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Nitrite and Oral *Lactobacillus*: A Systematic Review of Antibacterial Effects and Clinical Associations

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ABSTRACT

Nitrite has been proposed as an endogenous oral antimicrobial factor, but its relationship with caries-associated *Lactobacillus* remains unclear. This systematic review evaluated the antibacterial effects of nitrite on oral *Lactobacillus* and associations between salivary nitrite-related measures and bacterial levels. PubMed, Web of Science, and Scopus were searched through 5 August 2026, supplemented by citation searching and exploratory semantic search. Eligible studies included *in vitro* experiments and cross-sectional studies reporting *Lactobacillus*-specific outcomes. Risk of bias was assessed using QUIN and JBI checklists. Findings were synthesized narratively because of methodological heterogeneity. Five studies were included: three *in vitro* experiments and two cross-sectional studies. All experimental studies reported inhibition of *Lactobacillus*, with two demonstrating greater inhibition at lower pH and higher nitrite concentrations. One disk-diffusion study reported concentration-dependent inhibition by potassium nitrite and greater inhibition when combined with fluoride. Clinical findings differed: one study reported an adjusted inverse association between salivary nitrite and *Lactobacillus* levels, whereas the other found no significant difference in bacterial counts between comparison groups. Experimental studies had moderate risk of bias; observational studies had moderate and high risk, respectively. Limitations included a small, heterogeneous evidence base and single-reviewer screening and extraction. Nitrite inhibits oral *Lactobacillus* under controlled experimental conditions, particularly when acidified, but clinical evidence does not establish causality or effectiveness for caries prevention. Prospective studies and multispecies biofilm models are needed.

Keywords: Nitrite; *Lactobacillus*; dental caries; oral microbiome; antibacterial activity; systematic review

INTRODUCTION

Dental caries is the result of a dynamic imbalance between demineralization and remineralization processes in tooth enamel within the oral environment. It is one of the most prevalent chronic diseases worldwide, affecting individuals across all age groups, particularly children and vulnerable populations. If left untreated, dental caries can significantly impair quality of life [1,2]. Among schoolchildren, greater caries experience has also been associated with poorer oral health-related quality of life [3]. According to the WHO (2022), more than one-third of the global population is affected by dental caries, with untreated dental caries in permanent teeth being the most prevalent health condition worldwide and affecting more than 2 billion people. In primary teeth, dental caries is the most common chronic disease of childhood, affecting approximately 514 million children worldwide [4].

Dental caries arises from the interaction of several factors within the oral environment, including a susceptible host, cariogenic microorganisms, a suitable substrate, and time [1]. Among these factors, cariogenic microorganisms, particularly acidogenic bacteria, play a crucial role in the initiation and progression of dental caries [5]. Through the fermentation of dietary carbohydrates, these bacteria produce organic acids that lower local plaque pH and promote enamel demineralization [6]. *Streptococcus mutans* is one of the most extensively studied cariogenic bacteria and is strongly associated with the initiation of dental caries because of its ability to form biofilms and rapidly produce organic acids [7,8]. In a study of 50 children aged 6–12 years with dental caries, *S. mutans* was detected in 94% of participants, highlighting its frequent occurrence in carious lesions [9]. However,

as carious lesions progress and become deeper, *Lactobacillus* spp., including *Lactobacillus acidophilus*, become increasingly associated with lesion progression [5].

Lactobacillus spp. is important acidogenic and aciduric bacteria associated with the progression of carious lesions [1,5]. Their ability to tolerate low pH and produce lactic acid facilitates their persistence in deep carious lesions and may contribute to lesion progression [10,11]. In children with early childhood caries (ECC), *Lactobacillus* spp. levels are significantly higher in saliva and dental plaque than in caries-free children, indicating their potential as markers of caries activity [12]. In addition, their ability to adhere to collagen and form biofilms through exopolysaccharide (EPS) production may contribute to their cariogenic potential [13-15].

Beyond bacterial and dietary factors, salivary chemistry also plays a modulatory role in the oral microbial ecosystem. Dietary nitrate, abundant in vegetables and leafy greens, is concentrated in saliva via the enterosalivary circulation and subsequently reduced to nitrite by nitrate-reducing oral commensal bacteria [16,17]. In oral *Veillonella* species, nitrite production from nitrate is linked to lactate metabolism and is enhanced under acidic and anaerobic conditions in the presence of lactate [18]. Under the acidic conditions typical of cariogenic biofilms, nitrite can be further converted to nitric oxide (NO), a reactive antimicrobial molecule [16]. This nitrate–nitrite–NO pathway has been proposed as an endogenous host-defense mechanism against cariogenic bacteria, with several in vitro and clinical studies demonstrating an inverse relationship between salivary/plaque nitrite concentration and the growth or survival of *Streptococcus mutans* [16,19-23].

