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Probiotics in Prevention of Dental Caries: A Systematic Review

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Abstract
Aim:*To conduct a systematic review of the literature on how efficient the probiotics helps in the prevention of dental caries and to determine its effect on caries risk factors.*
Methodology:*Records were searched from Cochrane, PubMed and Google Scholar from (1971-2015 August). Out of 721 articles originally identified, 82 records were considered potentially eligible and sought for further assessment. 32 articles met the inclusion criteria and these studies were assessed independently for methodology and performance.*
Results:*Out of 32 included studies, 16 short term studies showed significant reduction in streptococcus counts, 8 short term studies showed reduction in streptococcus count in saliva and/or plaque and 3 long term studies had no effect on streptococcus mutans count in saliva and/or plaque. Only 3 studies showed decrease in caries progression. Only 2 studies showed reduction in lactobacilli counts in saliva. Only 2 studies showed that probiotics had no significant effect on buffering capacity and plaque pH.*
Conclusion:*This systematic review revealed that 75 % of the selected studies, probiotics had demonstrated the capacity to reduce streptococcus counts in saliva and/or plaque in short-term periods. Further studies on the long term or synergetic effect of the probiotic organisms on the caries causative bacteria, caries progression and optimum dosage of the probiotic organisms are still need to be explored.*
Keywords:*Probiotics, dental caries, type of probiotics, plaque pH., streptococcus mutans, lactobacilli and buffering capacity.*

I. Introduction

The term probiotic, meaning "for life," is derived from the Greek language. It was first used by Lilly and Stillwell in 1965 to describe "substances secreted by one microorganism which stimulates the growth of another" and thus was contrasted with the term antibiotic[1]. It can be defined as "Live microorganisms that when administered in adequate amounts confer a health benefit on the host" (FAO/WHO 2001)[2].

In 1907 the Ukrainian born biologist & Nobel laureate Eliemetchniikoff realized that consumption of Bulgarian yoghurt (which contains lactic acid bacteria) was good for health. Metchnikoff worked at the Pasteur Institute in Paris& had discovered Lactobacillus bulgaricus, a strain he later introduced into commercial production of sour milk products in France & throughout Europe. He devoted the last decade of his life to the study of Lactic acid producing bacteria as a means of increasing human longevity[3]. The increased popularity of using probiotic bacteria supplements to improve gastrointestinal health has prompted interest in the utility of this approach for oral applications[4]

One of the most important threats to oral health is dental caries. Dental caries is a multifactorial, infectious,microbial disease of calcified portion of teeth characterisedby dissolution of organic substance followed bydisintegration of inorganic substances.Dental caries although not a life endangering disease, is extremely troublesome, incapacitating & expensive. Itcan directly or indirectly be responsible for pain, infection,facial disfigurement, chewing & speech impairment, andloss of esthetics, as well as malnutrition.So dealing with dental caries should be directed towards custom made preventive protocol & not justendless surgical repair of sign & symptoms of dental caries.

Prevention of dental caries has been attempted withfluoride in different form, but excess body fluoride canalso cause skeletal and dental fluorosis. Conventionalprevention of dental caries is also focused on the removalof dental plaque by in discriminate use of antibacterial

Mouth rinse but it may not be totally effective as it createsopen, non-competitive surfaces for pathogens to re-populatethe oral cavity. Other professional prevention of dentalcaries includes application of pit & fissure sealant, onsusceptible pits and fissures of tooth, but are costly. Various other approaches including Chemo-prophylactic agents, Antibiotics, Caries Vaccines, Sugar Substitutes, Fluorides, Restorative materials have been in use; however the anti-caries effects of these approaches are still limited. So a newer preventive approach should be tried for dentalcaries. Previous studies have suggested that probioticapproach can be effective in selectively inhibiting oralpathogens or modulate the microbial composition of dentalplaque thereby reduce the incidence of dental caries.

Hence the hypothesis behind this systematic review was that the administration of probiotic strains might play a role in the caries lesion prevention and in the control of caries-related risk factors.

II. Methodology

Focused question: what is the clinical impact of probiotic strains in prevention of caries lesion and in control of caries- related risk factors?

Selection Criteria:
Inclusion criteria:
Type of studies: Randomized clinical trials.
Subjects: Anyone who received probiotics as a preventive agent for dental caries.
Randomized clinical trials will be included only when they
(1) Assess the in-vivo role of probiotics on caries lesion development and risk factors.
(2) Consider human studies without any medical condition.
(3) Were published in English language
(4) Involve any type of probiotic with any type of administration method.

Exclusion criteria:
(1) All in-vitro studies,
(2) All studies not focusing on probiotics administration for caries prevention.
(3) All studies where probiotics are administered for other reasons were excluded.

Types of outcome measures:
- Outcome variables are levels of streptococcus mutans, lactobacillus, and yeasts in saliva and plaque samples after the probiotic intervention.
- Change in Plaque P^H, buffering capacity was considered.
- There will be no restriction regarding the method for measuring any of the outcomes.
- There is no restriction in amount, type of strain used as intervention.
- There is no restriction in route of administration used and treatment duration.

