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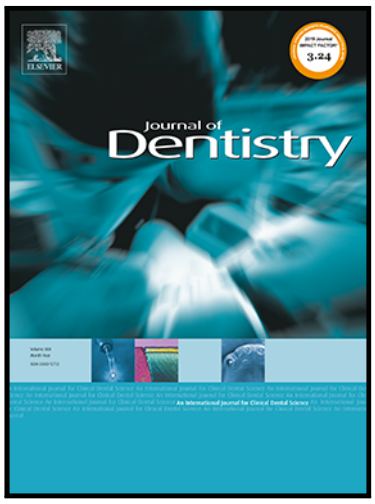

Effect of Probiotics Duration, Delivery Vehicle, and Dose on caries-related variables: A Systematic Review and Meta-Analysis

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Effect of Probiotics Duration, Delivery Vehicle, and Dose on caries-related variables: A Systematic Review and Meta-Analysis.

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Abstract

Background: Probiotics have been proposed as a promising adjunctive strategy for caries prevention. However, uncertainty remains regarding the most effective protocol of administration. In this context, the aim of this review was to evaluate the effects of probiotic administration on caries-related variables, with particular attention to duration of use, delivery vehicle, daily dose, and strain characteristics where the available data allowed.

Methods: PubMed and Scopus were searched for randomized controlled trials on probiotics in healthy subjects. Studies comparing probiotics with placebo, no treatment, or different probiotic regimens were included, focusing on outcomes related to mutans streptococci (MS), Lactobacillus spp. (LB), salivary buffer capacity, and plaque pH. Meta-analysis was conducted when at least three sufficiently comparable studies were available. Planned subgroup analyses or meta-regression by delivery vehicle, dose, strain category, and age group were considered but were not performed when subgroup sizes were too small or reporting was insufficient.

Results: Forty-six studies met the inclusion criteria, and 13 were included in the meta-analysis. Probiotic administration was generally associated with reductions in MS counts, particularly when delivered through yogurt, milk, and lozenges. Meta-analysis demonstrated significant reductions at 7 days (effect size -0.56; 95%CI: -0.83 to -0.28), 14 days (-0.81), and 28 days (-0.92; 95%CI: -1.69 to -0.15), suggesting a time-dependent effect despite substantial heterogeneity. For LB counts, the pooled effect was not significant at 2 weeks (0.29; 95%CI: -0.33 to 0.91) but became significant at 1 month (0.43; 95%CI: 0.10 to 0.75). No significant effect was observed in salivary buffer capacity. Daily doses ranged from 10^6 to 10^{10} CFU, with no clear dose-response relationship.

Conclusions: Probiotics may contribute to caries prevention mainly through reductions in MS counts, particularly after repeated administration. However, substantial heterogeneity in strains, doses, delivery vehicles, populations, and outcome measures prevents identification of a clearly superior vehicle, dose, or strain. Increases in Lactobacillus spp. counts should be interpreted cautiously, because lactobacilli may act as probiotic organisms in some contexts but are also associated with caries progression in acidic biofilm environments.

Clinical Significance: Probiotics may be a promising adjunct for caries prevention by reducing salivary mutans streptococci, especially after 2-4 weeks of use. However, no optimal strain,

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dose, or delivery vehicle can yet be recommended, and probiotics should complement, not replace, established preventive strategies.

Keywords: Probiotics; Dental caries; mutans streptococci; Lactobacillus spp.; Salivary buffer capacity; Systematic review and meta-analysis.

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1. Introduction

Dental caries is a lifelong non-communicable condition and remains the most common non-communicable disease worldwide, representing a major international public health concern [1,2]. Despite the widespread use of fluoride, dental caries remains highly prevalent from an early age [3]. The random-effects pooled prevalence of early childhood caries (ECC) is globally estimated at 49% ($_{95\%}$CI: 0.44-0.55), with wide variation across countries and continents [4]. In permanent dentition, the overall prevalence of dental caries has been reported to be almost 53% [5]. In Europe, the Decayed, Missing, and Filled Teeth (DMFT) index in 12-year-olds ranges from more than three to less than one, with significantly lower caries experience observed in higher-income countries [6]. A similar association between socioeconomic factors and caries has also been observed in adult populations [7]. Carbohydrate intake, particularly sugar intake, has been a major concern in the development of dental caries. In addition to fluoride use and sugar reduction, topical antibacterial agents such as chlorhexidine have been used to manage cariogenic microflora. However, these substances lack selectivity against cariogenic bacteria and may disrupt the oral microbial ecosystem without providing long-term benefits in caries prevention [8].

The modulation of resident oral microorganisms based on the ingestion of probiotic containing products has emerged as an adjunctive strategy for the prevention and treatment of oral diseases [9]. Back in 1908, Metchnikoff put forward the idea of probiotics and suggested that the long life of Bulgarian peasants was the result of their use of fermented milk products containing viable bacteria. The theory was that the harmless bacteria in fermented products were competing with harmful pathogens [10]. Compared with pharmacological agents, probiotics are natural additives with few reported side-effects. They are defined as live microorganisms that confer a health benefit on the host when

administered in appropriate quantities [11,12]. However, as the optimal dosage has not been clearly established, the definition remains somewhat ambiguous.

In the oral health context, food products containing live microorganisms are able to modify the biofilm environment toward a less acidogenic plaque [13–17]. Many different Lactobacilli strains have shown the ability to inhibit the growth of *Streptococcus mutans*, but the effect depends on the specific strain, sequence of colonization, and growth mode [13,15,16]. Several probiotic strains were found to be able to produce bacteriocins or other antibacterial substances, and the effect is usually pH dependent. *Lactobacillus brevis* CD2 is a functional *Lactobacillus* strain with biochemical properties, mainly related to the activity of arginine deiminase. This enzyme catalyzes arginine and influences the biosynthesis of polyamines [putrescine, spermidine, and spermine]. Polyamines are polycations found in high concentrations in both physiological and neoplastic cells. In the presence of glycerol, *Lactobacillus reuteri* produces reuterin, which has broad antimicrobial activity. Probiotics, mostly *Lactobacilli* or *Bifidobacteria*, are aciduric and acidogenic as well as the oral microorganisms involved in caries process [18–20]. Research on probiotics has primarily focused on *Lactobacillus* or *Bifidobacterium* strains. However, findings across studies have been inconsistent, with conflicting results regarding their effects on oral health, dose per day and the modes for their administration. Evidence suggests that the efficacy varies among species and that combinations of strains may exert a synergistic effect, enhancing overall outcomes [15,16,21,22]. Different dosages have also been reported. The best delivery medium or the optimal dosage, however, has yet to be established.

This systematic review and meta-analysis was designed to evaluate whether the available evidence allows assessment of how duration of administration, delivery vehicle,

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daily dose, and probiotic strain characteristics influence caries-related outcomes in healthy populations.

2. Materials and methods

Protocol and registration

The study protocol was registered on the PROSPERO database with registration number (PROSPERO - CRD42020222655). This review has been conducted and reported according to the Cochrane Handbook of Systematic Reviews of Interventions [23,24] and the guidelines of Preferred Reporting Items for Systematic Review and Meta-Analysis [PRISMA] [25]. The PRISMA checklist is displayed in Supplementary file S1. The research question was "What is the most effective delivery vehicle for probiotics to maximize their impact on oral health, and what constitutes the optimal daily dosage?"

The PICO model was used to structure the clinical research question by defining the inclusion criteria. Participants (P) were described as children or adults; Intervention (I) was described as probiotic, replacement therapy, bacterial interference, or bacteriotherapy; Comparison group (C) was either a control group (no treatment or different probiotic strain/s) or a placebo group. Outcome (O) was caries risk, cariogenic bacteria, mutans streptococci (MS), Lactobacillus spp. (LB), modification of plaque composition, salivary buffer capacity, or plaque pH. In this review, mutans streptococci (MS) refers to the group of cariogenic streptococci that includes Streptococcus mutans and related species. The term Streptococcus mutans is used only when the original study specifically assessed that species.

Eligibility criteria

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The inclusion criteria comprised randomized controlled clinical trials evaluating the effects of probiotics on caries-related outcomes and/or risk factors (such as cariogenic bacteria and plaque pH). Only studies assessing the impact of probiotics at the end of the intervention period were considered. Eligible studies included human participants without medical conditions, published in English, involving subjects aged 12 years and above, and employing a placebo or control group for comparison. The following outcomes were considered: (1) the effect of probiotics on salivary or biofilm levels of MS and/or LB, assessed as either primary or secondary outcomes, and (2) the effect of probiotics on plaque acidogenicity. Studies were excluded if they investigated outcomes not related to caries risk, such as the effects of probiotics on fungi, halitosis, periodontal diseases, or other oral conditions. Studies were excluded if the comparison group received any active substance, such as xylitol, chlorhexidine, or other bioactive agents.

Search strategy

A systematic search in the PubMed, and Scopus databases was performed. Two reviewers (C.S. and H.A.) were assigned to identify the eligible studies. The search was supervised by an expert third party (P.L.). The search included the literature up to 24th March 2026. Additional searches of the cited references in the included literature and grey literature were also considered. Duplicates from both databases were treated only once.

The search strategy was developed based on the elements of the PICOs model. The search strips are available in Supplementary file S2. The software Rayyan was used for literature management (http://rayyan.qcri.org).

