Unlocking the potential of probiotic administration in caries management: a systematic review

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Abstract

Background

The use of prebiotics and/or probiotic bacteria with the potential to modulate the oral ecosystem may play an important role in the prevention and management of dental caries. To assess the evidence of the potential of pre/probiotics both in the prevention and treatment of dental caries, and therefore as adjuvant approaches in caries management, we focused on the PICO question “In individuals with caries, after probiotic administration, is there an improvement in outcomes directly related to caries risk and development?".

Methods

An extensive systematic search was conducted in electronic databases PubMed, Web of Science, Scopus and Cochrane, to identify articles with relevant data. This systematic review included trials complying the following inclusion criteria: studies performed in Humans; published in English; including the observation of patients with caries, with clear indication of the probiotic used and measurement of outcomes directly involved with the cariogenic process, including quantification of bacteria with cariogenic potential. To evaluate methodological quality of the studies, the critical assessment tool Joanna Briggs Institute was used.

Results

Eight hundred and fifty articles, potentially relevant, were identified. Following PRISMA guidelines 14 articles were included in this systematic review. Outcomes related to improvement of caries risk and development parameters were salivary counts of *S. mutans* and *Lactobacillus*, salivary pH and buffer capacity, new caries and caries activity, reduction of the cariogenic organisms content, assessed through sequencing approaches and salivary concentration of antimicrobial peptides. The probiotic most often referred with beneficial results in dental caries outcomes is *Lacticaseibacillus rhamnosus* being the most recommended to be included in clinical studies related to oral health. Regarding the most used administration vehicle, in studies with positive effects on the caries management, probiotic supplemented milk is considered the best administration vehicle.

Conclusions

Evidence suggests a beneficial effect of probiotic supplemented milk (*Lacticaseibacillus rhamnosus*) as an adjuvant for caries prevention and management. However, comparable evidence is scarce and better designed and comparable studies are needed.

Background

Tooth decay is one of the most common chronic infectious diseases and results from a synergistic and complex interaction between bacteria, diet and susceptible host factors such as teeth and saliva (1). The development of caries lesions on tooth tissues involves dynamic biological processes, in which acids produced by bacterial fermentation of dietary carbohydrates affect the demineralization of tooth tissues. Repeated acidification leads to the selection of acid-producing and highly acid-resistant organisms, which in turn destroys the pH homeostasis and causes the demineralization-remineralization balance to proceed in towards tooth mineral loss. This disturbance of the oral environment is caused by the availability of fermentable carbohydrates in the diet on which acidogenic and acid tolerant microorganisms thrive upon. Therefore, changes in the composition and biochemical activity of oral biofilms are important determinants of the aetiology of dental caries (2). Excessive acidification of the oral environment by aciduric species such as Lactobacillus spp. and *Streptococcus mutans* (*S. mutans*) is directly associated with the development of dental caries. However, species with low acid tolerance, such as *Streptococcus salivarius* (*S. salivarius*) and *Streptococcus gordonii* (*S. gordonii*), produce a large amount of alkali, which plays an important role in the acid-base balance of the oral cavity. Tooth demineralization can progress from enamel to dentin but caries progression in the less mineralized dentin is not associated a pH as low as that observed for enamel caries (3) and therefore, the microbiota of dentin caries differs from that of enamel caries and includes *Lactobacillus, Bifidobacterium, Scardovia, Actinomyces* and *Prevotella* species, capable of acidic and proteolytic activity (4). The use of prebiotics and/or probiotic bacteria with the potential to modulate the oral ecosystem, may play an important role in the prevention and management of dental caries. This topic has attracted the interest of several research groups in the last decades. The World Health Organization defines probiotics as “Live microorganisms which, when administered in adequate amounts, confer a health benefit to the host” (5). Traditionally, probiotic bacteria usually *Lactobacillus ssp*. and *Bifidobacterium ssp*, have been used in the prevention and treatment of gastrointestinal infections or diseases including caries (6, 7).

The mechanism of action underlying probiotic therapy in the oral cavity is based on the hypothesis that harmless bacteria could occupy a niche in a biofilm that would otherwise be colonized by pathogenic or opportunistic microorganism (8, 9). However, this effect cannot be generalized as each individual's microbiome is unique, and probiotic effects are difficult to predict. The mechanisms of action are not fully understood but it is thought that probiotic bacteria interact with the commensal microbiota in different ways such as interference with other microorganisms and the ability to exclude or inhibit pathogens, modulating host immune responses with local and systemic effects (10). The effect in the oral cavity will result from the interaction of probiotic bacteria with the biofilm, inhibiting and hindering the growth of pathogens through the production of hydrogen peroxide and bacteriocins and by stimulating the immune response that locally results in increased production of IgA and stimulation of phagocytosis (11). Another mechanism suggested is that probiotics may prevent cariogenic dental plaque formation directly through adhesion to the tooth surface(12) or indirectly through free electron neutralization (13).

The definition of prebiotic has evolved, with the most recent update of "substrate that is used selectively by host microorganisms conferring a health benefit" (14). In terms of caries prevention and management, this would include nutrients for microorganisms that inhibit acidogenic and aciduric microbes and/or enhance pH recovery by generating alkali from these same nutrients (15, 16). The two main sources of alkali in the oral cavity are urea and arginine, which, when metabolized by some oral bacteria, result in the production of ammonia and lead to an increase in pH (16). Thus, prebiotic and probiotics demonstrate the potential to have preventive and therapeutic effects and can then be used to prevent or even treat the disease after it is installed. Regarding oral health, probiotics can prevent the oral biofilm from being environmentally "stressed", enhancing the symbiosis associated with health, as well as "repairing" a dysbiotic biofilm associated with disease(17).