In contrast, evidence regarding the relationship between nitrite and *Lactobacillus*, a genus increasingly implicated in the progression rather than initiation of carious lesions, remains considerably more limited and inconsistent across both experimental and clinical contexts. *In vitro* studies have reported inhibitory effects of nitrite on *Lactobacillus* species, including *L. acidophilus* and *L. casei*. Acidified sodium nitrite showed pH- and concentration-dependent activity in broth-based models, whereas potassium nitrite also inhibited *L. acidophilus* in a disk-diffusion model [19,24,25]. Clinical evidence on whether salivary or plaque nitrite concentration is similarly associated with reduced *Lactobacillus* counts remains scarce [16,26]. Given the established role of *Lactobacillus* in caries progression and the incompletely characterized nature of its interaction with nitrite across experimental and clinical settings, a systematic evaluation of the existing evidence is warranted. This review therefore aims to systematically assess whether nitrite, through direct experimental exposure or endogenous salivary/plaque concentration, correlates with reduced *Lactobacillus* growth or counts, and to examine whether this relationship is comparable to the established inverse relationship between nitrite and *S. mutans*. This systematic review was conducted following the PRISMA 2020 guidelines to ensure a transparent and reproducible synthesis of the available evidence [27].

METHODS AND ANALYSIS

Eligibility Criteria

Eligibility was defined according to the PICO framework, summarized in Table 1.

- *Population:* human saliva or plaque samples (for clinical/observational studies) or reference/ laboratory strains of oral cariogenic bacteria (for *in vitro* studies), with a specific focus on *Lactobacillus* spp., including *L. acidophilus* and *L. casei*.
- *Exposure:* nitrite, nitrate, or nitric oxide (NO), whether administered experimentally under controlled *in vitro* conditions or measured endogenously in saliva or dental plaque.
- *Comparator:* where applicable, lower or absent nitrite/nitrate/NO exposure, such as untreated controls in *in vitro* studies or lower-concentration /nitrite-negative groups in clinical studies. As several included clinical studies were descriptive or correlational in design without a predefined comparator group, studies without an explicit comparator were also eligible provided that a quantitative or semi-quantitative measure of nitrite/nitrate/NO exposure was reported.
- *Outcome: Lactobacillus* growth, survival, colony-forming unit (CFU) counts, or salivary/plaque *Lactobacillus* levels.
- *Study design: in vitro* controlled experimental studies and cross-sectional observational/clinical studies were both eligible for inclusion.

TABLE 1: PICO Framework for Study Eligibility.

PICO Element	Definition
Population	Human saliva/plaque samples (clinical/observational studies) or reference/laboratory strains of oral cariogenic bacteria (*in vitro* studies), with a specific focus on *Lactobacillus* spp., including *L. acidophilus* and *L. casei*
Exposure	Nitrite, nitrate, or nitric oxide (NO), administered experimentally under controlled *in vitro* conditions or measured endogenously in saliva or dental plaque
Comparator	Untreated controls (*in vitro*) or lower-concentration/nitrite-negative groups (clinical); studies without a formal comparator were also eligible provided nitrite/nitrate/NO exposure was reported quantitatively or semi-quantitatively
Outcome	*Lactobacillus* growth, survival, colony-forming unit (CFU) counts, or salivary/plaque *Lactobacillus* levels
Study Design	*In vitro* controlled experimental studies and cross-sectional observational/clinical studies

Exclusion Criteria
Studies were excluded if they: (1) evaluated nitrite/nitrate/NO exclusively in relation to Streptococcus mutans or other cariogenic bacteria without any measurement of Lactobacillus; (2) evaluated nitrite/nitrate/NO and caries indices without any bacterial outcome; (3) were conducted in non-oral systems (e.g., gastric, intestinal, vaginal, or systemic models); (4) were narrative or systematic reviews/meta-analyses (retained as background references rather than primary data sources); (5) were not published in English; or (6) were not original research articles.

Information Sources and Search Strategy
A systematic search was conducted across three electronic databases: PubMed, Web of Science, and Scopus, supplemented by citation searching and semantic search. Searches across all databases were last conducted on 5 August 2026. No date restriction was applied to publication year.

PubMed
Two complementary search strings were used to maximize sensitivity, and their results were combined and deduplicated:

- Query 1 (PICO/caries-focused): ("salivary nitrite" OR "nitrite" OR "nitrite concentration" OR "endogenous nitrite" OR "Nitrites"[Mesh]) AND ("Lactobacillus" OR "lactobacilli count" OR "*Lactobacillus* acidophilus" OR "bacterial load" OR "Colony Count, Microbial"[Mesh]) AND ("dental caries" OR "caries activity" OR "dmft" OR "DMFT" OR "Dental Caries"[Mesh]) — 4 records retrieved.
- Query 2 (broader oral/biofilm context): nitrite AND *lactobacillus* AND (oral OR dental OR saliva OR "oral cavity" OR biofilm OR plaque) — 15 records retrieved.

Web of Science
TS= (nitrite AND *lactobacillus* AND (oral OR dental OR saliva OR "oral cavity" OR biofilm OR plaque)) — 18 records retrieved.

