

Effectiveness of probiotic-containing mouth rinse in reducing halitosis among dental prosthesis wearers: An in-vivo study

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Aim: This study aimed to compare the effectiveness of chlorhexidine and probiotic-containing mouthwashes in reducing halitosis among RPD and FPD wearers.

Materials and methods: This randomized, double-blind controlled trial included 40 participants (20 RPD, 20 FPD wearers). Participants were assigned to Group I (chlorhexidine mouthwash) or Group II (probiotic mouthwash). Pre- and post-experiment halitosis levels were assessed using organoleptic breath assessment by an examiner and self-evaluation by participants. Wilcoxon signed ranks test, Mann-Whitney U test, and chi-square test were used for statistical analysis ($P < 0.05$ considered significant).

Results: Both mouthwashes significantly reduced halitosis in RPD wearers ($P < 0.05$), with no significant difference between groups. However, probiotic mouthwash demonstrated a significantly greater reduction in halitosis among FPD wearers ($P < 0.05$) when assessed by the examiner.

Conclusion: Probiotic mouthwash was found to be as effective as chlorhexidine in RPD wearers and superior in FPD wearers. These findings suggest that probiotics can serve as an alternative to chlorhexidine, particularly for fixed prosthesis users, without associated side effects.

Keywords: Halitosis, Dental Prostheses, Removable Partial Denture, Fixed Partial Prosthesis, Chlorhexidine.

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Introduction

Halitosis is characterized by malodorous breath caused by the release of volatile sulfur compounds (VSCs) due to the degradation of food and salivary proteins by anaerobic bacteria.¹⁻³ It is perceived both by the affected individual and by those around them². Halitosis tends to worsen with advancing age and is strongly correlated with various oral disorders, such as dental caries and periodontitis.⁴

Globally, the prevalence of halitosis is 31.8%, with a reported prevalence of 29% in developed countries and 39.8% in low- and middle-income countries.² Halitosis is a common complaint among prosthesis users, even in the absence of xerostomia, as observed in orthodontic⁵ and prosthetic patients wearing fixed and removable devices. Removable appliances, composed of both metal and plastic components, serve as sites for plaque accumulation, significantly contributing to the chain of events leading to halitosis.²

A study by Costacurta et al. concluded that halitosis levels were significantly higher in patients wearing removable dentures, due to increased salivary β -galactosidase activity and food stagnation². Among fixed prosthesis wearers, halitosis is commonly attributed to food retention, plaque formation, and periodontal inflammation.³ A study by Alzoman et al., using an Oral Chroma device, found that 65.9% of patients with crowns exhibited halitosis, compared to 32.69% of patients without fixed prostheses.⁴ The likely causes included subgingival margins, over-contouring, and leaky crowns.

Various chemical plaque control measures, such as mouthwashes, reinforce mechanical plaque control, including brushing, flossing, and tongue scraping, to create an anti-plaque and anti-gingivitis oral environment. Chlorhexidine gluconate (CHX) has been regarded as the gold standard antimicrobial agent due to its proven antiplaque and antigingivitis

effects.⁶ Rosenberg et al. reported that rinsing with 0.2% CHX resulted in a 43% reduction in VSC levels and a 50% reduction in organoleptic ratings within a single day, leading to a lower degree of halitosis.⁷ However, long-term use of CHX mouthwash is discouraged due to its side effects, including tooth discolouration, taste alteration, oral mucosal irritation, and burning sensation.⁷ Ahmad et al. confirmed that prolonged use of CHX mouth rinses led to increased dental stains, allergic reactions, and burning mouth sensations.⁸

Given these concerns, probiotics have been explored as an alternative for halitosis management. The concept of probiotics was first introduced by Lilly and Stillwell (1965) as substances produced by microorganisms that promote the growth of other beneficial organisms.⁹ Henker et al. first demonstrated the potential of probiotics in treating halitosis by successfully managing a case of intestinal malodor with a non-pathogenic strain of *Escherichia coli*. Since then, multiple studies have explored the role of probiotic strains, including *Streptococcus salivarius* K12, *Weissella cibaria*, and *Lactobacillus salivarius* WB21, in managing halitosis.¹⁰