The objective of this review is to assess the evidence of the potential of pre/probiotics both in the prevention and treatment of dental caries and therefore as adjuvant approaches in caries management.

Methods

This systematic review was conducted following the Preferred Reporting Items for Systematic reviews and Meta-analysis (PRISMA) guidelines and has been recorded in OSF Registries, with the registration DOI: https://doi.org/10.17605/OSF.IO/VF5NG. The focused question was determined according to the Population, Intervention, Comparison and Outcome (PICO) strategy (Table 1).

Table 1
Question PICO answered in this study.

PICO	
P*opulation*	Individuals with caries
I*ntervention*	Probiotic /Prebiotic administration
C*omparison*	Before and after probiotic / prebiotic administration
O*utcomes*	Improvement of clinical and/or microbiological parameters related to caries risk and development

Outcomes related to improvement of parameters related to caries risk and development analyzed/considered were salivary counts of *S. mutans* and *Lactobacillus* in probiotic group, increase in salivary pH, appearance or inactivation of caries, composition of the oral microbiome consistent with a healthier microbiota, salivary concentration of levels of antimicrobial peptides defensins.

An extensive systematic search of available studies in literature was conducted in electronic databases PubMed, Web of Science, Scopus and Cochrane on November 1st, 2022, to identify articles with relevant data to answer the PICO question "In individuals with caries, after probiotic administration, is there an improvement in outcomes directly related to caries risk and development?". The search carried out using the following terms and query: (caries OR dental caries OR tooth decay) AND (probiotics OR prebiotics). Due to the diversity of studies, the search was narrowed to include the main genera described in the literature with cariogenic potential (such as S*treptococcus*, *Lactobacillus*, *Actinomyces*, *Prevotella*, *Bifidobacterium* and Scardovia). (18) However, within the genus *Streptococcus*, other species were investigated to include not only the cariogenic properties (such as S. mutans and *S. sobrinus*) but also carioprotective species such as *S. salivarius*.

The results of the different bases were combined to eliminate duplicated documents and articles were screened by tittle and abstract for eligibility. When the title or abstract did not provide sufficient information regarding the inclusion criteria, full text was obtained and analysed. Two researchers independently participated in all processes (PL and AG), from article checking, data collection and risk of bias analysis. Disagreement regarding inclusion of specific articles between the reviewers was discussed with a third author (MC). To evaluate methodological quality of the studies, the critical assessment tool - Joanna Briggs Institute (JBI) was used (19).

This systematic review included randomized controlled trials and clinical trials complying with the following inclusion criteria: studies performed in Humans; published in English; including the observation of patients with caries, with clear indication of the probiotic used and measurement of outcomes directly involved with the cariogenic process, including quantification of bacteria with cariogenic potential, such as those referred to in the search key (*Streptococcus, Lactobacillus, Actinomyces*, *Prevotella* and *Scardovia*). Studies that did not meet all the inclusion criteria or in which the population observed had systemic pathologies that may influence the results such as diabetic, immunosuppressed or polymedicated patients were excluded. *In vitro* and animal studies were also excluded. The same reviewers (PL and AG) collected the data independently, in tables structured in Excel spreadsheets with essential information such as: Pubmed ID when available, title, probiotic in study, baseline characteristics of the study population, study design, probiotic dosage, vehicle of administration, cariogenic bacteria quantified, quantification method, outcomes and conclusions.

Results

A systematic literature search identified de 850 articles potentially relevant, with 183 publications from PubMed database, 365 from Scopus, 240 from Web of Science and 62 from Cochrane. Duplicated documents were excluded, for a total of 156, leaving 694 articles in study. Based on the information provided in the title and abstract and after article selection and full text analysis, 14 articles were considered in the current review (Fig. 1).

The main reasons for non-inclusion were as follows: 216 were *in vitro* studies, 139 articles were not related to the topic of probiotics and caries, 33 were animal studies or used bovine enamel on devices used by humans, 196 were reviews and 19 articles were focused on a caries-free population. Six studies addressed diabetics or polymedicated geriatric patients and were therefore excluded. Moreover, 71 articles with no full text available were also excluded. Most of these studies are randomized controlled trial (RCT) protocols registered but without published results.

The studies selected were analysed regarding the quality of the study according to the JBI criteria and the results of the analysis are presented in Table 2. Almost all aspects of the analysis were fulfilled except for 5 articles (20–24) which did not include patients follow up in the analysis. It should be noted that in one article (25) there is no real blinding, since the control group is given chewing gum and the experimental group is given yogurt, due to the different" nature" of the treatment, both subjects and researchers knew who was in the control and experimental group. Another article (26) refers to be a double blind, randomized controlled trial, but does not describe in detail how selection, randomization and blinding were performed.

Table 2. Results of the analysis of the Joanna Briggs Institute appraisal checklist for critical evaluation of randomized controlled studies.

	1. Was true randomization used for assignment of participants to treatment groups?	2. Was allocation to groups concealed?	3. Were treatment groups similar at the baseline?	4. Were participants blind to treatment assignment?	5. Were those delivering the treatment blind to treatment assignment?	6. Were treatment groups treated identically other than the intervention of interest?	7. Were outcome assessors blind to treatment assignment?	8. Were outcomes measured in the same way for treatment groups?	9. Were outcomes measured in a reliable way?	10. Was follow-up complete and, if not, were differences between groups in terms of their follow-up adequately described and analyzed?	11. Were participants analyzed in the groups to which they were randomized?
Campus, 2014	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	In part	Yes
Rungsri, 2017	Yes	In part	In part	In part	In part	In part	In part	Yes	Yes	Yes	Yes
Villavicencio, 2018	Yes	Yes	In part	Yes	In part	Yes	Yes	Yes	Yes	No	Yes
Zare Javid, 2019	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Manmontri, 2019	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Ferrer, 2019	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Gedam, 2019	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Shaalan, 2021	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	In part	Yes
Wattanarat, 2021	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Piwat, 2020	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Ratna Sudha, 2020	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Gandhi, 2020	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Sandoval, 2021	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

A summary of the analysis of the RCT assessed in Table 2 and a quasi-experimental pilot study (20) are presented in Table 3.