Study selection, data extraction and synthesis

Data collection and synthesis were independently performed by two authors (SC and HA). A first screening of studies was performed based on titles and abstracts. Disagreements

between the two authors were resolved through discussion or, if necessary, with the involvement of a third reviewer (PL). After full-text assessment, irrelevant articles were excluded, and the reasons for exclusion were documented. Data were extracted using an ad hoc designed data extraction form and the following information was listed for all the included studies: authors, year and journal of publication, country where the study was performed, population, sample size, sex and age of participants, probiotic strain/s used and its concentration, mode of administration, dose per day, identification of the test and comparison groups, intervention period and time of follow-up, primary and secondary outcomes and related results (Supplementary file S3). Numerical data were extracted and rounded up to two decimals; if this was not possible, data were extracted as they were reported in the papers.

Risk of bias assessment

Two reviewers (SC and HA) assessed each study independently. Risk of bias was assessed according to the Cochrane Risk of Bias 2 (RoB 2) tool (28), which considers six categories encompassing seven domains: (1) selection bias, including random sequence generation and allocation concealment; (2) performance bias, referring to the blinding of participants and personnel; (3) detection bias, involving the blinding of outcome assessment; (4) attrition bias, related to incomplete outcome data; (5) reporting bias, concerning selective outcome reporting; and (6) other potential sources of bias. Any disagreements were resolved by discussion until consensus was reached or including a third expert party (PL), if needed. For each domain, the risk of bias was scored as low risk of bias, high risk of bias or same concerns. The articles were regarded as having a low risk of bias, when all seven domains were judged as having a low risk or when up to one domain was estimated as having some concerns; if two or three domains where valued unclear, the article was classified as having

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moderate risk of bias; finally, if at least one of the seven criteria were assessed as having a high risk of bias or more than three criteria being unclear were categorized at high-risk of bias.

Data analysis

Stata SE(R) 19 software (StataCorp LLC, 4905 Lakeway Drive, College Station, TX 77845, USA) was employed for meta-analysis. If at least three studies included comparable subjects, interventions, and outcomes, a meta-analysis was performed. To evaluate the effectiveness of the intervention, the difference between pre-treatment (mean +/- SDt0) and post-treatment (mean +/- SDt1) of the key variables was calculated. For each study, the mean change from baseline to final assessment for both the treatment and control groups was determined. The mean change in the treatment group (Delta test) was defined as the difference between the post-intervention mean and the baseline mean, and the same calculation was performed for the control group (Delta control). The treatment effect for each study was then expressed as the difference in mean changes between the two groups (Delta diff = Delta test - Delta control). The standard deviation (SD) of the change scores for each group was estimated using the baseline and final SDs, assuming a correlation coefficient of 0.5 between baseline and final measurements. The variance of the mean change for each group was obtained by dividing the squared SD of the change by the corresponding sample size. The standard error (SE) of the difference between groups was then calculated as the square root of the sum of these variances. The results of each meta-analysis were presented graphically using forest plots. When meta-analysis was not feasible, a narrative synthesis of the findings from the included studies was performed in accordance with PRISMA guidelines.

Exploratory subgroup analyses or meta-regression for delivery vehicle, daily dose expressed as log CFU, probiotic strain category, and age group were planned when the data

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structure allowed. However, several subgroups included fewer than three sufficiently comparable studies, and reporting of dose, strain composition, and age strata was inconsistent. Therefore, formal subgroup analyses or meta-regression for these variables were not considered statistically robust and were not performed; these factors were instead addressed descriptively and as potential sources of heterogeneity.

3. Results

The study selection is presented in (Figure 1). After screening databases, 3269 records were identified, with 2947 remaining after the removal of duplicates. After title and abstract screening (Supplementary file S4), 2872 articles were excluded for reasons related to the exclusion criteria. The full text of one article could not be found, then a total of 74 articles plus one identified by a hand search were screened for full text and eligibility (Supplementary file S5). Of those excluded, 29 records were excluded because of comparison group, age limitation, or different outcome. One record was identified from citation searching. As a result, 46 articles were eligible for this review; among them, 13 were included in meta-analysis.

Characteristics of included studies

The majority of studies were conducted in India [26–36], followed by Turkey [37–43] and Thailand [44–50]. Nine studies were carried out in Northern Europe (Sweden, Finland, and Denmark) [51–58], and six in Southern Europe (Spain, Italy, and Greece) [59–64] (Table1). Most studies reported receiving research funding from universities or governmental institutions [18,42,44–49,51,52,55,58,62,65–67], while in many cases private companies provided the necessary materials free of charge, including the probiotic strains under investigation [34,37,38,40,41,43,53,54,56,57,63,68,69] (Supplementary file S6).

The sample size of the included studies ranged from 18 to 122 subjects. Most studies involved adult patients [18,29,32,33,35,37–41,48,51,53–59,62,64,67,68], while 18 studies were conducted on children aged 12 to 18 years [26,27,30,31,34,42–45,47,49,52,60,61,66,70–72], and five studies [28,46,63,65,69] included both children and adults. Among the studies on adults, the majority involved subjects younger than 40 years, whereas only four studies [56,59,62,65] included older adults (Table 1). Overall, women were more prominently represented in studies reporting participants' sex data (Table 1).

Most studies compared probiotic strain with placebo. In three studies [31,33,70], the comparison were control group who received no treatment. Six studies also included comparisons between two different probiotic products [27,28,42], two distinct probiotic strains administered via the same vehicle [40,64], and a multi-strain probiotic complex delivered through two different vehicles [36] (Table 1).

The probiotics tested primarily to the *Lactobacillus ssp*, *Bifidobacterium ssp*, and *Bacillus ssp*; in some cases, *Streptococcus* species were also evaluated. *L. reuteri*, *L. rhamnosus*, *B. lactis*, *L. paracasei*, *B. animalis*, *L. acidophilus* was among the most frequently represented. In most studies, combinations of different strains of the same probiotic or complexes of distinct probiotic strains were tested. In two studies the probiotic strain was administered in combination with maltitol [44] or xylitol [68]. The daily dose of probiotic administered ranged in magnitude from 10^6 to 10^{10} colony-forming units (CFU) and the intervention period ranged between 7 days and 18 months. In ten studies, the specific probiotic strain administered [27,28] or the daily dose was not reported [26–28,31,35,42,45,61,65,66,70] (Table 1).

Regarding the vehicles used for probiotic administration, the most commonly employed in the studies were lozenges [31,37,40,49,53,55,56,58–60,63,68], milk

[30,44,45,47,48,71] or dairy-based products, like cheese [18,51], yogurt [26–28,35,39,42,61,66,67,69] or ice cream [27,32,34,41]. In some cases, probiotics were added to vehicles commonly used to maintain good oral hygiene, such as mouthwash [29,33,52,54,72] toothpaste [28,42] or chewing gum [38,57] (Table 1).

Mutans streptococci counts

A total of 40 studies evaluated the impact of probiotic administration on MS counts in saliva and/or dental plaque. Across the dataset, a consistent trend emerged: probiotic interventions were generally associated with reductions in MS levels, whereas control groups tended to show either no change or slight increases (Table 2).

Significant improvements were most consistently observed when probiotics were administered through yogurt, milk, or lozenges. For instance, yogurt-based interventions over periods of 7 to 14 days frequently resulted in significant bacteria reductions ($p<0.01$) [26,61]. Similarly, milk-based delivery over longer durations (*e.g.*, from 3 to 6 months) showed sustained reductions in *S. mutans* count, with consistent statistically significant findings across multiple follow-up assessments [30,44–48,71]. Lozenges also demonstrated efficacy, especially in short-term studies (*e.g.*, 10–28 days) [41,49] (Table 2). By contrast, the control groups generally showed minimal or no improvement. When comparing intergroup differences, many studies demonstrated statistically significant reductions in the probiotic group compared to controls. These differences were most pronounced when the time of administration continues until 3 weeks, 6 weeks, and 12 weeks [28–32,38,42,44–47,49,51,67,71] (Table 2). It should be noted that outcomes varied depending on the probiotic strain and dosage, however, the extreme variability between studies does not allow us to draw conclusions on these two aspects.

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The delivery method played a crucial role in outcomes. Yogurt and milk were the most effective vehicles [26,30,44–48,61,71], especially when administered over longer periods. Lozenges and mouthwash showed mixed results, with some studies reporting significant reductions [31,41,49] and others showing no effect [53,63,68]. Toothpaste formulations also yielded positive outcomes, particularly in pediatric populations [28,42] (Table 2).

Regarding the duration of administration, short-term studies (1-2 weeks) often showed immediate reductions in MS, while mid-term (3-6 weeks) and long-term (12-24 weeks) administrations demonstrated sustained effects. Notably, studies with multiple follow-up points (*e.g.*, 12, 24, and 52 weeks) provided stronger evidence of the lasting impact of probiotics, especially when administered via milk (Table 2).

