

In vitro antimicrobial evaluation of probiotic combination of *Lactobacillus reuteri* and *Streptococcus salivarius* against *Porphyromonas gingivalis*

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ABSTRACT

Background: The increasing prevalence of antimicrobial resistance and adverse effects associated with conventional chemotherapeutic agents has led to growing interest in probiotics as alternative therapeutic agents in periodontal therapy.

Aim: To evaluate the antimicrobial efficacy of a combination of *Lactobacillus reuteri* and *Streptococcus salivarius* against *Porphyromonas gingivalis*.

Materials and Methods: Antimicrobial activity was assessed using agar well diffusion for ZOI and broth microdilution method for MIC and MBC determination. Plates were incubated under anaerobic conditions at 37°C for 48–72 hours. Descriptive analysis was performed.

Results: The combination of probiotics demonstrated the highest antimicrobial activity with the lowest MIC (0.04 mg/ml) and MBC (0.01 mg/ml). Individual strains showed moderate activity, with higher MIC and MBC values. Agar plate analysis revealed a dose-dependent increase in inhibition zones, with maximum inhibition observed in the combination group. Log CFU analysis further confirmed enhanced antimicrobial activity in the combination group.

Conclusion: The probiotic combination exhibited superior antimicrobial and bactericidal activity against *P. gingivalis*, suggesting its potential as an adjunct in periodontal therapy.

Keywords: Probiotics, *Lactobacillus reuteri*, *Streptococcus salivarius*, *Porphyromonas gingivalis*, MIC, MBC

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INTRODUCTION

The oral cavity consists of distinct microenvironments such as saliva, gingival crevicular fluid, tongue, buccal mucosa, teeth, and gingival epithelium, each of which supports a complex and dynamic microbial population. Under normal conditions, these microorganisms exist in a balanced

state, contributing to oral health. However, disruption of this equilibrium can result in microbial dysbiosis, which is closely linked to disease development [1]. Periodontal disease represents a chronic inflammatory condition primarily initiated by pathogenic microorganisms within dental plaque biofilms[2]. It commonly begins as gingivitis, a

reversible inflammation limited to the gingival tissues. Without appropriate periodontal health, the condition may advance to periodontitis, a destructive and irreversible disease marked by the breakdown of the tooth-supporting structures, including the periodontal ligament, alveolar bone, and cementum [3].

Among the key pathogens, *Porphyromonas gingivalis* is recognized as a keystone organism capable of modulating host immune responses, disrupting microbial homeostasis, and promoting a dysbiotic environment [4]. Evidence from in vitro studies has demonstrated that *P. gingivalis* can trigger apoptosis in a variety of cell types, including fibroblasts, endothelial cells, and lymphocytes. This induction of programmed cell death is considered a major factor in the progressive breakdown of periodontal tissues observed clinically. While some investigations report that it promotes apoptotic pathways in epithelial cells, others suggest that it may inhibit apoptosis, indicating a complex and possibly concentration dependent interaction [5]. The global burden of periodontal disease continues to rise, with increasing evidence linking it to systemic conditions such as cardiovascular diseases, diabetes mellitus, and adverse pregnancy outcomes [6]. Conventional periodontal therapy primarily involves mechanical debridement supported by adjunctive antimicrobial agents such as chlorhexidine and systemic antibiotics. However, prolonged use of these agents is associated with adverse effects including staining, taste alteration, disruption of normal microbiota, and the emergence of antimicrobial resistance [7]. In recent years, probiotics have gained considerable attention as a biologically safe and effective alternative for modulating oral microbiota. Probiotics are defined as live microorganisms that confer health benefits to the host when administered in adequate amounts. They act through multiple mechanisms, including competitive inhibition of pathogens, production of antimicrobial substances, modulation of host immune responses, and maintenance of microbial homeostasis [8]. Among the various probiotic strains, *Lactobacillus reuteri* has been extensively studied for its ability to produce reuterin, an antimicrobial compound with broad-spectrum activity against oral pathogens [9]. *Lactobacillus reuteri* has been shown to mitigate local oxidative stress, decrease the load of pathogenic microorganisms within the subgingival