Scopus
TITLE-ABS-KEY (nitrite AND *lactobacillus* AND (oral OR dental OR saliva OR "oral cavity" OR biofilm OR plaque)) — 37 records retrieved; after applying database filters for document type (article only) and language (English only), 33 records remained for screening.

Supplementary and Citation Searching
Reference lists and the "similar articles" function associated with key included studies were manually reviewed, identifying 4 additional candidate records.

Semantic Search
Scopus AI was additionally queried using a natural-language prompt reflecting the research question, as an exploratory supplementary check. This did not identify any eligible studies beyond those already retrieved through the Boolean search strategy.

All searches were restricted to original research articles published in English. No date restriction was applied.

Study Selection Process
Records retrieved from all sources were combined, and duplicates were removed. Titles and abstracts were screened against the predefined eligibility criteria. Full-text reports of potentially eligible records were retrieved and assessed for eligibility. Studies were excluded if they did not evaluate the exposure of interest, did not report extractable Lactobacillus-specific outcome data, did not involve an eligible oral population or experimental model, or were not primary research reports. Studies evaluating Streptococcus mutans only, without Lactobacillus-specific data, were also excluded. The study selection process and reasons for full-text exclusion are presented in the PRISMA 2020 flow diagram. Screening and eligibility assessment were performed by a single reviewer. The absence of independent duplicate screening and dual data extraction is acknowledged as a methodological limitation of this review and is discussed further in the Discussion section.

Data Extraction
Data from included studies were extracted by a single reviewer using a standardized extraction form, covering study identification (authors, year, country/setting), study design, population or sample characteristics (including species/strain for *in vitro* studies or participant demographics for clinical studies), exposure and measurement method for nitrite, nitrate, or NO (e.g., Griess assay, dry-test kit, experimental concentration), bacterial outcome measures (e.g., colony-forming units, optical density, inhibition zone diameter, or salivary/plaque *Lactobacillus* levels), caries-related outcomes where reported (e.g., DMFT, caries experience), and main findings relevant to the review question. Extracted data were compiled into a structured evidence table (Table 2 and Supplementary Table S1) to facilitate narrative synthesis grouped by study design.

Risk of Bias Assessment
Risk of bias in included *in vitro* studies was assessed using the QUIN (Quality Assessment Tool for *In Vitro* Studies) checklist, comprising 12 criteria scored as 0 (not specified), 1 (inadequately specified), or 2 (adequately specified). The overall QUIN score was calculated as [total score / (2 × 12)] × 100, with scores >70% classified as low risk of bias, 50–70% as moderate risk, and <50% as high risk of bias. Risk of bias in included observational/clinical studies was assessed using the JBI Critical Appraisal Checklist for Analytical Cross-Sectional Studies, comprising 8 items rated as Yes, No, or Unclear. Consistent with JBI guidance, no aggregate numerical score was calculated; overall risk of bias (low, moderate, or high) was instead judged qualitatively based on the pattern of domain-level responses [28,29]. Risk of bias assessments were performed by a single reviewer.

Synthesis Methods
Given the small number of included studies (n = 5) and substantial heterogeneity in study design, exposure measurement methods, and outcome definitions,

a quantitative meta-analysis was not performed. Findings were instead synthesized narratively, grouped by study design (*in vitro* vs. clinical/ observational), to characterize the direction and consistency of the relationship between nitrite/ nitrate/NO and *Lactobacillus* outcomes.

RESULTS

Study Selection

The database search identified 16 unique records from PubMed (4 + 15, after removing 3 within-database duplicates), 18 from Web of Science, and 37 from Scopus (33 after document type/language filtering). An additional 4 records were identified through supplementary citation searching. After combining all sources (71 records), removing 7 cross-database duplicates and 4 non-English sources, 60 unique records were screened by title and abstract. Of the 60 screened records, 53 were excluded at the title/abstract stage. Seven records identified through database searching and four records identified through citation searching were assessed in detail for eligibility (11 records in total). Of these, two database-derived records were excluded because they did not report Lactobacillus-specific outcomes. One of these studies, which focused exclusively on S. mutans, was retained as a background reference for the Introduction and Discussion. All four citation-searching-derived records were excluded because they lacked Lactobacillus-specific outcome data. Five studies met all eligibility criteria and were included in the qualitative synthesis.