Three forest plots (Figure 2 A,B,C) were constructed, each aggregating studies with similar intervention period: 1 week, 2 weeks and 4 weeks. The forest plot corresponding to a 1-week administration (Supplementary file S7) includes six studies [26,27,35,46,48,65] one of which included two different delivery vehicles, compared with placebo, and demonstrates a pooled effect size of –0.56 with a 95% confidence interval (–0.83, –0.28), indicating a statistically significant reduction in MS counts. Despite the favorable direction of effect, the analysis reveals substantial heterogeneity (I^2=97.47%), suggesting variability in study design, probiotic strains, and delivery methods. The test for overall effect was significant (z=–3.92, p<0.01), supporting the conclusion that even short-term probiotic administration can yield measurable reductions in MS levels.

The 2-week administration plot (Supplementary file S8) synthesizes data from five studies [46,48,60,66,67] and yields a pooled effect size of -0.81, reflecting a stronger average reduction than observed at 7 days. Individual study effects range from -0.35 to -1.01 and, while heterogeneity remains present, the consistency in direction and magnitude of effect

supports the robustness of the findings. This suggests that extending probiotic administration to two weeks may enhance its antimicrobial efficacy against MS.

The 4-week administration plot (Figure 2-A) includes four studies [78,97,106,112] and shows the most pronounced effect, with a pooled estimate of –0.92 [–1.69, –0.15]. This result underscores the cumulative benefit of sustained probiotic exposure. However, heterogeneity remains high (I^2= 87.78%), and the confidence interval is wide, reflecting greater variability in outcomes. The test for overall effect was statistically significant (z=–2.35, p=0.02), confirming the better efficacy of probiotics over a 4-week period.

Across all three time points (1, 2, or 4 weeks), the direction of effect consistently favored probiotic interventions, with increasing magnitude over time. These findings suggest a time-dependent relationship between probiotic administration and reduction in MS levels. While heterogeneity was notable, the consistent statistical significance and negative effect sizes support the potential of probiotics as a short-term strategy for modulating oral microbiota and reducing caries-related microbial risk.

Results on Lactobacillus spp. counts

Thirty-four clinical studies evaluated the impact of various probiotic strains and delivery methods on salivary LB counts, involving both pediatric and adult populations (Table 3). Significant increases in LB counts were observed in several trials, particularly those involving *L. paracasei* and *L. rhamnosus* strains delivered via milk at different durations of administration ($p<0.05$ to $p<0.001$) [44-47,50], suggesting that both strain and delivery method may influence measured probiotic effects (Table 3). These findings should be interpreted as changes in Lactobacillus spp. counts rather than as direct evidence of clinical benefit.

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Lozenge-based delivery of *L. reuteri* strains yielded mixed results. Romani Vestman et al. [55,56] reported significant increases at 1 and 3 months ($p< 0.01$), while Sinkiewicz et al. [57] observed significance only at 4 months ($p<0.01$). Conversely, another study using the same probiotic [40] did not report significant changes, indicating variability in outcomes possibly due to dosage, duration, or population differences (Table 3).

Yogurt-based delivery showed limited efficacy. Although Zare Javid et al. [67] reported significant increases ($p<0.01$) with *B. lactis* Bb12, other studies using similar strains [34,58,69] did not demonstrate significant effects. Similarly, ice cream as a delivery vehicle yielded inconsistent results: while one study [37] reported significant findings ($p<0.01$), another [43] did not (Table 3).

Capsule and bottle-based delivery of multi-strain formulations (e.g., *L. sporogens*, *L. bifidum*, *L. bulgaricus*, *L. acidophilus*, *L. casei*, *L. rhamnosus*) in Montalto et al. (64) resulted in significant intragroup changes ($p=0.02$ and $p< 0.01$, respectively), suggesting potential for complex formulations (Table 3).

Overall, the most consistent and significant increases in Lactobacillus spp. counts were associated with milk-based delivery of *L. paracasei* SD1 and *L. rhamnosus* SD11, particularly in younger populations. Lozenges containing *L. reuteri* and *L. rhamnosus* also showed promising results, although results were more variable (Table 3). Because lactobacilli may include both administered probiotic strains and resident acidogenic species, increases in LB counts should not automatically be interpreted as beneficial without strain-level and clinical outcome information.

The meta-analysis was performed at 2 and 4 weeks of intervention, including four [46,48,60,67] and six [78,103,106,112,114,115] studies, respectively. At the 2 weeks of intervention (Supplementary file S9), the pooled effect size (0.29; $_{95\%}$CI: –0.33 to 0.91)

indicated a non-significant increase in LB counts (z=0.91, p=0.36). The confidence interval crossed zero, suggesting that short-term probiotic administration does not reliably enhance salivary LB levels. Moreover, heterogeneity was substantial (I^2=82.93%; Q(3)=14.16, p<0.01), reflecting considerable variability among designs of the studies.

In contrast, the 4-week intervention period analysis (Figure 2.B), revealed a statistically significant pooled effect size (0.43; $_{95\%}$CI:0.10 to 0.75, z=2.56, p=0.01), suggesting that extending probiotic administration to four weeks results in a measurable increase in LB counts. Despite the positive outcome, heterogeneity remained high (I^2=85.93%, Q(5)=23.76, p<0.01). Nevertheless, the direction of effect was consistently favorable in most trials, supporting the hypothesis that probiotic efficacy is time dependent.

Results on buffer capacity of saliva or plaque pH

Ten studies evaluated the buffer capacity of saliva [31,42,44,46,59,60,62,65,68,70] and one the acidogenity of dental plaque [52] (Table 4).

Among the most studied strains, *L. reuteri* DSM 17938 and ATCC PTA 5289 were evaluated in multiple trials [52,60,70], demonstrating significant intragroup changes both in plaque-pH [52] and salivary buffer capacity [70] when administered via mouthwash or tablets (p<0.05), but not consistently in other form. *L. brevis* CD2 [59] and *L. paracasei* [46,68] showed limited efficacy, with only one study reporting intergroup significance at six weeks [59] (p< 0.05).

Other strains such as *S. dentisani* 7746 [62] and combination of *S. oralis*, *S. uberis*, and *S. rattus* [31] yielded mixed results. Notably, the latter combination demonstrated significant intragroup and intergroup differences (p<0.01), suggesting potential synergistic effects. *L. lactis*, *Leuconostoc*, and *S. thermophilus* administered via yogurt [42] also showed promising intragroup changes (p=0.32), although intergroup comparisons were not reported.

Lozenges were the most commonly used form in four studies [31,59,60,68]; however, their efficacy was inconsistent. For example, *L. reuteri* lozenges [60] did not produce significant pH changes, whereas combination of *S. oralis* KJ3sm; *S. uberis* KJ2sm; *S. rattus* JH145 lozenges [31] did. Mouthwash formulations [52] appeared more effective than lozenges [60] for *L. reuteri*, suggesting that direct contact with plaque may enhance probiotic activity. Toothpaste and yogurt [42] showed comparable efficacy, although the bacteriocin-based toothpaste had slightly stronger intragroup effects. Milk-based deliver [44,46] showed delayed or non-significant effects, possibly due to dilution or reduced retention time in the oral cavity. Gel formulations [62] demonstrated time-dependent efficacy, with significant changes only at later time of intervention period (4 and 6 weeks). The cake-based delivery [65] was novel but did not yield significant results.

Forest plot (Figure 2.C) summarized the results of four clinical trials [31,44,46,60] evaluating the effect of probiotic interventions on buffer capacity of saliva after 1 month of administration. The pooled estimate suggested no statistically significant effect of probiotics on buffer capacity of saliva at 1 month ($z=0.07$, $p=0.95$). This was further supported by the narrow confidence intervals crossing the null value in most studies. The included studies demonstrated low heterogeneity ($I^2=27.68\%$; $Q(3)=4.07$; $p=0.25$), indicating that the variability in effect sizes was primarily attributable to sampling error rather than genuine differences in treatment effects.

Assessment of the risk of bias

The risk of bias assessment was conducted for all studies included, using the Cochrane Risk of Bias 2.0 tool, which evaluates five domains: randomization process (D1), deviations from intended interventions (D2), missing outcome data (D3), measurement of the outcome (D4), and selection of the reported result (D5).

A total of 46 studies were assessed, of which 28 were analyzed using the Intention-to-treat (ITT) approach and 18 using the Per-protocol (PP) approach. The ITT approach was applied for the studies who reported no dropouts, allowing all randomized participants to be analyzed as originally allocated.

Among the ITT studies (Figure 3.A), one was judged as high risk [70], one as having some concerns [61], while the remaining 26 studies were classified as low risk. The most frequent source of bias in this group was related to selection of the reported result (D5), as the majority of studies didn't have a pre-registered protocol.

The PP studies (Figure 3.B) showed a less favorable risk of bias profile compared to ITT studies. Of the 18 PP studies, 1 was rated at high risk [62], 4 were judged as having some concerns [30,51,60,63] and the remaining studies at low risk. The single high-risk study was primarily affected by missing outcome data (D3), which significantly compromised its reliability. Consistent with the ITT analyses, the domain most associated with bias in PP studies was D5 (selection of the reported result), highlighting that this aspect constitutes a recurring methodological challenge across studies.

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4. Discussion

This systematic review and meta-analysis evaluated the efficacy of different probiotic strains and administration protocols in modulating oral microbiota, specifically targeting mutans streptococci (MS), Lactobacillus spp. (LB), salivary buffer capacity, and plaque pH. The findings suggest that probiotics may contribute to caries prevention mainly by reducing MS counts, particularly with sustained use. However, the available evidence does not allow identification of an optimal delivery vehicle, daily dose, or strain category, and the original research question can therefore only be partially answered.

