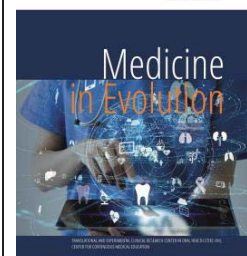


# Clinical Effects of Oral Probiotics on Plaque Accumulation and Gingival Inflammation in Young Adults



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**Doina Chioran<sup>1†</sup>, Vanessa Bolchis<sup>2,3†</sup>, Diana Florina Nica<sup>1</sup>, Octavia Balean<sup>2,3</sup>, Lucian Floare<sup>2,3</sup>, Oana Pirvu<sup>3</sup>, Delia Abrudan-Luca<sup>2,3</sup>**

<sup>1</sup>Department of Anesthesiology and Oral Surgery, School of Dental Medicine, "Victor Babes" University of Medicine and Pharmacy of Timisoara, 2A Eftimie Murgu Place, 300041 Timisoara, Romania]

<sup>2</sup>Clinic of Preventive, Community Dentistry and Oral Health, "Victor Babes" University of Medicine and Pharmacy, Eftimie Murgu Sq. no. 2, 300041, Timisoara, Romania

<sup>3</sup>Translation and Experimental Clinical Research Center in Oral Health (TEXC-OH), , 14 A Tudor Vladimirescu Ave., 300173, Timisoara,

<sup>†</sup>These authors contributed equally to this work.

Correspondence to:

Name: Octavia Balean

E-mail address: [baleen.octavia@umft.ro](mailto:baleen.octavia@umft.ro)

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## Abstract

**Background/Objectives:** Oral dysbiosis is recognized as a major contributing factor to the development of biofilm-associated oral diseases, including dental caries and gingivitis. Probiotics have emerged as promising adjunctive agents capable of modulating the oral microbiota and supporting oral health. The aim of this study was to evaluate the effects of a chewable multi-strain probiotic formulation on salivary pH, plaque accumulation, and gingival inflammation in healthy young adults. **Methods:** Thirty participants aged 18–25 years were allocated to a probiotic group (n = 15) or a control group (n = 15) and monitored for 28 days. The probiotic group received chewable tablets containing \*Streptococcus salivarius\*, \*Lactobacillus acidophilus\*, \*Lactobacillus plantarum\*, \*Lactobacillus reuteri\*, and \*Lactobacillus paracasei\* for 14 days, followed by a 14-day washout period. Salivary pH, Plaque Index (PI), and Gingival Index (GI) were assessed at baseline, Day 14, and Day 28. **Results:** No significant differences in salivary pH were observed between groups throughout the study period (p > 0.05). In contrast, probiotic supplementation resulted in significantly lower PI values at Day 14 (p = 0.042) and significantly lower GI values at Day 14 (p = 0.020) and Day 28 (p = 0.033) compared with the control group. Within-group analyses also demonstrated significant reductions in plaque accumulation and gingival inflammation following probiotic administration. **Conclusion:** Short-term supplementation with a multi-strain probiotic formulation improved plaque control and gingival health without significantly affecting salivary pH, supporting the potential role of probiotics as adjunctive tools in preventive oral healthcare.

**Keywords:** probiotics; oral microbiome; salivary pH; plaque index; gingival index; oral health; preventive dentistry

## INTRODUCTION

The oral cavity represents one of the most diverse microbial habitats in the human body, harboring a highly complex ecosystem composed of bacteria, fungi, viruses, and protozoa that coexist in a dynamic equilibrium with the host. Following the gastrointestinal tract, the oral environment contains one of the largest and most diverse microbial communities, with more than 700 bacterial species identified to date. These microorganisms colonize various oral surfaces, including teeth, gingival tissues, the tongue, buccal mucosa, and saliva, forming highly organized biofilms that play a fundamental role in maintaining oral and systemic health [1].

Oral biofilms are structured microbial communities embedded within a self-produced extracellular polymeric matrix and attached to biological or inert surfaces. Unlike planktonic microorganisms, bacteria within biofilms exhibit complex interactions that allow them to adapt to environmental fluctuations, regulate local pH and redox potential, and establish cooperative or competitive relationships with neighboring species. Under physiological conditions, these microbial communities contribute to maintaining oral homeostasis by preventing pathogen colonization, modulating immune responses, and supporting the integrity of oral tissues. Consequently, the oral microbiome should not be regarded merely as a collection of microorganisms but rather as an essential component of the oral ecosystem that actively participates in health maintenance [1].

