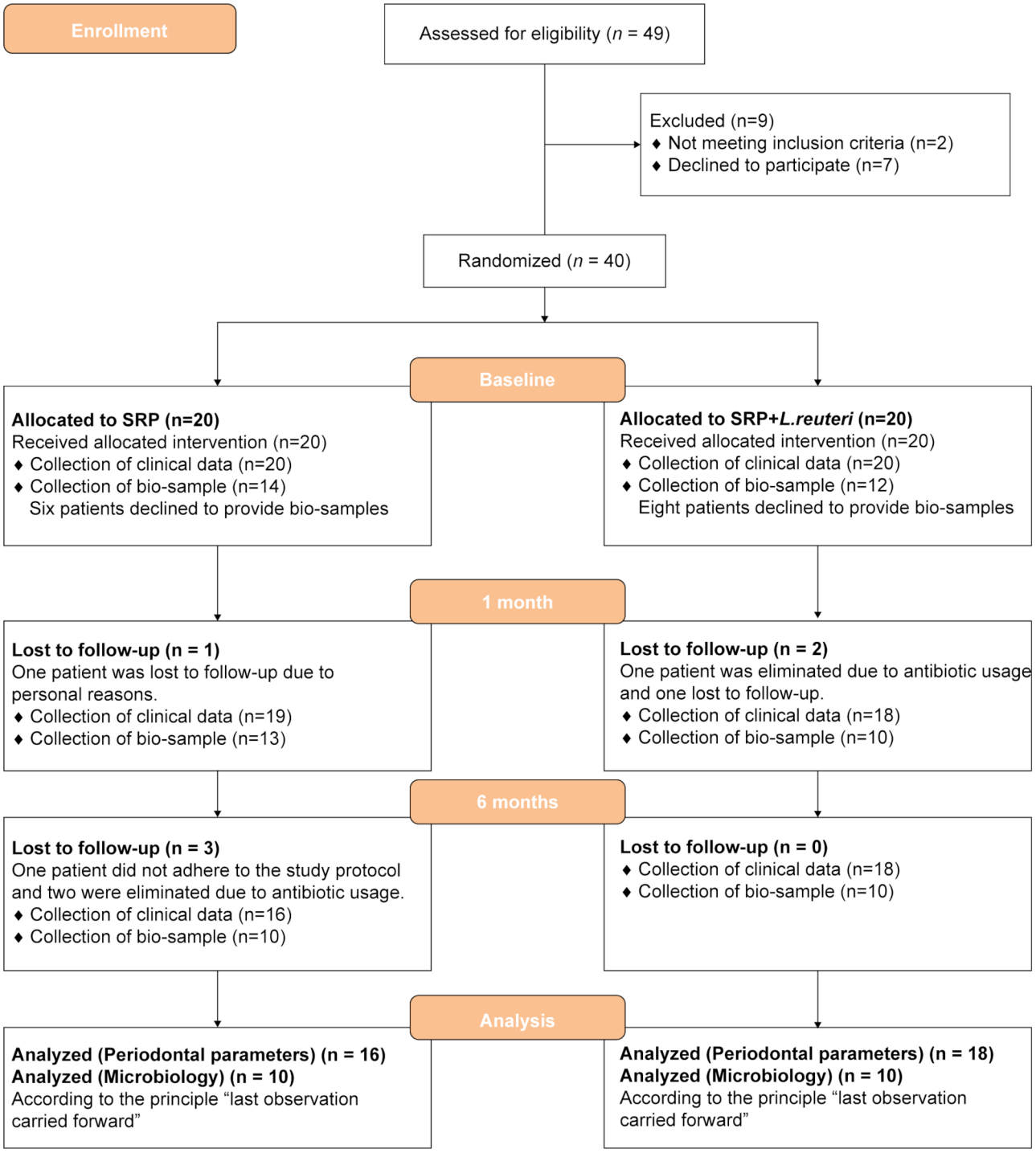

Fig. 1 CONSORT flow chart of the study

clustering based on sequence similarity [28]. It follows a series of steps, including "dereplication" (clustering at 100% similarity), to acquire representative sequences with single-base accuracy. This innovative method greatly enhances data accuracy and species resolution. The primary focus of DADA2 is denoising, followed by using amplicon sequence variants to create an operational taxonomic unit Table [29].

Please refer to Additional File 1 for further information regarding the Library Preparation and Bioinformatics sections.

Endpoints

The primary endpoint of this trial is the intergroup difference in the proportion of volunteers who achieve the clinical endpoint for therapy (≤ 4 sites with PD ≥ 5 mm) six months after treatment.

It also had several prespecified secondary endpoints, such as clinical parameters, including PD, BI, PLI, CAL, and microbial 16 S rRNA gene sequencing results. All patients were re-evaluated 1 and 6 months after treatment to assess clinical efficacy. The occurrence of adverse reactions during the treatment was also recorded.

Statistical analysis

Sample size calculation was based on variables from similar studies [13, 14, 16]. The hypothesized difference of the mean PD change in 6 months was 1 mm, with a standard deviation of 0.3 and upper and lower equivalence limits of 0.3 and − 0.3, respectively. The sample size for each group was determined to be 19 using PASS 15.0 software (NCSS Statistical Software, Kaysville, UT, USA). The study included 40 cases, with a power value exceeding 0.8.

In the analysis, the relative sequence variants (RSVs) were clustered at the species level, and the species readings were transformed into centered log ratios. The data were visualized using the "pheatmap" package version 1.0.12 [30]. All other between-group differences in continuous variables were assessed using a two-sided Mann–Whitney U test. The Wilcoxon signed-rank test was used to assess changes in continuous variables between two groups before and after treatment visits. The *chi-square* test or Fischer's exact test was used for categorical variables. The primary microbiological variances between the two groups at each time point were assessed using the linear discriminant analysis (LDA) effect size (LEfSe) method [31]. This method combines a standard statistical significance test with supplementary tests for biological consistency and effect correlation to identify the features most likely to explain differences between categories. The association between pathogenic bacteria and periodontal clinical indicators was analyzed using redundancy analysis ("vegan" package, version 2.5-7) [32], a method to evaluate the relationships between microorganisms and environmental factors [33]. A clustering correlation heatmap was used to determine the statistical correlations between microbiota and periodontal clinical indicators (FastSpar 0.10) [34]. To evaluate the degree of dysbiosis, Subgingival Microbial Dysbiosis Index (SMDI) was computed at the genus level, as described by Chen et al. [35, 36]. This index provides a measure of the extent of periodontitis-associated dysbiosis.