The main limitation affecting interpretation was the substantial clinical and methodological heterogeneity among the included studies. Potential sources of variability include differences in probiotic strains and strain combinations, daily dose and viability, duration of administration, delivery vehicles, participant age, baseline caries risk, fluoride exposure, oral hygiene habits, dietary background, follow-up periods, and microbiological assessment methods. Consequently, pooled estimates should be interpreted cautiously, and findings regarding vehicle and dose should be considered exploratory rather than definitive.

The results showed that the vehicles used for probiotic delivery were mostly dairy products, such as milk, cheese, yogurt, and curd. In addition to their potential probiotic effects, milk products themselves may reduce plaque acidogenicity [73], while calcium and phosphate have anticariogenic effects [74] and may increase enamel remineralization [75]. Approximately two-thirds of probiotic interventions delivered through dairy products were associated with significant reductions in MS levels in saliva or plaque. Some studies also reported significant increases in LB levels following administration via dairy products. Probiotics delivered through non-dairy products such as lozenges, chewing gum, gel, or mouthwash also showed positive effects in some studies, but the findings were inconsistent. Overall, the evidence did not demonstrate superiority of one delivery vehicle over another. Compliance, retention time in the oral cavity, and dose-response relationships should therefore be considered in future trials.

According to the FAO/WHO, probiotics are defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host [12]. However, the definition is subject to ambiguity because the necessary amount is not specified. No consensus emerged regarding the optimal probiotic dose for promoting oral health, and heterogeneity was evident across the included studies. Although most studies employed a

dose of 10^8 colony-forming units (CFU), around half failed to demonstrate a significant effect at the conclusion of the trial period. A higher dose was used in two studies [30,62], yet one of these studies did not demonstrate a positive effect on microbial counts. Therefore, clinical trials should verify that the probiotic product contains viable bacteria throughout the intervention period. In addition, probiotic bacteria should be evaluated for stability during manufacture and storage to ensure viability and functional properties [76-78]. Furthermore, most clinical studies reported the starting dose, but this may have varied throughout the study. Some studies relied on a single daily dose, while others used multiple doses as the administration regimen. The rationale for different administration regimens was generally not explained, apart from following manufacturers' instructions. A recent review investigating optimum dose in relation to general health, without including oral outcomes, has been published [79]. Evidence on this issue in oral health remains limited. In a pilot study of eleven subjects, Ferrer et al. [62] demonstrated the benefit of multiple doses in increasing salivary buffer capacity compared with a single dose after short-term use. Furthermore, it has been suggested that dosage delivered through regular dietary intake may be insufficient for therapeutic efficacy and that food processing may further impair bacterial viability [80]. As a result, the effective dose is likely influenced by probiotic strain, outcome variable, delivery vehicle, and administration regimen, indicating that no specific dose can currently be generalized.

Most publications in this review focused on lactobacilli- or bifidobacteria-derived probiotics. According to a systematic review of diverse probiotic strains, Lactobacillus species provide more consistent evidence of therapeutic effects for caries prevention compared with Bifidobacterium and Streptococcus genera [81]. Lactobacilli can produce low-molecular-weight bacteriocins that inhibit a wide range of bacterial species, including oral streptococci

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[82]. However, the role of lactobacilli in caries is complex. Lactobacilli are acidogenic and aciduric microorganisms frequently associated with caries progression, particularly in acidic biofilm environments and dentine caries. Therefore, increased LB counts should not be considered inherently beneficial. The interpretation depends on the specific strain, metabolic activity, persistence in the oral cavity, interaction with the resident biofilm, and whether detected increases reflect administered probiotic strains or proliferation of resident cariogenic lactobacilli.

Evidence remains insufficient to determine whether single-species or multi-species probiotic formulations are more effective in achieving oral health benefits. Juntunen et al. [83] demonstrated that the combination of two strains could improve probiotic adherence in a synergistic manner in the intestinal context. In the oral cavity, it has also been demonstrated that the success of one lactobacilli strain does not necessarily imply that other strains will be similarly effective, and that combinations of strains may improve adherence synergistically [84]. In this review, combinations of two or more strains were used in 14 studies, more than half of which reported significant microbial reductions. Single-strain interventions also reported positive outcomes in saliva and dental plaque. The most pronounced effects were observed in studies using yogurt, milk, and lozenges as delivery vehicles; however, because of heterogeneity and small subgroup sizes, these findings cannot be interpreted as evidence of superiority.

These findings align with previous research indicating that selected Lactobacillus and Bifidobacterium strains can inhibit Streptococcus mutans through competitive exclusion, bacteriocin production, and modulation of oral pH [85,86]. Notably, L. reuteri, L. rhamnosus, and B. lactis were among the most frequently studied strains, with antimicrobial properties reported in earlier studies [87].

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Thirty-four studies assessed the impact of probiotics on salivary LB levels. Milk-based delivery of *L. paracasei* SD1 and *L. rhamnosus* SD11 showed the most consistent and significant increases, particularly in pediatric populations [45,47]. These findings suggest that both strain specificity and delivery method are important determinants of measured probiotic effects. However, lozenge-based delivery yielded mixed outcomes; while some studies reported significant increases [56], others did not [60], indicating variability potentially due to dosage, duration, population characteristics, or host factors. Given the dual role of Lactobacillus species in oral health, as both potential cariogenic microorganisms and probiotic protectors depending on context [88], LB outcomes should be discussed separately from clinical caries-preventive effects.

Ten studies investigated the buffer capacity of saliva and plaque acidogenity following probiotic administration. Mouthwash formulations, particularly those containing *L. reuteri*, demonstrated significant intragroup pH changes [52], suggesting that direct contact with plaque enhances probiotic activity. Conversely, lozenges and milk-based vehicles showed inconsistent effects, possibly due to reduced retention time or dilution in the oral cavity.

The meta-analysis at 1 month revealed no statistically significant effect on salivary buffer capacity, although heterogeneity was low, indicating that variability in outcomes may stem primarily from sampling error rather than true differences in treatment effects. These findings are supported by studies showing that environmental pH influences antimicrobial production by probiotics [89]. Forest plots constructed for 1, 2, and 4 weeks of administration revealed a time-dependent relationship between probiotic administration and MS reduction. The pooled effect sizes increased with duration, indicating cumulative benefits of sustained probiotic exposure. Similar trends were observed for LB counts, with significant increases only evident after 4 weeks of intervention.

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These results underscore the importance of intervention duration in achieving measurable outcomes, consistent with findings from other meta-analyses [90,91].

Risk of bias assessment revealed that most studies had some concerns, particularly regarding selection of the reported results due to lack of pre-registered protocols. Only a minority were rated as low risk, highlighting the need for improved methodological rigor in future trials. The Cochrane Risk of Bias 2.0 tool identified selection bias and missing outcome data as common issues, echoing concerns raised in other reviews [92]. Future trials should use standardized reporting of strain identity, viable dose at baseline and end of shelf-life, delivery vehicle, exposure frequency, participant age and caries risk, and follow-up duration. Such standardization would enable robust subgroup analyses and meta-regression for vehicle, dose, strain category, and age group.

5. Conclusions

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Probiotic administration appears to reduce MS counts, with a consistent time-dependent effect across studies and greater reductions after longer durations of use. Favorable outcomes were reported for delivery through milk, yogurt, and lozenges, particularly when administered for at least 2-4 weeks; however, the evidence is insufficient to identify any delivery vehicle as clearly superior.

The effect on LB counts was less consistent, with significant increases observed mainly after prolonged administration. Because lactobacilli may be either probiotic or associated with caries progression depending on strain and ecological context, these findings should not be interpreted as inherently beneficial without supporting clinical outcomes. No significant effect was found on salivary buffer capacity, indicating limited impact of probiotics on this parameter.

Considerable heterogeneity was observed across studies, likely due to variability in probiotic strains, doses, delivery vehicles, study populations, outcome definitions, and study designs. Formal subgroup analyses or meta-regression for vehicle, dose, strain category, and age group were not statistically robust because of sparse and inconsistently reported data. Consequently, no definitive conclusions can be drawn regarding the optimal probiotic strain, dosage, or mode of delivery.

Overall, probiotics may represent a promising adjunctive strategy for modulating oral microbiota and reducing caries-related microbial risk; however, further well-designed, standardized clinical trials are needed before clear clinical protocols can be established.

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Figure and Tables Legends

Figure 1. PRISMA flowchart.

Figure 2. A. Forest plot showing the effect sizes (with 95% confidence intervals) of studies evaluating salivary mutans streptococci (MS) counts after 4 weeks of administration; B. Forest plot showing the effect sizes (with 95% confidence intervals) of studies evaluating salivary Lactobacilli (LB) counts after 4 weeks of administration; C. Forest plot showing the effect sizes (with 95% confidence intervals) of studies evaluating buffer capacity of saliva after 4 weeks of administration.

Figure 3. A. Risk of bias assessment of the studies analyzed using the Intention-to-treat (ITT) approach; B. Risk of bias assessment of the studies analyzed using the Per-protocol (PP) approach.

Table 1. Sample, characteristics of population, study design, probiotics administration and outcomes evaluated of the included studies.