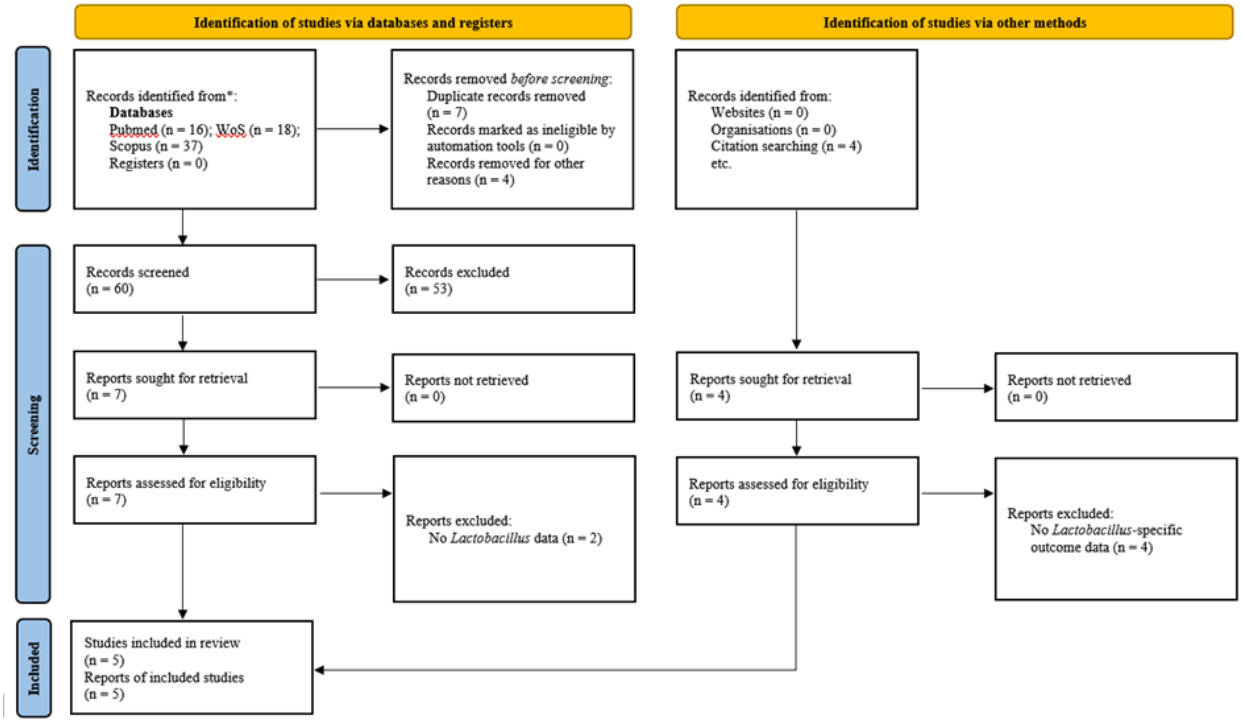


FIGURE 1: PRISMA 2020 Flow Diagram.

Study Characteristics

The five included studies comprised three *in vitro* experimental studies and two cross-sectional observational studies, conducted in the United Kingdom, China, South Korea, and Indonesia between 1999 and 2025.

TABLE 2: Overview of the Results.

Study ID	Design	Setting	Key exposure	Key outcome(s)
Xia *et al.*, 2006 [24]	*In vitro*	China	Sodium nitrite/ nitrate, pH 3.0–7.0	Growth inhibition (OD540), MIC/MBC for 6 oral strains including *L. acidophilus*
Silva Mendez *et al.*, 1999 [19]	*In vitro*	UK	Sodium nitrite, pH 2.0–7.0	Growth/survival of *S. mutans*, *A. naeslundii*, *L. casei*
Wicaksono *et al.*, 2025 [25]	*In vitro*	Indonesia	Potassium nitrite ± sodium fluoride	Inhibition zone diameter against *L. acidophilus*
Doel *et al.*, 2004 [16]	Cross-sectional	UK	Salivary nitrate/nitrite, nitrate-reductase capacity	*S. mutans* and *Lactobacillus* CFU, caries experience
Han *et al.*, 2013 [26]	Cross-sectional	South Korea	Salivary nitrite (Griess assay, labeled "NO")	Salivary *Lactobacillus* and *S. mutans* levels (Dentocult), DMFT/DT

Across the *in vitro* studies, nitrite demonstrated inhibitory activity against the tested oral bacteria [19,24,25]. In the two studies that manipulated pH, acidified nitrite produced dose- and pH-dependent inhibition; *L. acidophilus* was relatively more resistant than S. mutans in the study that directly compared them [19,24]. The two clinical studies differed in their exposure assessment, outcome measurement, and analytic approach. Doel et al. measured salivary nitrate and nitrite together with nitrate-reducing capacity and cultured *Lactobacillus* as CFU/mL, whereas Han et al. used a Griess assay to quantify nitrite-related activity and categorized salivary *Lactobacillus* levels using the Dentocult system [16,26]. Detailed characteristics and findings of the included studies are presented in Supplementary Table S1.

Risk of Bias in Included Studies

- *In Vitro Studies (QUIN)*

All three *in vitro* studies were rated as moderate risk of bias (QUIN scores 50.0–54.2%). Common strengths included clearly stated aims, well-described control groups and methodology, appropriate outcome measurement, and adequate statistical analysis. Common limitations included the absence of sample-size calculations, operator/assessor details, randomization, and blinding. Although some of these domains may be more challenging to implement in single-strain *in vitro* microbiological assays, their absence can still affect the transparency and internal validity of the reported findings.

- *Observational Studies (JBI)*

Risk of bias varied between the two observational studies. Han et al. (2013) was judged to have moderate risk of bias, with adequate reporting across most domains (inclusion criteria, confounding factor identification and adjustment, and outcome/statistical analysis), except for unclear validity of the nitrite/NO exposure measure [26]. Doel et al. (2004) was judged to have high risk of bias, driven primarily by inadequately reported eligibility criteria, lack of confounding adjustment, and insufficiently described caries and outcome assessment methods [16].

