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Original Article

Subgingival *Limosilactobacillus reuteri* in Diabetic Periodontitis: A Preliminary Randomized Split-Mouth Trial of Clinical and Microbiological Outcomes

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ABSTRACT

Diabetes and periodontitis are closely associated, and adjunctive probiotics may improve periodontal treatment outcomes. Evidence for locally administered *Limosilactobacillus reuteri* in patients with diabetes remains limited. To preliminarily evaluate the clinical and microbiological effects of subgingival *L. reuteri* as an adjunct to scaling and root planing (SRP), with HbA1c assessed descriptively. This randomized, blinded, split-mouth trial included five participants with diabetes and periodontitis, comprising 40 teeth (20 teeth per treatment condition). Test sites received SRP plus *L. reuteri*; control sites received SRP plus saline. Outcomes were assessed at baseline, 1 month, and 3 months. Clinical outcomes were analyzed using mixed-effects models or generalized estimating equations; microbiological findings were exploratory. Both conditions improved. At 3 months, mean probing pocket depth decreased from 7.20 to 2.75 mm at probiotic sites and from 7.15 to 4.85 mm at control sites. Longitudinal analyses indicated greater probing-depth reduction and clinical-attachment gain at probiotic sites, with lower bleeding on probing and residual surgical treatment need. HbA1c decreased descriptively. No statistically significant treatment-by-time interactions were observed for the assessed periodontal pathogens. Adjunctive subgingival *L. reuteri* showed promising short-term clinical outcomes compared with SRP plus saline. Given the five-participant sample and 3-month follow-up, results are preliminary and require confirmation in larger trials.

Keywords: Diabetes mellitus, Periodontitis, *Limosilactobacillus reuteri*, Probiotics, Scaling and root planing, Split-mouth randomized trial

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Introduction

Periodontitis is a chronic inflammatory disease driven by dysbiotic subgingival biofilms and dysregulated host responses, leading to progressive destruction of tooth-supporting tissues and possible tooth loss [1-4]. Diabetes is an important modifier of periodontal severity and healing: hyperglycemia can impair neutrophil function, alter collagen metabolism, and amplify inflammation [5-9]. The relationship is bidirectional, as periodontal inflammation may also increase systemic inflammatory burden and compromise glycemic control [5, 7-9].

Scaling and root planing (SRP) is the standard non-surgical treatment, but deep pockets may retain pathogenic biofilms after debridement, particularly in patients with diabetes [10-13]. Probiotics have therefore been investigated as adjuncts because of their antimicrobial and immunomodulatory effects [14-23]. *Limosilactobacillus reuteri* may inhibit periodontal pathogens through competitive adhesion and antimicrobial compounds, while also modulating host responses [14-23].

Evidence for locally delivered liquid *L. reuteri* in patients with diabetes remains limited, especially

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where clinical, microbiological, and glycemic outcomes are assessed longitudinally. This preliminary randomized, blinded, split-mouth trial explored the adjunctive effects of subgingival *L. reuteri* during SRP in adults with diabetes and periodontitis. HbA1c changes were assessed descriptively at the participant level.

Materials and Methods

Study design and participants

This preliminary randomized, blinded, split-mouth trial was conducted at the Dental Clinic, Faculty of Dentistry, University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam, from October 2025 to April 2026. The institutional review board approved the protocol (No. 3315/ĐHYD-HĐĐĐ; August 14, 2025). The trial was registered at ClinicalTrials.gov (NCT07652255) after data collection.

Five adults with diabetes contributed 40 eligible teeth (20 per condition). Participants were required to have at least 15 teeth and two eligible teeth in each half-mouth. Eligible teeth were selected from patients diagnosed with Stage III, Grade B or higher periodontitis and had PPD ≥6 mm, CAL ≥5 mm, radiographic bone loss of at least one-third of root length, and BOP. Exclusions included pregnancy or breastfeeding, smoking, recent antibiotics or anti-inflammatory drugs, recent periodontal or orthodontic treatment, and teeth with occlusal trauma, prosthetic involvement, pulpal disease, or active infection.

The prespecified minimum sample was 30 teeth (15 per condition), calculated from previously reported PPD/CAL differences with two-sided α=0.05, 80% power, and 10% attrition allowance. Forty teeth were enrolled. Because only five independent participants contributed clustered tooth-level observations, findings are exploratory. The design is shown in **Figure 1**.

Randomization and blinding

Following subgingival scaling and before root planing, each participant's right and left half-mouths were allocated by a concealed draw of folded slips labeled “right” and “left”; the selected side received probiotic and the contralateral side saline. A faculty member supervised allocation. No separate pre-generated sequence was used.