Table 2. Summary of the results of studies evaluating the influence of probiotics on salivary and/or plaque (*) S. mutans counts.

Table 3. Summary of the results of studies evaluating the influence of probiotics on salivary *Lactobacilli* counts.

Table 4. Summary of the results of studies evaluating the influence of probiotics on buffer capacity of saliva and plaque pH (*).

Declarations

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

For this type of study, formal consent is not required.

Conflict of interest

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The authors declare no potential conflict of interest with respect to the authorship and/or publication of this article.

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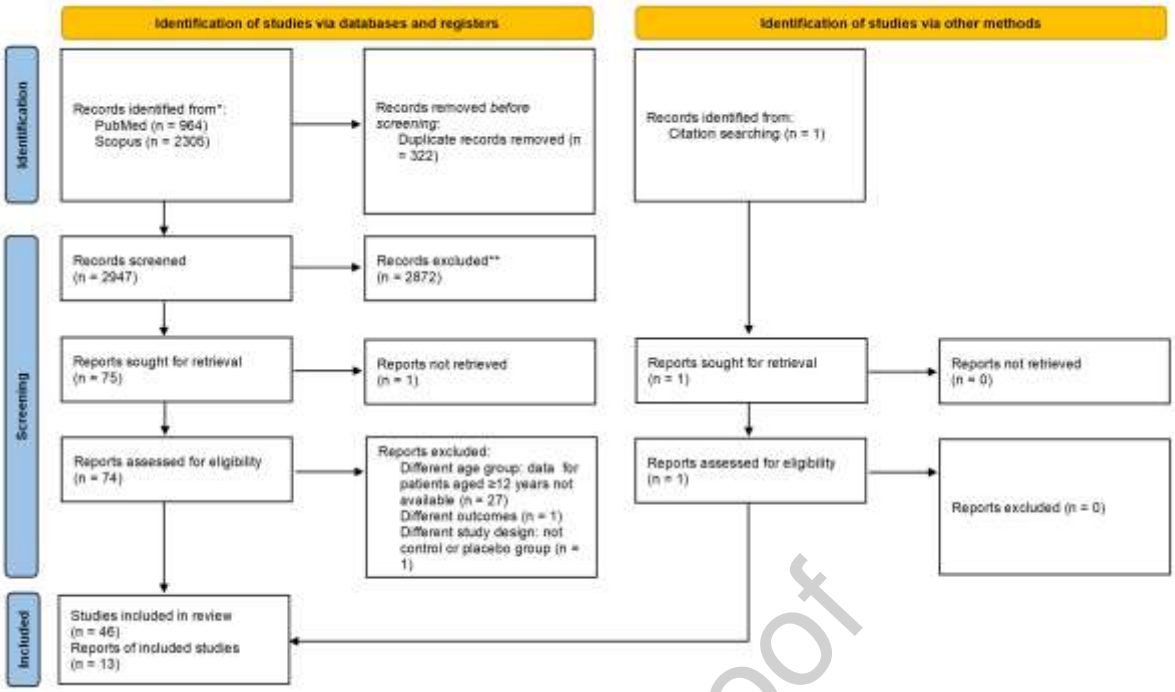

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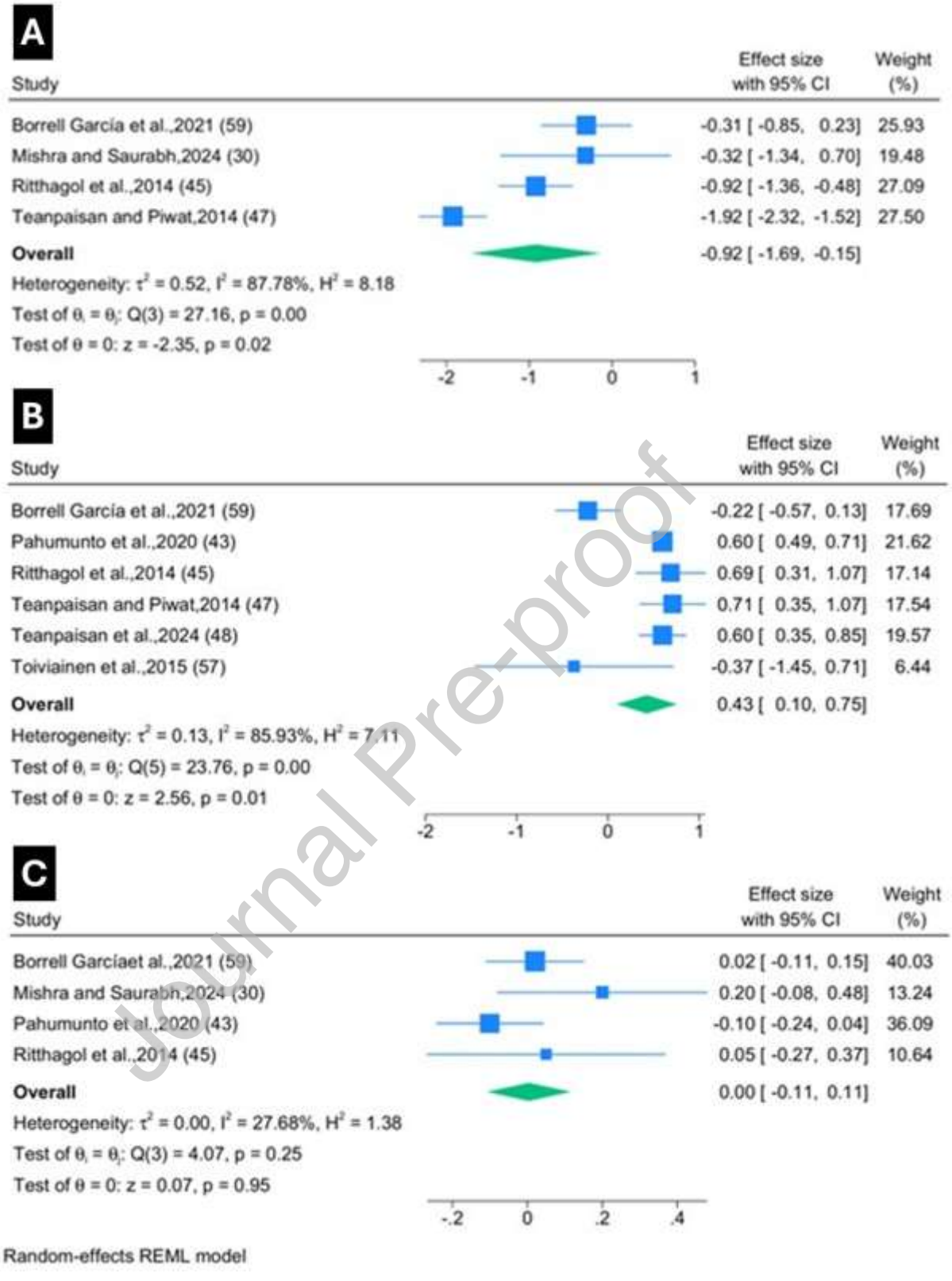

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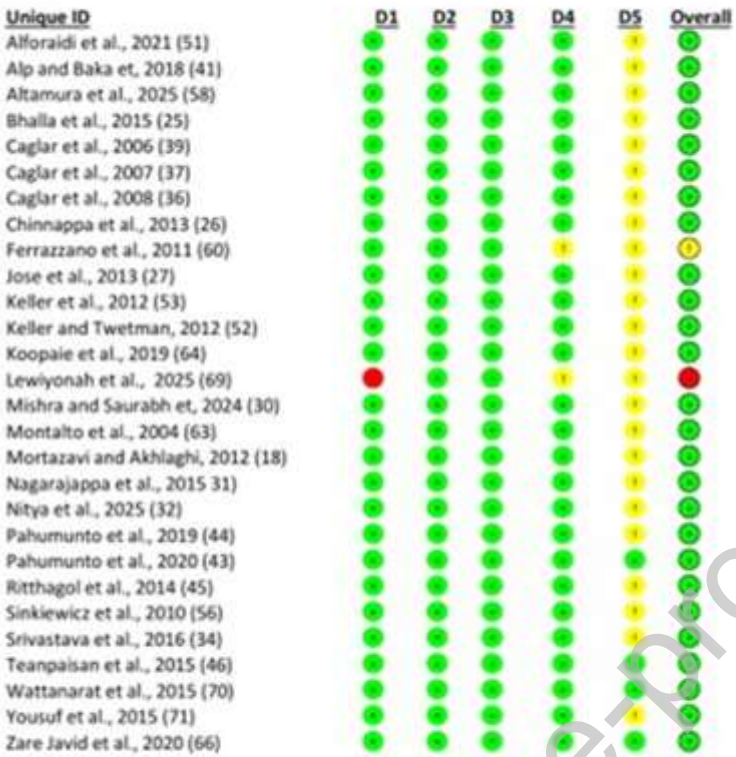