Lacticaseibacillus and *Bifidobacterium* are the most frequent probiotic studied, where the most common species are *Lacticaseibacillus rhamnosus* (20, 23, 26–28), *Lacticaseibacillus paracasei* (24, 29, 30) and *Bifidobacterium longum* (20, 23). Interestingly, two studies had reported the comparison of probiotics (*Bifidobacterium*, *Lacticaseibacillus rhamnosus* and *Lacticaseibacillus plantarum*) with the prebiotics Xylitol and Cinnamon Bark Oil (21, 25).

Most of the studies, reported the consumption of the probiotic as an ingredient of probiotic milk (20, 23, 26, 28–31), however, the administration via yogurts (24, 25), lozenges and oral tablets (22, 32), adhesive gel or patch (21, 33) and even mouthrinse (27) were found. The daily intake of such probiotics varies between 10^5-10^9 CFU/mL during the different intervention times.

Table 3
Primary characteristics of the 14 studies included in this systematic review.

Entry	Study	Study Desing	Participant number	Participant age (years)	Probiotic/Prebiotic	Administration vehicle	Intervention time
1	Campus *et al*, 2014	RCT	181	6–8	*Lactobacillus brevi*s CD2	lozenges	6 weeks of use + 2 weeks follow-up
2	Rungsri *et al*, 2017	RCT	41	20–25	*Lacticaseibacillus rhamnosus* SD11	milk	4 weeks of use + 8 weeks follow-up
3	Villavicencio *et al*, 2018	RCT	363	3–4	*Lacticaseibacillus rhamnosus, Bifidobacterium longum*	milk	9 months
4	Angarita-Díaz *et al*, 2019	Quasi-experimental pilot study	63	3–5	*Lacticaseibacillus rhamnosus, Bifidobacterium longum*	milk	3 months
5	Zare Javid *et al*, 2019	RCT	66	18–30	*Bifidobacterium lactis* Bb12	yogurt	2 weeks
6	Manmontri *et al*, 2019	RCT	286	1–5	*Lactobacillus paracasei* SD1	milk	6 months of use + 6 months follow-up
7	Ferrer *et al*, 2019	RCT	*59*	18–65	*Sreptococcus dentisani*	Bucco-adhesive gel	4 weeks of used + 2 weeks follow-up
8	Gedam *et al*, 2019	RCT	51	8–12	*Lacticaseibacillus rhamnosus, Lactobacillus acidophilus, Bifidobacterium longum, Saccharomyces boulardii*	mouthrinse	2 weeks of use + 4 weeks follow-up
9	Shaalan *et al*, 2021	RCT	96	> 65	Bifidobacterium, Xylitol	Yogurt/chewing gum	3 months
10	Wattanarat *et al*, 2021	RCT	286	1–5	*Lactobacillus paracasei* SD1	milk	6 months of use + 6 months follow-up
11	Piwat *et al*, 2020	RCT	469	1–5	*Lactobacillus paracasei* SD1	milk	6 months of use + 6 months follow-up
12	Ratna Sudna *et al*, 2020	RCT	48	5–15	*Bacillus coagulans* Unique IS2	Oral tablets	2 weeks
13	Gandhi *et al*, 2020	RCT	60	-10	Cinnamon Bark Oil, *Lacticaseibacillus rhamnosus*, *Lactobacillus plantarum*	Mucoadhesive pacth	2 weeks
14	Sandovalet *et al*, 2021	RCT	42	2–3	*Lactobobacillus rhamnosus* SP1	milk	10 months

Regarding intervention time, the discrepancy between studies is notorious. Studies with lower intervention times reported the use of probiotics for 2 weeks (22, 24, 28) or 2 weeks of probiotic usage followed by an additional period of 4 weeks for follow up (27).

However, more robust studies with 6 months of probiotic usage and an additional period of 6 months for follow up (29–32) or even 9 and 10 months of probiotic usage (23, 28) are the most common design.

The impact of probiotic administration on dental caries outcomes was evaluated in a range of different cohorts, such as children, adolescents, and adults all in good state of general systemic health. The studied population has different characteristics related to caries: from children with at least 1 decay (20) to individuals with 3–10 carious active lesions including white spot lesions and non-cavitated lesions on enamel surface (33). The caries identification and evaluation method were mainly the International Caries Detection and Assessment System (ICDAS II), however other clinical evaluation criteria were also considered such as plaque and gingival indexes.

In Table 4 the parameters evaluated, and the respective outcomes achieved in the studies selected are summarized.

Table 4
Evaluated parameters and the respective outcomes achieved in the 14 studies included in this systematic review.