Table 1 Demographic variables and periodontal diagnosis for the control and test groups before therapy

	Control ($n=16$)	Test ($n=18$)	*P*-value
Age	40.44 ± 2.33	37.39 ± 2.13	0.341
Female (%)	6 (37.5%)	10 (55.6%)	0.292
Stage IV (%)	6 (37.5%)	11 (61.1%)	0.169
Grade C (%)	12 (75.0%)	18 (100.0%)	0.024*
PD (mm)	4.30 ± 0.88	4.32 ± 0.76	0.953
CAL (mm)	3.30 ± 1.15	3.57 ± 0.97	0.867
BI	2.77 ± 0.74	2.90 ± 0.66	0.178
PLI	1.37 ± 0.54	1.29 ± 0.58	0.581
Tooth loss	1.92 ± 1.85	0.86 ± 0.86	0.075
Shallow pocket (%)	43.01 ± 21.07	42.8 ± 20.7	0.977
Medium pocket (%)	43.84 ± 13.47	44.16 ± 15.52	0.95
Deep pocket (%)	13.15 ± 12.07	13.04 ± 7.79	0.975

Results

Demographic and clinical analysis

In the test group, all samples were graded C. The difference in grades between the two groups may have resulted from varying dropout rates. Except for periodontitis grade, the groups did not significantly differ in terms of demographic and clinical variables before therapy (Table 1). One patient experienced diarrhea following probiotic administration, while the rest reported no significant complications. Clinical parameters, including PD, BI, and CAL, improved significantly after 1 and 6 months in both groups ($P<0.05$). After treatment, the percentage of shallow pockets (PD < 4 mm) exhibited an increasing trend, while those of the medium (7 mm > PD ≥ 4 mm) and deep (PD ≥ 7 mm) pockets decreased. Clinical parameters in the test group did not significantly improve compared with those in the control group. In the test group, the improvement in CAL was more significant after 6 months than after 1 month. Correspondingly, there was a statistically significant reduction in the percentage of medium pockets and a notable increase in shallow pockets after six months relative to the one-month evaluation. In contrast, the control group exhibited no significant differences over the same period. This finding suggests that probiotics may provide benefits when given with initial periodontal treatment over a prolonged period (Table 2).

Baseline distribution level tests were conducted using Fisher's exact test for categorical variables and the Mann–Whitney U test for continuous variables. PD: probing depth, BI: bleeding index, CAL: clinical attachment loss, PLI: plaque index, Shallow pocket: percentage of sites with PD < 4 mm, Medium pocket: percentage of sites with 7 > PD ≥ 4 mm, Deep pocket: percentage of sites with PD ≥ 7 mm. *$P<0.05$.

The distribution between the control and test groups at each time point was evaluated using Mann–Whitney U tests. Wilcoxon signed-rank tests were used to evaluate the changes within each group before treatment and at 1 and 6 months post-treatment. Fisher's exact test was used

Table 2 Clinical variables in the control and test groups at each time point

	Baseline	1 month	6 months
Achieved Clinical Endpoint			
Control ($n=16$)	0	7 (43.75%) *	8 (50%) $^{*\#}$
Test ($n=18$)	0	4 (22.22%) *	11 (61.11%) $^{*\#}$
P-value	-	0.18	0.515
PD (mm)			
Control ($n=16$)	4.30 ± 0.88	3.19 ± 0.59*	2.90 ± 0.47$^{*\#}$
Test ($n=18$)	4.32 ± 0.76	3.24 ± 0.46*	2.90 ± 0.44$^{*\#}$
P-value	0.928	0.826	0.994
CAL (mm)			
Control ($n=16$)	3.30 ± 1.15	2.56 ± 0.99*	2.45 ± 0.99*
Test ($n=18$)	3.57 ± 0.97	2.87 ± 0.98*	2.54 ± 1.18$^{*\#}$
P-value	0.462	0.385	0.802
BI			
Control ($n=16$)	2.77 ± 0.74	1.60 ± 0.51*	1.39 ± 0.46*
Test ($n=18$)	2.90 ± 0.66	1.63 ± 0.49*	1.49 ± 0.47*
P-value	0.576	0.891	0.421
PLI			
Control ($n=16$)	1.37 ± 0.54	1.07 ± 0.46	0.96 ± 0.42
Test ($n=18$)	1.29 ± 0.58	1.13 ± 0.44	1.12 ± 0.40
P-value	0.684	0.727	0.262
Shallow pocket (%)			
Control ($n=16$)	43.01 ± 21.07	72.72 ± 17.97*	81.93 ± 12.47*
Test ($n=18$)	42.8 ± 20.7	70.2 ± 16.25*	81.4 ± 10.39$^{*\#}$
P-value	0.977	0.671	0.893
Medium pocket (%)			
Control ($n=16$)	43.84 ± 13.47	23.25 ± 15.7*	15.58 ± 10.54*
Test ($n=18$)	44.16 ± 15.52	26.14 ± 15.76*	16.05 ± 9.17$^{*\#}$
P-value	0.95	0.597	0.89
Deep pocket (%)			
Control ($n=16$)	13.15 ± 12.07	4.03 ± 4.07*	2.49 ± 3.41*
Test ($n=18$)	13.04 ± 7.79	3.66 ± 3.22*	2.55 ± 3.4*
P-value	0.975	0.769	0.959

for categorical variables. $^{*}P<0.05$ vs. baseline; $^{\#}P<0.05$ vs. 1 month. PD: probing depth, BI: bleeding index, CAL: clinical attachment loss, PLI: plaque index, Shallow pocket: percentage of sites with PD < 4 mm, Medium pocket: percentage of sites with 7 mm > PD ≥ 4 mm, Deep pocket: percentage of sites with PD ≥ 7 mm.

Diversity analyses

In total, 938 RSVs were detected in salivary samples, while 983 RSVs were identified in subgingival biofilms from 60 samples. Sequencing depth did not correlate with probiotics at each sampling time. No significant differences between the test and control groups could be detected in terms of α diversity at all checkpoints (Fig. 2a, e, h and l). β diversity was analyzed using nonmetric multidimensional scaling (NMDS) and analysis of similarities (ANOSIM). NMDS stress was 0.18 and 0.183 in subgingival biofilms and saliva, respectively, between the test and control groups, indicating a minor difference (Fig. 2f and m). The ANOSIM results were the same for both groups [subgingival biofilm ($R=0.18798$, $P=0.001$), saliva ($R=0.14791$, $P=0.001$)], indicating that *L. Reuteri* influenced the microbiota in subgingival biofilms and saliva (Fig. 2g and n).

Heatmap and LEfSe analysis

After treatment, the test group showed a significant decrease in *Tannerella forsythia* and *286 Streptococcus constellatus* levels in subgingival biofilms at 1 month relative to baseline. Additionally, the abundance of *Prevotella saccharolytica* significantly increased at 1 month compared with baseline levels in the test group. The control group showed a notable decrease in the abundance of *Olsenella sp. F0004* from baseline at 1 and 6 months and a decrease in that of *286 S. constellatus* only at 6 months (Fig. 3a). Meanwhile, the abundance of *Prevotella nanceiensis* within the salivary microorganism population notably increased at 6 months in both the test and control groups. Conversely, the abundance of *Streptococcus sp* did not notably decrease from baseline at 6 months in the control group (Fig. 3b).