microbiota, and thereby slow the progression of periodontitis [10]. Furthermore, its favorable biocompatibility and established safety profile support its potential use as an adjunctive therapeutic agent in the clinical management of periodontal disease [11]. Similarly, *Streptococcus salivarius*, a commensal oral bacterium, produces bacteriocins such as salivaricins and has been shown to inhibit pathogenic biofilm formation and reduce oral inflammation [12]. *Streptococcus salivarius*, originally isolated from the saliva for use within the oral cavity. Its antimicrobial efficacy observed in in vitro studies, particularly against *Streptococcus pyogenes* and other microorganisms associated with halitosis, is largely attributed to its ability to produce lantibiotic bacteriocins, which inhibit the growth of pathogenic species [13]. Individual probiotic strains have demonstrated beneficial effects and emerging evidence suggests that multi-strain probiotic formulations may exhibit enhanced efficacy due to synergistic interactions between strains [14]. However, there remains limited evidence evaluating the combined antimicrobial potential of *L. reuteri* and *S. salivarius* specifically against *Porphyromonas gingivalis*. Therefore, the present study was designed to assess the antimicrobial efficacy of this probiotic combination.

MATERIALS AND METHODS

Study Design

This in vitro study was approved by the Institutional Ethics Committee of SIBAR Institute of Dental Sciences, Guntur, Andhra Pradesh, India (Approval No. Pr. 573/IEC/SIBAR/2025). The experimental procedures were conducted at the Department of Microbiology, Arihant Hospital, Belagavi, Karnataka, India.

Microorganisms

The test organism used was *Porphyromonas gingivalis* which is obtained from authenticated microbial repositories. The probiotic organisms were derived from commercially available formulations, namely ProRespi™ K12 (Dr. Reddy's Laboratories, India) containing *Streptococcus salivarius* K12, and *Lactobacillus reuteri* (Trophomed Healthcare, India).

Culture of Porphyromonas gingivalis

Porphyromonas gingivalis was cultured under strict anaerobic conditions to mimic its natural subgingival environment. The organism was obtained from a standard laboratory strain and initially revived on enriched blood agar plates supplemented with hemin (5 µg/mL) and vitamin K₁ (1 µg/mL), which are essential growth factors for this fastidious anaerobe. The inoculated plates were incubated in an anaerobic chamber or jar system containing a gas mixture (80–85% N₂, 10% H₂, and 5–10% CO₂) at 37°C for 48–72 hours. Following incubation, well-isolated colonies exhibiting characteristic black pigmentation were selected and transferred into anaerobic broth. The bacterial suspension was standardized to match 0.5 McFarland turbidity standard (approximately 1.5 × 10⁸ CFU/mL) using a spectrophotometer, ensuring uniform inoculum density for subsequent antimicrobial assays [15].

Probiotic Culture Preparation

Commercially available probiotic tablets containing *Lactobacillus reuteri* and *Streptococcus salivarius* were procured and used as the source of probiotic organisms. The tablets were aseptically crushed and suspended in sterile saline, followed by inoculation into appropriate culture media. *Lactobacillus reuteri* was cultured in de Man, Rogosa, and Sharpe (MRS) broth at 37°C for 24–48 hours under anaerobic/microaerophilic conditions, while *Streptococcus salivarius* was cultured in brain heart infusion (BHI) broth at 37°C for 24 hours under 5% CO₂ conditions. Following incubation, the cultures were adjusted to a turbidity equivalent to 0.5 McFarland standards to ensure uniform bacterial concentration for antimicrobial assays. For the combination group, equal volumes of standardized suspensions of *L. reuteri* and *S. salivarius* were mixed in a 1:1 ratio under sterile conditions to obtain a homogeneous probiotic preparation. This combined

preparation was used immediately for antimicrobial testing. [16].

Agar Well Diffusion Method

Blood agar plates were inoculated with *P. gingivalis*. Wells were prepared and loaded with probiotic samples (individual and combination). Appropriate controls were included to validate the experimental outcomes. The negative control consisted of samples without any antimicrobial agent to assess the natural growth pattern of the test microorganisms. The positive control included a standard antimicrobial agent to confirm the susceptibility of the organisms and to serve as a benchmark for evaluating the antimicrobial efficacy of the test formulations. Plates were incubated anaerobically at 37°C for 48–72 hours, and zones of inhibition (ZOI) were measured in millimeters.