Entry	Study	Clinical Examination	Microbiological analysis	pH evaluation	Oral microbiome composition	Other parameters	Main findings
1	Campus *et al*, 2014	Reduction of bleeding on probing	Reduction on salivary *S. mutans* concentration	Reduction of plaque pH	N/A	N/A	*L. brevis* CD2 lozenges were effective in reducing important oral health variables.
2	Rungsri *et al*, 2017	No effects in DMFT, GI, and PI	Reduction on total bacteria and *S. mutans* concentration and increase of *Lactobacilli*. Persistence of *L. rhamnosus* SD11 in test group	No effects on pH	N/A	N/A	Daily consumption of fermented milk containing *L. rhamnosus* SD11 for 4 wk may have beneficial effects on oral health.
3	Villavicencio *et al*, 2018	No significant differences were attained	*Lactobacillus spp.*concentration reduction	Increase of saliva buffer capacity	N/A	N/A	Daily milk intake supplemented with *L. rhamnosus* and *B. longum* reduces the *Lactobacillus spp.* counts and increases the saliva buffer capacity in preschool children
4	Angarita-Díaz *et al*, 2019	Positive effect on carious lesions demineralisation/remineralisation	No significant effect on *S. mutans* concentration	No significant effect on pH variation	N/A	N/A	Clinical studies should continue to determine the functional foods effect of supplemented with probiotics with low acid production capacity and define ideal functional foods that promote children oral health.
5	Zare Javid *et al*, 2019	N/A	*S. mutans* and *Lactobacillus spp.*concentration reduction	N/A	N/A	N/A	Consumption of probiotic yogurt with *B. lactis* Bb12 may modify the oral biofilm
6	Manmontri *et al*, 2019	N/A	Reduction on salivary and plaque *S. mutans* concentrations. Increase of *Lactobacilli spp.* in saliva and plaque.	N/A	N/A	N/A	Daily or triweekly consumption of milk supplemented with *L. paracasei* SD1 may help prevent preschool children dental caries.

Entry	Study	Clinical Examination	Microbiological analysis	pH evaluation	Oral microbiome composition	Other parameters	Main findings
7	Ferrer *et al*, 2019	Decrease in plate index, gingival index. Increase salivary flow	Efficient colonization of *S. dentisani*	No significant effect on pH variation	Beneficial shift in bacterial composition, with a reduction of several cariogenic organisms.	Increase of salivary calcium and ammonium	The application of *S. dentisani* 7746 improved several clinical and microbiological parameters associated with oral health, supporting its use as probiotic to prevent tooth decay
8	Gedam *et al*, 2019	N/A	No significant differences in *S. mutans* concentration between groups	N/A	N/A	N/A	Probiotic mouthrinse was equally efficacious in CHX and NaF mouthrinses against *S. mutans*.
9	Shaalan *et al*, 2021	N/A	Decrease on *S. mutans* concentration between groups	N/A	N/A	N/A	Probiotic yogurt can be used as an alternative to xylitol in enhancing the oral condition and prevention from caries of geriatric patients
10	Wattanarat *et al*, 2021	No significant differences were attained	Decrease in *S. mutans* concentration between groups. Increase of *Lactobacillus spp.* Concentration.	N/A	N/A	Elevated salivary HNP1-3 levels in children with early childhood caries upon probiotic supplementation	In the severe-ECC status, consumption of *L. paracase*i significantly enhanced salivary HNP1-3 levels and but reduce *S. mutans* levels, resulting in reduction of caries progression
11	Piwat *et al*, 2020	Transitions of active caries to inactive caries and decrease of caries risk in probiotic group	N/A	N/A	N/A	N/A	Probiotic milk consumption can modestly prevent new caries, but considerably transform active caries to inactive lesions
12	Ratna Sudna *et al*, 2020	N/A	Reduction on salivary and plaque *S. mutans* and *Lactobacilli spp.* concentrations	No significant effect on pH variation	N/A	N/A	14-day administration chewable tablets with probiotic *B. coagulans* Unique IS2 can reduce cariogenic bacteria

Entry	Study	Clinical Examination	Microbiological analysis	pH evaluation	Oral microbiome composition	Other parameters	Main findings
13	Gandhi *et al*, 2020	N/A	Decrease in *S. mutans* concentration.	N/A	N/A	N/A	Cinnamon bark oil incorporated mucoadhesive patch is comparable to the probiotic incorporated patch due to its similarity in the reduction of salivary S. mutans counts.
14	Sandovalet *et al*, 2021	Increase in the number of teeth with carious lesions in the control group	N/A	N/A	N/A	Increase of salivary hβD-3 in probiotic group	Regular intake of probiotic-supplemented milk in preschool children with high caries risk decreased the occurrence of caries and the salivary levels of hβD-3

Several clinical parameters related to oral health were evaluated and compared between control and probiotic groups in some studies, these include bleeding on probing, gingival and plaque indexes and salivary flow. Among the studies where statistical differences between groups were observed, the reduction of bleeding on probing (32) and in plaque and gingival indexes (33) and the increase of salivary flow (33) were the results achieved. In some cases, authors went one step further by evaluating clinical parameters strictly related to caries management. These studies showed that the administration of probiotics caused positive effects on carious lesion demineralization or remineralization (20) the transition of active to inactive caries and the decrease of caries risk (29). Moreover, one study reported the increase of the number of caries lesion in the control group (28). Several studies analyze concentration of cariogenic S. mutans by culture methods. Most reported the decrease of salivary and/or plaque *S. mutans* counts in the probiotic group after the intervention period (21, 22, 24–26, 30–32). However, only 2 studies didn´t observed statistical differences between these groups (20, 27) Some authors have compared the levels of *Lactobacillus spp*. between groups. The increase of *Lactobacillus spp*. was observed in 3 studies (26, 30, 31), however the same number of studies reported a decrease in these bacteria genera(22–24) Interestingly, both *Lacticaseibacillus rhamnosus*(26) and *Streptococcus dentisani (S. dentisani)* (33), were still observed in samples collected in the follow up period. Moreover, when oral microbiome of the individual of each group was analyzed by sequency strategies, a beneficial shift in bacterial composition, with a reduction of several cariogenic organisms was attained in the probiotic group (33).

Salivary and plaque pH were also evaluated by some groups. Only one study reported the decrease in the plaque pH in the probiotic group (32), and 4 studies observed an increase in pH after the probiotic intervention (20, 22, 26, 33). One study has also reported the increase of buffer capacity in the probiotic group (23).

