Jordan Journal of Dentistry, Volume **3**, No. **4**, **2026**
DOI: https://doi.org/10.14525/JJD.v3i4.04

Jordan Journal of Dentistry

https://jjd.just.edu.jo

In Vitro Comparison of the Antimicrobial Efficacy of Fluoride, Xylitol, and Probiotic Toothpastes Against Selected Oral Pathogens

Omar A. Ba-Zar[1], Sadiqa Razack[1], Nigar Mehtiyeva[2], Rayan Adlan[3], Huda Abutayyem[4], Hafiz Ahmed[5], Kenan Abdulrazak Al Saadi[1]

1 Department of Dentistry, Dubai Health, Dubai, United Arab Emirates.
2 Dubai Health, Dubai, United Arab Emirates.
3 Department of Dentistry, Ministry of Health, Ras Al Khaimah, United Arab Emirates.
4 Department of Dentistry, Ajman University, Ajman, United Arab Emirates.
5 Department of Microbiology, Ras Al Khaimah Medical & Health Sciences University, Ras Al Khaimah, United Arab Emirates.

ARTICLE INFO

Article History:
Received: 26/2/2026
Accepted: 23/4/2026

Correspondence:
Omar A. Ba-Zar,
Department of Dentistry,
Dubai Health, Dubai,
United Arab Emirates.
omaradele97@gmail.com

ABSTRACT

Objectives: This study evaluated the antimicrobial activity of two probiotic toothpastes, one xylitol-containing toothpaste, and one fluoride-containing toothpaste against common oral pathogens, including Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis, and Candida albicans.

Materials and Methods: A double-blinded design was employed. Each toothpaste was coded and anonymized prior to testing. Toothpaste suspensions were evaluated through agar well diffusion, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and inhibition zone tests. Data was examined using SPSS version 28.0 and the Kruskal-Wallis test to assess group differences, where $p < 0.05$ was considered statistically significant.

Results: Fluoride toothpaste exhibited the strongest antimicrobial activity across all tested microorganisms, with consistently low MIC and MBC values and the largest inhibition zones. Xylitol toothpaste demonstrated moderate activity, particularly against S. mutans and E. faecalis. Probiotic formulations showed limited overall efficacy, but displayed measurable inhibition against S. mutans.

Conclusions: Within the limitations of this in vitro study, fluoride toothpaste was the most effective, followed by xylitol and probiotic formulations. Although probiotics exhibited minimal direct antimicrobial action, their observed activity against S. mutans suggests potential adjunctive benefits. Further in vivo and clinical studies are warranted to validate these findings.

Keywords: Antimicrobial activity, Fluoride toothpaste, Probiotic toothpaste, Xylitol, Streptococcus mutans.

1. Introduction

Dental caries is one of the most common infectious diseases worldwide, causing progressive damage to tooth enamel, dentin, and cementum through bacterial activity (1). This disease develops through the interaction of cariogenic bacteria, dietary sugars, and time (2). Dental plaque is a complex bacterial biofilm that contains cariogenic bacteria, such as Streptococcus mutans, which initiates the carious process in association with other bacteria in the plaque (3,4). Other microorganisms, including *Staphylococcus aureus*, *Enterococcus faecalis*, and *Candida albicans*, can also contribute to oral infections and adhere to tooth surfaces (5).

Control of dental plaque and bacterial growth is essential for the prevention of oral diseases. Toothbrushing remains the most effective method for mechanical plaque removal (6). Toothpastes serve as delivery systems for active and therapeutic agents to the oral cavity (6). The efficacy of a toothpaste depends largely on its active ingredients. Fluoride is one of the most proven additives that strengthen enamel and inhibits bacterial activity, thus reducing caries development (7,8). Xylitol is a natural sugar alcohol that reduces S. mutans levels in plaque and saliva and disrupts how bacteria use sugars, helping prevent cavities (9,10).