Participants, the clinical examiner, and the operator were blinded to treatment identity. Coded probiotic and saline solutions were supplied in visually identical opaque containers. The examiner did not consult previous measurements during subsequent assessments.

Periodontal treatment and intervention

At baseline, participants underwent clinical, microbiological, and HbA1c assessments, supragingival scaling, and individualized oral-hygiene instruction. One week later, subgingival scaling was performed under local anesthesia when required using an ultrasonic scaler and Gracey curettes.

During debridement, periodontal pockets on both sides were irrigated with 1% povidone-iodine and rinsed with distilled water. Following randomization, root planing was performed, followed by subgingival administration of the assigned solutions. Test sites received subgingival liquid *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 (BioGaia Prodentis®, BioGaia AB, Stockholm, Sweden); control sites received sterile 0.9% saline using the same syringe-based protocol. Periodontal dressing was applied to both sides. The assigned intervention was repeated at 1 month.

Clinical assessment

A calibrated examiner assessed PlI, GI, BOP, PPD, and CAL at baseline, 1 month, and 3 months using a UNC-15 probe. Customized stents supported reproducible positioning; PPD, CAL, and BOP were recorded at six sites per tooth.

Examiner calibration used an acceptable ICC threshold of 0.80. HbA1c was measured at all three visits at the same laboratory and treated as a participant-level descriptive outcome.

Microbiological assessment

Subgingival plaque was collected from the same predetermined deepest baseline pocket on each designated tooth at baseline, 1 month, and 3 months. All 40 teeth were sampled at all visits.

A sterile size-30 paper point was inserted for approximately 30 seconds. Samples were placed in phosphate-buffered saline, transported cold to the laboratory within 4 hours, and stored at −80 °C.

DNA was extracted using the BXN IVD NK DNARNAprep MAGBEAD kit. Multiplex qPCR was performed on a CFX96 system (Bio-Rad) with species-specific primers and TaqMan probes to quantify *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Fusobacterium nucleatum*.

Statistical analysis

Analyses used Stata 16.0. Continuous variables are summarized as mean (SD) or median (interquartile range), as appropriate.

Linear mixed-effects models assessed continuous periodontal outcomes, with treatment, time, and treatment-by-time interaction as fixed effects and

participant as a random effect. Generalized estimating equations assessed binary outcomes while accounting for within-participant correlation.

Microbial counts were log10-transformed and analyzed separately by species using mixed-effects models. Estimates, 95% confidence intervals, and two-sided P values are reported. Microbiological analyses were exploratory.

$P < 0.05$ was considered statistically significant. Given the small number of participants, estimates are interpreted cautiously and not as confirmatory evidence.