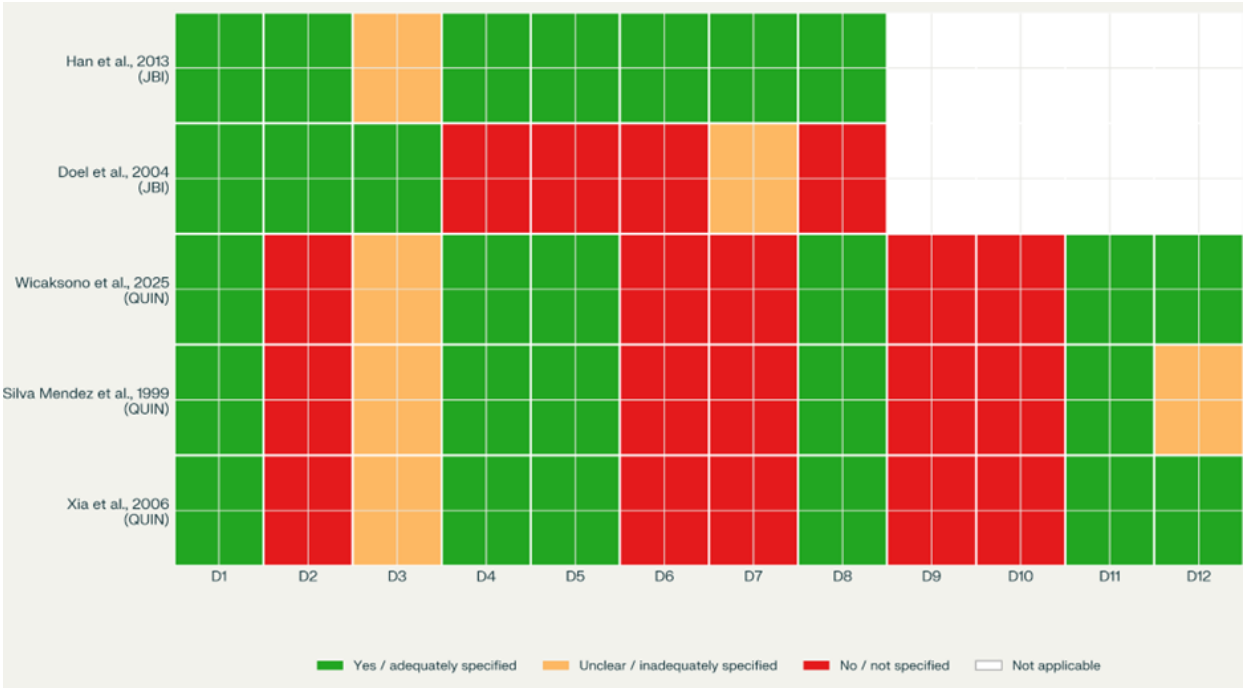


FIGURE 2: Risk-Of-Bias Assessment of Included Studies. The JBI Critical Appraisal Checklist for Analytical Cross-Sectional Studies Was Applied to Observational Studies (Domains D1–D8), and the Quin Tool Was Applied to *In Vitro* Studies (Domains D1–D12). Green Indicates "Yes" Or Adequately Specified, Orange Indicates "Unclear" Or Inadequately Specified, Red Indicates "No" Or Not Specified, And White Cells Indicate Domains Not Applicable to That Assessment Tool.

Synthesis of Results

- *In Vitro Evidence*

Three studies demonstrated inhibitory activity of nitrite against Lactobacillus species under controlled experimental conditions. In the two broth-based studies that manipulated pH, acidified sodium nitrite exerted bacteriostatic and/or bactericidal effects, with inhibition increasing at lower pH and higher nitrite concentrations. Lactobacillus acidophilus and *L. casei* were inhibited under these experimental conditions, although *L. acidophilus* appeared relatively more resistant than S. mutans in the study that compared them directly [19,24]. A third study reported concentration-dependent inhibition of *L. acidophilus* by potassium nitrite in a disk-diffusion model and found greater inhibition when nitrite was combined with fluoride than with either low-dose agent alone [25].

- *Clinical Evidence*

Only two cross-sectional studies met the eligibility criteria. Although both studies assessed salivary nitrate/nitrite-related measures and Lactobacillus outcomes, they differed substantially in exposure definition, outcome measurement, and analytic approach. Doel et al. (2004) reported that the combination of high salivary nitrate concentration

and high nitrate-reducing capacity was associated with lower caries experience; however, the study did not report a statistically significant difference in Lactobacillus counts between groups [16]. Han et al. (2013) reported that higher salivary nitrite concentrations, labeled by the authors as "NO," were associated with lower salivary Lactobacillus levels after adjustment for confounders (□=0.049). Han et al. found no significant association between nitrite-related measures and S. mutans levels or caries experience (DMFT/DT) [26].

