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Review Article

Potential role of *Lactobacillus reuteri* in the management of dental black stain: A systematic review of clinical and microbiological evidence

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Abstract

Dental black stain (DBS) is an atypical extrinsic pigmentation predominantly observed in youth. It is often associated with a low frequency of caries but with contemporary esthetic concerns. The aim of this systematic literature review was to evaluate the efficacy of *Lactobacillus reuteri* as a probiotic in the treatment and prevention of DBS. A systematic literature review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines. Studies were eligible if they reported original research investigating *Lactobacillus spp*. primarily *Lactobacillus reuteri* in relation to DBS, DBS-associated microorganisms, or relevant oral microbial and clinical outcomes. Observational, clinical intervention, and in vitro or ex vivo studies were eligible, while non-original publications and studies unrelated to *L. reuteri* or DBS were excluded. PubMed, Scopus, ScienceDirect, EBSCO, and SpringerLink were searched from inception to May 30, 2025, supplemented by reference-list screening. Risk of bias was independently assessed by two reviewers using design-appropriate interventions, experimental models, and outcome measures; studies were grouped into epidemiological or observational, clinical, and experimental evidence, with mechanistic findings synthesized across relevant studies using a qualitative narrative approach. The findings indicate that *L. reuteri* strains, such as DSM 17938, ATCC PTA 5289, LR08, and LC382415, demonstrate antimicrobial efficacy against chromogenic bacteria associated with DBS, including Streptococcus mutans, *Actinomyces* spp., and *Prevotella* spp. Reuterin and cell-free supernatants have been documented in vitro to reduce biofilm formation and pathogen viability. Clinical experiments showed a decrease in the recurrence and severity of black stain with the addition of *L. reuteri* after scaling. Moreover, its advantageous safety profile and regulation of oral microbiota suggest its potential as a long-term biofilm management approach. Although these results are encouraging, broad generalization is limited by variations in study design, probiotic dosage, and assessment measures. Metagenomic analyses and effective future clinical trials are necessary to understand microbiological processes and create standard procedures. In general, *L. reuteri* is a viable non-invasive adjunct for DBS management that improves oral microbial balance without having the negative side effects of traditional antimicrobials.

Keywords: Black-stain, *L. reuteri*, microbiota, biofilm, probiotic

Introduction

Dental black stain (DBS), an extrinsic discoloration that mostly affects children, is characterized by dark lines or dots that are usually found on the cervical third of the tooth crown. Clinically, it

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is recognized as a harmless condition, yet it can lead to esthetic concerns and social problems [1]. The pathophysiology of black stain is complicated and involves chromogenic bacteria like *Actinomyces* and *Prevotella* species producing hydrogen sulfide, which then reacts with salivary iron to generate deposits of ferric sulfide [2]. Black stain occurrence and dental caries prevalence have been shown to be uncorrelated in epidemiological research [3-5]. The results indicate that the microbial composition related to black stain may provide a defense against cariogenic bacteria [3-5]. The microbial ecosystem that forms biofilms with black stain is thought to either compete with or prevent the formation of species that cause dental cavities, including *Streptococcus mutans* [2].

Probiotics, particularly *Lactobacillus reuteri*, are expected to have a role in controlling oral microbiota. Reuterin and other bioactive substances produced by *L. reuteri* have shown antimicrobial properties [6]. Research has shown that *L. reuteri* can reduce pathogenic bacterial species, inhibit biofilm development, and prevent black stain from recurring after scaling [6-8]. Furthermore, research conducted in vitro and in clinical trials shows that *L. reuteri* has selective activity against oral infections without interfering with beneficial commensals [9]. Its ability to inhibit early colonizers such as *Streptococcus sanguinis* and its potential as an additional agent in root canal disinfection indicate its considerable relevance in dental biofilm management [10].

The aim of this systematic review was to evaluate the effectiveness of *Lactobacillus* strains, especially *L. reuteri*, in the management and prevention of DBS in children and to summarize the potential biological mechanisms underlying the effects.