The LEfSe method was used to compare microbial differences between the test and control groups at each time point. The analysis of subgingival biofilms after 1 month revealed a notable difference between the groups. The test group had a higher abundance of specific bacteria, including *Bacteroidota*, *Bacteroidia*, *Bacteroidales*, *Quinella*, *Anaerovibrio*, *Prevotellaceae*, *Olsenella*, *Phascolarctobacterium*, and *Collinsella*, which were statistically dominant according to LDA linear regression analysis. In contrast, certain bacterial groups such as *Paenibacillaceae*, *Alistipes*, *Muribaculum*, *Comamonadaceae*, *Rikenella*, *Lactobacillaceae*, *Limosilactobacillus*, *Hungateiclostridiaceae*, *Chryseobacterium*, *Sphingomonas sp*, and *Alphaproteobacteria* showed a competitive edge in the control group (Fig. 3d). The results of the comparisons of subgingival biofilms between the two groups at baseline and after 6 months, as well as saliva at all time points, are shown in Fig. 3c, e and h.

The microbiome was further assessed utilizing the SMDI. This index quantifies the pathogenic potential of the periodontal microbiome, with higher values indicating increased pathogenicity. In this investigation, a notable reduction in the SMDI was observed in both saliva and subgingival biofilms following treatment. However, the SMDI values returned to elevated levels after six months, with no statistically significant differences detected between the groups (Fig. 3i and j).

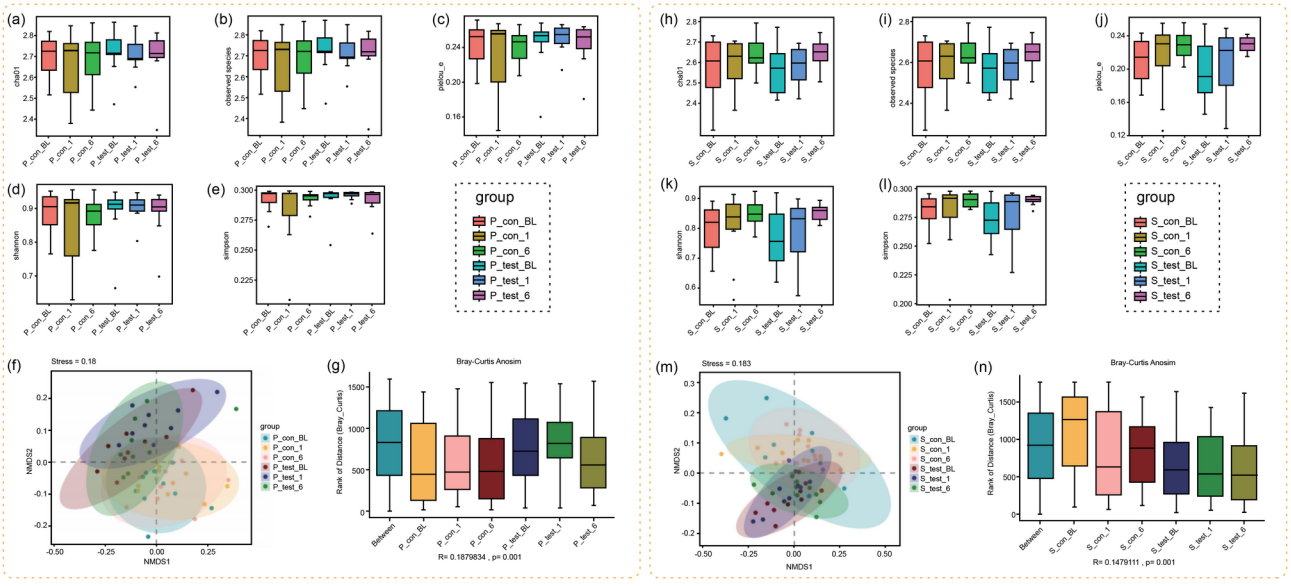

Fig. 2 Changes in the abundance of the different taxonomic groups. Comparison of α diversity between the control and test groups at baseline and at 1 and 6 months. (**a**) Chao1, (**b**) observed species, (**c**) pielou_e, (**d**) Shannon, and (**e**) Simpson indices in the subgingival biofilm communities. (**h**) Chao1, (**i**) observed species, (**j**) pielou_e, (**k**) Shannon, and (**l**) Simpson indices in the saliva communities. β-diversity was analyzed by nonmetric multidimensional scaling (NMDS) and analysis of multivariate similarities (ANOSIM). Distinct bacterial communities can be clearly visualized in the NMDS plot based on dimensionality reduction using distance ranking order for (**f**) subgingival and (**m**) saliva samples. Ellipses indicate 95% confidence intervals for each represented group. ANOSIM was used to analyze the differences in the composition of (g) subgingival and (**n**) salivary microbiota between the test and control groups over time

Relationship between microorganisms and clinical indicators

Redundancy analysis was used to assess the association between periodontal inflammation indicators and microorganisms. Each data point represents a sample, and the closer the two points are, the more similar the community structures of the samples. Arrows represent influencing factors, with acute and obtuse angles indicating positive and negative correlations, respectively. The length of the rays indicates factor influence (Fig. 4a and b). Regarding salivary plaque, the microorganisms *T. forsythia*, *Treponemadenticola*, *Streptococcus pneumoniae*, and *Streptococcus sp_106-03c* positively correlated with the PD, BI, and PLI indicators. *T. forsythia*, *T. denticola*, and *Streptococcus sp.* 106-03c exhibited the most significant correlations with PD and BI (Fig. 4a).

In subgingival biofilms, *P. saccharolytica*, *Streptococcus sp.*, *Alloprevotella tannerae*, *Treponema socranskii*, *T. forsythia*, and *Porphyromonas endodontalis* positively correlated with PD, BI, and subgingival plaque weight (BW). *T. socranskii* and *T. forsythia* strongly correlated with PD and BI (Fig. 4b).

Cluster correlation heatmaps were then employed to analyze the association between microbial abundance and periodontal clinical indicators. A significant positive correlation was identified between *S. constellatus* and PLI in salivary plaques. Additionally, *Streptococcus sp_106-03c* significantly positively correlated with both PD and BI. In contrast, *P. nanceiensis* significantly negatively correlated with the PD, BI, and PLI (Fig. 4c). Meanwhile, in subgingival biofilms, *Selenomonas noxia* negatively correlated with the PD, BI, and PLI, whereas *Olsenella sp. F0004* positively correlated with the PD (Fig. 4d).

Discussion

The study revealed that *L. reuteri* provided significantly benefit on CAL and medium pocket reduction on 6 months, which are not found in patient undergoing NSPT alone. We tend to link this finding to the effect of probiotics on the microbiota. SMDI was employed to evaluate the pathogenicity of microbial communities within subgingival biofilm and saliva. The results indicated a reduction in the SMDI of subgingival biofilm one month post-treatment, suggesting a shift towards a non-pathogenic and normative microbiota. This reduction was more pronounced in the group receiving probiotics, potentially accounting for the observed differences in clinical outcomes at later stages. However, the decrease in SMDI was not sustained at the six-month mark. This lack of persistence may be attributed to the absence of re-debridement during the review period, a methodological decision made to prevent interference with probiotic colonization, which contrasts with approaches taken in previous studies [37–39].