Overall, the *in vitro* evidence consistently supports an inhibitory effect of nitrite on Lactobacillus growth under controlled experimental conditions, with the strongest evidence for acidified nitrite in the two broth-based studies. In contrast, clinical evidence for an inverse association between salivary nitrite-related measures and Lactobacillus levels is limited, based primarily on one cross-sectional study, and remains insufficient for firm conclusions regarding clinical relevance or active caries status.

DISCUSSION

Principal Findings

The present systematic review identified five eligible studies, comprising three *in vitro* experimental studies and two cross-sectional observational studies. Overall, the findings indicate that nitrite has inhibitory activity against Lactobacillus species under controlled *in vitro* conditions, with the strongest evidence for acidified nitrite in the two broth-based experimental studies. Across the experimental studies, nitrite inhibited the growth or survival of *L. acidophilus* and *L. casei* [19,24,25]. In the two broth-based studies, Silva Mendez et al. and Xia et al. manipulated pH and reported dose- and pH-dependent inhibition by acidified nitrite; in Xia et al., *L. acidophilus* was relatively more resistant than S. mutans [19,24].

In contrast, the available clinical evidence was limited and did not permit a firm conclusion regarding the association between salivary nitrite-related measures and Lactobacillus levels *in vivo*. Han et al. reported an inverse association between salivary nitrite concentrations, labeled by the authors as "NO," and salivary Lactobacillus levels after adjustment for age, sex, salivary flow rate, and buffering capacity [26]. However, Doel et al. found lower caries experience among children with both high salivary nitrate concentrations and high nitrate-reducing capacity, but did not report a statistically significant difference in Lactobacillus counts between this group and the comparison group [16]. These clinical studies also differed in their exposure definitions, outcome measurement methods, and analytic approaches. Therefore, although experimental evidence supports an inhibitory effect of nitrite, particularly under acidified *in vitro* conditions, on Lactobacillus growth and survival, the clinical evidence remains preliminary and insufficient to establish an *in vivo* association or a clinically meaningful relationship with caries status.

Interpretation and Mechanisms

The inhibitory activity of acidified nitrite against Lactobacillus observed in the included *in vitro* studies is biologically plausible. Dietary nitrate is concentrated in saliva and can be reduced to nitrite by nitrate-reducing bacteria in the oral cavity [30]. When nitrite encounters an acidic environment, it forms nitrous acid, which can generate nitric oxide (NO) and other reactive nitrogen intermediates with antimicrobial activity [16,26]. This pathway is particularly relevant in cariogenic biofilms, where bacterial carbohydrate metabolism produces localized decreases in pH. Nitrite-derived antimicrobial activity may therefore represent a potential chemical host-defense mechanism acting in acidic oral niches.

The experimental findings support the importance of acidic conditions for nitrite activity. Silva Mendez et al. exposed *L. casei* to sodium nitrite at concentrations of 0.2–10 mM and pH values of 2.0–7.0, reporting dose-dependent inhibition of growth and impaired recovery after exposure [19]. An effect was evident at 0.2 mM, which the authors identified as approximating fasting salivary nitrite concentrations [19]. Similarly, Xia et al. observed that the inhibitory effect of sodium nitrite increased as pH decreased, whereas sodium nitrate at equivalent concentrations had no antimicrobial effect [24]. For *L. acidophilus*, the minimum inhibitory concentration of nitrite was 1 mM at pH 3.0, 2 mM at pH 4.0–4.5, and 10 mM at pH 5.0; no inhibition was observed at pH 6.0–7.0 at concentrations below 10 mM. These findings indicate that the effect of nitrite is conditional on both concentration and environmental acidity, rather than being a uniform property of nitrite across oral conditions [19,24].

The chemical basis of this pattern is likely related to acidification of nitrite. In acidic environments, nitrite can form nitrous acids and subsequently yield NO and related nitrogen oxides. NO and NO-derived species can act through nitrosative and oxidative stress, including interference with protein function, disruption of iron–sulfur clusters, and damage to nucleic acids and bacterial signaling pathways. However, neither Silva Mendez et al. nor Xia et al. directly measured nitrous acid, NO, or other reactive nitrogen species. Therefore, these mechanisms should be regarded as biologically plausible explanations, rather than mechanisms directly demonstrated by the included studies [31,32].

The relative resistance of *L. acidophilus* is also important when interpreting the *in vitro* evidence. Xia et al. ranked S. mutans as the organism most susceptible to acidified nitrite, whereas *L. acidophilus* was among the more resistant organisms tested. *L. acidophilus* also retained growth in acidic conditions without nitrite, supporting its recognized aciduric phenotype [24]. This pattern is compatible with the ability of acid-tolerant lactic acid bacteria to maintain intracellular pH through proton extrusion and other stress-response mechanisms, including the F0F1-ATPase system.

Such acid-adaptation mechanisms may contribute to persistence of *Lactobacillus* in low-pH carious environments and may reduce its apparent susceptibility to acidified nitrite [5,33].