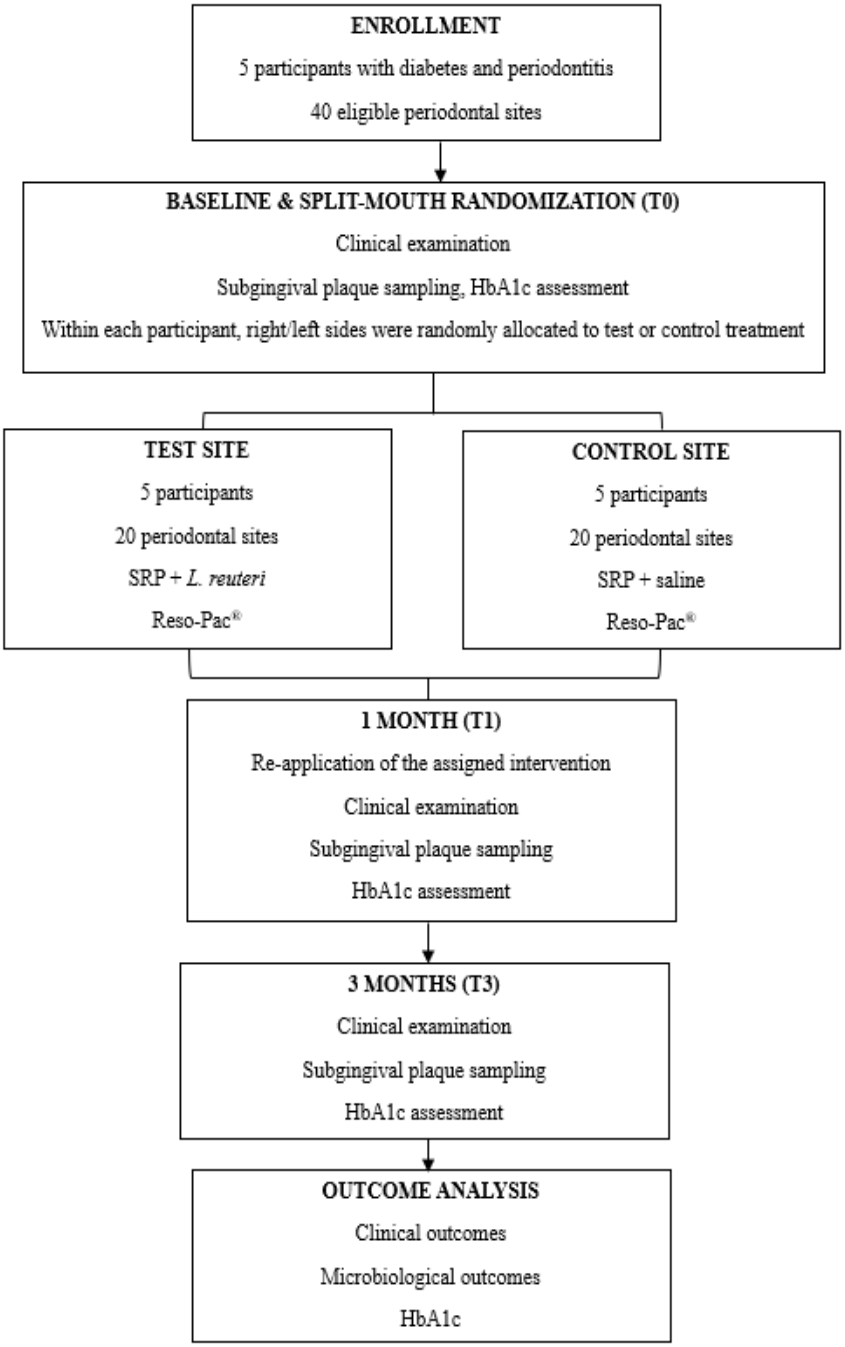


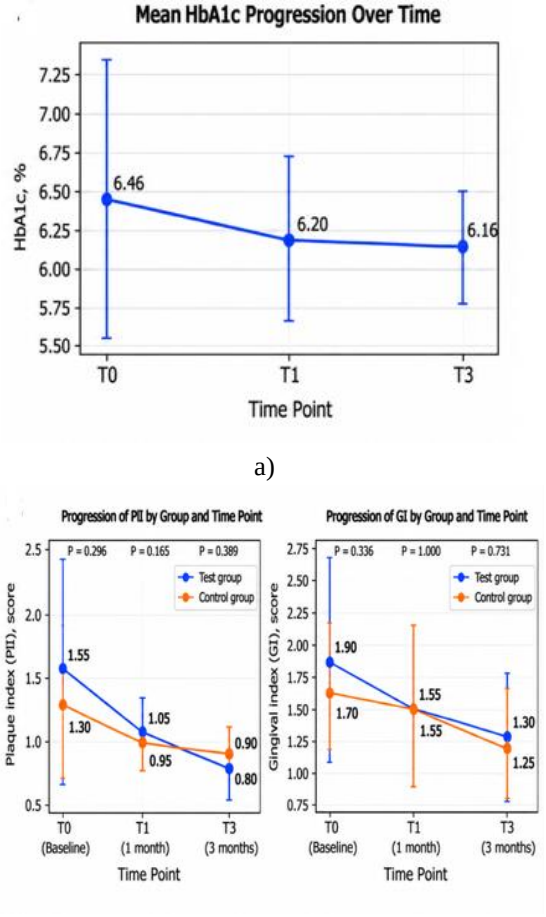
Figure 1. Study flow and split-mouth design. Within each participant, right and left sides were randomly allocated to SRP plus *Limosilactobacillus reuteri* (test) or SRP plus saline (control). Clinical and microbiological outcomes were assessed at baseline, 1 month, and 3 months.

Results and Discussion

Five participants contributed 40 teeth (20 per condition); no treatment-related adverse events occurred.

Mean HbA1c decreased descriptively from 6.46 (SD 0.90%) at baseline to 6.20 (0.58%) at 1 month and 6.16 (0.47%) at 3 months. Participants with HbA1c ≥7.0% decreased from 2/5 to 0/5 at both follow-ups. As

HbA1c was measured per participant, these results are descriptive **(Figure 2)**.



a)

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Figure 2. (a) Mean HbA1c levels at baseline (T0), 1 month (T1), and 3 months (T3). (b) Mean plaque index (PlI) and gingival index (GI) values over time in the test and control groups. Error bars indicate standard deviations.