At least, two studies had evaluated the levels of antimicrobial peptides defensins that provide the first line of host defense against a broad spectrum of microorganisms, HNP1-3 and hβD-1(28, 34). In both cases an increase of these defensins levels was achieved in the probiotic group.

Discussion

Oral microorganisms form complex and dynamic communities that play a crucial role in maintaining oral health. This eubiotic equilibrium may be lost when interaction between microbes and the host is significantly altered, leading to a dysbiotic state which underlies oral and systemic (35). Dental caries is caused by cariogenic microorganisms (such as *S. mutans* and *Lactobacillus* spp.) in plaque biofilms that can ferment dietary carbohydrates producing acids, lowering pH and leading to mineral loss from the hard tissue of teeth and finally cavities (36) Thus, maintaining a healthy oral microbiome is essential for preventing dental caries. The balanced and diverse oral microbiome can help prevent the overgrowth of cariogenic bacteria and maintain a neutral pH in the mouth. Several factors including diet, hygiene practices, genetics, and environmental can, in fact, influence the composition and diversity of the oral microbiome (35, 36).

Regular brushing, along with a balanced and healthy diet and antimicrobial measures with fluoride exposure, can help maintain a healthy oral microbiome and prevent the development of caries (37). However, the toxicity associated with excess of fluoride or other antibacterial drugs, may cause microecological damage, resulting in re-colonization with secondary opportunistic pathogen and other pathologies consequences. It is in this context that safer and more efficient methods are needed to effectively prevent caries without significant adverse effects (38). Probiotics are well-known in health promotion and have been extensively studied(39). In oral health promotion, oral probiotics should be able to adhere and colonize oral tissue including hard, non-shedding surfaces and become a part of the biofilm. Moreover, oral probiotics should not be able to lead to sugar fermentation, avoiding pH decrease and therefore caries development (40). Several studies have reported the potential of the use of probiotics in caries management and development, however it is not easy to find comparable data to support the generalized use of probiotics as adjuvants for treatment and/or prevention of caries. There is no lack of studies published and according to PubMed, in the last 5 years 8 systematic reviews were published focusing on the study of the probiotics' use in oral health management, namely in caries prevention and/or treatment in preschool children with or without caries caries (41–48). However, the comparison between studies is difficult due to heterogeneity in study design, outcomes measured and procedures/methods to assess, caries status, microbial and biochemical changes, for instance. Although most studies refer to a significant positive effect in caries prevention with probiotics use, the main conclusions even in the reviews are that more scientific evidence is needed. To be able to make more accurate comparisons, in this review, a single study design was used.

The results show that the probiotic most often referred with beneficial results in dental caries outcomes is *Lacticaseibacillus rhamnosus* (23, 26, 28), being the most recommended to be included in clinical studies related to oral health. *L. rhamnosus* has been the object of several studies for its application as a powerful probiotic for human health(49, 50) This fact is due to its capacity to endure to stressful environments, such low pH, to adhere or compete for colonization in the oral cavity, modulating the innate and adaptive immune responses (26). Moreover, *Lacticaseibacillus rhamnosus* strains are capable of secreting antimicrobial substances that can inhibit other bacteria strains and can be incorporated into varied delivery food vehicles.

Regarding the most used administration vehicle, in studies with positive effects on the caries management, probiotic supplemented milk is considered the best administration vehicle. Milk is a model ‘nutraceutical’, that is food that conveys immunological and other health benefits together with the nutritional contribution (2). Milk constituents are known to modulate the developing microbiota within the infant gastrointestinal tract. Of the functional ingredients present in milk, oligosaccharides are probably the most important, since they act as prebiotics (2). Although most of the studies published are focused on the effect human milk oligosaccharides (HMOs) present in human milk in the gastrointestinal tract, it is important to highlight that HMOs play a crucial role in supporting the growth of beneficial gut bacteria, possess anti-adhesive effects that reduce the binding of pathogenic bacteria and modulating effects on immunological processes. Even though the diversity of oligosaccharides content of human milk cannot be successfully reproduced on a large scale, the bioactivities of oligosaccharides from bovine and human milk are similar (2, 26, 51). Thus, it is possible to assume that the synergy between oligosaccharides from milk and probiotic *L. rhamnosus* could have a beneficial impact on oral microbiota. Moreover, the consumption of probiotic supplemented milk could be an advantage for the children's population, since milk is widely used in this age group, and it could effectively contribute to the prevention and even to stop the progression of dental caries.

It is important to emphasize the results achieved with probiotic *S. dentisani* (33). The fact that the use of this specie could modulate oral microbiome, with a beneficial shift in bacterial composition, leading to a reduction of cariogenic organisms makes it a powerful probiotic for caries management and further randomized clinical trials are needed to provide better support for these findings.

The results achieved with *S. dentisani*, demonstrate the importance of use, in future works, *s*equencing approaches to evaluate the effective community changes and new bacteria after probiotic exposure.

As far as intervention time, due to the heterogeneity of data, further randomized clinical trials are recommended to provide better understanding the influence of intervention time on the probiotic effects. Nevertheless, and considering the studies included in this systematic review, longer intervention times (up to 6 months) with follow-ups could come out in more robust results in decrease of caries risk.