The subgingival biofilms in the test group showed a significant decrease in the abundance of *T. forsythia* and *P.*

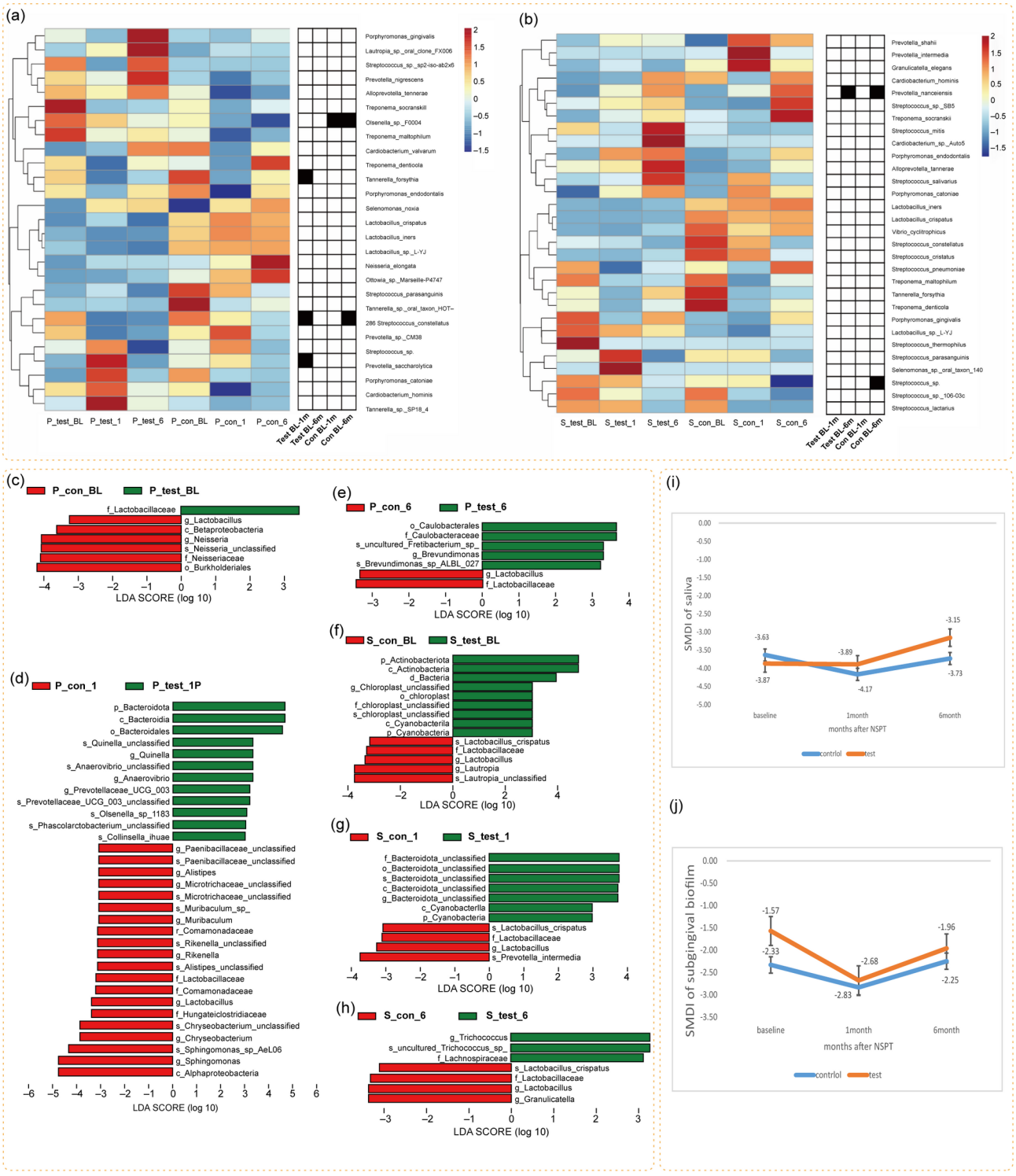

Fig. 3 (See legend on next page.)

saccharolytica after 1 month, while the control group did not exhibit the same result. Additionally, the abundance of *S. constellatus* in the test group notably decreased after 1 month, a change not observed in the control group. Probiotics accelerated this change, which was noticeable only after 6 months in the control group.

a. *T. forsythia* is recognized as a primary causative factor of periodontitis and is classified as a member

(See figure on previous page.)

Fig. 3 Heatmap and linear discriminant analysis (LDA) present variations in microbial taxa between groups and within groups, respectively. Heatmap illustrating the variations in ribosomal sequences (RSVs) within the (**a**) subgingival and (**b**) saliva communities. The columns present variations from the baseline to 6 months for each time point in both the test and control groups. The rows present RSVs categorized at the species level. The RSVs are organized according to similar microbial dynamics within the test group. Black boxes are positioned directly behind the corresponding changes for both test and control groups to indicate statistically significant within-group differences between pre-treatment and 1 and 6 months post-treatment. The heatmap displays the 27 most abundant RSVs in subgingival communities and the 30 most abundant RSVs in saliva communities, excluding unclassified RSVs from the list. LDA scores for differentially abundant taxa in subgingival communities at (**c**) baseline, (**d**) 1 month, and (**e**) 6 months, and saliva communities at (**f**) baseline, (**g**) 1 month, and (**h**) 6 months. LDA scores were used to assess the effect size and ranking of each differentially abundant taxon. Differences in relative species abundances were assessed using a significance level of 0.05 for the Kruskal–Wallis test and a threshold of 2.0 for logarithmic LDA scores. The LDA score or effect size is displayed on a logarithmic scale below the bars. Each group is distinguished by a specific color at the top of the figure. The subgingival microbial dysbiosis index (SMDI) values prior to and following treatment are depicted in panels (**i**) and (**j**) for the salivary and subgingival biofilms, respectively, to evaluate the pathogenic potential of the periodontal microbiome. In this context, a more negative index value denotes a more normobiotic or symbiotic microbiome composition, while a more positive index value denotes a greater extent of dysbiosis

of the red complex [40]. *L. reuteri* effectively aids NSPT and decreases the quantity and percentage of *T. forsythia*. This finding aligns with the outcomes of prior in vitro and in vivo experiments [41, 42] and may represent a potential avenue for beneficial intervention in periodontal disease.

b. *P. saccharolytica* has recently been isolated and detected in the oral cavity. It can undergo glycolysis, producing acetic and succinic acids as fermentation end products [43]. In 2020, its potential role as a significant pathogen contributing to the severity of caries disease in children was recognized [44]. Numerous studies have shown that different *Prevotella* species are pathogens in periodontal diseases, such as periodontitis, gingivitis during pregnancy, and acute necrotizing ulcerative gingivitis. These species include *P. intermedia*, *P. melaninogenica*, *P. bivia*, *P. nigrescens*, and *P. disiens* [45, 46]. However, *P. saccharolytica* was identified and characterized later. No research has yet established a correlation between *P. saccharolytica* and periodontitis. In the test group in this study, *P. saccharolytica* exhibited an increasing trend in abundance after treatment, indicating the necessity for additional investigations.

c. *S. constellatus* was initially discovered in the gastrointestinal tract in the 1970s and has often been recognized as a significant contributor to "refractory" periodontitis [47]. Research indicates that a higher proportion of *S. constellatus* in subgingival biofilms is linked to a higher likelihood of periodontal disease progression after initial treatment (CAL within 1 year of treatment and/or more than three sites with CAL exceeding 2.5 mm) [48]. Research indicated that *S. constellatus* in the periodontal pockets of patients with severe periodontitis showed resistance to common antibiotics, such as ciprofloxacin, doxycycline, and metronidazole [49]. Furthermore, research has indicated that *S. constellatus* may cause brain abscesses triggered by periodontitis [50]. Genetic recombination events within periodontal pockets may lead to mutations in *S. constellatus*, enabling it to cause abscesses in remote locations, such as the brain [50]. *L. reuteri* can reduce *S. constellatus* prevalence early on, improving periodontal therapy effectiveness and reducing the risk of complications, such as extraoral infections.