Plaque and gingival indices improved in both groups **(Figure 2)**. PPD and CAL also improved, with larger reductions at probiotic-treated sites. At 3 months, mean PPD was 2.75 mm at test sites and 4.85 mm at control sites **(Figure 3; Table 1)**.

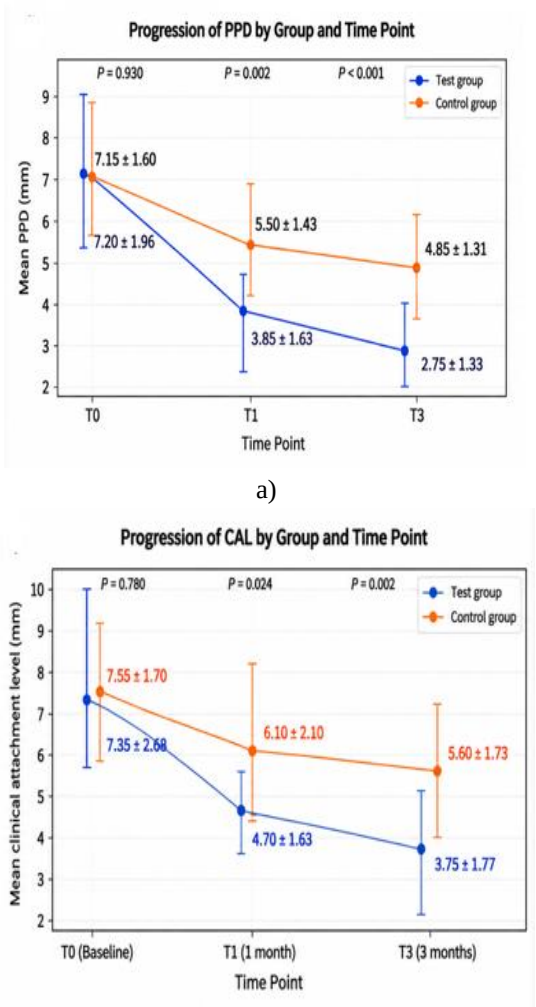

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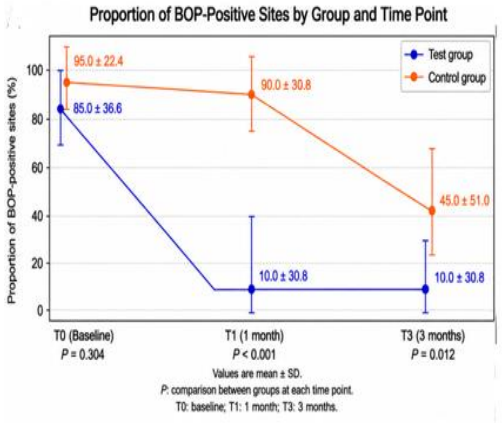
Figure 3. (a) Mean probing pocket depth (PPD) and (b) clinical attachment level (CAL) over time in probiotic-treated and saline-treated control sites. Values are means; error bars indicate standard deviations.

Table 1. Linear mixed-effects and generalized estimating equation estimates for clinical periodontal outcomes. LMM, linear mixed-effects model; GEE, generalized estimating equation; PPD, probing pocket depth; CAL, clinical attachment level; BOP, bleeding on probing; CI, confidence interval. Models included participant-level clustering as described in Methods.

Outcome	Effect	Estimate (β)	SE	95% CI	P value
PPD (mm)	Intercept (reference: control group at T0)	0.05	0.361	-0.92 to 1.02	0.919
	Time (T1 vs. T0)	-1.65	0.215	-2.05 to -1.25	< 0.001
	Time (T3 vs. T0)	-2.30	0.215	-2.70 to -1.90	< 0.001
	Treatment group × Time (T1)	-1.70	0.304	-2.26 to -1.14	< 0.001
	Treatment group × Time (T3)	-2.15	0.304	-2.71 to -1.59	< 0.001
CAL (mm)	Intercept (reference: control group at T0)	-0.20	0.514	-1.21 to 0.81	0.697
	Time (T1 vs. T0)	-1.45	0.296	-2.03 to -0.87	< 0.001