Recently, probiotics have been incorporated into oral care products as an alternative or adjunct to chemical antimicrobials. Probiotics are defined as live microorganisms that provide health benefits when administered in adequate amounts (11). They may influence the oral microbiota by competing with pathogenic bacteria, producing antimicrobial substances, and modulating the microbial environment. Early studies suggested that probiotic toothpastes may benefit oral health; however, evidence regarding their direct antimicrobial effects remains limited (12,13).

Given the increasing demand for alternative oral care formulations, comparative studies are needed to clarify their relative effectiveness. Therefore, the objective of this study was to assess and compare the antimicrobial effectiveness of two probiotic toothpastes, one xylitol-containing toothpaste, and one fluoride-containing toothpaste against S. mutans, S. aureus, E. faecalis, and C. albicans under in vitro conditions.

2. Materials and Methods

2.1 Ethical Authorization

This research was approved by the Institutional Ethics Committee. All experimental procedures followed established biosafety and ethical standards (14).

2.2 Toothpaste Samples

Four commercially available toothpastes were selected and coded anonymously as A-D to keep researchers unaware of which product was being tested. Those toothpastes are:

- Toothpaste A (Fluoride-based): Contained 1450 ppm (parts per million) sodium fluoride (NaF) as the main active ingredient.
- Toothpaste B (Xylitol-based): Contained 10% xylitol as the main active ingredient.
- Toothpaste C (Charcoal-probiotic formulation): Contained heat-killed (inactivated) Lactobacillus paracasei bacteria (1×10^7 cells per gram) with tapioca maltodextrin and activated charcoal.
- Toothpaste D (Probiotic formulation): Contained heat-killed (inactivated) Lactobacillus paracasei bacteria (1×10^7 cells per gram) with tapioca maltodextrin.

Test suspensions were prepared by mixing 1 g of each toothpaste with 1 mL of sterile distilled water, followed by centrifugation to obtain a homogeneous supernatant for antimicrobial testing.

2.3 Test Microorganisms

Reference strains of oral pathogens were acquired from the American Type Culture Collection (ATCC): Streptococcus mutans (ATCC 25175), Staphylococcus aureus (ATCC 25923), Enterococcus faecalis (ATCC 29212), and Candida albicans (ATCC 90028). All microorganisms were cultured and maintained under standard growth conditions prior to testing (14).

2.4 Standardization of Inoculum

Bacterial and fungal suspensions were standardized according to the 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). Turbidity was confirmed visually against a McFarland reference under controlled lighting conditions (15).

2.5 Agar Well Diffusion

Antimicrobial activity was assessed using the agar well diffusion method. Mueller-Hinton agar was used for bacterial isolates, while Sabouraud dextrose agar was used for *C. albicans*. Standardized inocula were evenly spread on agar plates. Wells of 4 mm diameter and 3 mm depth were created, and 50 µL of toothpaste suspensions were introduced into each well. Cefuroxime (for bacterial isolates) and fluconazole (for *C. albicans*) served as positive controls. Plates were incubated at 37 °C for 18-24 hours, and inhibition zones were measured in millimetres.

2.6 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Determination

MIC and MBC values were determined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (16). Serial dilutions of toothpaste suspensions were prepared and incubated at 37 °C for 24 hours. MIC was defined as the lowest concentration showing no visible microbial growth. MBC was determined by subculturing aliquots from clear tubes onto fresh agar plates, with the lowest concentration showing no colony growth recorded as the MBC.

2.7 Statistical Analysis

All experiments were performed three times. Data was presented as mean ± standard deviation (SD). Statistical analysis was performed using SPSS, version 28.0 (IBM Corp., Armonk, NY, USA). Intergroup comparisons were conducted using the Kruskal–Wallis test, and $p < 0.05$ was considered statistically significant (17).

3. Results

3.1 Agar Diffusion Assay (Zones of Inhibition)

The agar well diffusion test demonstrated clear differences among toothpaste formulations (Figure 1A-D), with example plates shown in Figure 2A-D.