The healthy oral microbiota is characterized by a balanced composition of commensal microorganisms capable of limiting the growth of pathogenic species. Beneficial bacterial genera, including *Lactobacillus* and *Bifidobacterium*, have been identified as important contributors to microbial stability and host protection. Studies based on culture-dependent and molecular techniques have demonstrated that these microorganisms colonize the oral cavity early in life, partly through maternal transmission and exposure to breast milk. Species such as *Bifidobacterium bifidum*, *Bifidobacterium dentium*, and *Bifidobacterium longum* have been isolated from oral samples and are generally associated with beneficial effects on host health. These microorganisms contribute to the maintenance of ecological balance by competing with pathogens for nutrients and adhesion sites while simultaneously supporting immune regulation [2].

Despite the resilience of the oral microbiome, numerous endogenous and exogenous factors can disrupt this delicate balance and promote dysbiosis. Oral dysbiosis refers to qualitative and quantitative alterations in microbial composition that favor the proliferation of pathogenic microorganisms and increase susceptibility to disease. The transition from health to disease is not generally associated with the emergence of a single pathogen but rather with ecological shifts involving multiple microbial species and changes in their metabolic activity. Such alterations may result in the development of common oral diseases, including dental caries, gingivitis, and periodontitis, which continue to represent significant public health concerns worldwide [3].

Dental caries remains one of the most prevalent chronic diseases globally and affects individuals across all age groups. According to the ecological plaque hypothesis, frequent exposure to fermentable carbohydrates promotes environmental acidification, resulting in selective pressure favoring acidogenic and aciduric bacterial species such as *Streptococcus mutans* and various *Lactobacillus* species. This shift toward an acidic microbiome suppresses beneficial microorganisms that thrive under neutral pH conditions and contributes to progressive demineralization of dental tissues. Similarly, gingivitis and periodontal diseases are associated with dysbiotic changes characterized by increased bacterial load, altered microbial composition, and persistent inflammatory responses within periodontal tissues [1].

The development of oral dysbiosis is influenced by a broad range of factors. Prolonged or inappropriate antibiotic therapy may alter microbial diversity and facilitate the emergence of opportunistic pathogens. Dietary habits, particularly high consumption of refined sugars and processed carbohydrates, can significantly affect microbial metabolism and ecological balance. Additional factors such as smoking, alcohol consumption, hormonal changes, systemic diseases, stress, geographical variations, genetic predisposition, and inadequate oral hygiene practices further contribute to microbial disruption and disease susceptibility. Emerging evidence has also demonstrated that psychological stress may influence microbial diversity and community structure, potentially increasing vulnerability to oral diseases through both behavioral and biological pathways [3,4].

Given the multifactorial nature of oral diseases and the increasing concerns regarding antimicrobial resistance, interest has grown in alternative and adjunctive approaches capable of promoting oral health without disrupting microbial balance. Among these strategies, probiotics have emerged as particularly promising candidates. The World Health Organization defines probiotics as live microorganisms that, when administered in adequate amounts, confer health benefits on the host. Although traditionally associated with gastrointestinal health, probiotics have increasingly been investigated for their potential applications in oral medicine and dentistry [5].

The rationale for probiotic use in oral health is based on their capacity to modulate microbial communities and influence host immune responses. Rather than eliminating microorganisms indiscriminately, probiotics contribute to restoring ecological balance by promoting beneficial bacterial populations and suppressing pathogenic species. This ecological approach is particularly attractive because it aligns with contemporary concepts of microbiome-centered disease prevention and management. As a result, probiotics are increasingly viewed as biological agents capable of supporting oral health through mechanisms that extend beyond simple antimicrobial activity [5].

One of the most extensively studied properties of probiotics is their anti-inflammatory potential. Several *Lactobacillus* and *Bifidobacterium* strains have demonstrated the ability to modulate both innate and adaptive immune responses. These microorganisms can stimulate immunoglobulin A production, regulate cytokine secretion, and reduce the expression of pro-inflammatory mediators such as interleukin-1 $\beta$  and tumor necrosis factor-alpha. Through these mechanisms, probiotics may contribute to reducing gingival inflammation and preserving periodontal tissue integrity. Furthermore, by promoting epithelial repair and strengthening mucosal barriers, probiotic bacteria help maintain host-microbe homeostasis and protect oral tissues from inflammatory damage [5].