Methods

Study design and literature search strategy

This systematic review aims to summarize the efficacy and mechanisms of action of *L. reuteri* in the treatment of DBS. The systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines using Patient, Intervention, Control, Outcome (PICO) based keywords in five electronic databases: PubMed, Scopus, ScienceDirect, EBSCO, and SpringerLink. The search included the period from inception to May 30, 2025. The keywords were arranged with various synonyms, including both text words and medical subject headings (MeSH Terms) for each PICO component: “probiotics”, “Lactobacillus”, “tooth”, and “black stains”. The search was supplemented by screening reference lists of relevant articles. The following Boolean keywords were used: ((“*Lactobacillus reuteri*” OR “*Limosillactobacillus reuteri*” OR “probiotic” OR “probiotics” OR “*L. reuteri*”) AND (“dental black stain” OR “black stain”).

Eligibility criteria

This systematic review included studies that met the following criteria: (1) original research articles; (2) full-text articles written in English; (3) studies investigating the application or effectiveness of *L. reuteri* as a cell or containing in probiotics in relation to DBS, including its effects on DBS deposits or recurrence, or on the oral biofilm microbiota associated with DBS; (4) studies evaluating these outcomes in children with DBS or in laboratory models using human-derived oral biofilm or microorganisms relevant to DBS. Studies were excluded if they were: (1) review articles; (2) published in languages other than English; (3) studies not involving *L. reuteri*; (4) studies that did not involve human subjects or oral biofilm or microbiota models relevant to DBS; or (5) studies that were not relevant to biofilm mechanisms associated with oral health or DBS.

Screening and study selection

All identified records were imported into a reference manager and duplicates were removed. Two reviewers independently screened titles and abstracts against the predefined eligibility criteria. Potentially eligible articles were then independently assessed in full text to determine final inclusion. Reasons for exclusion at the full-text stage were recorded. Any disagreements between the reviewers were resolved through discussion and consensus.

Data synthesis

Given the heterogeneity in study designs, interventions, and outcome measures, a meta-analysis was not performed. Findings were synthesized qualitatively using a narrative approach and grouped by evidence type: observational or epidemiological studies, clinical interventions, and in vitro or ex vivo experimental studies. Mechanistic findings on antimicrobial activity, biofilm modulation, and changes in oral microbiota were synthesized across relevant studies.

Risk of bias assessment

Risk of bias was independently assessed by two reviewers using design-specific appraisal tools. Randomized clinical trials were evaluated using the Cochrane Risk of Bias 2 (RoB2) tool; observational studies and case reports were assessed using the appropriate Joanna Briggs Institute (JBI) critical appraisal checklists; and in vitro or ex vivo studies were evaluated using a modified Office of Health Assessment and Translation (OHAT) tool. Disagreements were resolved by discussion and consensus. Risk-of-bias judgments were used to inform the narrative interpretation of the evidence rather than as criteria for study exclusion, with observational and laboratory findings considered primarily supportive or mechanistic evidence.

Results

Study selection

A total of 209 records were identified through the database search. After removal of four duplicates, 205 unique records remained. Following preliminary screening, 14 non-research articles were excluded, leaving 191 records. After further screening, 50 records were excluded, leaving 141 reports for full-text assessment. Of these, 111 articles were excluded because they were irrelevant to the review, while 20 were excluded because they did not directly address *L. reuteri* in relation to DBS or relevant DBS-associated mechanisms. Consequently, 10 studies were included in the systematic review, comprising one randomized clinical study, one in vivo intervention study, four in vitro experimental studies, and four observational studies. The study selection process is presented in **Figure 1**.