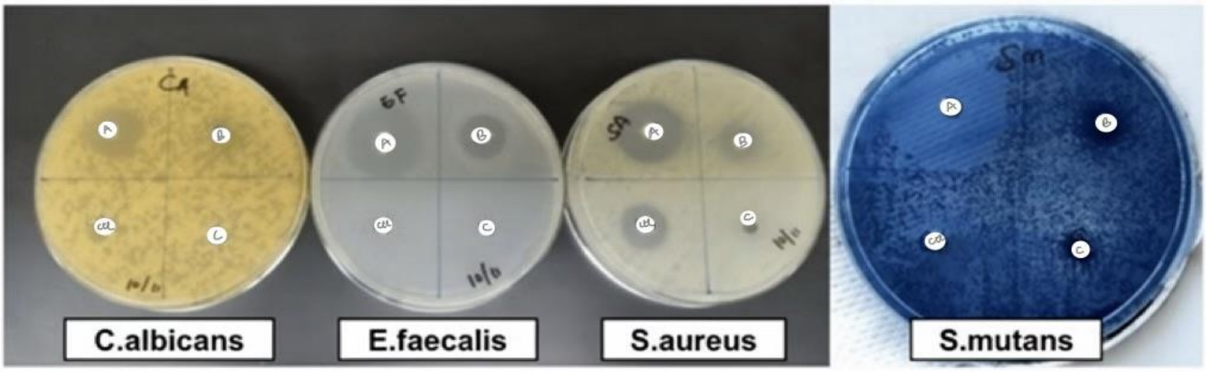

Figure 1. Well diffusion method showing mean inhibition zones against test microorganisms produced by toothpastes A, B, C, and D. *E. faecalis*, *S. aureus,* and *S. mutans* versus cefuroxime, and *C. albicans* versus fluconazole

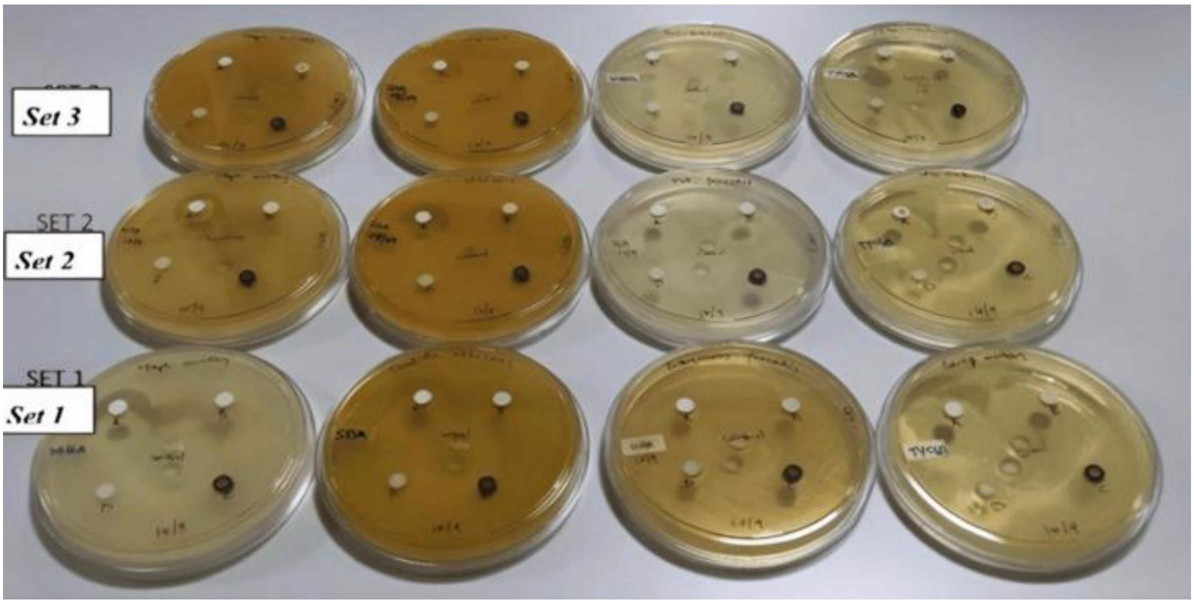

Figure 2. Representative agar diffusion assay plates performing *multiple tests in sets* showing inhibition zones produced by toothpastes A, B, C, and D against the tested microorganisms

Fluoride toothpaste (A) consistently produced the largest inhibition zones against all microorganisms tested, including Candida albicans (21.0 ± 1.73 mm), Staphylococcus aureus (19.67 ± 0.33 mm), Streptococcus mutans (30.67 ± 1.15 mm), and Enterococcus faecalis (23.33 ± 1.15 mm).