In addition to their immunomodulatory effects, probiotics possess significant antimicrobial properties. Various probiotic strains produce organic acids, hydrogen peroxide, bacteriocins, and other antimicrobial peptides capable of inhibiting the growth of pathogenic microorganisms. They also compete with pathogens for adhesion sites and nutrients, thereby reducing colonization and biofilm formation. These mechanisms are particularly relevant in the oral cavity, where biofilm accumulation plays a central role in the pathogenesis of both caries and periodontal diseases. By influencing microbial composition and biofilm dynamics, probiotics may contribute to a healthier oral environment and reduced disease risk [5].

Another important characteristic of probiotics is their antioxidant activity. Oxidative stress has been increasingly recognized as a contributing factor in the pathogenesis of oral inflammatory diseases. Reactive oxygen species generated during chronic inflammation may damage host tissues and perpetuate disease progression. Probiotic microorganisms have demonstrated the capacity to reduce oxidative stress through multiple pathways, including the production of antioxidant enzymes, enhancement of antioxidant absorption, modulation

of inflammatory mediators, and regulation of microbial metabolism. Such effects may provide additional benefits in maintaining oral tissue health and preventing disease progression [2].

Recent investigations have specifically focused on the influence of probiotics on salivary characteristics and clinical oral health parameters. Saliva plays a crucial role in maintaining oral homeostasis through its buffering capacity, antimicrobial properties, and contribution to remineralization processes. Alterations in salivary pH may significantly affect microbial ecology and caries susceptibility. Several studies have suggested that probiotic supplementation can enhance salivary pH and buffering capacity by promoting alkali-generating metabolic pathways involving compounds such as arginine and urea. These processes contribute to the production of ammonia, favoring a less acidic environment and supporting the maintenance of ecological balance within the oral cavity [6-9].

Furthermore, clinical trials have demonstrated beneficial effects of probiotic supplementation on plaque accumulation and gingival inflammation. Significant reductions in plaque index and gingival index scores have been reported following the administration of probiotic tablets or lozenges, suggesting that probiotics may serve as valuable adjuncts to conventional oral hygiene measures. Particular attention has been directed toward strains such as *Lactobacillus reuteri*, *Lactobacillus paracasei*, and *Streptococcus salivarius* K12 and M18, which have shown promising results in reducing oral biofilm formation, decreasing gingival inflammation, and inhibiting cariogenic bacteria, including *Streptococcus mutans* [6,8,10-12].

Despite the growing body of evidence supporting the beneficial effects of probiotics in oral health, significant heterogeneity remains regarding probiotic strains, administration protocols, treatment duration, and clinical outcomes. Moreover, studies involving young adults remain relatively limited, particularly in Eastern European populations. Therefore, additional research is needed to clarify the clinical relevance of probiotic supplementation and its impact on key oral health parameters [1,2].

Maintaining a balanced oral microbiome has emerged as a central objective in contemporary preventive dentistry, given its fundamental role in preserving oral homeostasis and preventing biofilm-associated diseases. While conventional preventive approaches primarily focus on mechanical plaque control and antimicrobial agents, increasing attention has been directed toward probiotic supplementation as a strategy capable of modulating the oral ecosystem without disrupting its natural microbial equilibrium. Despite the growing body of evidence supporting the beneficial effects of probiotics on oral health, the extent to which these microorganisms influence clinically relevant parameters such as salivary pH, plaque accumulation, and gingival inflammation remains incompletely understood, particularly in young adults with generally good oral health status.

Although several clinical trials have investigated the effects of probiotics on oral health outcomes, the available evidence remains inconsistent. Variations in probiotic strains, treatment duration, dosage, and study populations have produced heterogeneous results regarding changes in salivary pH, plaque accumulation, and gingival inflammation [13,14]. Moreover, relatively few studies have focused on healthy young adults, a population in which early preventive interventions may contribute to the long-term maintenance of oral health. Consequently, additional clinical investigations are required to better understand the effectiveness of probiotic supplementation as a microbiome-modulating strategy in preventive dentistry [13,15-17].

### *Aim and objectives*

The present study was undertaken to evaluate the effects of a chewable probiotic formulation containing *Streptococcus salivarius*, *Lactobacillus acidophilus*, *Lactobacillus*

paracasei, and other beneficial probiotic strains on selected parameters associated with oral health in young adults. Growing interest in microbiome-based approaches for disease prevention has highlighted the importance of probiotics as potential modulators of the oral ecosystem, capable of influencing both microbial composition and host-related factors involved in the maintenance of oral health. In this context, the study focused on assessing the impact of probiotic administration on salivary pH, a key determinant of the oral environment that plays a crucial role in the balance between demineralization and remineralization processes, as well as on clinical indicators of oral hygiene and periodontal health, including the Plaque Index and Gingival Index.