Conclusion

According with this study, the evidence suggests a beneficial and promising effect on dental caries outcomes by the usage of probiotic supplemented milk with *Lacticaseibacillus rhamnosus* as an adjuvant approach to clinical intervention and daily oral hygiene routines. Knowledge in this field would benefit from well-designed studies with a systematic assessment of caries, caries risk and microbial quantification and identification to elicit a systematic comparison between probiotic composition, vehicles and administration strategy.

abbreviations

S. mutans- *Streptococcus mutans*

S. salivarius- *Streptococcus salivarius*

S. gordonii- *Streptococcus gordonii*

S. dentisani- *Streptococcus dentisani*

PRISMA- Preferred Reporting Items for Systematic reviews and Meta-analysis

PICO- Population, Intervention, Comparison and Outcome

JBI - Joanna Briggs Institute

RCT - randomized controlled trial

ICDAS- International Caries Detection and Assessment System

HMOs- human milk oligosaccharides

Declarations

Ethics approval and consent to participate

Not applicable to a systematic review.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

Authors declare no competing interests.

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Authors' contributions

PCLopes collected and processed the data and drafted the manuscript.

ATPCGomes collected and processed the data and drafted the manuscript.

AKMendes collected and processed the data.

LBlanco revised the final document.

MJCorreia designed the study and revised the final document.

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References

1. Takahashi N, Nyvad B. Caries Ecology Revisited: Microbial Dynamics and the Caries Process. Caries Res. 2008;42(6).
2. Takahashi N. Oral Microbiome Metabolism: From “Who Are They?” to “What Are They Doing?”. J Dent Res. 2015 Dec;94(12).
3. Hashimoto K, Sato T, Shimauchi H, Takahashi N. Profiling of dental plaque microflora on root caries lesions and the protein-denaturing activity of these bacteria. Am J Dent. 2011 Oct;24(5).
4. Tanner ACR, Kent RL, Holgerson PL, Hughes C V., Loo CY, Kanasi E, et al. Microbiota of severe early childhood caries before and after therapy. J Dent Res. 2011 Nov;90(11):1298–305.
5. Sanders ME. Probiotics: definition, sources, selection, and uses. Clin Infect Dis. 2008 Feb 1;46 Suppl 2:S58-61; discussion S144-51.
6. Guandalini S. Probiotics for prevention and treatment of diarrhea. J Clin Gastroenterol. 2011 Nov;45 Suppl:S149-53.
7. Whelan K, Quigley EMM. Probiotics in the management of irritable bowel syndrome and inflammatory bowel disease. Curr Opin Gastroenterol. 2013 Mar;29(2):184–9.
8. Twetman S. Treatment protocols: Nonfluoride management of the caries disease process and available diagnostics. Dent Clin North Am. 2010;54(3):527–40.
9. Twetman S, Stecksén-Blicks C. Probiotics and oral health effects in children. Int J Paediatr Dent. 2008 Jan;18(1):3–10.
10. Servin AL. Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. FEMS Microbiol Rev. 2004 Oct;28(4):405–40.