Xylitol formulation (B) produced inhibition zones against S. mutans (23.33 ± 1.15 mm) and E. faecalis (12.67 ± 1.15 mm), but showed no activity against C. albicans or S. aureus.

Probiotic toothpaste (D) showed limited activity against S. mutans (14.67 ± 1.52 mm) and E. faecalis (8.0 ± 0.00 mm), while the charcoal-probiotic formulation (C) showed no detectable activity against any tested organism.

Positive controls performed as expected: cefuroxime produced zones of 25-35 mm for bacterial strains, and fluconazole produced zones of 20-28 mm for C. albicans. Statistical comparisons are shown in Figure 3.

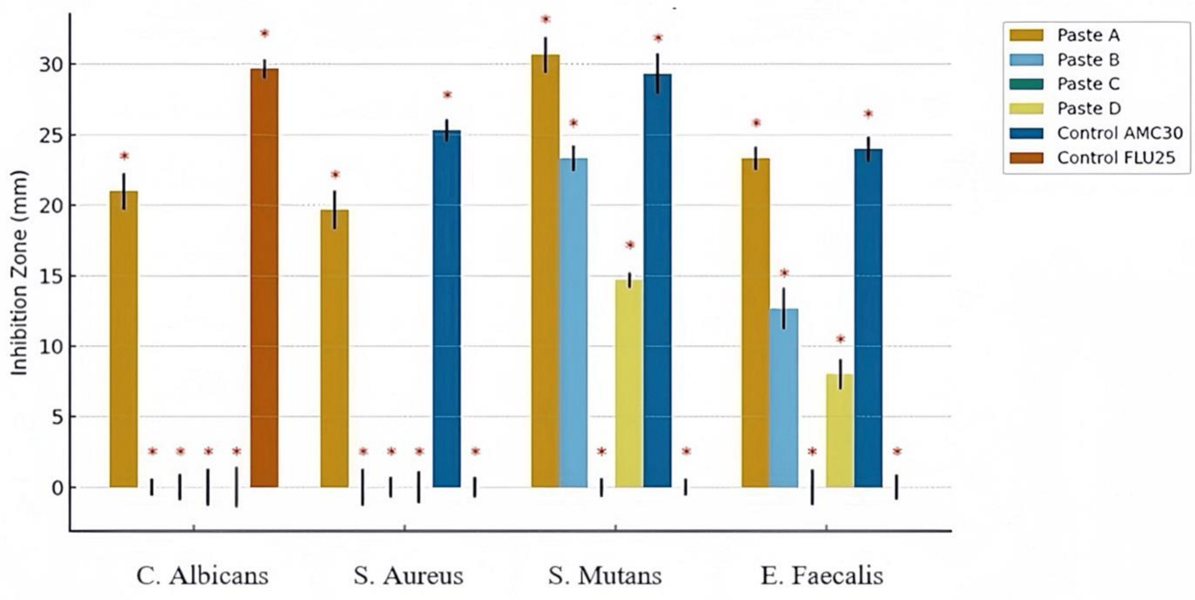

Figure 3: Zones of inhibition produced by different toothpastes and control agents against oral pathogens. Zones of inhibition (mean ± SD) formed by toothpastes A-D and control agents against *C. albicans*, *S. aureus*, *S. mutans*, and *E. faecalis.* Error bars depict standard deviation. Asterisks (*) denote statistically significant differences when compared to controls *(Kruskal-Wallis test, p < 0 05)*

3.2 MIC and MBC Assays

The antimicrobial activity of the four toothpaste formulations was further evaluated using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), as summarized in Table 1 and described in Figure 4.