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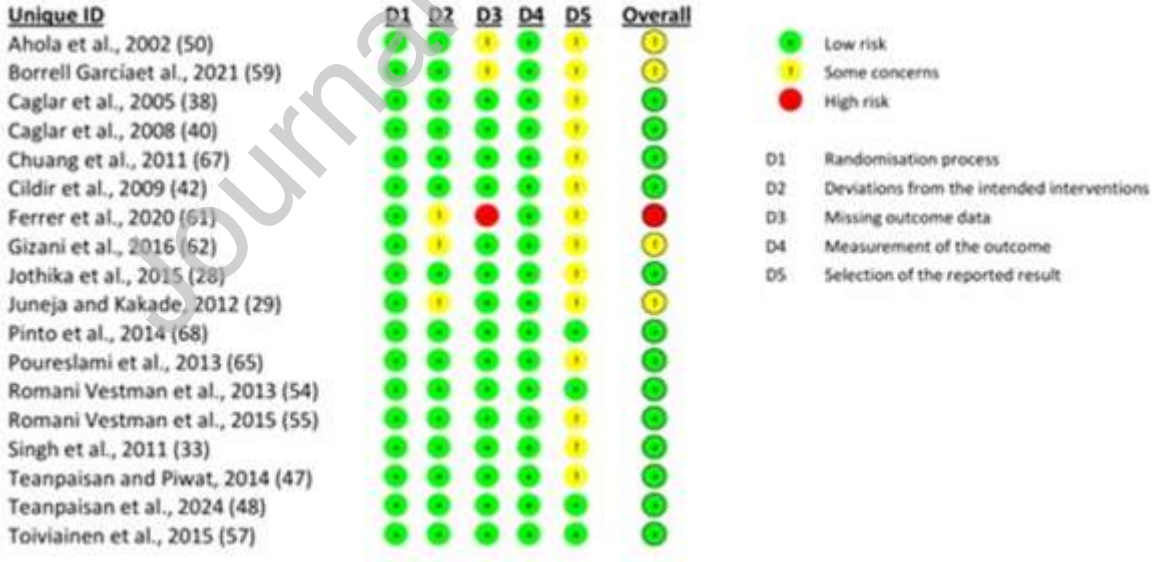

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Table 1. Sample, characteristics of population, study design, probiotics administration and outcomes evaluated of the included studies.

Author	Age range/ mean (SD)	Study design	Probiotics strain	Delivery	Amount (CFU/day)	Duration	Outcomes
Ahola et al.[51]	18-35	Probiotic *vs* PL	*L. rhamnosus* GG & LC 705	Cheese	$1.43*10^9$	3 weeks	MS count
Alforaidi et al.[52]	16-18	Probiotic *vs* PL	*L. reuteri* DSM 17938 & ATCC PTA 5289	Mouthwash	$2{,}00*10^8$	3 weeks	Plaque pH
Alp et al.[42]	12-17	Probiotic toothpaste *vs* probiotic yogurt *vs* no treatment	Bacteriocin extracted from lactic acid bacteria	Toothpaste	na	6 weeks	MS count LB count
			L. lactis subsp.; *Leuconostoc sp.; Lactobacillus sp.*; *S. thermophilus*	Yogurt	na		Buffer capacity of saliva
Altamura et al.[59]	20-75	Probiotic *vs* PL	*L. brevis* CD2	Lozenges	$4{,}00*10^9$	4 weeks	Buffer capacity of saliva
Bhalla et al.[26]	12-14	Probiotic *vs* PL	*B. lactis* Bb-12	Yogurt	na	1 week	MS count
Borrell García et al.[60]	12-18	Probiotic *vs* PL	*L. reuteri* DSM 17938 & ATCC PTA 5289	Lozenges	$1.00*10^8$	4 weeks	MS count LB count Buffer capacity of saliva
Caglar et al.[39]	21-24	Probiotic *vs* PL	*Bifidobacterium* DN-173 010	Yogurt	$1.40*10^{10}$	2 weeks	MS count LB count
Caglar et al.[40]	21-24	Probiotic *vs* PL	*L. reuteri* ATCC 55730	Staw	$1.00*10^8$	3 weeks	MS count
				Lozanges	$1.00*10^8$		LB count
Caglar et al., [38]	21-24	Probiotic *vs* PL	*L. reuteri* ATCC 55730 & ATCC PTA 5289	Chewing gum	$3.00*10^8$	3 weeks	MS count
Caglar et al.[41]	20	Probiotic *vs* PL	*L. reuteri* ATCC 55730 & ATCC PTA 5289	Lozenges	$1.10*10^8$	1.5 week	MS count LB count
Caglar et al.[37]	20	Probiotic *vs* PL	*B. lactis* Bb-12	Ice cream	$5.30*10^8$	1.5 week	MS count LB count
Chinnappa et al.[27]	12-14	Probiotic ice cream *vs* probiotic yogurt *vs* PL	na	Ice cream	na	1 week	MS count
				Yogurt			
Chuang et al.[68]	20-26	Probiotic + xylitol *vs* xylitol	*L. paracasei* GMNL-33	Lozenges	$9.00*10^8$	2 weeks	MS count LB count Buffer capacity of saliva
Cildir et al.[43]	12-16	Probiotic *vs* PL	*B. animalis subsp. lactis* DN-173010	Ice cream	$4.00*10^{10}$	2 weeks	MS count LB count

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Ferrazzano et al.[61]	12-18	Probiotic *vs* PL	*L. bulgaricus; S. thermophilus*	Yogurt	na	2 weeks	MS count LB count
Ferrer et al.[62]	18-65	Probiotic *vs* PL	*S. dentisani* 7746	Gel	$1,25*10^9$	4 weeks	Buffer capacity of saliva
Gizani et al.[63]	15.9 (3.9)	Probiotic *vs* PL	*L. reuteri* DSM 17938 & ATCC PTA 5289	Lozenges	$2.00*10^8$	72 weeks	MS count LB count
Jose et al.[28]	14-19	Probiotic toothpaste *vs* probiotic yogurt *vs* no treatment	na	Toothpaste	na	4 weeks	MS count
			L. acidophilus; B. lactis	Yogurt	$2.00*10^8$		
Jothika et al.[29]	18-25	Probiotic *vs* PL	*L. acidophilus; L. rhamnosus; B. longum; S. boulardii*	Mouthwash	$2.50*10^9$	4 weeks	MS count
Juneja & Kakade[30]	12-15	Probiotic *vs* PL	L. rhamnosus HTC 70	Milk	$2.34*10^9$	3 weeks	MS count
Keller & Twetman[53]	26	Probiotic *vs* PL	*L. reuteri* DSM 17938 & ATCC PTA 5289	Lozenges	$3.00*10^8$	2 weeks	MS count LB count
Keller et al.[54]	19-35	Probiotic *vs* PL	*L. reuteri* DSM 17938 & ATCC PTA 5289	Mouthwash	$2.00*10^8$	6 weeks	MS count
Koopaie et al.[65]	15-73	Probiotic *vs* PL	*H. coagulans*	Cake	na	1 week	MS count Buffer capacity of saliva
Lewiyonah et al.[70]	12-15	Probiotic *vs* no treatment	*Lactobacillus reuteri* DSM 17938; *Lactobacillus reuteri* ATCC PTA 5289	Tablets	na	1 week	MS count Buffer capacity of saliva
Mishra & Saurabh[31]	12-14	Probiotic *vs* no treatment	*S. oralis* KJ3sm; *S. uberis* KJ2sm; *S. rattus* JH145	Lozenges	na	4 weeks	MS count Buffer capacity of saliva
Montalto et al.[64]	23–37	Probiotic bottle *vs* probiotic capsule *vs* PL	*L. sporogens; L. bifidum; L. bulgaricus; L. termophilus; L. acidophilus; L. casei; L. rhamnosus*	Bottle	$1.88*10^9$	6 weeks	MS count LB count
				Capsule	$1.88*10^9$		
Mortazavi & Akhlaghi[18]	18-37	Probiotic *vs* PL	*L. casei* LAFTI-L26	Cheese	$1.00*10^8$	2 weeks	MS count LB count
Nagarajappa et al.[32]	18-22	Probiotic *vs* PL	*Bifidobacteria*	Ice cream	$6.00*10^7$	2.5 weeks	MS count LB count
Nitya et al.[33]	20-35	Probiotic *vs* no treatment	*M. hydrocarbonoclasticus; Pseudoaltero monas spp.*	Mouthwash	$5.00*10^8$	1 week	MS count
Pahumunto et al.[45]	12-14	Probiotic *vs* PL	*L. paracasei* SD1	Milk	na	12 weeks	MS count LB count
Pahumunto et al.[44]	13-14	Probiotic + maltitol *vs*	*L. rhamnosus* SD11	Milk	$1.00*10^{10}$	4 weeks	MS count LB count