Previous studies on the relationship between *L. reuteri* and periodontitis have primarily concentrated on individual bacteria or small groups. Accordingly, the present study thoroughly examined the microbial composition of salivary and subgingival dental biofilms using sequencing technology. Despite presenting similar abundances, the microbial composition of both groups continued to exhibit distinct changes post-treatment. Significant variation was observed at the species/genus level and pathogenic microorganisms. After 1 month of treatment, subgingival dental biofilms in the test group exhibited a notable rise in the abundance of *Phascolarctobacterium*. Research indicates that a higher abundance of *Phascolarctobacterium* in the gut microbiota is associated with a reduced risk of head and neck cancer recurrence [51]. Furthermore, *Olsenella* has been linked to endodontic conditions [52], and *Collinsella* in subgingival biofilm-intestinal flora has been associated with hypertension [53]. The impact of *L. reuteri* on these microorganisms may have potential implications for oral and systemic health, necessitating further research.

Reuterin, a compound produced by *L. reuteri*, is widely acknowledged for its potent antimicrobial properties that enable the inhibition of pathogenic microorganisms, such as gram-positive and gram-negative bacteria and fungi [54, 55]. Numerous studies have demonstrated the bacteriostatic effects of reuterin. For example, Jørgensen et al. [56] reported that *L. reuteri* almost completely inhibited *Candida albicans* and *Candida smoothii* growth in a co-culture. Moreover, the extracellular polysaccharides produced by *L. reuteri* are essential for biofilm formation and epithelial cell adhesion [57]. These extracellular polysaccharides inhibit *Escherichia coli* attachment to porcine epithelial cells. Moreover, they can result in a significant decrease in TNF-α expression in RAW246.7 cells [58].

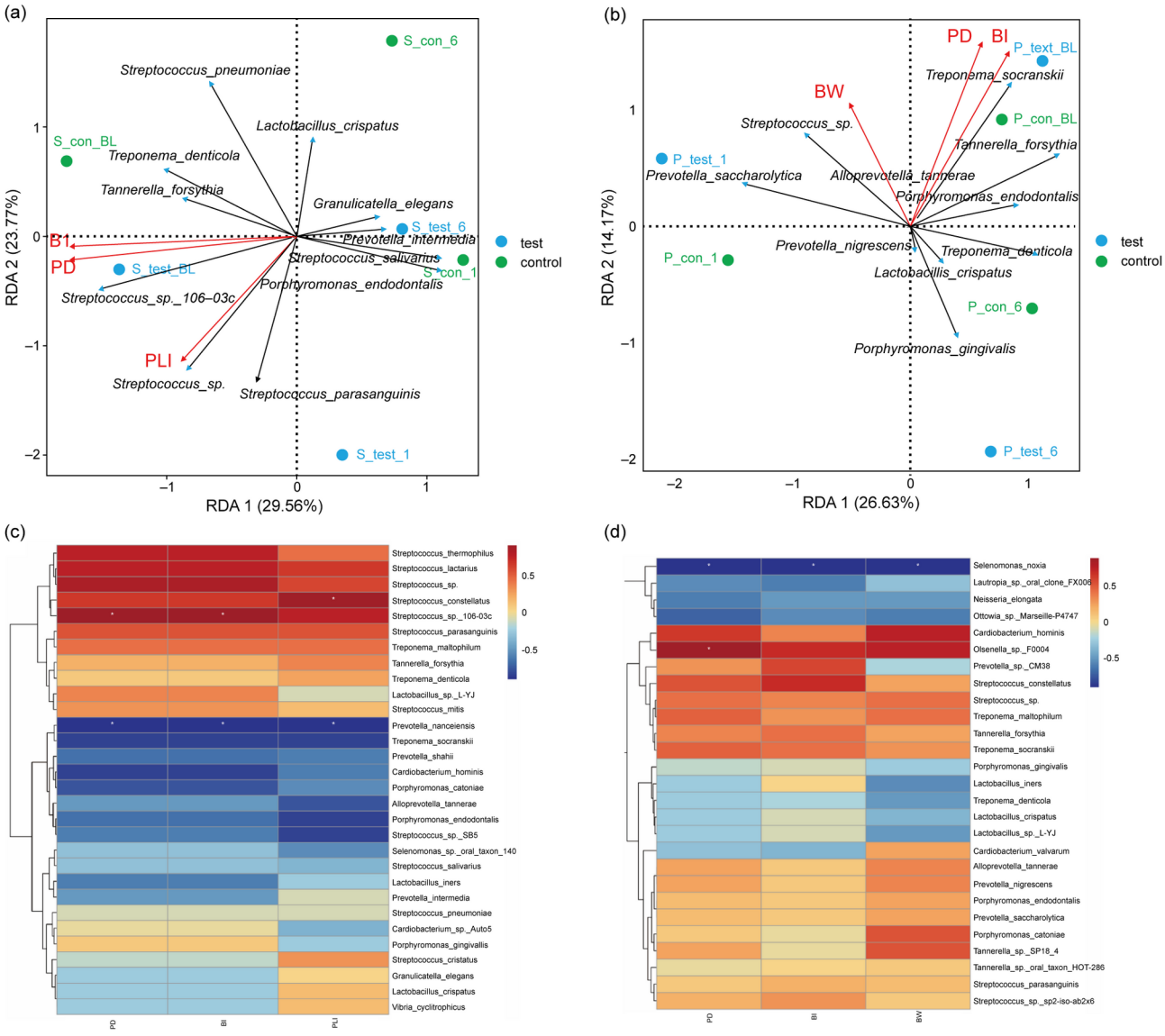

Fig. 4 Redundancy analysis to examine the association between microorganisms and periodontal clinical indicators in (**a**) salivary and (**b**) subgingival samples. Each point represents a sample, and the closer the distance between two points, the more similar the community structures of the samples are. The arrows represent various influencing factors, with acute and obtuse angles indicating positive and negative correlations, respectively. The ray length indicates the significance of each factor. Projected point positioning on the arrow approximates the magnitude of the factor's value in the sample. Clustered correlation heatmaps were utilized to analyze the statistical relationships between microorganisms and periodontal clinical indicators in (**c**) salivary and (**d**) subgingival samples. PD: probing depth, BI: bleeding index, PLI: plaque index, BW: subgingival plaque weight. $^{*}P < 0.05$

The association between saliva and subgingival biofilm bacteria and clinical markers was also studied. While probiotics did not affect this association, pathogenic microorganisms and clinical markers were favorably correlated. Significant causative microorganisms such as *T. forsythia*, *T. denticola*, and other pathogenic agents had a more pronounced effect on PD and BI, demonstrating a notably strong positive correlation with BI. This finding indicates that probing bleeding, as the primary and most noticeable indicator of periodontal inflammation, is the first clinical parameter to show changes in response to inflammatory conditions, which is inherently linked to an elevation in pathogen levels.