Ta**ble 1:** Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of toothpaste formulations against test microorganisms

Microorganism	**Parameter**	**Toothpaste A (Fluoride)**	**Toothpaste B (Xylitol)**	**Toothpaste C (Charcoal-Probiotic)**	**Toothpaste D (Probiotic)**
C. albicans	MIC (mg/mL)	0.125	NA	NA	NA
	MBC (mg/mL)	0.25	NA	NA	NA
S. aureus	MIC (mg/mL)	0.063	NA	NA	NA
	MBC (mg/mL)	0.13	NA	NA	NA
S. mutans	MIC (mg/mL)	0.031	0.25	NA	0.5
	MBC (mg/mL)	0.063	0.5	NA	1.0
E. faecalis	MIC (mg/mL)	0.063	0.5	NA	1.0
	MBC (mg/mL)	0.13	1.0	NA	>1.0

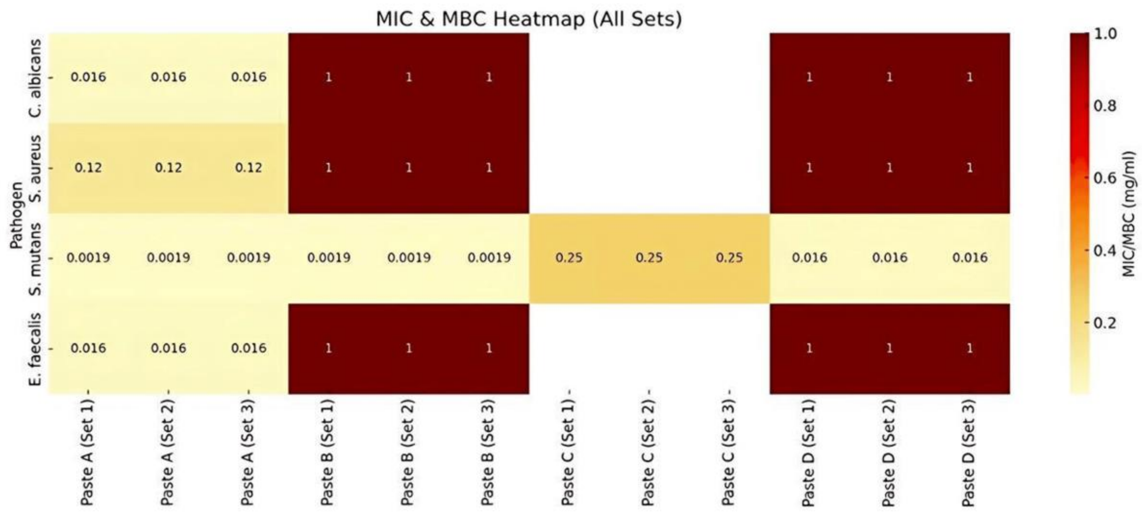

Figure 4: Heatmap of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values for toothpastes A-D across three experimental sets. Color scale: 0 (light yellow) to 1.0 (dark red), representing MIC/MBC values *(mg/mL)*, as shown in the figure legend. Columns represent Paste A (Set 1-3), Paste B (Set 1-3), Paste C (Set 1-3), and Paste D (Set 1-3). Rows correspond to *C. albicans, S. aureus, S. mutans,* and *E. faecalis*. Data represents three independent experimental sets. Lighter shades correspond to lower MIC/MBC values (stronger antimicrobial activity), whereas darker shades indicate higher concentration requirements and weaker antimicrobial effects

4. Discussion

This study evaluated the antimicrobial effectiveness of four toothpaste formulations against selected oral bacteria and fungi. It was found that fluoride toothpaste showed the highest activity, xylitol toothpaste showed moderate activity, and probiotic toothpastes showed minimal effect.