The investigation sought to determine whether regular consumption of the probiotic supplement could contribute to the stabilization of salivary conditions, reduce the accumulation of dental biofilm, and diminish gingival inflammatory responses. These parameters were selected due to their well-established association with the development and progression of common oral diseases, including dental caries and gingivitis, and because they provide clinically relevant information regarding the effectiveness of preventive interventions aimed at improving oral health outcomes.

Beyond the evaluation of individual clinical endpoints, the study was designed to explore the broader implications of probiotic supplementation in supporting oral microbial homeostasis. By promoting the growth and activity of beneficial microorganisms while limiting the proliferation of potentially pathogenic species, probiotics may contribute to the ecological balance of the oral cavity and enhance its resilience against disease-associated dysbiosis. Consequently, the present research aimed to provide a comprehensive assessment of both biological and clinical responses to probiotic administration, offering further insight into the potential role of probiotics as a non-invasive, preventive strategy for maintaining oral health. Through the integration of salivary and periodontal parameters, the findings may contribute to the expanding body of evidence supporting the incorporation of microbiome-oriented approaches into contemporary preventive dentistry and oral healthcare practices.

## MATERIALS AND METHODS

This controlled clinical trial was conducted to evaluate the effects of oral probiotic supplementation on salivary pH, plaque accumulation, and gingival inflammation in young adults. The study included 30 participants aged between 18 and 25 years, who were recruited after providing written informed consent. Participants were allocated into two parallel groups consisting of 15 individuals each and were monitored over a 28-day period. The intervention period consisted of 14 days of probiotic administration, followed by a 14-day observation period.

The study protocol was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Victor Babeş University of Medicine and Pharmacy Timișoara (Approval No. 103/03.09.2024 rev2026). Written informed consent was obtained from all participants prior to enrollment.

Eligible participants were healthy young adults without severe chronic or autoimmune diseases and willing to comply with the study protocol. Exclusion criteria included antibiotic therapy within the previous four weeks, current use of probiotic supplements, pregnancy or breastfeeding, known hypersensitivity to probiotic ingredients, and a history of periodontal treatment involving scaling and root planing before enrollment.

Participants assigned to the test group received chewable probiotic tablets (Swanson® Oral Probiotic Formula, Swanson Health Products, Fargo, ND, USA) containing a proprietary blend of 3 billion colony-forming units (CFU) per serving. The formulation included BLIS K12 *Streptococcus salivarius*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus*

reuteri, and Lactobacillus paracasei. BLIS K12 is a patented strain of Streptococcus salivarius specifically selected for its potential benefits in oral health maintenance. Participants were instructed to chew and slowly dissolve one tablet daily in the oral cavity immediately after evening tooth brushing for 14 consecutive days. Food and beverage consumption was prohibited after tablet administration.

The control group did not receive probiotic supplementation and was instructed to maintain its regular oral hygiene routine, including tooth brushing twice daily throughout the study period. No additional dietary restrictions or oral hygiene interventions were introduced in either group.

Clinical and salivary assessments were performed at baseline (Day 0), after completion of probiotic administration (Day 14), and at the end of the follow-up period (Day 28). The primary outcomes included salivary pH, Plaque Index (PI), and Gingival Index (GI).

Unstimulated whole saliva samples were collected under standardized conditions. Participants were instructed to refrain from eating, drinking, smoking, chewing gum, or performing oral hygiene procedures for at least two hours before sample collection. Salivary pH was measured immediately after collection using a calibrated digital pH meter according to the manufacturer’s recommendations.

Dental plaque accumulation was assessed using the Silness–Löe Plaque Index (PI), which evaluates the thickness of plaque deposits along the gingival margin on selected tooth surfaces. Gingival inflammation was evaluated using the Löe–Silness Gingival Index (GI), based on visual signs of inflammation and bleeding tendency following gentle probing. All clinical examinations were performed using standardized assessment criteria in order to ensure consistency throughout the study period.

Data were recorded in a dedicated database and analyzed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). Descriptive statistics were calculated for all variables and are presented as mean ± standard deviation (SD). Comparisons between groups were performed using the independent-samples Student’s t-test, whereas longitudinal comparisons within the same group were analyzed using the paired Student’s t-test. Statistical significance was established at a p-value < 0.05.

## RESULTS

Salivary pH represents an important indicator of oral environmental balance, influencing enamel integrity, microbial composition, and the susceptibility to dental caries. Throughout the study period, salivary pH values remained within the physiological range in both the probiotic and control groups, suggesting the maintenance of a favorable oral environment.