Introducing probiotics into the oral cavity could potentially lead to the introduction of unintended microbes, altering the delicate balance of the oral microbiome. However, probiotics typically used in oral care are strains that have been studied and shown to have a beneficial role in promoting oral health. These probiotics, such as *Lactobacillus* and *Bifidobacterium*, are selected for their ability to improve the balance of beneficial microbes in the mouth rather than disrupting it. A critical factor to consider is that these strains usually do not persist long-term in the oral cavity and are more likely to provide transient benefits without permanently altering the microbiome (Gänzle & Hammes, 2015)¹¹ Furthermore, studies show that probiotics are unlikely to introduce harmful microbes or disrupt the balance of the oral microbiome, as well-researched probiotic

strains do not typically cause significant shifts in the microbial community (Dani & Roldan, 2018; Hernandez et al., 2015)¹²

While the oral microbiome is dynamic and influenced by a variety of factors (e.g., diet, and oral hygiene habits), studies have demonstrated that probiotics can help restore or maintain the balance of the microbiome rather than disrupt it. Research suggests that probiotics may reduce the overgrowth of pathogenic bacteria (like *Streptococcus mutans*, which is associated with tooth decay) while promoting the growth of beneficial microbes (Chalmers & Kline, 2017)¹³. Therefore, a well-chosen probiotic mouthwash is unlikely to cause significant disruption to the oral microbiome, especially if it is used intermittently rather than continuously.

Probiotics offer a strategic alternative by competing with pathogenic bacteria for adhesion sites, producing antimicrobial substances, forming protective biofilms, and neutralising acidic pH.^{1, 2} Thakkar et al. found that probiotic mouth rinse was more effective against plaque accumulation at both 14 days of use and 3 weeks post-discontinuation.¹⁴

A 14-day study period is indeed short, but it is important to understand that probiotic effects, particularly in the oral cavity, may be transient. The primary goal of the study could have been to observe the immediate effects of probiotic mouthwash on oral health markers such as plaque, gingivitis, and microbial composition. It is common to measure short-term effects in such studies, especially since probiotics may not persist in the mouth long-term. Studies on probiotics have demonstrated improvements in oral health within 7-14 days, which supports the use of short-term study periods to assess these effects (Lee et al., 2017¹⁵; Roland & Huppertz, 2016)¹⁶

Shorter study periods allow for easier recruitment, lower participant drop-out rates, and more immediate data collection. Additionally, given that the effects of probiotics on the oral microbiome

might be seen within the first few days to weeks, the 14-day period could still provide useful insights into how the probiotic interacts with the mouth's ecosystem in the short term. Longer periods (e.g., several months) are more resource-intensive and might not yield dramatically different results since the probiotic effect is likely transient (Marsh, 2012)¹⁷

In oral health studies, particularly those focusing on probiotics, there is evidence that short-term use (7 to 14 days) is sufficient to observe beneficial changes in microbial populations or oral hygiene parameters. For example, research on probiotic lozenges or mouthwashes has demonstrated improvements in oral health outcomes, such as reduced plaque accumulation or decreased levels of harmful bacteria, within this time frame (Niv & Goldstein, 2017)¹⁸. Longer studies could be conducted, but the current research suggests that the observed effects are typically seen in the short term.

While the 14-day study period may seem brief, it is in line with many other studies investigating the short-term impacts of probiotics on the oral microbiome and oral health. The primary goal of the study is likely to determine whether there are immediate or short-term benefits of using a probiotic mouthwash, with the understanding that probiotics are typically transient in the oral cavity. As for the potential introduction of unintended microbes, evidence suggests that well-researched probiotics can help maintain a healthy balance in the oral microbiome without causing significant disruptions (Gänzle & Hammes, 2015¹⁹; Dani & Roldan, 2018)²⁰.