Search Strategy: The main important available data bases will be searched MEDLINE (1994-2015), GOOGLE SCHOLAR (1987-2015), and COCHARANE CENTRAL (1994-2015).

The search was focused on using the keywords: probiotic bacteria, prevention of dental caries, cariogenic bacteria, Streptococcus mutans, Lactobacillus which were used as isolated or in combinations using different Boolean operators.Comparisons of different searches were carried out to delete the repeated studies. Then abstracts of all available articles were examined. All studies, which appeared to meet the inclusion criteria, were obtained in the full text format. Application of the Cochrane Collaboration tool for evaluating the risk of bias was done. Then selected were grouped into high risk bias and low risk bias articles.

III. Results

The initial search resulted in 721 articles; however, 639 of these articles were excluded after reviewing the abstracts because they did not have the proper clinical trial design or because they were duplicates. After analyzing the full text from 82 clinical trials, 50 articles were excluded because they did not fulfill all the selection criteria. Our final review included 32 articles.

Table 1 Articles excluded from the study

Author name	Title of article	Reason for exclusion
Burton et al	Influence of the probiotic Streptococcus salivarius strain M18 on indices of dental health in children: a randomized double-	Complete oral health improvement

	blind, placebo-controlled trial	
A. Shimada, et al.	Oral lactic acid bacteria related to the occurrence and/orprogression of dental caries in Japanese preschool children	Not a randomized study
OmidSavabi et al	Effects of biosurfactant produced by Lactobacillus casei on gtfB, gtfC, and ftf gene expression level in S. mutans by real-time RT-PCR	Not a randomized study
RasoulSalehi et al	Effects of Lactobacillus reuteri-derived biosurfactant on the gene expression profile of essential adhesion genes (gtfB, gtfC and ftf) of Streptococcus mutans	Not a randomized study
Ying-ChiehTsai,etal	Inhibitory effect of Lactobacillus salivarius on Streptococcus mutans biofilm formation	Not a randomized study
Keller *et al.*	Effect of chewing gums containing the probiotic bacterium Lactobacillus reuteri on oral malodour.	Oral malodour
Wang *et al.*	Fermented milk supplemented with probiotics and prebiotics can effectively alter the intestinal microbiota and immunity of host animals.	Intestinal health
Allen et al.	A multicentrerandomised controlled trial evaluating lactobacilli and bifidobacteria in the prevention of antibiotic-associated diarrhoea in older people admitted to hospital: The PLACIDE study protocol.	Diarrhoea
Iniesta et al.	Probiotic effects of orally administered Lactobacillus reuteri-containing tablets on the subgingival and salivary microbiota in patients with gingivitis. A randomized clinical trial.	Gingival health
Slawik et al.	Probiotics affect the clinical inflammatory parameters of experimental gingivitis in humans.	Gingival health
Vandenplas et al.	The synbioticfoodsupplementProbiotical vs. placebo for acute gastroenteritis in children.	Acute gastroenteritis
Burton et al.	Evaluation of safety and human tolerance of the oral probiotic Streptococcus salivarius K12: A randomized, placebo-controlled, double-blind study	Safety and tolerance
Krauss-Silva et al.	A randomised controlled trial of probiotics for the prevention of spontaneous preterm delivery associated with bacterial vaginosis: Preliminary results.	Preterm delivery
Hummelen et al.	Effect of 25 weeks probiotic supplementation on immune function of HIV patients.	Human immunodeficiency virus (HIV)
Harini et al.	Efficacy of a probiotic and chlorhexidine mouth rinses: A short-term clinical study.	Gingival health
Saxelin et al.	Persistence of probiotic strains in the gastrointestinal tract when administered as capsules, yoghurt, or cheese.	Gastrointestinal persistence
Hummelen et al.	Lactobacillus rhamnosus GR-1 and L. reuteri RC-14 to prevent or cure bacterial vaginosis among women with HIV.	Bacterial vaginosis
Arroyo et al.	Treatment of infectious mastitis during lactation: Antibiotics versus oral administration of Lactobacilli isolated from breast milk.	Infectious mastitis
Grossi et al.	Treatment of infectious mastitis during lactation: Antibiotics versus oral administration of Lactobacilli isolated from breast milk.	Diarrhoea
Sierra et al.	Intestinal and immunological effects of daily oral administration of Lactobacillus salivarius CECT5713 to healthy adults.	Intestinal effect
Sinkiewicz et al.	Influence of dietary supplementation with Lactobacillus reuteri on the oral flora of healthy subjects.	Gingival health
Mayanagi et al.	Probiotic effects of orally administered Lactobacillus salivarius WB21-containing tablets on periodontopathic bacteria: A double-blinded, placebo-controlled, randomized clinical trial	Gingival health
Dommels et al.	Survival of Lactobacillus reuteri DSM 17938 and Lactobacillus rhamnosus GG in the human gastrointestinal tract with daily consumption of a low-fat probiotic spread.	Intestinal persistence
Ranganathan et al.	Probiotic dietary supplementation in patients with stage 3 and 4 chronic kidney disease: A 6-month pilot scale trial in Canada	Kidney disease
Twetman et al.	Short-term effect of chewing gums containing probiotic Lactobacillus reuteri on the levels of inflammatory mediators in gingival crevicular fluid.	Gingival health
Basu et al.	Efficacy of high-dose Lactobacillus rhamnosus GG in controlling acute watery diarrhea in Indian children: A randomized controlled trial.	Diarrhoea
Staab et al.	The influence of a probiotic milk drink on the development of gingivitis: A pilot study.	Gingival health
Mao et al.	Effect of a lactose-free milk formula supplemented with bifidobacteria and streptococci on the recovery from acute diarrhoea.	Diarrhoea