At baseline, the mean salivary pH was  $6.75 \pm 0.42$  in the probiotic group and  $6.65 \pm 0.21$  in the control group. Following the 14-day intervention period, a slight increase in salivary pH was observed in both groups, reaching  $6.82 \pm 0.21$  in the probiotic group and  $6.80 \pm 0.25$  in the control group. At the end of the follow-up period (Day 28), the mean salivary pH values were  $6.77 \pm 0.30$  and  $6.71 \pm 0.15$ , respectively (Table 1).

Table 1. Comparison of salivary pH values between the probiotic and control groups during the study period

		Day 0 ± SD	Day 14 ± SD	Day 28 ± SD
<b>Probiotic group</b>	Mean ± SD	6,75± 0,42	6,82± 0,21	6,77± 0,3
	N	15	15	15
<b>Control grup</b>	Mean ± SD	6,65± 0,21	6,80± 0,25	6,77± 0,15
	N	15	15	15
<i>p-value</i>		0,457	0,814	1,000

Although minor fluctuations were observed over time, salivary pH remained relatively stable in both groups. Statistical analysis revealed no significant differences between the probiotic and control groups at any of the evaluated time points (Day 0:  $p = 0.457$ ; Day 14:  $p = 0.814$ ; Day 28:  $p = 1.000$ ), indicating that probiotic supplementation did not produce a measurable effect on salivary pH under the conditions of the present study.

The temporal evolution of salivary pH values in the probiotic and control groups is illustrated in Figure 1. As shown, both groups exhibited similar trends throughout the study period, with values remaining within the range generally associated with oral health and ecological stability.

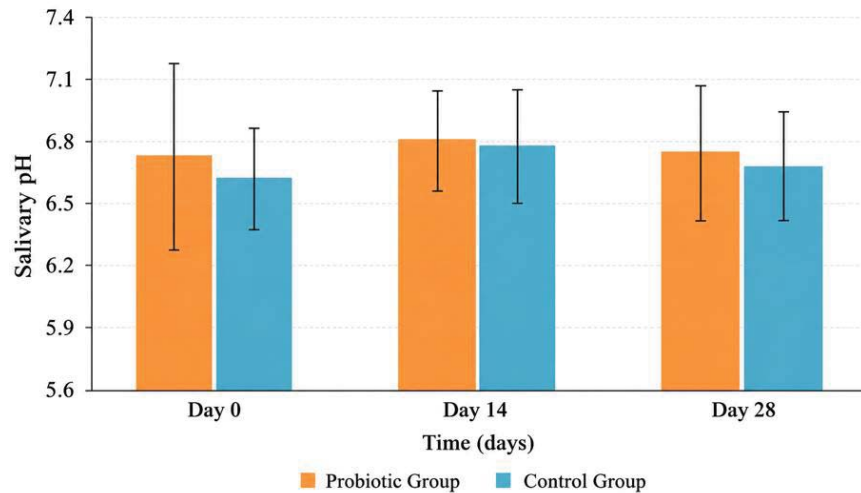


Figure 1. Temporal evolution of salivary pH values in the probiotic and control groups during the study period

The Plaque Index (PI) was evaluated at baseline, after completion of the 14-day probiotic administration period, and at the end of the follow-up period (Day 28) in order to assess changes in dental biofilm accumulation over time.

At baseline, the mean PI value was  $0.97 \pm 0.44$  in the probiotic group and  $1.21 \pm 0.48$  in the control group. Although the control group presented slightly higher plaque accumulation, the difference between groups was not statistically significant ( $p = 0.165$ ), indicating a comparable oral hygiene status at study initiation.

Following the intervention period, a substantial reduction in plaque accumulation was observed in both groups. However, participants receiving probiotic supplementation exhibited a greater decrease in PI values compared with those in the control group. At Day 14, the mean PI decreased to  $0.42 \pm 0.40$  in the probiotic group, whereas the control group showed a mean value of  $0.78 \pm 0.52$ . This difference reached statistical significance ( $p = 0.042$ ), suggesting that probiotic administration may have contributed to a more effective reduction in dental biofilm during the active supplementation period (Table 2).