This study had some limitations. First, the sample size was small due to some patients being lost during follow-up or being unable to provide biological samples because of the COVID-19 pandemic, as well as their use of antibiotics, among other reasons. Therefore, larger sample sizes and multicenter studies are needed to further explore the effects of probiotics in periodontal therapy. Another point of contention is the initial analysis time point of 1 month in this study, which potentially impacted the clinical and microbiologic outcomes. Nonetheless, prior

research has indicated that notable improvements can be observed at 3 weeks when using *L. reuteri* [13]. In the current investigation, sampling occurred before maintenance treatment to prevent potential confounding effects from supragingival cleansing. Additionally, the LDA conducted at the 1-month mark revealed the most substantial differences between groups. Third, the abundance of pathogenic bacteria, such as *T. forsythia*, significantly decreased in the experimental group. Therefore, residual deep periodontal pockets were treated, potentially influencing the microbial community at the 6-month mark. Fourth, using pooled samples, each comprising subgingival biofilms from four teeth, prevented the evaluation of differences at specific sites. Instead, we assessed these differences based on the average clinical indicators of the sampled loci. Despite these limitations, the strengths of this study enabled a comprehensive analysis of the microbiome and offered insights into the effects of probiotic interventions.

Conclusion

The use of *L. reuteri* in combination with NSPT did not directly improve clinical indicators. However, it showed potential benefits in terms of modifying the microbial composition of subgingival biofilms and enhancing treatment sensitivity. We recommend conducting future research with longer durations and larger sample sizes to further validate the effects of probiotics in periodontal therapy.

Abbreviations

NSPT	Non-surgical periodontal therapy
PD	Probing depth
BI	Bleeding index
CAL	Clinical attachment loss
PLI	Plaque index
PCR	Polymerase chain reaction
DADA2	Divisive Amplicon Denoising Algorithm
RSVs	Relative sequence variants
NMDS	Nonmetric multidimensional scaling
ANOSIM	Analysis of similarities
LDA	Linear discriminant analysis
LEfSe	Linear discriminant analysis effect size
SMDI	Subgingival Microbiome Dysbiosis Index

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12903-025-06084-1.

Supplementary Material 1

Supplementary Material 2

Acknowledgements

Not applicable.

Author contributions

P.H. and L.L. were involved in the formal analysis, validation, and methodology. P.H. and L.D. contributed to the experimental practices and data curation. L.D., P.K., and R.J. were responsible for project administration. P.H., L.D., and J.L. conducted data analysis. L.L. secured funding for the project. P.H. wrote the original manuscript, and all authors read and approved the final manuscript.

Funding

This study was supported by the Foundation of Shanghai Science and Technology Committee (Project: 20Y11904200) and the Shanghai Municipal Health Commission (Grant/Award Number: 202240196).

Data availability

Data is provided within the manuscript. Additional detailed data supporting the findings are available upon request from the corresponding author, subject to privacy and ethical considerations.

Declarations

Ethics approval and consent to participate

This study was approved by the human subjects ethics board of the Stomatological Hospital of Tongji University (Approval Number [2019]-R-011) and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013. All participants provided their informed consent to engage in the experiment.

Consent for publication

All relevant data are provided informed consent to engage in the experiment and for the subsequent publication of the findings by signing an informed consent document.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Periodontics, Shanghai Engineering Research Center of Tooth Restoration and Regeneration, Stomatological Hospital and Dental School of Tongji University, 399 Yanchangzhong Road, Jingan District, Shanghai 200072, China

Received: 21 January 2025 / Accepted: 29 April 2025
Published online: 22 May 2025

References

1. Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, et al. Periodontitis: consensus report of workgroup 2 of the 2017 world workshop on the classification of periodontal and Peri-Implant diseases and conditions. J Periodontol. 2018;89(Suppl 1):S173–82. https://doi.org/10.1002/JPER.17-0721.
2. Trindade D, Carvalho R, Machado V, Chambrone L, Mendes JJ, Botelho J. Prevalence of periodontitis in dentate people between 2011 and 2020: A systematic review and meta-analysis of epidemiological studies. J Clin Periodontol. 2023;50:604–26. https://doi.org/10.1111/jcpe.13769.
3. Curtis MA, Diaz PI, Van Dyke TE. The role of the microbiota in periodontal disease. Periodontol 2000. 2020;83:14–25. https://doi.org/10.1111/prd.12296.
4. Haffajee AD, Teles RP, Socransky SS. The effect of periodontal therapy on the composition of the subgingival microbiota. Periodontol 2000. 2006;42:219–58. https://doi.org/10.1111/j.1600-0757.2006.00191.x.
5. Teughels W, Newman MG, Coucke W, Haffajee AD, Van Der Mei HC, Haake SK, et al. Guiding periodontal pocket recolonization: A proof of concept. J Dent Res. 2007;86:1078–82. https://doi.org/10.1177/154405910708601111.
6. Mombelli A, Schmid B, Rutar A, Lang NP. Persistence patterns of Porphyromonas gingivalis, Prevotella intermedia/nigrescens, and Actinobacillus actinomyetemcomitans after mechanical therapy of periodontal disease. J Periodontol. 2000;71:14–21. https://doi.org/10.1902/jop.2000.71.1.14.
7. Nibali L, Zavattini A, Nagata K, Di Iorio A, Lin GH, Needleman I, et al. Tooth loss in molars with and without furcation involvement– A systematic review and meta-analysis. J Clin Periodontol. 2016;43:156–66. https://doi.org/10.1111/jcpe.12497.
8. Mendonça C, Marques D, Silveira J, Marques J, de Souza RF, Mata A. Effects of probiotic therapy on periodontal and peri-implant treatments: an umbrella review. JDR Clin Transl Res. 2024;23800844241240474. https://doi.org/10.1177/23800844241240474.