Similarly, Noordin and Kamin tested probiotic mouth rinse in adults and concluded that it significantly reduced plaque accumulation and improved gingival health.²¹

From a prosthodontic perspective, evidence supporting the role of probiotic mouthwashes in prosthesis wearers remains scarce. To the best of our knowledge, this is

the first study comparing probiotic and chlorhexidine mouthwashes for halitosis reduction in prosthesis wearers.

This study aimed to evaluate the effect of chlorhexidine mouthwash and probiotic mouthwash on halitosis in patients wearing removable partial dentures (RPDs) and fixed partial prostheses (FPDs).²⁰ The null hypothesis stated that both types of mouthwash would have the same effect on halitosis in RPD and FPD wearers.

Materials and Methods

This study was conducted as a double-blind controlled clinical trial to evaluate the effect of probiotic-containing mouthwash and 0.2% chlorhexidine mouthwash on halitosis in removable partial denture (RPD) and fixed partial prosthesis (FPD) wearers. The research proposal was submitted to the Ethical Committee for approval and clearance (IPDC/THESIS/2022/6118(6)). A total of 40 patients were selected, and written informed consent was obtained before participation.

The inclusion criteria consisted of healthy adults aged 30–60 years, without any gastrointestinal disorders, and wearing fixed or removable prostheses. Participants were required to have at least 20 permanent teeth, a gingival probing depth of <3 mm, and more than 10% of sites with a gingival and plaque index score of 1. The exclusion criteria included a history of antibiotic use in the past 3–4 weeks, orthodontic appliance wearers (both removable and fixed), smokers, individuals with deep-fissured tongues, and those already using regular mouth rinses.

The sample size was estimated based on data from Mohan Jothika et al., assuming a halitosis prevalence of 30–40%. The final sample size for each group was 13, considering the effect size of 0.84.

Participants were divided into two groups: Group I (0.2% Chlorhexidine Mouthwash - Control group) and Group II (Probiotic Mouthwash - Experimental group). Each group was further subdivided into 10 RPD wearers and 10 FPD wearers, assigned

through simple random sampling using a random number table.

Pre-experiment and post-experiment breath analysis were performed. The pre-experiment phase included an assessment of gingival health and plaque accumulation using the Modified Gingival Index (MGI), Plaque Index (PI), and Probing Depth (PD). All patients underwent professional oral prophylaxis before the experiment. Following this, a breath analysis was conducted. Organoleptic breath assessment (ORG1) was performed by a blinded and calibrated examiner (dentist), and self-assessment of breath (ORG2) was conducted by the participants (patients).

For preparation and administration of 0.2% chlorhexidine mouthwash, participants were instructed to dilute 15 mL of chlorhexidine mouthwash (B.P) in a 1:1 ratio and rinse for 5 minutes daily after brushing for 14 days. Similarly, for probiotic mouthwash, participants used 10 mL of mouth-rinse containing probiotics (DAROLAC, 2g; Aristo Pharmaceuticals, Chennai, India) for 5 minutes daily after brushing for 14 days.

The organoleptic test was conducted under standardized conditions. Participants were instructed to keep their mouths completely closed for 3 minutes, breathing only through the nose. They were then asked to exhale slowly through the mouth from a distance of 10 cm from the examiner's nose. For self-assessment, participants were asked to lick their wrists, allow them to dry, and then smell the area (ORG2). The calibrated examiner also performed an assessment and assigned a score (ORG1).

The odour intensity ratings followed the Rosenberg and McCulloh scale:

- 0 = No odour present
- 1 = Barely noticeable odor
- 2 = Slight but noticeable odour
- 3 = Moderate odour
- 4 = Strong offensive odour
- 5 = Extremely foul odour

The entire methodology is represented using a flow diagram (Figure 1) Brief description of the methodology.