STATISTICAL ANALYSIS

The MIC, MBC, and log CFU values are presented descriptively, indicating enhanced antimicrobial activity and a concentration-dependent reduction in bacterial counts in the combination group compared to individual strains.

RESULTS

The antimicrobial activity of probiotic formulations against *Porphyromonas gingivalis* was evaluated using agar well diffusion, MIC, MBC, and log CFU analysis.

Agar Diffusion Findings

Both *Lactobacillus reuteri* and *Streptococcus salivarius* demonstrated moderate zones of inhibition, while the combination group exhibited the largest zones, showing a dose-dependent increase (Fig 1). The combination group demonstrated significantly lower MIC and MBC values, indicating strong synergistic and bactericidal activity (Fig 2).

Figure 1: Blood agar plates showing antimicrobial activity at different concentrations.



Figure2: Comparative visualization showing enhanced antimicrobial activity in the combination group.



Table 1: MIC and MBC Values of Probiotic Strains Against Porphyromonas gingivalis

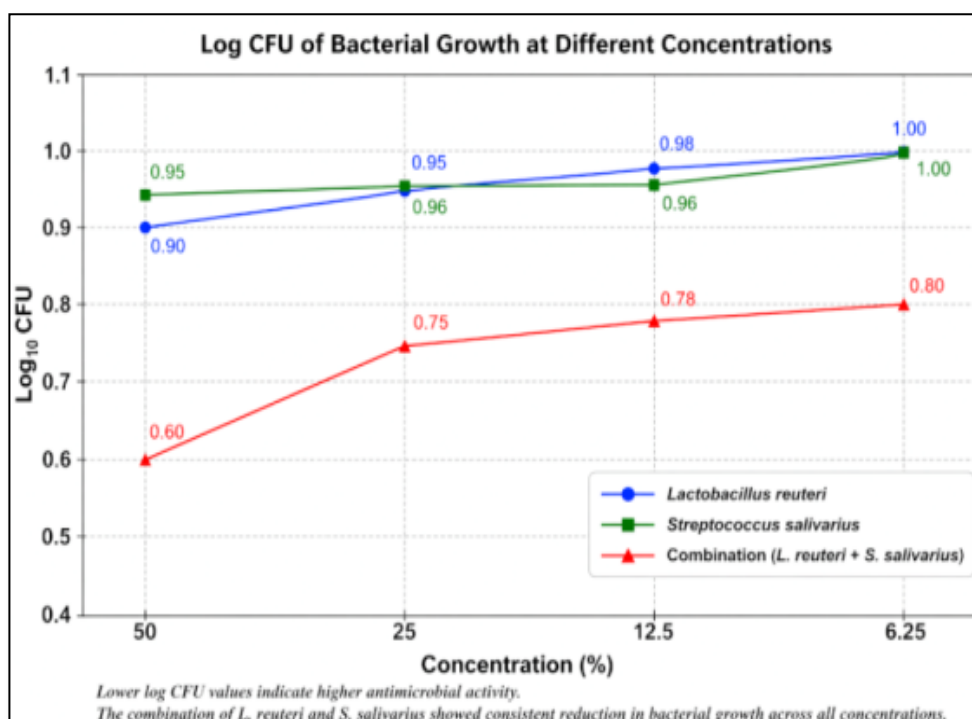
Probiotic Group	MIC (mg/ml)	MBC (mg/ml)
Lactobacillus reuteri	0.12	0.50

Streptococcus salivarius	0.13	0.60
Combination	0.04	0.01

Log CFU Analysis

The log CFU analysis demonstrated a concentration-dependent reduction in bacterial counts across all groups. The combination of *Lactobacillus reuteri* and *Streptococcus salivarius* exhibited the lowest log CFU values at all tested concentrations, with maximum inhibition observed at 50% concentration (0.60). In contrast, *Lactobacillus reuteri* showed moderate antimicrobial activity, while *Streptococcus salivarius* demonstrated minimal inhibitory effect. These findings indicate a synergistic interaction between the two probiotic strains.