	Time (T3 vs. T0)	-1.95	0.296	-2.53 to -1.37	< 0.001
	Treatment group × Time (T1)	-1.20	0.418	-2.02 to -0.38	0.004
	Treatment group × Time (T3)	-1.65	0.418	-2.47 to -0.83	< 0.001
BOP positivity	Intercept (reference: control group at T0)	0.950	0.285	0.392 to 1.508	< 0.001
	Treatment group at T0	-0.100	0.402	-0.889 to 0.689	0.804
	Time (T1 vs. T0)	-0.050	0.098	-0.241 to 0.141	0.608
	Time (T3 vs. T0)	-0.500	0.098	-0.691 to -0.309	< 0.001
	Treatment group × Time (T1)	-0.700	0.138	-0.971 to -0.429	< 0.001
	Treatment group × Time (T3)	-0.250	0.138	-0.521 to 0.021	0.070
Surgical treatment need	Intercept (reference: control group at T0)	1.000	0.215	0.578 to 1.422	< 0.001
	Treatment group at T0	0.000	0.304	-0.596 to 0.596	1.000
	Time (T1 vs. T0)	-0.250	0.074	-0.395 to -0.105	0.001
	Time (T3 vs. T0)	-0.550	0.074	-0.695 to -0.405	< 0.001
	Treatment group × Time (T1)	-0.600	0.104	-0.805 to -0.395	< 0.001
	Treatment group × Time (T3)	-0.350	0.104	-0.555 to -0.145	0.001

BOP decreased in both groups, with a greater reduction at probiotic-treated sites **(Figure 4; Table 1)**.



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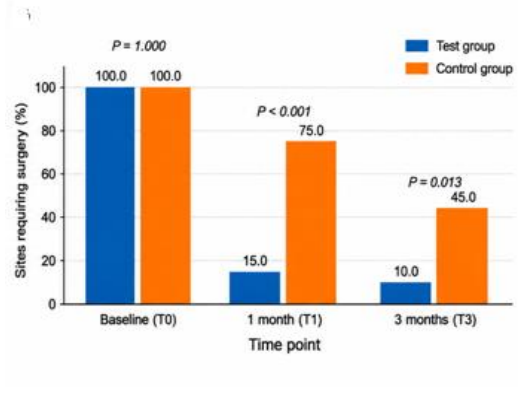

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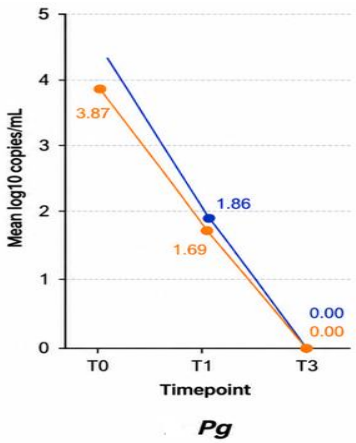
Figure 4. (a) Percentage of bleeding-on-probing-positive sites and (b) percentage of sites requiring periodontal surgery at baseline, 1 month, and 3 months. Surgical treatment need was defined as residual PPD ≥5 mm.

Residual sites requiring periodontal surgery decreased in both groups, with fewer such sites at probiotic-treated sites during follow-up **(Figure 4; Table 1)**.

Mixed-effects and GEE analyses, accounting for repeated measurements and participant clustering, indicated greater PPD reduction and CAL gain and lower BOP positivity and surgical-treatment need at probiotic-treated sites. Given the five-participant sample, these estimates are preliminary **(Table 1)**.

P. gingivalis and *T. forsythia* counts decreased over time, whereas *F. nucleatum* counts remained relatively stable. Significant overall time effects were observed for *P. gingivalis* and *T. forsythia*, but not for *F. nucleatum*. No significant treatment-by-time interactions were detected for any of the three species. These microbiological findings should be interpreted as exploratory **(Figure 5; Table 2)**.



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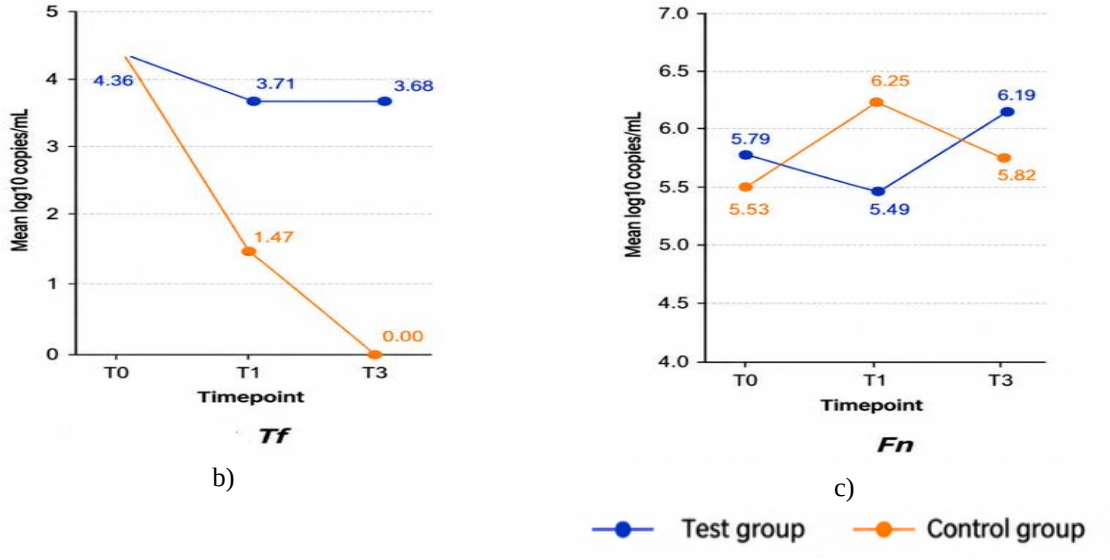