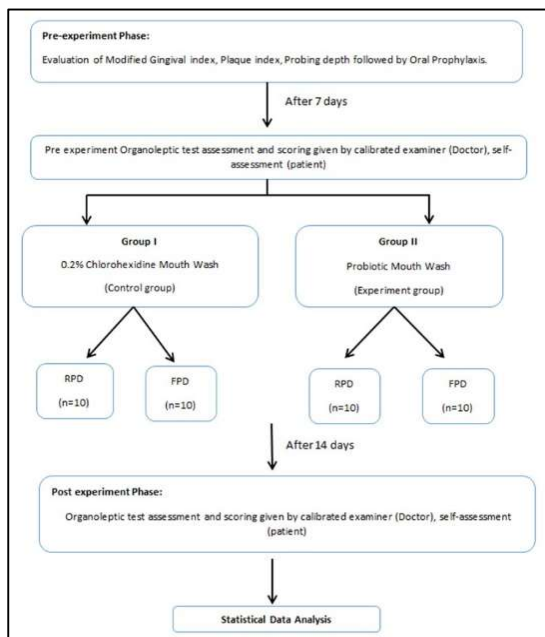


Figure 1: Brief description of the methodology.

The inter-examiner agreement was assessed using Cohen's kappa coefficient, which was found to be 0.82, indicating strong agreement. For statistical analysis, data were analyzed using SPSS software (version 22). Wilcoxon signed-rank test and chi-square test were used to compare pre-and post-experiment values within the same group. The Mann-Whitney U test and chi-square test were applied to compare pre-and post-experiment values between the two groups. A P-value <0.05 was considered statistically significant.

Results

The Mann-Whitney U test was used for intergroup comparisons, while the Wilcoxon signed-rank test was applied for intragroup comparisons to evaluate changes in halitosis scores from the pre- to post-treatment phase. The chi-square test was performed to compare categorical variables. The level of statistical significance was set at $P < 0.05$.

At the pre-experiment stage, among both FPD and RPD wearers, no statistically significant difference was observed in mean

halitosis scores between the two study groups when assessed by both patients and doctors. However, at the post-experiment stage, the mean halitosis score was found to be higher in Group I (chlorhexidine) compared to Group II (probiotic) when assessed by the patient among FPD wearers. This difference, however, did not reach statistical significance.

When assessed by the doctor among FPD wearers, a statistically significant difference was observed between the two groups. Group II (probiotic mouthwash) demonstrated significantly lower halitosis scores than Group I (chlorhexidine mouthwash). Among RPD wearers, lower mean halitosis scores were observed in Group II when assessed by both the doctor and the patient. However, this difference was not statistically significant. Both mouthwashes were found to be equally effective in reducing halitosis in RPD wearers (Tables 1 and 2).

Table 1: Intergroup Comparison (Pre-experimental)

Group	Mean Halitosis Score (Doctor)	SD (Doctor)	Mean Halitosis Score (Patient)	SD (Patient)	p-value
Chlorhexidine (Group I)	3.2	0.5	3.1	0.4	0.78
Probiotic (Group II)	3.3	0.6	3.2	0.5	

SD -standard deviation, * $p < 0.05$ is statistically significant

Table 2 : Intergroup Comparison (Post-experimental)

Group	Mean Halitosis Score (Doctor)	SD (Doctor)	Mean Halitosis Score (Patient)	SD (Patient)	p-value
Chlorhexidine (Group I)	2.1	0.4	2.3	0.3	0.03*
Probiotic (Group II)	1.5	0.5	1.7	0.4	

SD -standard deviation, * $p < 0.05$ is statistically significant

A significant reduction in mean halitosis scores from the pre- to post-treatment phase was observed within both study groups and subgroups (RPD and FPD wearers) when assessments were made by both the patient and the doctor (Tables 3-5). A significant difference was found in halitosis intensity scores between Group I

and Group II when assessed by the patient during the pre-treatment phase. The majority of Group I subjects had a score of 3, whereas the majority of Group II subjects had a score of 4.