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		PL + maltitol					Buffer capacity of saliva
Pinto et al.[69]	10-30	Probiotic *vs* PL	*B. animalis subsp. lactis* DN-173010	Yogurt	$2.00*10^{10}$	2 weeks	MS count LB count
Poureslami et al.[66]	15-17	Probiotic *vs* PL	*Lactobacilli*	Yogurt	na	2 weeks	MS count
Ritthagol et al.[46]	19,22 (3,7)	Probiotic *vs* PL	*L. paracasei* SD1	Milk	$7.50*10^{9}$	4 weeks	MS count LB count Buffer capacity of saliva
Romani Vestman et al.[55]	19-35	Probiotic *vs* PL	*L. reuteri* DSM 17938; *L. reuteri* ATCC PTA 5289	Lozenges	$4.00*10^{8}$	6 weeks	MS count LB count
Romani Vestman et al.[56]	20-66	Probiotic *vs* PL	*L. reuteri* DSM 17938 & PTA 5289	Lozenges	$4,00*10^{8}$	12 weeks	LB count
Singh et al.[34]	12-14	Probiotic *vs* PL	*B. lactis* Bb-12 ; *L. acidophilus* La-5	Ice cream	$2.00*10^{6}$	10 days	MS count LB count
Sinkiewicz et al.[57]	> 18	Probiotic *vs* PL	*L. reuteri* ATCC PTA 5289 & ATCC 55730	Chewing gum	$4,00*10^{8}$	12 weeks	LB count
Srivastava et al.[35]	20-25	Probiotic *vs* PL	*L. acidophilus*	Yogurt	na	1 week	MS count
Teanpaisan & Piwat[48]	18-25	Probiotic *vs* PL	*L. paracasei* SD1	Milk	$7.50*10^{9}$	4 weeks	MS count LB count
Teanpaisan et al.[47]	12-14	Probiotic *vs* PL	*L. paracasei* SD1	Milk	$5.00*10^{10}$	24 weeks	MS count LB count
Teanpaisan et al.[49]	13-14	Probiotic *vs* PL	*L. rhamnosus* SD11	Lozenges	$1.00*10^{8}$	4 weeks	MS count LB count
Toiviainen et al.[58]	24,0 (3,0)	Probiotic *vs* PL	*L. rhamnosus* GG, *B. animalis subsp. lactis* BB-12	Lozenges	$2.00*10^{9}$	4 weeks	MS count LB count
Wattanarat et al.[71]	13-15	Probiotic *vs* PL	*L. paracasei* SD1	Milk	$3.75*10^{9}$	24 weeks	MS count LB count
Yousuf et al.[72]	12-15	Probiotic A *vs* probiotic B *vs* PL	*B. clausii* *L. acidophilus*; *B. longum*; *B. bifidum*; *B. lactis*	Mouthwash	$2.00*10^{10}$ $2.00*10^{9}$	2 weeks	MS count
Zare et al.[67]	18-30	Probiotic *vs* PL	*B. lactis* Bb12	Yogurt	$3.00*10^{9}$	2 weeks	MS count LB count

N: number; M: male; F: female; SD: standard deviation; CFU: colony forming unit; MS: Mutans Streptococci; LB: Lactobacilli; PL: Placebo. Time was standardized to weeks across all studies to enable comparability.

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Table 2. Summary of the results of studies evaluating the influence of probiotics on salivary and/or plaque (*) S. Mutans Streptococci counts.

Author, year	N of subject (probiotic/comparison)	PROBIOTIC		COMPARISON		INTERGROUP comparison (probiotic *vs* control)
		Delivery	INTRAGROUP comparison (baseline *vs* follow-up)	Comparison	INTRAGROUP comparison (baseline *vs* follow-up)	
Ahola et al., 2002[51]	74 (38/36)	Cheese	na	Placebo	na	p=NS at 3 w. p=0.04 at 6 w.
Alp et al., 2018[42]	45 (15 at group)	Toothpaste	p<0.01 at 3 w. p<0.01 at 6 w.	No treatment	p=0.16 at 3 w. p=0.05 at 6 w.	p<0.01
		Yogurt	p<0.01 at 3 w. p<0.01 at 6 w.			P<0.01
Bhalla et al., 2015[26]	30 (15 at group)	Yogurt	p<0.01	Placebo	p=NS	p<0.01
Borrell García et al., 2021[60]	27 (12-15)	Lozenges	↑ p=na	Placebo	↑ p=na	p>0.05 at 2, 4, 6 w.
Caglar et al., 2005[39]	21 (x-over)	Yogurt	p<0.05	Placebo	p=NS	na
Caglar et al., 2006[40]	90 (30 at group)	Staw	p<0.05	Placebo	p=NS	na
		Tablet	p<0.01		p=NS	na
Caglar et al., 2007[38]	80 (20 at group)	Chewing gum	p<0.05	Placebo	p=NS	p<0.05
Caglar et al., 2008[41]	20 (10 at group)	Lozenges	p=0.02	Placebo	p=NS	p=0.02
Caglar et al., 2008[37]	24 (x-over)	Ice cream	p<0.01	Placebo	p=0.09	na
Chinnappa et al., 2013[27]	30 (10 at group)	Ice cream	↓ p=na	Placebo	↑ p=na	p<0.01
		Yogurt	↓ p=na			p<0.01
Chuang et al., 2011[68]	78 (42/36)	Lozenges	p=0.40 at 2 w. p=0.08 at 4 w.	Placebo	p=0.80 at 2 w. p=1.00 at 4 w.	p=0.88 at 2 w. p=0.22 at 4 w.
Cildir et al., 2009[43]	24 (x-over)	Ice cream	p<0.05	Placebo	p=NS	na
Ferrazzano et al., 2011[61]	84 (42 at group)	Yogurt	p<0.01 at 1, 2 w.	Placebo	p=NS at 1, 2 w.	OR=6.60 at 1 w. OR=4.89 at 2 w.
Gizani et al., 2016[63]	85 (42/43)	Lozenges	p=NS	Placebo	p=NS	p=na
Jose et al., 2013[28]	60 (20 at group)	Toothpaste	p=0.05*	No treatment	p=0.70*	p<0.01*
		Yogurt	p=0.05*		p=0.70*	p<0.01*
Jothika et al., 2015[29]	26 (13 at group)	Mouthwash	↑ p=NS at 1, 2 4 w. *	Placebo	↑ p<0.001 at 1, 2 4 w.*	p<0.05 at 1, 2 4 w.*
Juneja and Kakade, 2012[30]	40 (18 at group)	Milk	↓ p<0.01 at 3 w. p<0.01 at 6 w.	Placebo	↓ p=0.38 at 3 w. p<0.01 at 6 w.	p=0.005 at 3 w. p<0.01 at 6 w.
Keller and Twetman, 2012[53]	18 (x-over)	Lozenges	p=NS	Placebo	p=NS	p=na
Keller et al., 2012[54]	62 (32/30)	Mouthwash	p=NS at 6, 12 w.	Placebo	p=NS at 6, 12 w.	p=NS at 6, 12 w.

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Koopaie et al., 2019[65]	40 (x-over)	Cake	↓	Placebo	↓	na
Lewiyonah et al.,2025[70]	40 (20 at group)	Tablets	↑ p=NS	No treatment	↓ p<0.01	na
Mishra and Saurabh, 2024[31]	40 (20 at group)	Lozenges	↓ p=na	No treatment	↓ p=na	p=0.001
Montalto et al., 2004[64]	35 (16/14/5)	Bottle	p=NS	Placebo	p=NS	p=na
		Capsule	p=NS			p=na
Mortazavi and Akhlaghi, 2012[18]	60 (29/31)	Cheese	p<0.01	Placebo	p=0.16	p=0.46
Nagarajappa et al., 2015[32]	30 (15 at group)	Ice cream	↓ p<0.01	Placebo	p=NS	p<0.05
Nitya et al., 2025[33]	20 (10 at group)	Mouthwash	p<0.01	No treatment	p=0.48	na
Pahumunto et al., 2019[45]	40 (20 at group)	Milk	↓ p<0.01 at 12 w. p<0.01 at 24 w. p<0.01 at 52 w.	Placebo	↑ p<0.01 at 12 w. p=0.04 at 24 w. p=0.02 at 52 w.	p<0.01 at 12, 24, 52 w.
Pahumunto et al., 2020[44]	123 (62/61)	Milk	↓ p<0.05 at 4, 8 w.	Placebo	p=NS	p<0.05 at 4, 8 w.
Pinto et al., 2014[69]	26 (x-over)	Yogurt	↓ p=NS ↓ p=NS*	Placebo	↓ p=NS ↓ p=NS*	p=NS p=NS*
Poureslami et al., 2013[66]	25 (x-over)	Yogurt	↓ p<0.01	Placebo	p=NS	p<0.01
Ritthagol et al., 2014[46]	30 (15 at group)	Milk	↓ p<0.01	Placebo	↑ p=NS	p<0.001
Romani Vestman et al., 2013[55]	62 (32/30)	Lozenges	↑ p<0.05 at 1, 6 12, 24 w. (p=NS subjects by *L. reuteri*)	Placebo	↑ p<0.05 at 1, 6 12, 24 w	p=NS at 1, 6 12, 24 w.
Singh et al., 2011[34]	14 (x-over)	Ice cream	p<0.01	Placebo	p=NS	p=na
Srivastava et al., 2016[35]	60 (30 at group)	Yogurt	↓ p<0.05	Placebo	p=NS	p=0.01
Teanpaisan and Piwat, 2014[48]	37 (20/17)	Milk	↓ p<0.01 at every follow-up	Placebo	↓ p=0.05 at 2 w. p=NS at 1, 2, 4 w.	na
Teanpaisan et al., 2015[47]	122 (59/63)	Milk	↓ p<0.01 at 12. 24. 36 w. p=NS at 52 w.	Placebo	p=NS at 12, 24 w. ↑ p=0.01 at 36, 52 w.	p<0.01 at 12 w. p=0.01 at 24 w. p=NS at 36, 52 w.
Teanpaisan et al., 2024[49]	121 (61/60)	Lozenges	↓ p<0.05 at 4 w. p=NS at 8 w.	Placebo	↑ p=na	p< 0.05 at 4, 8 w.
Toiviainen et al., 2015[58]	60 (29/31)	Lozenges	↓ p=NS	Placebo	↑ p=NS	p=NS
Wattanarat et al., 2015[71]	60 (30 at group)	Milk	↓ p<0.01 at 12 w p<0.01 at 24 w. p=NS at 52 w.	Placebo	p=NS at 12, 24 52 w.	p<0.01at 12 w. p<0.01 at 24 w. p=NS at 52 w.
Yousuf et al., 2015[72]	33 (11 at group)	Mouthwash (A)	↓ p=0.02 at 1, 2, 3 w.	Placebo	↓ p=0.02 at 1, 2, 3 w.	na
		Mouthwash (B)	↓ p=0.02 at 1, 2 w. p=NS at 3 w.			na
Zare Javid et al., 2020[67]	66 (33 at group)	Yogurt	↓ p<0.01	Placebo	↓ p=NS	p<0.01

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NS: not significant; na: not available; w.: weeks. Time was standardized to weeks across all studies to enable comparability

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Table 3. Summary of the results of studies evaluating the influence of probiotics on salivary Lactobacilli counts.