Graph 1: Log CFU values of bacterial growth following treatment at different concentrations.



DISCUSSION

The present *in vitro* study evaluated the antimicrobial efficacy of *Lactobacillus reuteri*, *Streptococcus salivarius*, and their combination against *Porphyromonas gingivalis*. The findings demonstrated that while individual probiotic strains exhibited moderate antimicrobial activity, their combination showed significantly enhanced inhibitory and bactericidal effects. The moderate antimicrobial activity of *Streptococcus salivarius* observed in this study can be attributed to its production of bacteriocins such as salivaricins and hydrogen peroxide, which inhibit the growth of pathogenic oral microorganisms [17]. However, its relatively higher MBC value suggests that its effect is

primarily bacteriostatic rather than bactericidal, consistent with previous studies [18].

Lactobacillus reuteri demonstrated moderate antimicrobial activity, which may be explained by the production of reuterin, organic acids, and other antimicrobial metabolites [19]. These compounds are known to inhibit bacterial growth and interfere with biofilm formation. However, *P. gingivalis*, being a strict anaerobe with proteolytic metabolism, may exhibit resistance to acidic environments, thereby limiting the bactericidal effect of *L. reuteri* alone [20]. The combination of *L. reuteri* and *S. salivarius* demonstrated a marked reduction in MIC and MBC values, indicating a strong synergistic interaction. This enhanced antimicrobial activity may be due to complementary mechanisms of action, including

combined bacteriocin production, organic acid release, and competitive inhibition of pathogen adhesion [21]. Multi-strain probiotic formulations have been shown to exert additive or synergistic effects, resulting in improved pathogen suppression compared to single-strain therapies [22].

The observed MBC/MIC ratio (~2) confirms the bactericidal nature of the probiotic combination, in accordance with CLSI criteria. This finding is clinically significant, as bactericidal agents are preferred in the management of periodontal infections characterized by high bacterial load and tissue invasion [23]. Furthermore, the log CFU analysis demonstrated a clear concentration-dependent reduction in bacterial counts, with maximum inhibition observed at higher concentrations. This aligns with recent studies highlighting that probiotic efficacy is dose-dependent and influenced by bacterial viability and metabolic activity [24].

Probiotics are known to interfere with key virulence factors of *P. gingivalis*, including gingipains, biofilm formation, and quorum sensing pathways. Additionally, the ecological plaque hypothesis supports the concept that restoration of microbial balance through beneficial bacteria can effectively suppress pathogenic species and maintain periodontal health. Recent clinical and in vitro studies have further supported the role of probiotics as adjuncts in periodontal therapy, demonstrating reductions in plaque accumulation, gingival inflammation, and periodontal pathogens. Notably, multi-strain probiotic formulations have consistently shown superior clinical outcomes compared to single-strain interventions [25].

Despite these promising findings, the present study has certain limitations. Being an in vitro study, it does not fully replicate the complex oral environment, which includes host immune responses, salivary factors, and multispecies biofilms. Therefore, further in vivo studies and randomized clinical trials are necessary to validate these findings and determine optimal dosage, formulation, and delivery methods.

CONCLUSION

Within the limitations of this in vitro study, the combination of *Lactobacillus reuteri* and *Streptococcus salivarius* demonstrated superior antimicrobial and bactericidal activity against *Porphyromonas gingivalis* compared to individual

strains. The observed synergistic effect highlights the potential of multi-strain probiotic formulations as adjuncts in periodontal therapy.