Characteristics of included literature and risk of bias assessment

Ten studies published between 2003 and 2025 were included in the systematic review [3-8,10-13]. The included studies comprised one randomized clinical study [6], one in vivo study [8], four in vitro experimental studies [7,8,10,13], and four observational studies [3-5,12]. The observational studies consisted of three cross-sectional studies [3,4,12] and one population-based cohort study [5]. The results of the studies varied from the prevalence and correlation of black stain with caries incidence, antimicrobial activity, changes in oral microbiota to microbial inhibition and biofilm disruption. Sample sizes and intervention types varied across the included studies. Some studies evaluated probiotic strains, such as *L. reuteri* DSM 17938 [6] or reuteri extracts [7], whereas others investigated microbial composition [11] or analyzed patient records [12]. The main characteristics of the included studies are summarized in **Table 1**.

Quality assessment of included studies

Methodological quality was assessed using design-specific tools: the JBI checklists for cross-sectional, cohort, and case-report studies; RoB2 for randomized clinical trials; and a modified OHAT tool for in vitro and ex vivo studies (**Table 2**). Overall, study quality ranged from moderate to high. The cohort study by França-Pinto *et al.* [5] and the cross-sectional study by Martínez *et al.* [4] showed high methodological quality, whereas Gasparetto *et al.* [3] and Mathews and Ravikumar [12] were rated moderate, mainly because of limited control of confounding and smaller sample sizes. The randomized trial by D'Errico *et al.* [6] raised some concerns related to sample size, allocation concealment, and short follow-up.

Most laboratory studies demonstrated low risk of bias, with standardized protocols, appropriate controls, replicated experiments, and validated outcome measures. The mixed in vitro/in vivo study by Wahba *et al.* [8] and the case report by Yu *et al.* [11] were rated moderate. Overall, the evidence was methodologically acceptable but remained limited by few randomized trials, small clinical samples, heterogeneous probiotic formulations, and short follow-up periods.