Wicaksono et al. further demonstrated that potassium nitrite inhibited *L. acidophilus* in a concentration-dependent manner in a disk-diffusion model, with inhibition beginning at 0.85% potassium nitrite [25]. The authors also observed larger inhibition zones when nitrite was combined with sodium fluoride, including inhibition at a low-dose combination for which each agent alone produced no detectable zone. This finding may suggest complementary antibacterial activity; however, the study did not report medium pH, measure NO formation, assess biofilm behavior, or use clinically realistic salivary exposure conditions. It therefore supports the general antibacterial potential of nitrite against *L. acidophilus* but does not directly confirm an acidified nitrite or nitrate–nitrite–NO mechanism *in vivo* [25].

Taken together, the results suggest that nitrite-related effects may be selective rather than uniform across oral microorganisms. The evidence supports inhibition of *Lactobacillus* under simplified and often acidic experimental conditions, but it does not establish that comparable inhibition occurs within mature, multispecies carious biofilms. Similarly, reduced growth of S. mutans or nitrite-mediated effects in single-strain models cannot be assumed to translate directly to *Lactobacillus*, which appears relatively acid tolerant in the available experimental evidence.

Translational Gap between Experimental and Clinical Evidence

The consistent *in vitro* inhibitory activity of nitrite should not be interpreted as direct evidence of an equivalent clinical effect in the oral cavity. The experimental studies used simplified single-strain models and controlled exposure conditions. Silva Mendez et al. and Xia et al. acidified broth cultures with hydrochloric acid and exposed bacteria to defined sodium nitrite concentrations, allowing pH-dependent antimicrobial activity to be evaluated directly [19,24]. In contrast, Wicaksono et al. used a disk-diffusion model with potassium nitrite and did not report medium pH or quantify nitric oxide formation [25]. These approaches are useful for demonstrating antibacterial potential, but they do not reproduce the spatially heterogeneous pH, nutrient availability, oxygen gradients, salivary clearance, and multispecies interactions present in dental plaque biofilms [34].

The two clinical studies were also not designed to test causality. Doel et al. evaluated naturally occurring salivary nitrate/nitrite-related measures and nitrate-reducing capacity, whereas Han et al. used a Griess assay to quantify nitrite but described the measure as salivary “NO” [16,26]. Neither study prospectively manipulated nitrate or nitrite exposure, and both were cross-sectional. Consequently, reverse causation and residual confounding cannot be excluded. Dietary nitrate intake, oral hygiene, fluoride exposure, salivary flow and buffering capacity, plaque pH, antimicrobial use, socioeconomic factors, and the composition of the oral microbiome may influence both nitrite-related measures and *Lactobacillus* levels. The observed inverse association in Han et al. is therefore hypothesis-generating but cannot establish that higher salivary nitrite directly reduces *Lactobacillus* colonization or caries progression *in vivo* [16,26].

Strengths and Limitations

This review has several strengths. First, the search was conducted across three major bibliographic databases, PubMed, Web of Science, and Scopus, and was supplemented by citation searching and a semantic-search check. The search strategy included both a caries-focused query and a broader oral/biofilm query, thereby improving the likelihood of identifying evidence relevant to direct experimental nitrite exposure as well as salivary nitrate/nitrite-related measures. Study selection, data extraction, risk-of-bias assessment, and reporting were structured according to the PRISMA 2020 framework, and the study-selection process was reported using a PRISMA flow diagram [27].

Second, the review applied design-appropriate risk-of-bias instruments. The QUIN tool, which was developed and validated specifically for *in vitro* dental studies, was used to assess the three experimental studies, while the JBI Critical Appraisal Checklist for Analytical Cross-Sectional Studies was applied to the two observational studies. This approach allowed study limitations to be evaluated in relation to the distinct methodological requirements of controlled laboratory experiments and observational clinical research, rather than treating all included evidence as methodologically equivalent [28,29].

Several limitations should also be considered. The evidence base was small, comprising only five eligible studies, including three *in vitro* investigations and two cross-sectional observational studies. This limited number of studies, together with substantial heterogeneity in bacterial species, laboratory models, nitrite formulations, nitrite concentrations, pH conditions, exposure duration, salivary measurement methods, and *Lactobacillus* outcome definitions, precluded quantitative pooling and limited the certainty of the overall conclusions. The two clinical studies also differed materially: Doel et al. evaluated salivary nitrate, nitrite, and nitrate-reducing capacity alongside culture-based *Lactobacillus* CFU, whereas Han et al. used a Griess assay to measure nitrite but described the measure as salivary “NO” and categorized *Lactobacillus* using Dentocult [16,26].

The *in vitro* evidence should also be interpreted cautiously. Although all three experimental studies reported an inhibitory effect of nitrite against *Lactobacillus*, the models used reference strains and planktonic broth cultures or agar disk-diffusion methods rather than mature multispecies cariogenic biofilms.