Figure 5. Mean log10 bacterial counts of (a) *Porphyromonas gingivalis*, (b) *Tannerella forsythia*, and (c) *Fusobacterium nucleatum* at baseline, 1 month, and 3 months.

Table 2. Linear mixed-effects model estimates for changes in subgingival bacterial counts. Values are regression coefficients (β) for log10-transformed bacterial counts; T0 was the reference time point and the control group was the reference treatment. SE, standard error; CI, confidence interval.

Bacterial species	Fixed effect	Estimate (β)	SE	95% CI	P value
P. gingivalis	Test vs control at T0	-0.117	0.620	-1.332 to 1.097	0.850
	T1 vs T0	-1.431	0.542	-2.494 to -0.369	0.008
	T3 vs T0	-2.003	0.542	-3.065 to -0.940	< 0.001
	Test × T1	0.319	0.767	-1.183 to 1.822	0.677
	Test × T3	0.381	0.767	-1.122 to 1.883	0.620
T. forsythia	Test vs control at T0	0.341	0.655	-0.943 to 1.625	0.603
	T1 vs T0	-1.797	0.541	-2.858 to -0.736	0.001
	T3 vs T0	-1.996	0.541	-3.057 to -0.935	< 0.001
	Test × T1	0.613	0.765	-0.887 to 2.113	0.423
	Test × T3	0.180	0.765	-1.320 to 1.681	0.814
F. nucleatum	Test vs control at T0	0.266	0.349	-0.419 to 0.951	0.447
	T1 vs T0	0.299	0.298	-0.286 to 0.884	0.316
	T3 vs T0	-0.020	0.298	-0.605 to 0.564	0.945
	Test × T1	-0.767	0.422	-1.595 to 0.060	0.069
	Test × T3	-0.072	0.422	-0.899 to 0.755	0.864

The present preliminary split-mouth study suggests that adjunctive subgingival administration of *Limosilactobacillus reuteri* may provide additional clinical benefits when combined with non-surgical periodontal therapy in patients with diabetes mellitus. Greater reductions in periodontal inflammation, probing pocket depth (PPD) and residual surgical treatment need, along with greater clinical attachment level (CAL) gains, were observed at probiotic-treated sites compared with saline-treated control sites. However, given the small number of participants and the clustered split-mouth design, these findings should be interpreted as preliminary and hypothesis-generating rather than confirmatory.

Among the evaluated outcomes, the reduction in bleeding on probing (BOP) is clinically relevant because BOP reflects periodontal inflammatory activity and is related to the inflammatory processes underlying periodontal tissue destruction [3, 4, 24, 25] Previous clinical studies have reported clinical and

biochemical effects associated with adjunctive *L. reuteri* therapy [14-16]. In particular, modulation of inflammatory mediators has been proposed as one potential mechanism underlying the clinical effects of probiotic therapy [16, 24, 25]. These mechanisms provide biological plausibility for the greater reduction in BOP observed at probiotic-treated sites; however, inflammatory mediators were not directly measured in the present study, and mechanistic interpretations therefore remain speculative.

Improved local inflammatory control may contribute to periodontal wound healing and stability. Diabetes mellitus is associated with altered host inflammatory responses and impaired periodontal tissue homeostasis, which may adversely affect periodontal healing and increase susceptibility to periodontal breakdown [5-9, 26]. The greater CAL improvement observed at probiotic-treated sites may therefore be compatible with improved local healing conditions. Nevertheless, because the present study did not directly assess cellular or inflammatory mechanisms, a causal biological pathway cannot be established.