Author, year	N of subject (probiotic/comparison)	PROBIOTIC		COMPARISON		INTERGROUP comparison (probiotic *vs* control)
		Delivery	INTRAGROUP comparison (baseline *vs* follow-up)	Comparison	INTRAGROUP comparison (baseline *vs* follow-up)	
Alp and Baka et, 2018[42]	45 (15 at group)	Toothpaste	p=NS at 3 w. p=0.06 at 6 w.	No treatment	p=NS at 3, 6 w.	p=NS at 3, 6 w.
		Yogurt	p=0.01 at 3 w. p=NS at 6 w			p=NS at 3, 6 w.
Borrell García et al., 2021[60]	27 (12-15)	Lozenges	na	Placebo	na	p=NS at 2, 4 w.
Caglar et al., 2005[39]	21 (x-over)	Yogurt	p=NS	Placebo	p=NS	na
Caglar et al., 2006[40]	90 (30 at group)	Straw	p=NS	Placebo	p=NS	na
		Tablet	p=NS	Placebo	p=NS	na
Caglar et al., 2008[41]	20 (10 at group)	Lozenges	p=NS	Placebo	p=NS	p=NS
Caglar et al., 2008[37]	24 (x-over)	Ice cream	p<0.01	Placebo	p=NS	na
Chuang et al., 2011[68]	78 (42/36)	Lozenges	p=NS at 2, 4 w.	Placebo	p=NS at 2, 4 w.	p=NS 2, 4 w.
Cildir et al., 2009[43]	24 (x-over)	Ice cream	p=NS	Placebo	p=NS	na
Ferrazzano et al., 2011[61]	84 (42 at group)	Yogurt	p=NS at 1, 2 w.	Placebo	p=NS at 1, 2 w.	p=NS at 1, 2 w.
Gizani et al., 2016[63]	85 (42/43)	Lozenges	p<0.05	Placebo	p<0.05	p=NS
Keller and Twetman, 2012[53]	18 (x-over)	Lozenges	p<0.05	Placebo	p=NS	na
Montalto et al., 2004[64]	35 (16/14/5)	Bottle	p=0.02	Placebo	p=NS	na
		Capsule	P<0.01	Placebo	p=NS	na
Mortazavi and Akhlaghi, 2012[18]	60 (29/31)	Cheese	p=NS	Placebo	p=NS	p=NS
Nagarajappa et al., 2015[32]	30 (15 at group)	Ice cream	p=NS	Placebo	p=NS	p=NS
Pahumunto et al., 2019[45]	40 (20 at group)	Milk	p<0.05 at 12, 24, 52 w.	Placebo	p=NS at 12, 24, 52 w.	p<0.01 at 12, 24, 52 w.
Pahumunto et al., 2020[44]	123 (62/61)	Milk	p<0.05 at 4, 8 w.	Placebo	p=NS at 4, 8 w.	p<0.05 at 4, 8 w.
Pinto et al., 2014[69]	26 (x-over)	Yogurt	p=NS*	Placebo	p=NS*	p=NS*
Ritthagol et al., 2014[46]	30 (15 at group)	Milk	p<0.01 at 1, 2, 3, 4 w.	Placebo	p=NS at 1, 2, 3, 4 w.	p<0.01 at 1, 2, 3, 4 w.
Romani Vestman et al., 2013[55]	62 (32/30)	Lozenges	p< 0.05 at 1, 6 w., 3, 6 mo.	Placebo	p<0.05 at 1, 6, 12, 24 w.	p<0.05 at 1 w. p=NS at 6, 12, 24 w.

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Romani Vestman et al., 2015[56]	41 (21/20)	Lozenges	p<0.01 at 4, 12 w. p<0.05 at 8 w. p=NS at 16 w.	Placebo	p=NS at 4, 8, 12, 16 w.	p<0.01 at 4 and 12 w. p<0.05 at 8 w. p=NS at 16 w.
Singh et al., 2011[34]	14 (x-over)	Ice cream	p=NS	Placebo	p=NS	na
Sinkiewicz et al., 2010[57]	23 (11/12)	Chewing gum	p<0.01 at 16 w. p=NS at 12 w..	Placebo	P<0.01 at 12 w. p=NS at 16 w.	p=0.01 at 16 w. p=NS at 12 w.
Teanpaisan and Piwat, 2014[48]	37 (20/17)	Milk	p=NS at 1w. p<0.01 at 2, 3 w. p<0.05 at 4 w.	Placebo	p=NS at 1, 3, 4 w.; p<0.05 at 2 w.	p=NS at 1 w. p<0.05 at 2, 3, 4 w.
Teanpaisan et al., 2015[47]	122 (59/63)	Milk	p<0.01 at 3, 6, 9, 12 mo.	Placebo	p<0.05 at 12, 52 w.	p=0.03 at 12 w. P<0.01 at 24 w. p=0.04 at 36 w. p=0.03 at 52 w.
Teanpaisan et al., 2024[49]	121 (61/60)	Lozenges	p<0.05 at 4, 8 w.	Placebo	p=NS at 4, 8 w.	p< 0.05 at 4, 8 w.
Toiviainen et al., 2015[58]	60 (29/31)	Lozenges	p=NS	Placebo	p=NS	p=NS
Wattanarat et al., 2015[71]	60 (30 at group)	Milk	p<0.05 at 3, 6 mo. p<0.01 at 12 mo.	Placebo	p=NS 12, 24 52 w.	p<0.05 at 12, 24 w. p<0.01 at 52 w.
Zare Javid et al., 2020[67]	66 (33 at group)	Yogurt	p<0.01	Placebo	p=NS	P<0.01

NS: not significant; na: not available; w.: weeks. Time was standardized to weeks across all studies to enable comparability.
* Same results on plaque detection

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Table 4. Summary of the results of studies evaluating the influence of probiotics on buffer capacity of saliva and plaque pH (*).

Author, year	N of subject (probiotic/comparison)	PROBIOTIC		COMPARISON		INTERGROUP comparison (probiotic *vs* control)
		Delivery	INTRAGROUP comparison (baseline *vs* follow-up)	Comparison	INTRAGROUP comparison (baseline *vs* follow-up)	
Alforaidi et al., 2021[52]	28 (13/14)	Mouthwash	p<0.05 at every follow-up*	Placebo	p=NS at 1, 3 w.*	p=NS at 1 w.* p=0.04 at 3 w.*
Alp and Baka et, 2018[42]	45 (15 at group)	Toothpaste Yogurt	p=0.03 at 3 w. p=0.01 at 6 w. p=0.31 at 3, 6 w.	No treatment	p=NS at 3, 6 w.	na na
Altamura et al., 2025[59]	30 (15 at group)	Lozenges	p=NS at 4, 6 w.	Placebo	p=NS at 4, 6 w.	p=NS at 4 w. p<0.05 at 6 w.
Borrell García et al., 2021[60]	27 (12-15)	Lozenges	na	Placebo	na	p=NS at 1, 2, 3, 4, 6 w.
Chuang et al., 2011[68]	78 (42/36)	Lozenges	p=NS	Placebo	p=NS	p=NS
Ferrer et al., 2020[62]	59 (26/26)	Gel	p=NS at 2 w p< 0.05 at 4 w p< 0.05 at 6 w	Placebo	p=NS at 2, 4 6 w.	p=NS at 2, 4, 6 w.
Koopaie et al., 2019[65]	40 (x-over)	Cake	p=0.07	Placebo	p=NS	p=NS
Lewiyonah et al.,[70]	40 (20 at group)	Tablets	↑p=0.04	No treatment	↓p=0.02	na
Mishra and Saurabh, 2024[31]	40 (20 at group)	Lozenges	p<0.01	No treatment	p=NS	P<0.01
Pahumunto et al., 2020[44]	123 (62/61)	Milk	na	Placebo	na	p=NS at 4 w. p<0.05 at 8 w.
Ritthagol et al., 2014[46]	30 (15 at group)	Milk	p=N	Placebo	p=NS	p=NS

NS: not significant; na: not available; w.: weeks.

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Declaration of Interest Statement

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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