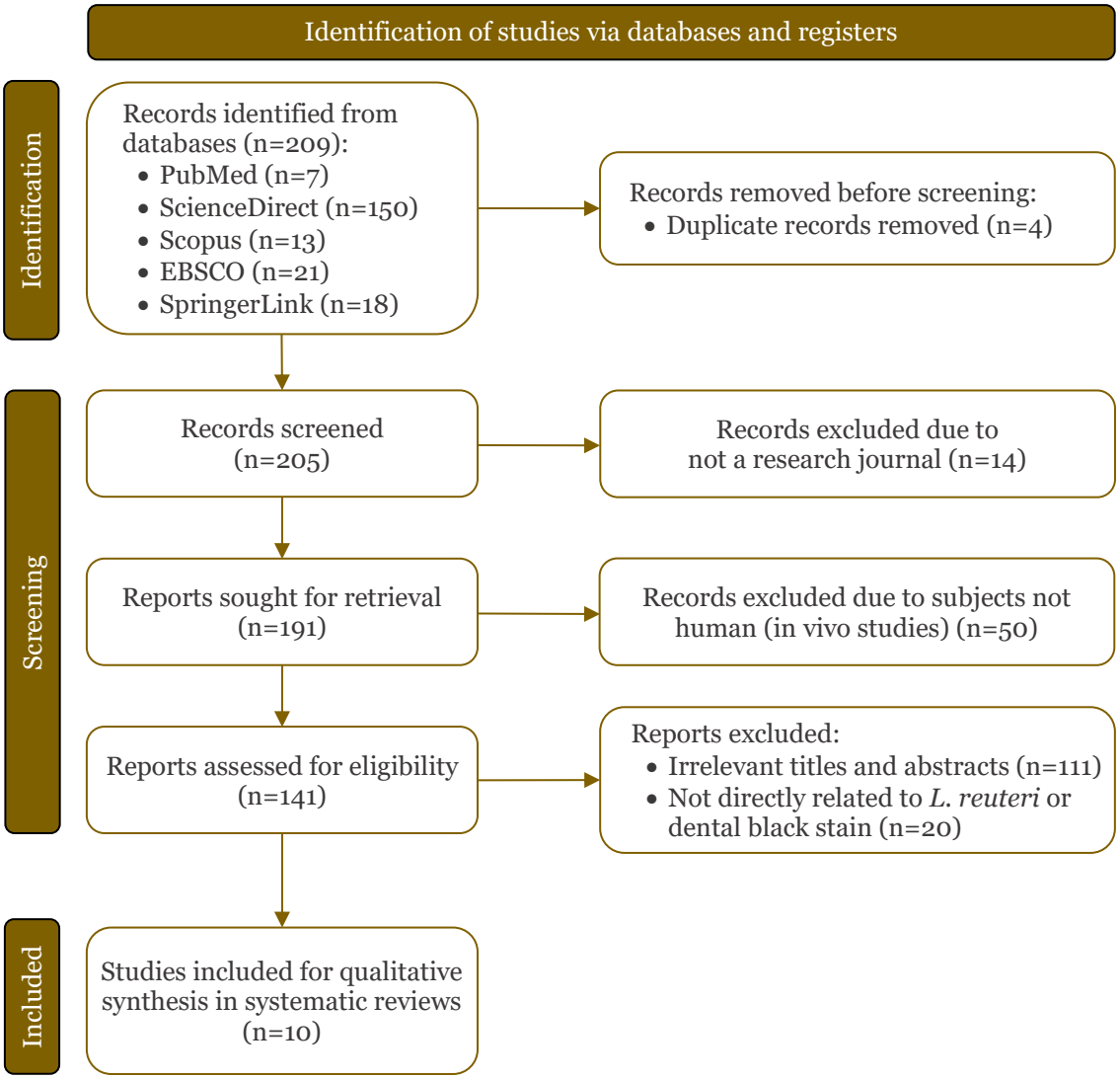


Figure 1. Literature searching and screening process.

Clinical efficacy and mechanistic effects of probiotics in dental black stain (DBS)

The included studies encompassed epidemiological investigations, clinical trials, pilot studies, in vitro experiments, ex vivo models, and microbiological analyses addressing the clinical and biological characteristics of DBS and the potential role of probiotics in its management. Reported outcomes encompassed clinical measures such as DBS prevalence, severity, recurrence and caries-related measures [3-6]. Microbiological outcomes included changes in bacterial growth, bacterial counts, biofilm formation, and oral microbiota composition [6-8,13]. Biochemical outcomes included changes in microbial metabolic activity, biofilm integrity, and bacterial gene expression [7,11]. Of the ten included studies, three directly evaluated probiotic interventions [6,8,11]. One directly investigated DBS recurrence [6], whereas the other two provided evidence on the microbial characteristics of DBS and the antibacterial or antibiofilm activities of probiotic strains against microorganisms associated with chromogenic biofilms [8,11].

Clinical evidence suggests that probiotics, particularly *L. reuteri* and *Streptococcus salivarius* M18, may reduce DBS recurrence following professional prophylaxis, potentially through modulation of the oral microbial community rather than direct removal of existing pigmentation [6]. A randomized clinical study reported lower recurrence and reduced stain severity among probiotic-treated participants compared with controls [6]. In addition, a recent pilot case study demonstrated alterations in the oral microbiome, including reductions in bacterial taxa associated with DBS formation following topical probiotic administration [11]. However, interpretation of these findings is limited by small sample sizes, short intervention periods, and heterogeneity in probiotic strains, formulations, and administration protocols.