At the final evaluation (Day 28), a slight increase in plaque accumulation was observed in the probiotic group, with the mean PI rising to  $0.57 \pm 0.36$ . In contrast, the control group demonstrated a modest reduction, reaching a mean PI value of  $0.74 \pm 0.56$ . Despite these changes, PI values in both groups remained lower than those recorded at baseline. The difference between groups at Day 28 was no longer statistically significant ( $p = 0.330$ ), indicating that the beneficial effect observed immediately after probiotic administration tended to diminish during the post-intervention period.

Table 2. Comparison of Plaque Index (PI) values between the probiotic and control groups during the study period

		Day 0	Day 14	Day 28
<b>Probiotic group</b>	Mean ± SD	0,97± 0,44	0,42± 0,40	0,57± 0,36
	N	15	15	15
<b>Control group</b>	Mean± SD	1,21± 0,48	0,78± 0,52	0,74± 0,56
	N	15	15	15
<b>p -value</b>		0,165	0,042*	0,330

The comparative analysis of PI values throughout the study period is presented in Table 2. As illustrated in Figure 2, both groups demonstrated a downward trend in plaque accumulation over time; however, the reduction was more pronounced in the probiotic group, particularly at the end of the supplementation period. The lowest mean PI value recorded during the study was observed in the probiotic group at Day 14, corresponding to the completion of probiotic administration.

The Gingival Index (GI) was assessed at baseline, after completion of the probiotic supplementation period (Day 14), and at the end of the follow-up period (Day 28) to evaluate changes in gingival health and the degree of gingival inflammation over time.

At baseline, the mean GI values were comparable between the two study groups, with no statistically significant differences observed. The probiotic group presented a mean GI of  $0.78 \pm 0.38$ , while the control group exhibited a mean value of  $0.74 \pm 0.44$  ( $p = 0.791$ ), indicating similar gingival conditions at the beginning of the study (Table 3).

Following the 14-day intervention period, both groups demonstrated an improvement in gingival status, reflected by a reduction in GI values. However, the decrease was substantially greater in participants receiving probiotic supplementation. At Day 14, the mean GI value decreased to  $0.33 \pm 0.34$  in the probiotic group, compared with  $0.63 \pm 0.32$  in the control group. This difference was statistically significant ( $p = 0.020$ ), suggesting a beneficial effect of probiotic administration on gingival health during the active supplementation period.

Table 3. Comparison of Gingival Index (GI) values between the probiotic and control groups during the study period

		Day 0	Day 14	Day 28
<b>Probiotic group</b>	Mean± SD	0,78± 0,38	0,33± 0,34	0,36± 0,30
	N	15	15	15
<b>Control group</b>	Mean± SD	0,74± 0,44	0,63± 0,32	0,59± 0,26
	N	15	15	15
<b>p -value</b>		0,791	0,020*	0,033*

At the final assessment (Day 28), GI values remained lower than baseline levels in both groups. The probiotic group maintained a lower mean GI value ( $0.36 \pm 0.30$ ) compared with the control group ( $0.59 \pm 0.26$ ). The difference between groups remained statistically significant ( $p = 0.033$ ), indicating that the beneficial effect observed following probiotic administration persisted throughout the follow-up period.

The comparative analysis of Gingival Index values recorded during the study period is presented in Table 3. As illustrated in Figure 2, both groups exhibited a progressive reduction in gingival inflammation over time; however, the decrease was more pronounced in the probiotic group at both post-baseline evaluations. The lowest GI values were observed among participants receiving probiotic supplementation, supporting the potential role of probiotics in improving gingival health and reducing signs of gingival inflammation.

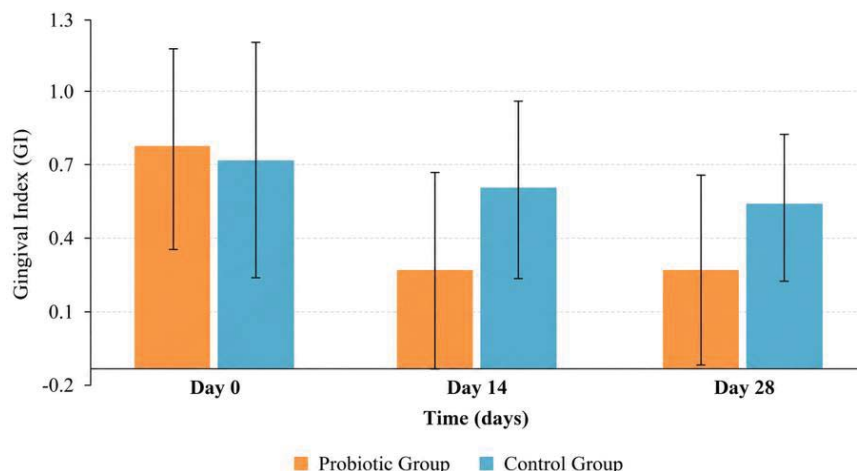


Figure 2. Temporal evolution of Gingival Index (GI) values in the probiotic and control groups during the study period. Error bars represent standard deviations