11. Lux R, A L I F O R N I A D E N T A L A S S O C I A T I O N JC. ECOLOGICAL APPROACHES TO ORAL BIOFILM MANAGEMENT: FUTURE PERSPECTIVES FOR DENTISTRY. 2017.
12. Hasslöf P, Stecksén-Blicks C. Chapter 10: Probiotic Bacteria and Dental Caries. Monogr Oral Sci. 2020;28:99–107.
13. Doron S, Gorbach SL. Probiotics: their role in the treatment and prevention of disease. Expert Rev Anti Infect Ther. 2006 Apr;4(2):261–75.
14. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol. 2017 Aug;14(8):491–502.
15. Burne RA, Marquis RE. Alkali production by oral bacteria and protection against dental caries. FEMS Microbiol Lett. 2000 Dec 1;193(1):1–6.
16. Liu YL, Nascimento M, Burne RA. Progress toward understanding the contribution of alkali generation in dental biofilms to inhibition of dental caries. Int J Oral Sci. 2012 Sep;4(3):135–40.
17. Zaura E, Twetman S. Critical Appraisal of Oral Pre- and Probiotics for Caries Prevention and Care. Caries Res. 2019;53(5):514–26.
18. Tanner ACR, Mathney JMJ, Kent RL, Chalmers NI, Hughes C V., Loo CY, et al. Cultivable Anaerobic Microbiota of Severe Early Childhood Caries. J Clin Microbiol. 2011 Apr;49(4).
19. Moola S, Munn Z, Tufanaru C, Aromataris E, Sears K, Sfetc R, et al. Chapter 7: Systematic Reviews of Etiology and Risk. In: JBI Manual for Evidence Synthesis. JBI; 2020.
20. Angarita-Díaz MP, Forero-Escobar D, Cerón-Bastidas XA, Cisneros-Hidalgo CA, Dávila-Narvaez F, Bedoya-Correa CM, et al. Effects of a functional food supplemented with probiotics on biological factors related to dental caries in children: a pilot study. Eur Arch Paediatr Dent. 2020 Feb;21(1):161–9.
21. Gandhi HA, Srilatha KT, Deshmukh S, Venkatesh MP, Das T, Sharieff I. Comparison of Antimicrobial Efficacy of Cinnamon Bark Oil Incorporated and Probiotic Blend Incorporated Mucoadhesive Patch against Salivary Streptococcus mutans in Caries Active 7-10-year-old Children: An In Vivo Study. Int J Clin Pediatr Dent. 2020;13(5):543–50.
22. Ratna Sudha M, Neelamraju J, Surendra Reddy M, Kumar M. Evaluation of the Effect of Probiotic Bacillus coagulans Unique IS2 on Mutans Streptococci and Lactobacilli Levels in Saliva and Plaque: A Double-Blind, Randomized, Placebo-Controlled Study in Children. Int J Dent. 2020;2020.
23. Villavicencio J, Villegas LM, Arango MC, Arias S, Triana F. Effects of a food enriched with probiotics on Streptococcus mutans and Lactobacillus spp. salivary counts in preschool children: a cluster randomized trial. J Appl Oral Sci. 2018 May 14;26:e20170318.
24. Zare Javid A, Amerian E, Basir L, Ekrami A, Haghighizadeh MH, Maghsoumi-Norouzabad L. Effects of the Consumption of Probiotic Yogurt Containing Bifidobacterium lactis Bb12 on the Levels of Streptococcus mutans and Lactobacilli in Saliva of Students with Initial Stages of Dental Caries: A Double-Blind Randomized Controlled Trial. Caries Res. 2020;54(1):68–74.
25. Shaalan O, Gad HMA, Riad MI. Comparison of Antibacterial Effect of Probiotic Yogurt and Xylitol-Containing Chewing Gum in Geriatric Patients: A Randomized Controlled Clinical Trial. Acta Stomatol Croat. 2021 Dec;55(4):380–9.
26. Rungsri P, Akkarachaneeyakorn N, Wongsuwanlert M, Piwat S, Nantarakchaikul P, Teanpaisan R. Effect of fermented milk containing Lactobacillus rhamnosus SD11 on oral microbiota of healthy volunteers: A randomized clinical trial. J Dairy Sci. 2017 Oct;100(10):7780–7.
27. Gedam KY, Katre AN. Efficacy of Probiotic, Chlorhexidine, and Sodium Fluoride Mouthrinses on Mutans Streptococci in 8- to 12-Year-Old Children: A Crossover Randomized Trial. Lifestyle Genom. 2022;15(1):35–44.
28. Sandoval F, Faleiros S, Cabello R, Díaz-Dosque M, Rodríguez G, Escobar A. The consumption of milk supplemented with probiotics decreases the occurrence of caries and the salivary concentration of hβD-3 in children. Clin Oral Investig. 2021 Jun;25(6):3823–30.
29. Piwat S, Teanpaisan R, Manmontri C, Wattanarat O, Pahumunto N, Makeudom A, et al. Efficacy of Probiotic Milk for Caries Regression in Preschool Children: A Multicenter Randomized Controlled Trial. Caries Res. 2020;54(5–6):491–501.
30. Wattanarat O, Nirunsittirat A, Piwat S, Manmontri C, Teanpaisan R, Pahumunto N, et al. Significant elevation of salivary human neutrophil peptides 1-3 levels by probiotic milk in preschool children with severe early childhood caries: a randomized controlled trial. Clin Oral Investig. 2021 May;25(5):2891–903.
31. Manmontri C, Nirunsittirat A, Piwat S, Wattanarat O, Pahumunto N, Makeudom A, et al. Reduction of Streptococcus mutans by probiotic milk: a multicenter randomized controlled trial. Clin Oral Investig. 2020 Jul;24(7):2363–74.
32. Campus G, Cocco F, Carta G, Cagetti MG, Simark-Mattson C, Strohmenger L, et al. Effect of a daily dose of Lactobacillus brevis CD2 lozenges in high caries risk schoolchildren. Clin Oral Investig. 2014 Mar;18(2):555–61.
33. Ferrer MD, Pérez S, Lopez AL, Sanz JL, Melo M, Llena C, et al. Evaluation of clinical, biochemical and microbiological markers related to dental caries. Int J Environ Res Public Health. 2021;18(11).
34. Wattanarat O, Makeudom A, Sastraruji T, Piwat S, Tianviwat S, Teanpaisan R, et al. Enhancement of salivary human neutrophil peptide 1-3 levels by probiotic supplementation. BMC Oral Health. 2015;15(1).
35. Peng X, Cheng L, You Y, Tang C, Ren B, Li Y, et al. Oral microbiota in human systematic diseases. Int J Oral Sci. 2022 Mar 2;14(1):14.
36. Chen X, Daliri EBM, Kim N, Kim JR, Yoo D, Oh DH. Microbial Etiology and Prevention of Dental Caries: Exploiting Natural Products to Inhibit Cariogenic Biofilms. Pathogens. 2020 Jul 14;9(7).
37. Pitts NB, Twetman S, Fisher J, Marsh PD. Understanding dental caries as a non-communicable disease. Br Dent J. 2021 Dec;231(12):749–53.
38. Shkembi B, Huppertz T. Impact of Dairy Products and Plant-Based Alternatives on Dental Health: Food Matrix Effects. Nutrients. 2023 Mar 18;15(6).