Table 3: Intragroup Comparison (Pre to Post-treatment)

Group	Mean Reduction (Doctor)	SD (Doctor)	Mean Reduction (Patient)	SD (Patient)	p-value
Chlorhexidine (Group I)	1.1	0.3	0.8	0.2	0.04*
Probiotic (Group II)	1.8	0.4	1.5	0.3	

SD -standard deviation, * $p < 0.05$ is statistically significant

Table 4: Intergroup Comparison of Halitosis Intensity (Pre-experimental)

Group	Mean Intensity Score (Doctor)	SD (Doctor)	p-value
Chlorhexidine (Group I)	4	0.5	0.85
Probiotic (Group II)	4	0.5	

SD -standard deviation, * $p < 0.05$ is statistically significant

Table 5: Intergroup Comparison of Halitosis Intensity (Post-experimental)

Group	Mean Intensity Score (Doctor)	SD (Doctor)	p-value
Chlorhexidine (Group I)	2	0.4	0.01*
Probiotic (Group II)	1	0.3	

SD -standard deviation, * $p < 0.05$ is statistically significant

In the post-treatment phase, no significant differences were observed in halitosis intensity scores between Group I and Group II when assessed by the patient. The majority of subjects in both groups had a score of 1.

Discussion

In this study, oral halitosis was evaluated in RPD and FPD wearers before and after the use of chlorhexidine and probiotic mouthwash, using the organoleptic test. The primary goal was to reduce malodor in patients with removable or fixed prostheses, as existing literature suggests higher levels of β -galactosidase, an indicator of volatile sulfur compound (VSC) levels, in such patients.²²

The organoleptic test is a subjective assessment method and one of the main tools for analyzing oral malodor. Other subjective methods include gas chromatography (GC) and sulphide monitoring (e.g., Halimeter). The Organoleptic Score, introduced by Rosenberg and McCulloch, is widely used for ranking halitosis severity². This test was chosen as the gold standard due to its cost-effectiveness, absence of equipment requirements, and ability to assess a wide range of odours.^{23, 24}

During the pre-experiment phase, oral health assessments, including modified gingival index (MGI), plaque index (PI), and probing depth (PD), were conducted, followed by oral prophylaxis for standardization. After seven days, the organoleptic test was performed. Participants were asked to keep their mouths closed for 3 minutes, then slowly exhale from a distance of 10 cm from the examiner's nose. For self-assessment, participants licked their wrists and smelled the dried area. The examiner (ORG1) and the patient (ORG2) assigned scores based on the 0 to 5 scale proposed by Rosenberg and McCulloch.⁷

In Group I (chlorhexidine mouthwash), 10 RPD wearers and 10 FPD wearers were instructed to dilute 15 mL of mouthwash in a 1:1 ratio and rinse for 5 minutes daily for 14 days. In Group II (probiotic mouthwash), 10 RPD wearers and 10 FPD wearers were instructed to use 10 mL of probiotic mouth rinse for 5 minutes daily for 14 days. The post-experiment organoleptic scores were recorded in the same manner as the pre-experiment scores.

Statistical analysis was conducted using the Wilcoxon signed-rank test and chi-square test for intragroup comparisons, while the Mann-Whitney U test and chi-square test were used for intergroup comparisons. At the pre-experiment stage, no statistically significant difference was observed between Group I and Group II in

mean halitosis scores, as assessed by both the doctor and the patient.

At the post-experiment stage, among FPD wearers, the mean halitosis score was higher in Group I compared to Group II, based on patient assessments. However, this difference did not reach statistical significance. In contrast, when assessed by the doctor, a significant difference was observed, with Group II (probiotic mouthwash) demonstrating a greater reduction in halitosis compared to Group I (chlorhexidine mouthwash).

Among RPD wearers, lower halitosis scores were observed in Group II (probiotic), based on assessments by both the doctor and the patient, but this difference was not statistically significant. Both types of mouthwash were equally effective in reducing halitosis in RPD wearers. A significant reduction in mean halitosis scores from pre- to post-treatment was observed within both study groups, as well as in subgroups (RPD and FPD wearers), based on assessments by both the doctor and the patient.