Table 4. Within-group comparison of Plaque Index (PI), Gingival Index (GI), and salivary pH values in the probiotic group during the study period

	Plaque Index			Gingival index			pH values		
	N	Mean± SD	<i>p</i>	N	Mean± SD	<i>p</i>	N	Mean± SD	<i>p</i>
Day 0	15	0,97± 0,44	0,003*	15	0,78± 0,38	0,004*	15	6,75± 0,42	0,573
Day14	15	0,42± 0,40		15	0,33± 0,34		15	6,82± 0,21	
Day 0	15	0,97± 0,44	0,017*	15	0,78± 0,38	0,005*	15	6,75± 0,42	0,883
Day 28	15	0,57± 0,36		15	0,36± 0,30		15	6,77± 0,30	
Day 14	15	0,42± 0,40	0,298	15	0,33± 0,34	0,801	15	6,82± 0,21	0,606
Day 28	15	0,57± 0,36		15	0,36± 0,30		15	6,77± 0,3	

## DISCUSSIONS

The present study evaluated the effects of a multi-strain oral probiotic formulation on salivary pH, plaque accumulation, and gingival inflammation in young adults. The findings demonstrated that probiotic supplementation was associated with significant improvements in clinical oral health parameters, particularly regarding plaque control and gingival status. Participants receiving probiotics exhibited significantly lower Plaque Index values at the end of the intervention period and significantly lower Gingival Index values at both post-baseline evaluations compared with the control group. In contrast, salivary pH remained relatively stable throughout the study and did not differ significantly between groups. Furthermore, the within-group analysis revealed that the reductions in plaque accumulation and gingival inflammation persisted during the washout period, suggesting that the beneficial effects of probiotic administration may extend beyond the active supplementation phase. These findings support the potential role of probiotics as adjunctive agents in preventive oral healthcare and contribute to the growing body of evidence highlighting the importance of microbiome-modulating strategies for maintaining oral health.

The findings of the present study demonstrated that probiotic supplementation was associated with significant improvements in both plaque accumulation and gingival health. A

significant reduction in Plaque Index values was observed following the 14-day intervention period, while Gingival Index values remained significantly lower than baseline levels even after the washout period. These results are consistent with those reported by Kavitha et al. [10] (2022), who evaluated the effects of probiotic lozenges in children aged 6–12 years and found statistically significant reductions in both plaque and gingival index scores following one month of probiotic administration. In their study, the probiotic group exhibited substantial improvements in oral hygiene and gingival status compared with the placebo group, supporting the potential role of probiotics as adjunctive agents in oral disease prevention. Similar to our findings, the authors suggested that probiotics may contribute to plaque control by inhibiting the growth of pathogenic microorganisms, reducing bacterial adhesion to tooth surfaces, and modulating the composition of the oral biofilm toward a less pathogenic microbial community. Furthermore, the observed reduction in gingival inflammation may be explained by the immunomodulatory properties of probiotic strains, which have been shown to influence local inflammatory responses and promote oral microbial homeostasis. Collectively, these findings reinforce the growing body of evidence supporting the use of probiotics as a complementary strategy for improving oral health and maintaining periodontal stability.

The present findings are also in agreement with those reported by García et al. (2021), [8] who investigated the effects of *Lactobacillus reuteri* DSM 17938 and ATCC PTA 5289 supplementation on oral health parameters in adolescents. Although the authors observed improvements in plaque, gingival, and bleeding indices, as well as a slight increase in salivary pH, these changes did not reach statistical significance. Nevertheless, the overall trend indicated a favorable effect of probiotic administration on oral health. Similarly, in the present study, probiotic supplementation was associated with lower plaque and gingival index values, while salivary pH remained relatively stable throughout the observation period. The differences observed between the two studies may be partly explained by variations in study design, probiotic strains, participant characteristics, duration of supplementation, and sample size. García et al. emphasized that the absence of statistically significant findings could be related to the limited number of participants included in the trial, highlighting the need for larger randomized clinical studies to better clarify the clinical impact of probiotics on oral health outcomes. Furthermore, the authors reported that the persistence of probiotic strains in the oral cavity appears to be temporary, which may explain why probiotic-associated benefits tend to diminish after discontinuation of supplementation. These observations are consistent with the slight increase in Plaque Index observed during the washout period in our study, despite values remaining below baseline levels.