Fluoride toothpaste (1450 ppm sodium fluoride) created the largest inhibition zones against all tested microorganisms, ranging from 19.67 mm against S. aureus to 30.67 mm against S. mutans. Several other studies have reported similar results. Verkaik et al. (18) reported zones of 18-32 mm against S. mutans, and Guven et al. (15) showed that fluoride was significantly more effective than herbal toothpastes (22-28 mm vs. 12-16 mm, $p < 0.001$). Fluoride acts by inhibiting bacterial enzymes involved in acid production, which contributes to caries development. It also enhances enamel resistance by forming fluorapatite, which is more resistant to acid than hydroxyapatite (19). The MIC values (0.031- 0.13 mg/mL) were consistent with those reported by Fernández et al. (20), who found similar values in biofilm studies. However, Moran et al. (21) reported that fluoride toothpaste had only modest immediate antibacterial effects in vivo, despite its well-established clinical efficacy in caries prevention. This difference may be explained by the fact that the present study evaluated antimicrobial activity under controlled conditions with continuous exposure, whereas in vivo conditions involve salivary clearance of fluoride (21).

Xylitol toothpaste showed moderate antimicrobial activity against S. mutans (23.33 mm) and E. faecalis (12.67 mm), but showed no activity against C. albicans or S. aureus. These results are partially consistent with previous research. Nayak et al. (22) reported similar MIC values (0.2–0.3 mg/mL) against S. mutans. Söderling et al. (10) showed that xylitol reduced S. mutans in clinical studies, mainly by interfering with bacterial metabolism rather than exerting direct bactericidal effects. Xylitol enters bacterial cells, but cannot be metabolized, resulting in futile metabolic cycles that deplete cellular energy (22).

However, Kontiokari et al. (23) found that xylitol could inhibit Candida at concentrations above 5%. This differs from the present findings, because 10% xylitol toothpaste was tested, while they used pure xylitol solutions at 15% or higher. In addition, Mäkinen et al. (9) showed that xylitol mainly acts by altering oral microbiota over prolonged use rather than through an immediate bactericidal effect, which a short-term in vitro assay cannot measure, as in the present study. Xylitol showed activity only against specific bacteria (S. mutans and E. faecalis), because these bacteria have specific transport systems for xylitol uptake (22).

Probiotic toothpastes showed very limited antimicrobial activity. The regular probiotic toothpaste (D) created small inhibition zones only against S. mutans (14.67 mm) and E. faecalis (8.0 mm). The charcoal-probiotic toothpaste (C) showed no detectable activity. These results are consistent with studies indicating that probiotics enhance oral health by modulating the microbial community rather than exerting direct antimicrobial effects (12,13). However, current results differ from those reported by Çaglar et al. (24), who found that probiotic bacteria created 18–24 mm zones against S. mutans and reduced oral microbial levels in patients.

This difference may be explained by the fact that the toothpastes used in the present study contained heat-killed probiotic bacteria (1×10^7 cells/g), whereas Çaglar et al. used viable probiotics at higher concentrations (10^8-10^9 cells/g). Living probiotics exert antimicrobial effects through the production of lactic acid, hydrogen peroxide, and antimicrobial peptides (bacteriocins) (25). Non-viable bacteria are unable to produce these substances, although they may still influence the immune response (26). Keller et al. demonstrated that viable probiotics produce reuterin, a strong antimicrobial compound that disappears when bacteria are heat-killed (27). Cildir et al. also showed that probiotic effects are observed primarily at higher concentrations ($\geq 10^8$ cells/mL), which is greater than the concentration used in the present study (28). These findings may explain the minimal antimicrobial activity observed with probiotic toothpastes.

Statistical analysis showed that fluoride toothpaste was significantly more effective than all other formulations ($p < 0.001$), with xylitol ranking second. Walsh et al. (29) reviewed 79 studies and found the same pattern; fluoride prevented 24% of caries, xylitol prevented 13%, and probiotics lacked sufficient evidence.