Table 1. Characteristics of included literature

Authors (year)	Study design	Population (P)	Intervention/exposure (I)	Comparator (C)	Outcomes (O)	Objective
Gasparetto *et al.*, 2003 [3]	Cross-sectional	263 Brazilian schoolchildren (6–12 years) with assessment of DBS	Not applicable: observational studies of DBS	Children without DBS according to DBS status	DBS prevalence and severity; DMFT	Evaluate the prevalence and clinical characteristics of DBS and its relationship with dental caries, contributing to the clinical characterization of DBS.
França-Pinto *et al.*, 2012 [5]	Population-based birth cohort	1,129 children aged 5 years	Not applicable: observational assessment of DBS	Children without DBS	dmf-s; DBS prevalence; caries risk	Investigate the association between DBS and dental caries over time, contributing to the epidemiological characterization of DBS in children.
Martínez *et al.*, 2016 [4]	Cross-sectional	706 Brazilian schoolchildren	Not applicable: observational assessment of DBS	Children without DBS	DMFT, relative risk of caries	Evaluate the relationship between DBS and caries experience in schoolchildren, contributing to the clinical characterization of DBS.
Mathews and Ravikumar, 2020 [12]	Cross-sectional	93 Indian children (6–12 years)	Not applicable: clinical examination for black stain and caries (DMFS/deft)	Comparison according to DBS status	Black stain prevalence was approximately 10%; coexistence with caries was uncommon and not statistically significant.	Investigate the prevalence of DBS and its relationship with dental caries in children, contributing to the epidemiological context of DBS.
D'Errico *et al.*, 2021 [6]	Pilot randomized clinical study	20 patients (8–24 years)	Professional prophylaxis with or without *L. reuteri* DSM 17938 + ATCC PTA 5289 for 2 months	Professional prophylaxis alone	Lower recurrence scores in probiotic group after 1 and 2 months.	Investigate the clinical effectiveness of *L. reuteri* in reducing DBS recurrence following professional prophylaxis.
Yang *et al.*, 2021 [13]	In vitro	*Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Streptococcus mutans*	Cell-free culture supernatant of *L. reuteri* AN417 tested against oral pathogens; growth, ATP, biofilm integrity and gene expression assessed.	Untreated or control condition	Supernatant inhibited growth, reduced ATP, disrupted biofilms and downregulated virulence genes despite absence of reuterin genes.	Investigate the antimicrobial and antibiofilm activity of *L. reuteri* cell-free culture supernatant against oral pathogenic bacteria, contributing to the understanding of potential biological mechanisms.
Widyarman *et al.*, 2020 [10]	In vitro	Mono- and dual-species biofilms (*S. mutans*, *S. sanguinis*)	Indonesian *L. reuteri* LC382415 tested at different concentrations with/without glycerol over 1–24 h.	Untreated or control biofilm	Significant reduction of mono- and dual-species biofilms; greatest inhibition observed during early biofilm formation.	Evaluate the ability of *L. reuteri* strain Indonesia to inhibit initial oral biofilm colonization.

Authors (year)	Study design	Population (P)	Intervention/exposure (I)	Comparator (C)	Outcomes (O)	Objective
Widyarman *et al*., 2023 [7]	In vitro and ex vivo	Human premolar root canal biofilms	Reuterin derived from Indonesian *L. reuteri* tested against *E. faecalis*, *P. gingivalis*, *F. nucleatum*, *C. albicans*; compared with NaOCl.	NaOCl	Reuterin significantly reduced multispecies biofilms and showed efficacy comparable with NaOCl at optimal concentration.	Provides mechanistic evidence supporting postbiotic antibiofilm activity.
Wahba *et al*., 2025 [8]	In vitro + in vivo	EBS bacteria and children with EBS	Three probiotics (S. salivarius M18, *L. reuteri LR08*, L. paracasei Lpc-37) tested against *A. actinomycetemcomitans* and *A. naeslundii* using MIC and salivary qPCR before/after 14 days.	Control condition	Dose-dependent inhibition observed in vitro; *A. actinomycetemcomitans* eliminated and *A. naeslundii* markedly reduced in vivo.	Evaluate the strongest mechanistic evidence that probiotics directly suppress EBS-associated bacteria.
Yu *et al*., 2025 [11]	Pilot case study	One adult with recurrent black stain	Topical multi-strain probiotic application followed by full-length 16S rRNA sequencing of saliva and biofilm.	No comparator	Reduced recurrence accompanied by decreased abundance of *Corynebacterium* and shifts in *Actinobacteriota* and *Firmicutes*.	Investigate the effect of topical probiotic treatment on DBS recurrence and oral microbiome composition, contributing to the assessment of microbiota modulation as a potential mechanism.