9. Lebeer S, Vanderleyden J, De Keersmaecker SCJ. Genes and molecules of lactobacilli supporting probiotic action. Microbiol Mol Biol Rev. 2008;72:728–64. https://doi.org/10.1128/MMBR.00017-08.
10. Ramos TCS, Boas DS, Nunes MLV, Ferreira CMM, Pannuti CL, Santamaria CM. Effect of systemic antibiotic and probiotic therapies as adjuvant treatments of subgingival instrumentation for periodontitis: A randomized controlled clinical study. J Appl Oral Sci. 2022;30:e20210583. https://doi.org/10.1590/1678-7757-2021-0583.
11. İnce G, Gürsoy H, İpçi ŞD, Cakar G, Emekli-Alturfan E, Yılmaz S. Clinical and biochemical evaluation of lozenges containing Lactobacillus reuteri as an adjunct to non-surgical periodontal therapy in chronic periodontitis. J Periodontol. 2015;86:746–54. https://doi.org/10.1902/jop.2015.140612.
12. Szkaradkiewicz AK, Stopa J, Karpiński TM. Effect of oral administration involving a probiotic strain of Lactobacillus reuteri on pro-inflammatory cytokine response in patients with chronic periodontitis. Arch Immunol Ther Exp (Warsz). 2014;62:495–500. https://doi.org/10.1007/s00005-014-0277-y.
13. Tekce M, Ince G, Gursoy H, Dirikan Ipci S, Cakar G, Kadir T, et al. Clinical and Microbiological effects of probiotic lozenges in the treatment of chronic periodontitis: A 1-year follow-up study. J Clin Periodontol. 2015;42:363–72. https://doi.org/10.1111/jcpe.12387.
14. Teughels W, Durukan A, Ozcelik O, Pauwels M, Quirynen M, Haytac MC. Clinical and Microbiological effects of Lactobacillus reuteri probiotics in the treatment of chronic periodontitis: A randomized placebo-controlled study. J Clin Periodontol. 2013;40:1025–35. https://doi.org/10.1111/jcpe.12155.
15. Grusovin MG, Bossini S, Calza S, Cappa V, Garzetti G, Scotti E, et al. Clinical efficacy of Lactobacillus reuteri-containing lozenges in the supportive therapy of generalized periodontitis stage III and IV, grade C: 1-year results of a double-blind randomized placebo-controlled pilot study. Clin Oral Investig. 2020;24:2015–24. https://doi.org/10.1007/s00784-019-03065-x.
16. Laleman I, Pauwels M, Quirynen M, Teughels W. A dual-strain lactobacilli reuteri probiotic improves the treatment of residual pockets: A randomized controlled clinical trial. J Clin Periodontol. 2020;47:43–53. https://doi.org/10.1111/jcpe.13198.
17. Pelekos G, Acharya A, Eiji N, Hong G, Leung WK, McGrath C. Effects of adjunctive probiotic L. reuteri lozenges on S/RSD outcomes at molar sites with deep pockets. J Clin Periodontol. 2020;47:1098–107. https://doi.org/10.1111/jcpe.13329.
18. Borrell García C, Ribelles Llop M, García Esparza MÁ, Flichy-Fernández AJ, Marqués Martínez L, Izquierdo Fort R. The use of Lactobacillus reuteri DSM 17938 and ATCC PTA 5289 on oral health indexes in a school population: A pilot randomized clinical trial. Int J Immunopathol Pharmacol. 2021;35:20587384211031107. https://doi.org/10.1177/20587384211031107.
19. Iniesta M, Herrera D, Montero E, Zurbriggen M, Matos AR, Marín MJ, et al. Probiotic effects of orally administered Lactobacillus reuteri-containing tablets on the subgingival and salivary microbiota in patients with gingivitis. A randomized clinical trial. J Clin Periodontol. 2012;39:736–44. https://doi.org/10.1111/j.1600-051X.2012.01914.x.
20. Pelekos G, Ho SN, Acharya A, Leung WK, McGrath C. A double-blind, paralleled-arm, placebo-controlled and randomized clinical trial of the effectiveness of probiotics as an adjunct in periodontal care. J Clin Periodontol. 2019;46:1217–27. https://doi.org/10.1111/jcpe.13191.
21. Ng E, Tay JRH, Saffari SE, Lim LP, Chung KM, Ong MMA. Adjunctive probiotics after periodontal debridement versus placebo: A systematic review and meta-analysis. Acta Odontol Scand. 2022;80:81–90. https://doi.org/10.1080/00016357.2021.1942193.
22. Liu J, Liu Z, Huang J, Tao R. Effect of probiotics on gingival inflammation and oral microbiota: A meta-analysis. Oral Dis. 2022;28:1058–67. https://doi.org/10.1111/odi.13861.
23. Gruner D, Paris S, Schwendicke F. Probiotics for managing caries and periodontitis: systematic review and meta-analysis. J Dent. 2016;48:16–25. https://doi.org/10.1016/j.jdent.2016.03.002.
24. Chapple ILC, Mealey BL, Van Dyke TE, Bartold PM, Dommisch H, Eickholz P, et al. Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: consensus report of workgroup 1 of the 2017 world workshop on the classification of periodontal and Peri-Implant diseases and conditions. J Periodontol. 2018;89(Suppl 1):S74–84. https://doi.org/10.1002/JPER.17-0719.
25. Fisher RA, Yates F. Statistical tables for biological, agricultural, and medical research. London: Oliver and Boyd; 1938.
26. Ramfjord SP. Indices for prevalence and incidence of periodontal disease. J Periodontol. 1959;30:51–9. https://doi.org/10.1902/jop.1959.30.1.51.
27. Mazza JE, Newman MG, Sims TN. Clinical and antimicrobial effect of stannous fluoride on periodontitis. J Clin Periodontol. 1981;8:203– 12. https://doi.org/10.1111/j.1600-051x.1981.tb02031.x
28. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from illumina amplicon data. Nat Methods. 2016;13:581–3. https://doi.org/10.1038/nmeth.3869.
29. Blaxter M, Mann J, Chapman T, Thomas F, Whitton C, Floyd R, et al. Defining operational taxonomic units using DNA barcode data. Philos Trans R Soc Lond B Biol Sci. 2005;360:1935–43. https://doi.org/10.1098/rstb.2005.1725.
30. Murtagh F, Legendre P. Ward's hierarchical agglomerative clustering method: which algorithms implement Ward's criterion? J Classif. 2014;31:274–95. https://doi.org/10.1007/s00357-014-9161-z.
31. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12:R60. https://doi.org/10.1186/gb-2011-12-6-r60.
32. Oksanen J, Blanchet FG, Friendly M, et al. Vegan community ecology package version 2.5-7. R project for statistical computing. Vienna, Austria; 2020.
33. ter Braak CJF, Verdonschot PFM. Canonical correspondence analysis and related multivariate methods in aquatic ecology. Aquat Sci. 1995;57:255–89. https://doi.org/10.1007/BF00877430.
34. Watts SC, Ritchie SC, Inouye M, Holt KE, FastSpar. Rapid and scalable correlation Estimation for compositional data. Bioinformatics. 2019;35:1064–6. https://doi.org/10.1093/bioinformatics/bty734.
35. Chen T, Marsh PD, Al-Hebshi NN. SMDI: an index for measuring subgingival microbial dysbiosis. J Dent Res. 2022;101:331–8. https://doi.org/10.1177/00220345211035775.
36. Chew RJJ, Tan KS, Chen T, Al-Hebshi NN, Goh CE. Quantifying periodontitis-associated oral dysbiosis in tongue and saliva microbiomes-An integrated data analysis. J Periodontol. 2025;96:55–66. https://doi.org/10.1002/JPER.24-0120.
37. Johnston W, Rosier BT, Carda-Diéguez M, Paterson M, Watson P, Piela K, Culshaw S. Longitudinal changes in subgingival biofilm composition following periodontal treatment. J Periodontol. 2023;94(9):1065–77. https://doi.org/10.1002/JPER.22-0749.
38. Hagenfeld D, Kleine Bardenhorst S, Matern J, Prior K, Harks I, Eickholz P, Ehmke B. Long-term changes in the subgingival microbiota in patients with stage III–IV periodontitis treated by mechanical therapy and adjunctive systemic antibiotics: A secondary analysis of a randomized controlled trial. J Clin Periodontol. 2023;50(8):1101–12. https://doi.org/10.1111/jcpe.13824.
39. Xu X, He L, Huo P, Jin D, Zhu Y, Lu H, Meng H. Potential short-term shift in oral microbiota of patients with stage III-IV periodontitis and type 2 diabetes treated by non-surgical periodontal therapy. J Dent Sci. 2025. https://doi.org/10.1016/j.jds.2025.01.014.
40. Sharma A. Virulence mechanisms of Tannerella forsythia. Periodontol 2000. 2010;54:106–16. https://doi.org/10.1111/j.1600-0757.2009.00332.x.
41. Baca-Castañón ML, De la Garza-Ramos MA, Alcázar-Pizaña AG, Grondin Y, Coronado-Mendoza A, Sánchez-Najera RI, et al. Antimicrobial effect of Lactobacillus reuteri on cariogenic bacteria Streptococcus gordonii, Streptococcus mutans, and periodontal diseases Actinomyces naeslundii and Tannerella forsythia. Probiotics Antimicrob Proteins. 2015;7:1–8. https://doi.org/10.1007/s12602-014-9178-y.
42. Salinas-Azuceno C, Martínez-Hernández M, Maldonado-Noriega J-I, Rodríguez-Hernández A-P, Ximenez-Fyvie L-A. Probiotic monotherapy with Lactobacillus reuteri (Prodentis) as a coadjutant to reduce subgingival dysbiosis in a patient with periodontitis. Int J Environ Res Public Health. 2022;19:7835. https://doi.org/10.3390/ijerph19137835.
43. Downes J, Tanner ACR, Dewhirst FE, Wade WG. Prevotella saccharolytica Sp. nov., isolated from the human oral cavity. Int J Syst Evol Microbiol. 2010;60:2458–61. https://doi.org/10.1099/ijs.0.014720-0.
44. Kalpana B, Prabhu P, Bhat AH, Senthilkumar A, Arun RP, Asokan S, et al. Bacterial diversity and functional analysis of severe early childhood caries and recurrence in India. Sci Rep. 2020;10:21248. https://doi.org/10.1038/s41598-020-78057-z.
45. Ibrahim M, Subramanian A, Anishetty S. Comparative pan genome analysis of oral Prevotella species implicated in periodontitis. Funct Integr Genomics. 2017;17:513–36. https://doi.org/10.1007/s10142-017-0550-3.
46. Nadkarni MA, Browne GV, Chhour KL, Byun R, Nguyen KA, Chapple CC, et al. Pattern of distribution of Prevotella species/phylotypes associated with healthy gingiva and periodontal disease. Eur J Clin Microbiol Infect Dis. 2012;31:2989–99. https://doi.org/10.1007/s10096-012-1651-5.
47. Holdeman LV, Moore WEC. New genus, Coprococcus, twelve new species, and emended descriptions of four previously described species of bacteria