These findings may assist dentists and patients in selecting toothpastes appropriately, especially given the increasing popularity of alternative products. Current results demonstrate that fluoride-based toothpastes are the most effective option for caries prevention, particularly for high-risk patients (8). Xylitol toothpastes may provide additional benefits for individuals seeking adjunctive protection or alternatives, but should not replace fluoride as the primary active ingredient. The weak effects of non-viable probiotic toothpastes suggest that current commercial products may not exert direct antimicrobial activity, although they may provide benefits through other mechanisms. For dentists, these findings support recommending fluoride toothpastes as the first-line option while maintaining realistic expectations regarding alternative formulations.

However, this lab study has important limitations. The current study tested bacteria floating freely in liquid, which differs from the real dental plaque environment, where bacteria form the major component of biofilms. Biofilm bacteria are 10-100 times harder to kill than floating bacteria (26). Also, the experimental conditions could not accurately simulate real oral cavity conditions such as saliva flow, pH changes, and immune responses that may affect the efficacy of toothpastes (30). Additionally, only four types of microorganisms were tested, whereas real dental plaque contains hundreds of bacterial species. The use of non-viable probiotics further limits the generalization of the findings to formulations containing viable probiotic bacteria. Future studies should test bacteria in biofilms, conduct patient trials, compare toothpastes with living probiotics at higher doses (10^8-10^9 cells/g), and investigate the combination of fluoride, xylitol, and probiotics.

5. Conclusions

Under the constraints of this in vitro research, toothpaste with fluoride exhibited the most significant antimicrobial effectiveness against Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis, and Candida albicans, affirming its position as the standard in preventive oral care. Xylitol-containing toothpaste showed moderate activity, particularly against S. mutans and E. faecalis, indicating potential value as an adjunctive agent.

Probiotic formulations exhibited limited direct antimicrobial effects, with measurable inhibition only against S. mutans, suggesting that their benefits may arise more from modulation of the oral microbiome rather than direct pathogen suppression. These findings highlight fluoride as the most reliable active ingredient for caries prevention, while supporting xylitol and probiotics as possible adjuncts.

Overall, fluoride toothpastes demonstrated greater antimicrobial activity than xylitol or non-viable probiotic toothpastes under in vitro conditions. Fluoride remains the most reliable ingredient for preventing caries, while xylitol can serve as an additional agent. Non-viable probiotic toothpastes may require reformulation with viable bacteria at higher concentrations to achieve meaningful antimicrobial effects. As demand for alternative toothpastes grows, studies comparing different products become essential for guiding dentists and informing clinical recommendations and health policy.

Conflict of Interests

No potential or actual conflict of interests relevant to this article was reported.