Table 2. Quality assessment of the selected articles

Study	Tool	Score	Quality
Gasparetto *et al*., 2003 [3]	JBI cross-sectional	6/8	Moderate
França-Pinto *et al*., 2012 [5]	JBI cohort	10/11	High
Martínez *et al*., 2016 [4]	JBI cross-sectional	8/8	High
Mathews and Ravikumar, 2020 [12]	JBI cross-sectional	5/8	Moderate
D'Errico *et al*., 2021 [6]	RoB2	Some concerns	Moderate
Yang *et al*., 2021 [13]	Modified OHAT	Low risk	High
Widyarman *et al*., 2020 [10]	Modified OHAT	Low risk	High
Widyarman *et al*., 2023 [7]	Modified OHAT	Low risk	High
Wahba *et al*., 2025 [8]	OHAT + RoB2	Moderate	Moderate
Yu *et al*., 2025 [11]	JBI Case Report	7/8	Moderate–high

JBI: Joanna Briggs Institute; OHAT: Office of Health Assessment and Translation; RoB2: risk of bias 2

Mechanistic studies further support the potential biological effects of probiotics in DBS [6-8,10,13]. In vitro [10,13] and ex vivo investigations [7] demonstrated that *L. reuteri*, its metabolites such as reuterin, and other probiotic strains inhibited bacterial growth, disrupted biofilm structure, reduced bacterial viability, suppressed biofilm-associated gene expression, and interfered with early microbial colonization. More recent studies [14] also reported inhibitory activity against bacteria associated with extrinsic black stains, including *Aggregatibacter actinomycetemcomitans* and *Actinomyces naeslundii*. These findings suggest that probiotics may suppress selected chromogenic or biofilm-forming microorganisms while potentially preserving the broader ecological balance of the oral microbiota.

Epidemiological studies [3-5,12] consistently reported an inverse association between DBS and dental caries. Although DBS is primarily an esthetic concern, these observations support the concept that DBS represents a distinct oral biofilm phenotype rather than a conventional pathogenic lesion [3-5]. Its characteristic microbial ecology may partly explain why ecological modulation of the oral microbiome, rather than broad-spectrum antimicrobial eradication, represents a biologically plausible strategy for its management.

Collectively, the available evidence indicates that probiotics may have potential as an adjunctive approach for recurrent DBS through modulation of oral biofilm ecology, suppression of chromogenic microorganisms, and inhibition of biofilm development. Nevertheless, the current evidence remains preliminary because of methodological heterogeneity, the limited number of randomized clinical trials, small study populations, and insufficient long-term follow-up. Well-designed multicenter randomized controlled trials using standardized probiotic formulations, clearly defined clinical outcomes, longer follow-up periods, and integrated microbiome and molecular analyses are required to establish clinical efficacy, optimal dosing regimens, treatment duration, and the mechanisms underlying probiotic effects in the prevention and management of DBS.

Discussion

This systematic review synthesized current evidence regarding the use of *L. reuteri* for the management of DBS, integrating findings from epidemiological, clinical, and laboratory studies. Although direct clinical evidence remains limited, the collective findings consistently suggest that *L. reuteri* may reduce black stain recurrence by modulating the oral microbial ecosystem rather than by indiscriminate bacterial elimination [6-8,10,11,13]. This represents an important shift from conventional management, which primarily relies on repeated professional polishing despite the high recurrence of DBS [6,8].

The present findings also support the emerging view that DBS is more than an esthetic condition caused by ferric sulfide deposition. Increasing microbiological evidence indicates that DBS represents a distinct oral biofilm characterized by a unique microbial composition and ecological balance [1,11,15]. Consequently, recurrence following mechanical stain removal is expected because the underlying microbial ecosystem remains largely unchanged. This ecological perspective provides a biological rationale for probiotic therapy, which aims to restore microbial homeostasis rather than simply remove visible pigmentation.