A greater reduction in PPD was also observed at probiotic-treated sites during follow-up. Similar clinical improvements following adjunctive *L. reuteri* therapy have been reported by Teughels *et al*. [14] and Tekce *et al*. [15]. Moreover, Martin-Cabezas *et al*. [17], in a systematic review and meta-analysis, reported additional clinical benefits of probiotics as adjuncts to non-surgical periodontal treatment. These observations are broadly consistent with subsequent systematic reviews and evidence syntheses evaluating probiotics in periodontal therapy [18-23]. Nevertheless, direct comparisons should be made cautiously. Previous studies differed in patient populations, probiotic formulations, administration protocols, follow-up periods, and study designs.

The observed improvement in periodontal inflammation may also be considered in the context of the well-established bidirectional relationship between periodontitis and diabetes mellitus [5-9, 26]. In the present study, HbA1c showed a modest downward trend during follow-up. Because only five participants were included and the study was neither designed nor powered to evaluate metabolic outcomes, this observation should not be interpreted as evidence that locally delivered probiotic therapy improves glycemic control. Systematic reviews have reported that periodontal treatment may contribute to modest improvements in glycemic control among patients with diabetes [23, 27]. A randomized trial has also demonstrated systemic effects following intensive periodontal treatment in patients with type 2 diabetes [28]. In addition, probiotic supplementation has been investigated for its effects on inflammatory and cardiometabolic parameters in patients with type 2 diabetes [29]. These studies provide biological and clinical context for the observed HbA1c trend but do not establish a causal relationship in the present study.

Another clinically relevant observation was the reduction in the proportion of residual sites meeting the prespecified criterion for surgical treatment need. Persistent residual periodontal pockets have been associated with subsequent periodontal disease progression and tooth loss during supportive periodontal care [11-13]. Consequently, a reduction in residual deep pockets following non-surgical treatment may have implications for subsequent periodontal management. This finding should nevertheless be considered exploratory because multiple teeth and periodontal sites were contributed by a small number of participants and the follow-up period was limited.

Microbiological changes were less pronounced and less consistent than the clinical improvements. Longitudinal analyses showed significant reductions over time in *P. gingivalis* and *T. forsythia*, whereas *F. nucleatum* showed no significant temporal change. However, no statistically significant treatment × time interactions were observed for any of the three species, suggesting that the present study did not provide sufficient evidence of an additional microbiological effect attributable to adjunctive *Limosilactobacillus reuteri* administration.

The lack of a differential microbiological effect despite the greater clinical improvements observed at probiotic-treated sites may have several explanations. SRP itself can substantially reduce the subgingival microbial burden, potentially limiting the additional detectable effect of *L. reuteri*. In addition, the small sample size and relatively short follow-up period may have limited the ability to detect modest between-group microbiological differences. Periodontitis is a polymicrobial dysbiotic disease; therefore, clinical improvement may not necessarily parallel changes in individual bacterial species. Moreover, the clinical benefits associated with *L. reuteri* may partly involve modulation of the local host inflammatory response and periodontal microenvironment rather than solely direct antimicrobial effects on the specific pathogens evaluated. In addition, the microbiological assessment focused on three selected periodontal species and used targeted qPCR rather than broad profiling of the subgingival community. Samples were obtained from the predetermined deepest baseline pocket at each assessment. Accordingly, the findings describe changes in these measured organisms at sampled sites and should not be generalized to the entire oral microbiome or to unmeasured microbial taxa [30, 31].

The clinical findings should be interpreted across the different dimensions of periodontal response rather than on the basis of a single endpoint. PPD reduction describes the change in pocket depth, whereas CAL gain provides information about the position of the periodontal attachment relative to the tooth reference. The concurrent improvement in these measures, together with reduced BOP, suggests that the observed response was not limited to a change in pocket measurements alone. Nevertheless, the apparent advantage should be interpreted cautiously because multiple teeth were contributed by only five participants. The number of tooth-level observations cannot replace an adequate number of independent participants.

These findings also inform the evaluation of adjunctive probiotics in periodontal care for people with diabetes. Local administration may offer a targeted approach by delivering the intervention directly to selected periodontal pockets. In this study, *L. reuteri* was used as an adjunct to SRP, while control sites received SRP with saline. Thus, the comparison addresses the additional contribution of the local adjunct under this protocol. It does not establish that probiotics can replace mechanical periodontal treatment or that similar responses would occur with other formulations, doses, or administration schedules. Those questions require direct evaluation in appropriately designed comparative trials.