In addition, only Silva Mendez et al. and Xia et al. evaluated acidified nitrite under controlled pH conditions; Wicaksono et al. did not report medium pH or measure nitric oxide formation [19,24,25]. The experimental results therefore demonstrate antibacterial potential, but cannot establish the magnitude, duration, or clinical relevance of any effect within the dynamic oral environment.

The certainty of the clinical evidence was additionally constrained by study design and risk of bias. Both clinical studies were cross-sectional, which prevents determination of temporal sequence and causality. Han et al. was judged to have moderate risk of bias, largely because the Griess assay measured nitrite rather than NO directly, whereas Doel et al. was judged to have high risk of bias because of incompletely reported eligibility criteria, limited information on caries and outcome assessment, and no reported adjustment for confounding [16,26]. Dietary nitrate exposure, fluoride use, oral hygiene, salivary flow, buffer capacity, plaque pH, medication or antibiotic use, socioeconomic conditions, and oral-microbiome composition may have influenced the observed measures and associations.

Finally, screening, eligibility assessment, and data extraction were conducted by a single reviewer, without independent duplicate screening or dual data extraction. This may have increased the risk of study-selection errors or extraction bias. The review was also limited to original research articles published in English, which may have introduced language and publication bias. These limitations, combined with the small and heterogeneous evidence base, mean that the conclusions should be interpreted as preliminary rather than definitive.

Clinical and Research Implications

The current evidence is insufficient to support the use of nitrate, nitrite, or nitrite–fluoride combinations as clinical interventions for caries prevention or for reducing oral *Lactobacillus* levels. Although the included *in vitro* studies indicate that nitrite can inhibit *Lactobacillus* under controlled experimental conditions, the available clinical evidence is limited to two cross-sectional studies and does not establish causality, effectiveness, optimal dosage, route of administration, safety, or long-term effects within the oral ecosystem. In particular, the inhibition observed with potassium nitrite and fluoride in a disk-diffusion model should not be interpreted as evidence that a nitrite–fluoride mouthrinse, toothpaste, or other topical product would be effective or safe in clinical use [25].

Future research should first clarify the clinical association between nitrate–nitrite metabolism and *Lactobacillus* in well-designed prospective longitudinal studies. Such studies should recruit clearly defined populations, document caries activity using standardized diagnostic criteria, and measure salivary and plaque nitrate, nitrite, and NO-related products separately using validated methods. Because the Griess reaction measures nitrite rather than nitric oxide directly, future studies should avoid using "NO" as an interchangeable term unless nitric oxide itself is measured using an appropriate direct method. Salivary/plaque *Lactobacillus* outcomes should also be reported using standardized quantitative methods, ideally alongside species-level molecular characterization, rather than relying exclusively on semi-quantitative culture categories.

Future studies should account for factors that can affect both nitrate–nitrite metabolism and caries-associated bacterial profiles. These include dietary nitrate intake, timing of food and drink consumption, oral hygiene practices, fluoride exposure, antimicrobial or antibiotic use, salivary flow rate, buffering capacity, plaque pH, socioeconomic conditions, and the composition of the oral microbiome. Repeated measurements over time would help establish whether higher nitrite-related measures precede reductions in *Lactobacillus* levels, or whether differences in bacterial ecology and oral-health status instead influence nitrate reduction and nitrite accumulation.

Experimental work should also move beyond single-strain planktonic culture and disk-diffusion models. Multispecies cariogenic biofilm models that include *Lactobacillus*, S. mutans, and nitrate-reducing commensals would allow researchers to examine whether nitrite affects microbial composition, acid production, pH dynamics, bacterial viability, and enamel demineralization under conditions that more closely resemble the oral cavity. Such studies should report pH, nitrate/nitrite concentrations, NO-related products, exposure duration, biofilm maturity, and clinically relevant comparator conditions. The potential interaction between nitrite and fluoride is worth further investigation, but it should be evaluated using dose-response experiments and safety assessments before any clinical application is considered.

CONCLUSION

This systematic review found that nitrite demonstrates inhibitory activity against *Lactobacillus* species *in vitro*. The strongest evidence for acidified nitrite was observed in broth-based experimental studies conducted under controlled low-pH conditions, while one disk-diffusion study also reported concentration-dependent inhibition by potassium nitrite. The available evidence indicates that *L. acidophilus* and *L. casei* can be inhibited by nitrite, although susceptibility varies according to nitrite concentration, pH, bacterial species, and experimental model. One *in vitro* study also reported that potassium nitrite combined with sodium fluoride produced greater inhibition of *L. acidophilus* than either low-dose agent alone; however, this finding was obtained using a single-strain disk-diffusion model and requires confirmation in clinically relevant biofilm systems.

In contrast, clinical evidence regarding the association between salivary nitrite-related measures and *Lactobacillus* levels is limited to two cross-sectional

studies and remains insufficient to establish causality or clinical relevance for caries prevention. Therefore, although acidified nitrite may have antibacterial potential against *Lactobacillus*, and nitrite–fluoride interactions warrant further investigation, well-designed prospective clinical studies and clinically relevant multispecies biofilm models are required before the role of nitrite in caries prevention or the potential application of nitrite-based interventions can be determined.

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