A consistent finding across the included epidemiological studies was the inverse association between DBS and dental caries [3-5,12]. Children presenting with black stain generally exhibited lower caries prevalence than stain-free controls, suggesting that DBS is associated with a microbial community that differs from conventional cariogenic plaque. Several mechanisms have been proposed, including reduced abundance of acidogenic bacteria, enrichment of iron-associated microorganisms, and alterations in enamel pellicle composition that may decrease enamel demineralization [1,15,16]. Although the exact mechanism remains unclear, these observations reinforce the concept that DBS reflects a distinct ecological biofilm rather than a simple accumulation of pathogenic bacteria.

This ecological interpretation is consistent with the ecological plaque hypothesis, which proposes that oral diseases arise from shifts in microbial community balance instead of infection by individual pathogens [16]. Accordingly, successful management of biofilm-associated conditions should focus on restoring microbial homeostasis rather than eliminating all microorganisms. The probiotic approach observed in the present review aligns well with this

concept, as *L. reuteri* consistently demonstrated the ability to suppress microorganisms associated with dysbiosis while maintaining the overall structure of the oral microbiome [6,8,11,17].

Notably, the included clinical studies demonstrated reduced recurrence of black stain following probiotic administration, whereas microbiological investigations reported decreases in chromogenic bacteria together with preservation of microbial diversity [6,8,11]. Unlike conventional antiseptics such as chlorhexidine, which may indiscriminately reduce both pathogenic and commensal microorganisms, probiotics appear to exert a more selective ecological effect [17,18]. This distinction is clinically important because preservation of microbial diversity is increasingly recognized as essential for maintaining oral health and preventing recolonization by opportunistic pathogens [17].

Collectively, these findings suggest that the clinical benefits of *L. reuteri* should be interpreted as a consequence of microbiome modulation rather than simple antimicrobial activity. By targeting the ecological processes underlying biofilm development, probiotic therapy may provide a more sustainable strategy for preventing recurrent DBS than repeated mechanical stain removal alone. This interpretation forms the biological basis for the mechanistic evidence discussed in the following section.

The findings of this review indicate that the beneficial effects of *L. reuteri* in managing DBS are primarily mediated through ecological modulation of the oral microbiome rather than broad-spectrum antimicrobial activity. Experimental evidence suggests that *L. reuteri* inhibits biofilm development through multiple complementary mechanisms, including competitive exclusion of pathogenic microorganisms, production of antimicrobial metabolites, disruption of extracellular polymeric substances (EPS), and regulation of bacterial communication systems [6-8,10,11,13,17]. These mechanisms collectively create an oral environment that is less favorable for the persistence of chromogenic bacteria associated with DBS. Competitive exclusion is considered one of the principal mechanisms by which probiotics influence oral biofilms. By occupying adhesion sites and competing for nutrients, *L. reuteri* can interfere with early bacterial colonization, thereby limiting subsequent biofilm maturation [15,19]. Consistent with this concept, a study demonstrated that the Indonesian strain LC382415 significantly inhibited mono- and dual-species biofilm formation by *S. mutans* and *S. sanguinis*, particularly during the initial stages of biofilm development [10]. Although these microorganisms are not directly responsible for black stain formation, disruption of early colonizers may alter microbial succession and ultimately influence the composition of mature chromogenic biofilms.

Another important mechanism involves the production of antimicrobial metabolites. Reuterin, one of the best-characterized metabolites of *L. reuteri*, exhibits broad-spectrum antibacterial activity by disrupting intracellular redox balance and inhibiting essential metabolic pathways [20]. A study further demonstrated that reuterin effectively reduced biofilms of *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Enterococcus faecalis*, and *Candida albicans*, supporting its potential as an antibiofilm agent [7]. Interestingly, Yang et al. reported that the AN417 strain retained potent antibacterial activity despite lacking genes responsible for reuterin synthesis, suggesting that extracellular carbohydrates and fatty acid metabolites also contribute to the antimicrobial effects of *L. reuteri* [13]. These findings highlight the strain-dependent nature of probiotic activity and emphasize that multiple metabolites likely act synergistically to suppress oral pathogens.