from human feces. Int J Syst Evol Bacteriol. 1974;24:260–77. https://doi.org/10.1099/00207713-24-2-260.
48. Colombo AP, Haffajee AD, Dewhirst FE, Paster BJ, Smith CM, Cugini MA, et al. Clinical and Microbiological features of refractory periodontitis subjects. J Clin Periodontol. 1998;25:169–80. https://doi.org/10.1111/j.1600-051X.1998.tb02424.x.
49. Rams TE, Feik D, Mortensen JE, Degener JE, van Winkelhoff AJ. Antibiotic susceptibility of periodontal Streptococcus constellatus and Streptococcus intermedius clinical isolates. J Periodontol. 2014;85:1792–8. https://doi.org/10.1902/jop.2014.130291.
50. Marques da Silva RM, Caugant DA, Josefsen R, Tronstad L, Olsen I. Characterization of Streptococcus constellatus strains recovered from a brain abscess and periodontal pockets in an immunocompromised patient. J Periodontol. 2004;75:1720–3. https://doi.org/10.1902/jop.2004.75.12.1720.
51. Al-Qadami G, Bowen J, Van Sebille Y, Secombe K, Dorraki M, Verjans J, et al. Baseline gut microbiota composition is associated with oral mucositis and tumour recurrence in patients with head and neck cancer: A pilot study. Support Care Cancer. 2023;31:98. https://doi.org/10.1007/s00520-022-07559-5.
52. Nardello LCL, Amado PPP, Franco DC, Cazares RXR, Nogales CG, Mayer MPA, et al. Next-generation sequencing to assess potentially active bacteria in endodontic infections. J Endod. 2020;46:1105–12. https://doi.org/10.1016/j.joen.2020.05.004.
53. Chen B-Y, Lin W-Z, Li Y-L, Bi C, Du L-J, Liu Y, et al. Roles of oral microbiota and oral-gut microbial transmission in hypertension. J Adv Res. 2023;43:147–61. https://doi.org/10.1016/j.jare.2022.03.007.
54. Engevik MA, Danhof HA, Shrestha R, Chang-Graham AL, Hyser JM, Haag AM, et al. Reuterin disrupts clostridioides difficile metabolism and pathogenicity through reactive oxygen species generation. Gut Microbes. 2020;12:1788898. https://doi.org/10.1080/19490976.2020.1795388.
55. Jones SE, Versalovic J. Probiotic Lactobacillus reuteri biofilms produce antimicrobial and anti-inflammatory factors. BMC Microbiol. 2009;9:35. https://doi.org/10.1186/1471-2180-9-35.
56. Jørgensen MR, Kragelund C, Jensen PØ, Keller MK, Twetman S. Probiotic Lactobacillus reuteri has antifungal effects on oral Candida species in vitro. J Oral Microbiol. 2017;9:1274582. https://doi.org/10.1080/20002297.2016.1274582.
57. Salas-Jara MJ, Ilabaca A, Vega M, García A. Biofilm forming Lactobacillus: new challenges for the development of probiotics. Microorganisms. 2016;4:35. https://doi.org/10.3390/microorganisms4030035.
58. Kšonžeková P, Bystrický P, Vlčková S, Pätoprstý V, Pulzová L, Mudroňová D, et al. Exopolysaccharides of Lactobacillus reuteri: their influence on adherence of E. coli to epithelial cells and inflammatory response. Carbohydr Polym. 2016;141:10–9. https://doi.org/10.1016/j.carbpol.2015.12.037.

Publisher's note