Funding Information

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

1. Pitts NB, Zero DT, Marsh PD, Ekstrand K, Weintraub JA, et al. Dental caries. Nat Rev Dis Primers. 2017;3:17030.
2. Takahashi N, Nyvad B. The role of bacteria in the caries process: Ecological perspectives. J Dent Res. 2011;90:294-303.
3. Loesche WJ. Role of Streptococcus mutans in human dental decay. Microbiol Rev. 1986;50:353-380.
4. Gross EL, Beall CJ, Kutsch SR, Firestone ND, Leys EJ, et al. Beyond Streptococcus mutans: Dental caries onset linked to multiple species by 16S rRNA community analysis. PLoS One. 2012;7:e47722.
5. Banu A, Noorul Hassan MM, Rajkumar J, Srinivasa S. Spectrum of bacteria associated with dental caries: Current concepts. Open Dent J. 2015;9:452-456.
6. Valkenburg C, Slot DE, Bakker EW, Van der Weijden FA. Does dentifrice use help remove plaque? A systematic review. J Clin Periodontol. 2016;43:1050-1058.
7. Featherstone JD. Prevention and reversal of dental caries: Role of low level fluoride. Community Dent Oral Epidemiol. 1999;27:31-40.
8. Marinho VC, Higgins JP, Sheiham A, Logan S. Fluoride toothpastes for preventing dental caries in children and adolescents. Cochrane Database Syst Rev. 2003;2003:CD002278.
9. Mäkinen KK, Bennett CA, Hujoel PP, Isokangas PJ, Isotupa KP, et al. Xylitol chewing gums and caries rates: A 40-month cohort study. J Dent Res. 1995;74:1904-1913.
10. Söderling E, Mäkinen KK, Chen CY, Pape HR Jr, Loesche W, et al. Effect of sorbitol, xylitol, and xylitol/sorbitol chewing gums on dental plaque. Caries Res. 1989;23:378-384.
11. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11:506-514.
12. Gruner D, Paris S, Schwendicke F. Probiotics for managing caries and periodontitis: Systematic review and meta-analysis. J Dent. 2016;48:16-25.
13. Haukioja A. Probiotics and oral health. Eur J Dent. 2010;4:348-355.
14. Nikawa H, Makihira S, Fukushima H, Nishimura H, Ozaki Y, et al. Candida albicans pathogenicity and oral microbial interactions. Int J Food Microbiol 2003;90:333-337.
15. Guven Y, Ustun N, Tuna EB, Aktoren O. Antimicrobial effect of newly formulated toothpastes and a mouthrinse on specific microorganisms: An in vitro study. Eur J Dent. 2019;13:172-177.
16. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-tenth edition. CLSI document M07-A10. Wayne, PA: CLSI; 2015.
17. Selvaraj K, Bharath N, Natarajan R, Dinesh S, Murugesan R. Statistical applications in biomedical research: Use of Kruskal-Wallis test. J Pharm Bioallied Sci. 2020;12:S595-S596.
18. Verkaik MJ, Busscher HJ, Jager D, Slomp AM, Abbas F, et al. Efficacy of natural antimicrobials in toothpaste formulations against oral biofilms in vitro. J Dent. 2011;39:218-224.
19. Buzalaf MA, Pessan JP, Honório HM, ten Cate JM. Mechanisms of action of fluoride for caries control. Monogr Oral Sci. 2011;22:97-114.
20. Fernández E, Sánchez MC, Llama-Palacios A, Figuero

E, Herrera D, et al. Antibacterial effects of toothpastes evaluated in an in vitro biofilm model. Oral Health Prev Dent. 2017;15:503-512.

21. Moran J, Addy M, Newcombe R. The antibacterial effect of toothpastes on the salivary flora. J Clin Periodontol. 1988;15:193-199.
22. Nayak PA, Nayak UA, Khandelwal V. The effect of xylitol on dental caries and oral flora. Clin Cosmet Investig Dent. 2014;6:89-94.
23. Kontiokari T, Uhari M, Koskela M. Effect of xylitol on growth of nasopharyngeal bacteria in vitro. Antimicrob Agents Chemother. 1995;39:1820-1823.
24. Çaglar E, Cildir SK, Ergeneli S, Sandalli N, Twetman S. Salivary mutans streptococci and lactobacilli levels after ingestion of the probiotic bacterium Lactobacillus reuteri ATCC 55730 by straws or tablets. Acta Odontol Scand. 2006;64:314-318.
25. Twetman S, Keller MK. Probiotics for caries prevention and control. Adv Dent Res. 2012;24:98-102.
26. Marsh PD, Head DA, Devine DA. Ecological approaches to oral biofilms: Control without killing. Caries Res. 2015;49:46-54.
27. Keller MK, Bardow A, Jensdottir T, Lykkeaa J, Twetman S. Effect of chewing gums containing the probiotic bacterium Lactobacillus reuteri on oral malodour. Acta Odontol Scand. 2012;70:246-250.
28. Cildir SK, Germec D, Sandalli N, Ozdemir FI, Arun T, et al. Reduction of salivary mutans streptococci in orthodontic patients during daily consumption of yoghurt containing probiotic bacteria. Eur J Orthod. 2009;31:407-411.
29. Walsh T, Worthington HV, Glenny AM, Marinho VC, Jeroncic A. Fluoride toothpastes of different concentrations for preventing dental caries. Cochrane Database Syst Rev. 2019;3:CD007868.
30. Amerongen AVN, Veerman ECI. Saliva: The defender of the oral cavity. Oral Dis. 2002;8:12-22.