Beyond direct antibacterial effects, *L. reuteri* may also promote oral microbial homeostasis by selectively suppressing pathogenic bacteria while preserving beneficial commensals [17]. This ecological modulation contrasts with conventional antiseptics such as chlorhexidine, which can indiscriminately reduce microbial diversity and contribute to ecological imbalance [17,21]. Preservation of microbial diversity is increasingly recognized as essential for maintaining oral health because resilient microbial communities are more resistant to colonization by opportunistic pathogens [17].

Overall, the available mechanistic evidence supports the clinical findings observed in this review. Rather than functioning as a conventional antimicrobial agent, *L. reuteri* appears to restore ecological balance within the oral microbiome through complementary actions on bacterial colonization, biofilm maturation, and microbial interactions. These properties provide

a strong biological rationale for its use as an adjunctive strategy in preventing recurrent DBS while supporting the broader concept of microbiome-based management of oral biofilm-associated conditions.

This systematic literature review presents several limitations due to the inherent characteristics of the included studies and the methodological constraints of the review process itself. First, only one of the included studies was a randomized clinical trial focused directly on *Lactobacillus reuteri*. The remaining studies were either observational, in vitro, in vivo, or non-randomized clinical trials, which inherently limits the strength of the evidence. This restricts the ability to draw strong causal conclusions and affects the overall quality of the findings. Second, substantial heterogeneity was observed across studies regarding probiotic strains, formulations (lozenges, extracts, supernatants), intervention durations, and outcome measures. This variability complicates direct comparison and synthesis of results, reducing the potential for meta-analysis and robust generalization. Third, many of the studies lacked long-term follow-up or post-treatment monitoring, particularly clinical and observational studies. This limits the understanding of the sustainability of *L. reuteri* colonization and its prolonged impact on black stain recurrence or prevention. Fourth, the in vitro studies, although mechanistically informative, cannot fully replicate the complex oral environment of the human oral microbiota. Therefore, the translational applicability of these findings remains speculative without supporting clinical trials. Fifth, observational studies are inherently susceptible to confounding variables and do not establish causal relationships. The inverse correlation between black stains and caries may be influenced by unmeasured factors such as dietary habits, oral hygiene practices, or genetic predispositions. Lastly, some studies focused on probiotics other than *L. reuteri*, reducing their direct relevance to this review's core objective. Although supportive of the probiotic concept, such studies dilute the specificity of the evidence related to *L. reuteri*'s role in black stain management.

Conclusion

This systematic review suggests that *L. reuteri* has potential as an adjunctive approach in the management and prevention of DBS, particularly through modulation of the oral microbial environment and biofilm-associated processes. However, the current evidence is insufficient to support *L. reuteri* as a standalone treatment or to establish standardized clinical protocols. In clinical practice, *Lactobacillus* strain is considered as a potential adjunct to conventional mechanical management, while its routine clinical use for DBS requires stronger clinical evidence. Further research should prioritize well-designed randomized clinical trials with adequate sample sizes, standardized *L. reuteri* strains and formulations, clearly defined treatment protocols, and longer follow-up to determine its clinical effectiveness and optimal role in preventing DBS recurrence.

Ethics approval

Not required

Acknowledgments

None.

Competing Interest

The authors declare no conflicts of interest concerning this study.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

Rayyan AI was used during the literature screening process for this study. Specifically, Rayyan AI was employed to assist in collecting, organizing, and sorting potentially relevant articles according to predefined inclusion and exclusion criteria. The AI tool was used solely as a supportive aid to facilitate article management and screening efficiency. Final decisions regarding article selection, eligibility assessment, and data interpretation were independently reviewed and determined by the authors. The authors take full responsibility for the accuracy, integrity, and content of this statement. The AI-based language tool, QuillBot, was used for language refinement (improving grammar, sentence structure, and readability of the manuscript). We confirm that all AI-assisted processes were critically reviewed to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were solely made by the authors.

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