DECLARATIONS

Conflict of Interest: None

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REFERENCES

1. Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation. *Nat Rev Immunol*. 2015 Jan;15(1):30-44. doi: 10.1038/nri3785. PMID: 25534621; PMCID: PMC4276050.
2. Arigbede AO, Babatope BO, Bamidele MK. Periodontitis and systemic diseases: A literature review. *J Indian Soc Periodontol*. 2012 Oct;16(4):487-91. doi: 10.4103/0972-124X.106878. PMID:23493942; PMCID: PMC3590713.
3. Ochôa C, Castro F, Bulhosa JF, Manso C, Fernandes JCH, Fernandes GVO. Influence of the Probiotic *L. reuteri* on Periodontal Clinical Parameters after Nonsurgical Treatment: A Systematic Review. *Microorganisms*. 2023 May 30;11(6):1449. doi: 10.3390/microorganisms11061449. PMID:37374951; PMCID: PMC10303645.
4. Lamont RJ, Koo H, Hajishengallis G. The oral microbiota: dynamic communities and host interactions. *Nat Rev Microbiol*. 2018 Dec;16(12):745-759. doi: 10.1038/s41579-018-0089-x. PMID: 30301974; PMCID: PMC6278837. How KY, Song KP, Chan KG. *Porphyromonas gingivalis*: overview. *Front Microbiol*. 2016;7:53.
5. Jun-jun Zhao, Long Jiang, Ya-qin Zhu, Xi-ping Feng, Effect of *Lactobacillus acidophilus* and *Porphyromonas gingivalis* on proliferation and apoptosis of gingival epithelial cells, *Advances in Medical Sciences*, Volume 64, Issue 1, 2019, Pages 54-57.
6. Tonetti MS, Jepsen S, Jin L, Otomo-Corgel J. Impact of the global burden of periodontal diseases on health, nutrition and wellbeing of mankind: A call for

- global action. J Clin Periodontol. 2017 May;44(5):456-462. doi: 10.1111/jcpe.12732. Epub 2017 May 8. PMID: 28419559.
7. Lee SY, Nam EJ. Clinical Efficacy of 1% CHX Gluconate Gel and 0.12% CHX Solution: A Randomized Controlled Trial. Int J Environ Res Public Health. 2022 Jul 30;19(15):9358. doi:10.3390/ijerph19159358. PMID: 35954713; PMCID: PMC9368169.
8. Dr. Seelam Hadhassa Vardhini, Dr. Deepa Anumala, Dr. Blessi Sravya Bandi, Dr. Payyavula Sri Sai Charan, Dr. Chennamsetti Ramya and Dr. Ravindranath Dulipallla (2025) Comparative evaluation of effectiveness of green tea mouth rinse with probiotic mouth rinse on oral halitosis and gingivitis. World Journal of Pharmaceutical Science and Research, 4(5),141-150. <https://doi.org/10.5281/zenodo.17234404>
9. Liu Z, Cao Q, Wang W, Wang B, Yang Y, Xian CJ, Li T, Zhai Y. The Impact of Lactobacillus reuteri on Oral and Systemic Health: A Comprehensive Review of Recent Research. Microorganisms. 2024 Dec 30;13(1):45. doi: 10.3390/microorganisms13010045. PMID: 39858814; PMCID: PMC11767923.
10. Ram J, Awan KH, Freitas CMT, Bhandi S, Licari FW, Patil S. Clinical effects of Lactobacillus reuteri probiotic in chronic periodontitis - a systematic review. Eur Rev Med Pharmacol Sci. 2024 Mar;28(5):1695-1707. doi: 10.26355/eurrev_202403_35584. PMID: 38497853.
11. Wang, Y., Tang, Y., Huang, Q. et al. Engineered Lactobacillus reuteri for scavenging reactive oxygen species and modulating oral microflora in periodontitis therapy. Int J Oral Sci 18, 16 (2026). <https://doi.org/10.1038/s41368-025-00418-z>
12. Burton JP, Wescombe PA, Moore CJ, Chilcott CN, Tagg JR. Safety assessment of the oral cavity probiotic Streptococcus salivarius K12. Appl Environ Microbiol. 2006 Apr;72(4):3050-3053. doi:10.1128/AEM.72.4.3050-3053.2006. PMID: 16598017; PMCID: PMC1449041.
13. Williams MD, Smith L. Streptococcus salivarius and Ligilactobacillus salivarius: Paragons of Probiotic Potential and Reservoirs of Novel Antimicrobials. Microorganisms. 2025 Feb 28;13(3):555. doi:10.3390/microorganisms13030555. PMID: 40142448; PMCID: PMC11944278.
14. Chapman CM, Gibson GR, Rowland I. Health benefits of probiotics: are mixtures more effective than single strains? Eur J Nutr. 2011 Feb;50(1):1-17. doi: 10.1007/s00394-010-0166-z. Epub 2011 Jan 13. PMID: 21229254. Timmerman HM, et al. Multi-strain synergy. Antonie Van Leeuwenhoek. 2004.
15. Seers CA, Mahmud ASM, Huq NL, Cross KJ, Reynolds EC. Porphyromonas gingivalis laboratory strains and clinical isolates exhibit different distribution of cell surface and secreted gingipains. J Oral Microbiol. 2020 Dec 9;13(1):1858001. doi:10.1080/20002297.2020.1858001. PMID: 33391630; PMCID: PMC7733959.
16. Temmerman R, Pot B, Huys G, Swings J. Identification and antibiotic susceptibility of bacterial isolates from probiotic products. Int J Food Microbiol. 2003 Feb 25;81(1):1-10. doi: 10.1016/s0168-1605(02)00162-9. PMID: 12423913.
17. Markowiak P, Śliżewska K. Effects of Probiotics, Prebiotics, and Synbiotics on Human Health. Nutrients. 2017 Sep 15;9(9):1021. doi: 10.3390/nu9091021. PMID: 28914794; PMCID: PMC5622781.
18. Gaspar C, Donders GG, Palmeira-de-Oliveira R, Queiroz JA, Tomaz C, Martinez-de-Oliveira J, Palmeira-de-Oliveira A. Bacteriocin production of the probiotic Lactobacillus acidophilus KS400. AMB Express. 2018 Sep 27;8(1):153. doi: 10.1186/s13568-018-0679-z. PMID: 30264211; PMCID: PMC6160374.
19. T. C. Chung, L. Axelsson, S. E. Lindgren & W. J. Dobrogosz (1989) In Vitro Studies on Reuterin Synthesis by Lactobacillus reuteri, Microbial Ecology in Health and Disease, 2:2, 137-144, DOI: 10.3109/08910608909140211
20. Santos TA, Scorzoni L, Correia R, Junqueira JC, Anbinder AL. Interaction between Lactobacillus reuteri and periodontopathogenic bacteria using in vitro and in vivo (G. mellonella) approaches. Pathog Dis. 2020 Nov 11;78(8):ftaa044. doi: 10.1093/femspd/ftaa044. PMID: 32845308.
21. Lundtorp-Olsen C, Markvart M, Twetman S, Belstrøm D. Effect of Probiotic Supplements on the Oral Microbiota-A Narrative Review. Pathogens. 2024 May 16;13(5):419. doi:

10.3390/pathogens13050419. PMID: 38787271; PMCID: PMC11124442.

22. Aleksijević LH, Aleksijević M, Škrlec I, Šram M, Šram M, Talapko J. Porphyromonas gingivalis Virulence Factors and Clinical Significance in Periodontal Disease and Coronary Artery Diseases. Pathogens. 2022 Oct 11;11(10):1173. doi: 10.3390/pathogens11101173.PMID:36297228;PMCID: PMC9609396.

23. Weinstein MP, Lewis JS 2nd. The Clinical and Laboratory Standards Institute Subcommittee on Antimicrobial Susceptibility Testing: Background, Organization, Functions, and Processes. J Clin Microbiol. 2020 Feb 24;58(3):e01864-19. doi: 10.1128/JCM.01864-19. PMID: 31915289; PMCID: PMC7041576.

24. Albuquerque-Souza E, Balzarini D, Ando-Suguimoto ES, Ishikawa KH, Simionato MRL, Holzhausen M, Mayer MPA. Probiotics alter the immune response of gingival epithelial cells challenged by Porphyromonas gingivalis. J Periodontal Res. 2019 Apr;54(2):115-127. doi: 10.1111/jre.12608. Epub 2018 Oct 4. PMID: 30284741.

25. Mendonça CD, Mata ADSPD, Azevedo LFR, Marques JF, Silveira JML, Marques DNDS. Probiotics in the non-surgical treatment of periodontitis: a systematic review and network meta-analysis. BMC Oral Health. 2024 Oct 15;24(1):1224. doi: 10.1186/s12903-024-05027-6.PMID:39407177; PMCID: PMC11481756.