A significant difference was found in halitosis intensity scores between Group I and Group II at the pre-experiment stage. The majority of Group I subjects had a score of 3, whereas the majority of Group II subjects had a score of 4 (Table 1). At the post-experiment stage, no significant differences were found in halitosis intensity scores between Group I and Group II, when assessed by the patient. The majority of subjects in both groups had a score of 1. A significant difference was observed in halitosis intensity scores between Group I and Group II in the post-treatment phase, based on doctor assessments. The majority of Group I subjects had a score of 2, while the majority of Group II subjects had a score of 1, indicating a greater reduction in halitosis in Group II (probiotic mouthwash). The null hypothesis, which stated that both mouthwashes would have the same effect on halitosis in RPD and FPD wearers, was rejected. Among RPD wearers, both chlorhexidine and probiotic mouthwashes

were equally effective in reducing halitosis. This may be attributed to difficulty in maintaining oral hygiene due to advanced age, comorbidities, and the material of the prosthesis (resin), which serves as a site for bacterial colonization. Among FPD wearers, probiotic mouthwash demonstrated a greater reduction in halitosis compared to chlorhexidine mouthwash. This may be due to the ability of probiotics to inhibit odour-causing bacteria, compete for adhesion sites, and neutralize acidic pH, leading to longer-lasting effects.

To the best of our knowledge, this is the first study evaluating the effectiveness of chlorhexidine and probiotic mouthwashes in RPD and FPD wearers before and after use. This study was designed to directly compare the effectiveness of chlorhexidine and probiotic mouthwashes in patients wearing removable and fixed prostheses. A probiotic mouthwash was used instead of probiotic ingestion to ensure direct antimicrobial effects within the oral cavity, providing a more localized and effective intervention against halitosis-causing bacteria. The study employed a double-blind, randomized design, which minimized bias and enhanced the reliability of the results. Additionally, self-assessment tests were included to promote patient awareness of halitosis, emphasizing the importance of oral hygiene motivation, particularly in individuals with fixed prostheses, where effective plaque control is essential for long-term oral health. Despite its strengths, this study had several limitations. The small sample size may have impacted statistical significance, particularly in subgroup comparisons between RPD and FPD wearers. The organoleptic test, although considered the gold standard for assessing oral malodor, remains subjective, as odour perception can vary among assessors. Additionally, the study lacked quantitative microbial analysis, which could have provided deeper insights into how probiotic mouthwash influences bacterial populations responsible for halitosis. Another limitation was the

potential for nasal desensitization (olfactory fatigue) among examiners, which could have affected the accuracy of odour perception over repeated assessments.

Future long-term clinical studies are necessary to further explore the relationship between halitosis, probiotic therapy, and prosthesis wearers. Expanding the sample size would improve statistical power, allowing for more definitive conclusions regarding the effectiveness of probiotic mouthwash in different prosthetic groups. Conducting microbiological analysis would help determine the specific mechanisms through which probiotics exert their halitosis-reducing effects, including their impact on bacterial composition and volatile sulfur compound production. Advanced diagnostic techniques, such as genomic sequencing, could identify specific bacterial strains associated with halitosis, paving the way for targeted probiotic formulations. Furthermore, personalized probiotic therapies could be explored as part of a precision medicine approach, where probiotics are tailored to an individual's oral microbiome composition, enhancing treatment efficacy and ensuring long-term benefits for patients with removable and fixed prostheses.

Conclusion

This study demonstrated that both chlorhexidine and probiotic mouthwashes were equally effective in reducing halitosis in RPD wearers, while probiotic mouthwash showed significantly greater effectiveness in FPD wearers. These findings suggest that probiotic mouthwash can serve as a viable alternative to chlorhexidine, particularly for fixed prosthesis users, without the associated side effects. Further research is needed to explore long-term effects and other therapeutic strategies for managing halitosis in dental prosthesis wearers.

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None.

Conflict of Interest

None.

Ethics approval

Inderprastha Dental College and hospital
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