39. Sharifi-Rad J, Rodrigues CF, Stojanović-Radić Z, Dimitrijević M, Aleksić A, Neffe-Skocińska K, et al. Probiotics: Versatile Bioactive Components in Promoting Human Health. Medicina (B Aires). 2020 Aug 27;56(9):433.
40. Saïz P, Taveira N, Alves R. Probiotics in Oral Health and Disease: A Systematic Review. Applied Sciences. 2021 Aug 31;11(17):8070.
41. Hao S, Wang J, Wang Y. Effectiveness and safety of Bifidobacterium in preventing dental caries: a systematic review and meta-analysis. Acta Odontol Scand. 2021 Nov;79(8):613–22.
42. Inchingolo AD, Malcangi G, Semjonova A, Inchingolo AM, Patano A, Coloccia G, et al. Oralbiotica/Oralbiotics: The Impact of Oral Microbiota on Dental Health and Demineralization: A Systematic Review of the Literature. Children (Basel). 2022 Jul 8;9(7).
43. Kairey L, Leech B, El-Assaad F, Bugarcic A, Dawson D, Lauche R. The effects of kefir consumption on human health: a systematic review of randomized controlled trials. Nutr Rev. 2023 Feb 10;81(3):267–86.
44. Meng N, Liu Q, Dong Q, Gu J, Yang Y. Effects of probiotics on preventing caries in preschool children: a systematic review and meta-analysis. J Clin Pediatr Dent. 2023 Mar;47(2):85–100.
45. Nadelman P, Magno MB, Masterson D, da Cruz AG, Maia LC. Are dairy products containing probiotics beneficial for oral health? A systematic review and meta-analysis. Clin Oral Investig. 2018 Nov;22(8):2763–85.
46. Poorni S, Srinivasan MR, Nivedhitha MS. Probiotic Streptococcus strains in caries prevention: A systematic review. J Conserv Dent. 2019;22(2):123–8.
47. Shi J, Wang Q, Ruan G, Chen Y, Zhao M, Shi D, et al. Efficacy of probiotics against dental caries in children: a systematic review and meta-analysis. Crit Rev Food Sci Nutr. 2022 May 24;1–18.
48. Twetman S, Jørgensen MR. Can probiotic supplements prevent early childhood caries? A systematic review and meta-analysis. Benef Microbes. 2021;12(3):231–8.
49. Mathipa-Mdakane MG, Thantsha MS. Lacticaseibacillus rhamnosus: A Suitable Candidate for the Construction of Novel Bioengineered Probiotic Strains for Targeted Pathogen Control. Foods. 2022 Mar 8;11(6):785.
50. Yefet E, Colodner R, Strauss M, Gam Ze Letova Y, Nachum Z. A Randomized Controlled Open Label Crossover Trial to Study Vaginal Colonization of Orally Administered Lactobacillus Reuteri RC-14 and Rhamnosus GR-1 in Pregnant Women at High Risk for Preterm Labor. Nutrients. 2020 Apr 19;12(4):1141.
51. Han D, Zulewska J, Xiong K, Yang Z. Synergy between oligosaccharides and probiotics: From metabolic properties to beneficial effects. Crit Rev Food Sci Nutr. 2022 Oct 31;1–23.

Figures

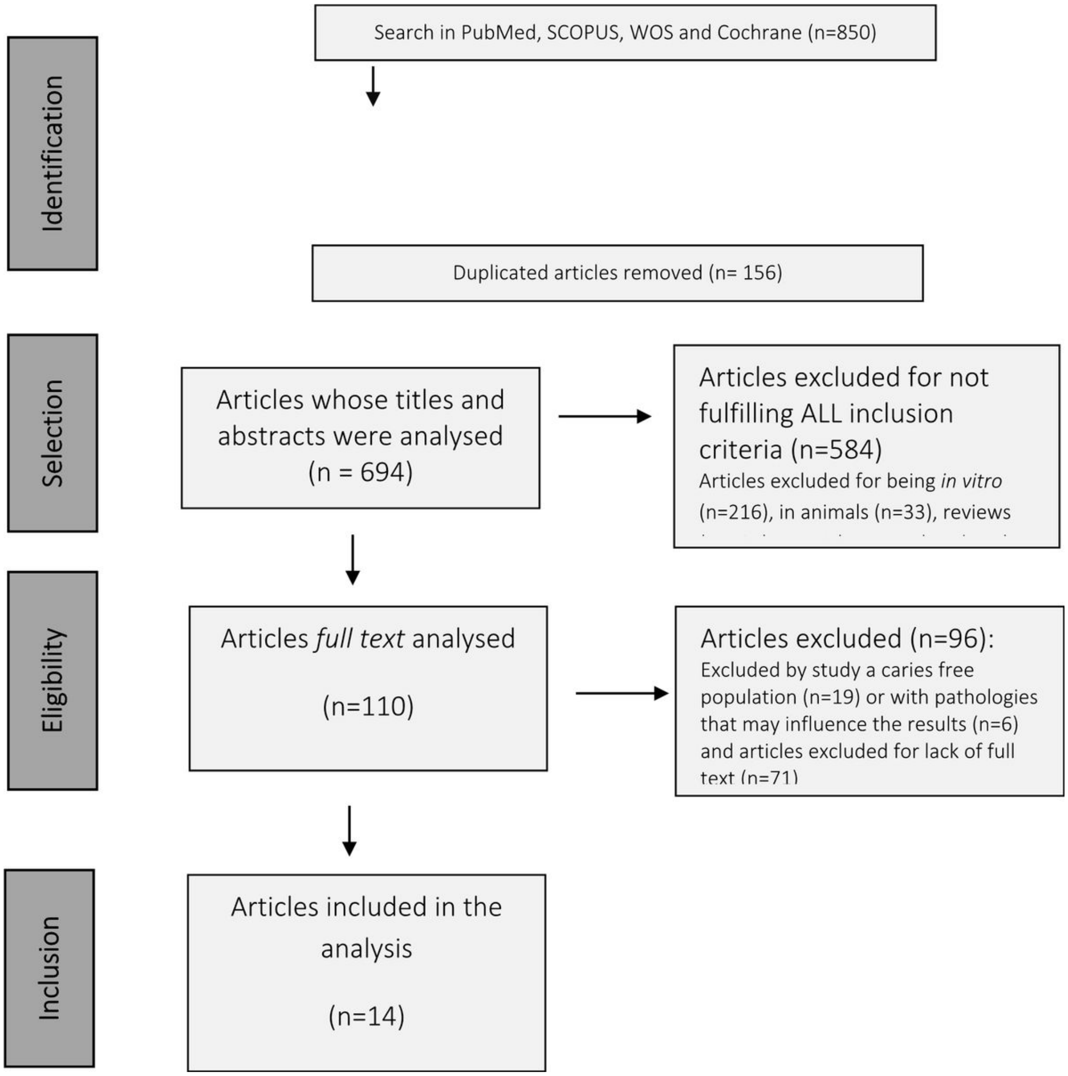

Figure 1

Overview of article selection procedure according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).