One of the notable findings of the present study was the absence of significant changes in salivary pH following probiotic supplementation. Although a slight increase in pH values was observed during the intervention period, the differences were not statistically significant. Similar observations have been reported by García et al. (2021), who found a modest increase in salivary pH among adolescents receiving *Lactobacillus reuteri* supplementation, without reaching statistical significance [8]. A possible explanation for these findings is that participants in both studies exhibited baseline salivary pH values within the physiological range, leaving limited room for measurable improvement. Furthermore, salivary pH is influenced by multiple factors, including diet, salivary flow rate, buffering capacity, and individual oral hygiene practices, which may mask subtle probiotic-related effects. These observations suggest that the beneficial impact of probiotics on oral health may be mediated primarily through modulation of the oral microbiota and inflammatory response rather than through substantial alterations in salivary pH.

The beneficial effects observed in the present study may be explained by several biological mechanisms attributed to probiotic microorganisms. Probiotic strains are capable of

competing with pathogenic bacteria for adhesion sites on oral surfaces, producing antimicrobial substances such as bacteriocins, and modulating the ecological balance of the oral microbiome. In addition, probiotics may influence host immune responses by reducing the production of pro-inflammatory cytokines and promoting a more balanced inflammatory environment within gingival tissues. These mechanisms may contribute to the reduction of dental biofilm accumulation and gingival inflammation observed among participants receiving probiotic supplementation. The persistence of improved Gingival Index values during the washout period may further suggest that probiotics induce temporary ecological and immunological changes that continue to exert beneficial effects even after supplementation has ceased.

Strengths of the present study include the controlled design, the use of validated clinical indices, the inclusion of a washout period, and the simultaneous evaluation of salivary and clinical oral health parameters.

Several limitations of the present study should be acknowledged when interpreting the results. First, the relatively small sample size may have limited the statistical power to detect subtle differences between groups, particularly regarding salivary pH. Second, the duration of probiotic administration and follow-up was relatively short, which restricts the ability to evaluate the long-term effects and sustainability of the observed improvements in oral health parameters. Third, the study relied exclusively on clinical indicators, without incorporating microbiological analyses of the oral biofilm or salivary biomarkers that could have provided additional insight into the mechanisms underlying the observed effects. Furthermore, the study population consisted of healthy young adults with generally good oral health, which may limit the generalizability of the findings to other populations, such as children, older adults, or individuals with active periodontal disease. Despite these limitations, the controlled study design, the inclusion of a washout period, and the simultaneous evaluation of salivary pH, plaque accumulation, and gingival inflammation represent important strengths that contribute to the understanding of the potential role of probiotics in preventive oral healthcare.

From a clinical perspective, the results of the present study support the potential incorporation of probiotics into preventive oral healthcare strategies. Although probiotics cannot replace conventional oral hygiene measures such as tooth brushing and professional plaque control, they may represent a valuable adjunctive approach for maintaining oral health. The observed improvements in plaque accumulation and gingival status suggest that probiotic supplementation may contribute to creating a more favorable oral environment and reducing early signs of periodontal inflammation. Such benefits may be particularly relevant for individuals at increased risk of plaque accumulation or gingival disease, as well as for populations seeking non-pharmacological strategies to support oral health maintenance.

## CONCLUSIONS

The findings of the present study suggest that short-term supplementation with a multi-strain chewable probiotic formulation may contribute to improvements in oral health among young adults. Probiotic administration was associated with significant reductions in both Plaque Index and Gingival Index values, indicating beneficial effects on dental biofilm control and gingival health. Notably, the improvement in gingival status persisted throughout the washout period, suggesting that the effects of probiotic supplementation may extend beyond the active intervention phase.

In contrast, salivary pH remained within the physiological range throughout the study and was not significantly influenced by probiotic administration. These findings indicate that the beneficial effects of probiotics may be mediated primarily through modulation of the oral

microbiota and host inflammatory responses rather than through substantial alterations in salivary physicochemical characteristics.

Overall, the results support the potential use of probiotics as adjunctive agents in preventive oral healthcare and highlight their role as microbiome-based strategies for maintaining oral health. Further randomized clinical trials with larger sample sizes, longer follow-up periods, and microbiological assessments are warranted to confirm these findings and better elucidate the mechanisms underlying the observed clinical benefits.

### *Conflicts of Interest*

The authors declare no conflict of interest.

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