Clinical and microbiological outcomes should also be distinguished. Clinical indices describe the condition of periodontal tissues and their response to treatment, whereas targeted qPCR evaluates selected bacterial species at sampled sites. Changes in these organisms may not capture broader shifts in microbial community structure. Accordingly, the absence of a significant treatment-by-time interaction does not prove that the intervention had no biological effect; it indicates that these data do not establish a differential effect on the three assessed pathogens. Future studies using broader microbiome characterization alongside standardized clinical assessments may clarify whether clinical improvements accompany changes in microbial community composition or function.

Study limitations

Several methodological considerations and limitations should be acknowledged. First, only five participants contributed the 40 analyzed teeth. Although the split-mouth design and statistical models accounted for within-participant clustering and repeated measurements, the number of independent biological units remained very small. Consequently, the small number of independent participants limits statistical power and the precision of treatment-effect estimates, even when clustering and repeated measurements are accounted for. It also increases uncertainty around the estimated effects and restricts generalizability. The multiple tooth- and site-level observations improve within-participant information but do not compensate for the limited number of independent participants. Therefore, the observed treatment effects should be interpreted as preliminary estimates rather than confirmatory evidence and require validation in adequately powered studies with large participant-level samples.

Second, the microbiological observations were clustered with participants and repeatedly assessed over time. Failure to account for this dependence could result in pseudoreplication and underestimated uncertainty. The longitudinal microbiological analyses were therefore performed using models that accounted for the repeated and clustered structure of the observations. Nevertheless, because the number of independent participants remained very small, these analytical approaches cannot fully overcome the limitation imposed by the participant-level sample size. The microbiological results should consequently be regarded as exploratory.

Third, the split-mouth design provides an important methodological advantage in this population because probiotic-treated and control sites were evaluated within the same patient with diabetes mellitus. Thus, each participant served as their own control, reducing inter-individual variability related to systemic condition, glycemic status, host inflammatory response, and other patient-level characteristics that could influence periodontal treatment outcomes. This within-patient comparison may improve the efficiency of treatment-effect estimation, particularly in a small preliminary study.

Nevertheless, this advantage must be balanced against the inherent possibility of carryover or cross-site effects between probiotic-treated and control sites within the same oral cavity. In the present study, *L. reuteri* was administered locally and directly into the selected periodontal pockets by subgingival irrigation rather than as a whole-mouth intervention. Following administration, the treated sites were covered with a periodontal dressing to facilitate local retention of the solution and minimize its immediate dispersion within the oral cavity. Despite these measures, intraoral dissemination of the probiotic or treatment-related biological effects across sites cannot be completely excluded. Such potential carryover could not be reliably quantified or eliminated statistically in the present small dataset. Therefore, although the split-mouth design reduces between-patient confounding, it

does not eliminate the possibility of cross-site effects. Between-treatment differences should therefore be interpreted cautiously. Future studies should prospectively incorporate strategies to minimize and evaluate potential carryover when split-mouth designs are used for locally administered probiotics.

Additional limitations include the short follow-up period, the absence of direct assessment of inflammatory biomarkers such as IL-1β, IL-6, TNF-α, and C-reactive protein, and microbiological evaluation restricted to selected periodontal pathogens rather than comprehensive microbiome profiling. Future randomized controlled studies should include substantially larger participant-level samples, longer follow-up periods, prespecified statistical approaches that appropriately account for clustering and repeated measurements, and strategies to evaluate potential carryover in split-mouth designs. Comprehensive microbiome profiling and inflammatory biomarker assessments would also help clarify the biological mechanisms underlying any clinical effects of adjunctive *L. reuteri* therapy.

Conclusion

Within the limitations of this preliminary split-mouth randomized study, adjunctive subgingival administration of *Limosilactobacillus reuteri* was associated with greater improvements in periodontal clinical outcomes following non-surgical periodontal therapy compared with SRP with saline in patients with diabetes. Improvements were observed particularly in bleeding, probing pocket depth, clinical attachment level, and the proportion of sites with residual deep pockets requiring further intervention. Microbiological findings showed favorable longitudinal trends, although these results should be interpreted cautiously given the small sample size. Changes in HbA1c were exploratory and do not allow conclusions regarding systemic effects. These preliminary findings support further investigation of locally administered *L. reuteri* as an adjunct to non-surgical periodontal therapy in larger, adequately powered randomized studies.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: The study protocol was reviewed and approved by the Institutional Review Board of the University of Medicine and Pharmacy at Ho Chi Minh City (Approval No. 3315/ĐHYD-HĐĐĐ, dated 14 August 2025). The study was registered at ClinicalTrials.gov (NCT07652255) after completion of data collection. Written informed consent was obtained from all participants prior to enrollment.

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