Shimauchi et al	Improvement of periodontal condition by probiotics with Lactobacillus salivarius WB21: A randomized, double-blind, placebo-controlled study.	Gingival health
Marcone et al.	Effectiveness of vaginal administration of Lactobacillus rhamnosus following conventional metronidazole therapy: How to lower the rate of bacterial vaginosis recurrences.	Bacterial vaginosis
Panigrahi et al.	Long-term colonization of a Lactobacillus plantarumsynbiotic preparation in the neonatal gut.	Neonatal gut colonization
Mohan et al.	Effects of Bifidobacteriumlactis Bb12 supplementation on body weight, fecal pH, acetate, lactate, calprotectin, and IgA in preterm infants.	Intestinal health
Ivory et al.	Oral delivery of Lactobacillus caseiShirota modifies allergen-induced immune responses in allergic rhinitis.	Allergic rhinitis
Htwe et al.	Effect of Saccharomyces boulardii in the treatment of acute watery diarrhea in Myanmar children: A randomized controlled study.	Diarrhoea
Larsson et al.	Human lactobacilli as supplementation of clindamycin to patients with bacterial vaginosis reduce the recurrence rate; a 6-month, double-blind, randomized, placebo-controlled study.	Bacterial vaginosis
Hatakka et al.	Probiotics reduce the prevalence of oral candida in the elderly—A randomized controlled trial.	Oral candida
Basu et al.	Effect of Lactobacillus rhamnosus GG in persistent diarrhea in Indian children: A randomized controlled trial.	Diarrhoea
Henker et al.	The probiotic Escherichia coli strain Nissle 1917 (EcN) stops acute diarrhoea in infants and toddlers.	Diarrhoea
Sugawara et al.	Perioperative synbiotic treatment to prevent postoperative infectious complications in biliary cancer surgery: A randomized controlled trial.	Biliary cancer surgery
Krasse et al.	Decreased gum bleeding and reduced gingivitis by the probiotic Lactobacillus reuter	Gingival health
Margreiter et al.	Therapeutic value of a Lactobacillus gasseri and Bifidobacteriumlongum fixed bacterium combination in acute diarrhea: A randomized, double-blind, controlled clinical trial.	Diarrhoea
Olivares et al.	Oral administration of two probiotic strains, Lactobacillus gasseri CECT5714 and Lactobacillus coryniformisCECT5711, enhances the intestinal function of healthy adults.	Intestinal health
Sarker et al.	Lactobacillus paracasei strain ST11 has no effect on rotavirus but ameliorates the outcome of nonrotavirus diarrhea in children from Bangladesh.	Diarrhoea
Schrezenmeir et al.	Benefits of oral supplementation with and without synbiotics in young children with acute bacterial infections.	Acute bacterial infections
Reid et al.	Nucleic acid-based diagnosis of bacterial vaginosis and improved management using probiotic lactobacilli.	Bacterial vaginosis
Morelli et al.	Utilization of the intestinal tract as a delivery system for urogenital probiotics.	Vaginal colonization
Reid et al.	Oral use of Lactobacillus rhamnosus GR-1 and L. fermentum RC-14 significantly alters vaginal flora: Randomized, placebo-controlled trial in 64 healthy women.	Vaginal colonization
Arvola et al.	Prophylactic Lactobacillus GG reduces antibiotic-associated diarrhea in children with respiratory infections: A randomized study.	Diarrhoea

Table 2. Studies with caries risk factors as outcome in children

Reference Study design	Subjects Age	Strain (Concentration)	Delivery System/ Treatment Duration	Groups	Outcome(s)	Results
Bhalla,et al.: et al[1] 2015	30 childern (12-14 years)	B.lactis 12	200 ml curd once daily/week	I (probiotic curd) II (plain curd)	MS in saliva (plate culturing)	Statistically significant reduction in MS immediately after 1 hr and after 1 week among probiotic group.
Sidhu GK et al[3] 2015	20 childern (10-15 years)	Probitic bacteria	curd 200 g (1 bowl)/1year	experimental group, control group	MS and LB counts in saliva(plate culturing)	No statistically significant MS and lactobacilli differences among groups. In experimental group,87%

						children showed decrease in SM and 67 % children showed increase in LB after 1 year follow up.
NozariA.,et al[4]. 2015	49 childern (6-12 years)	Bifidobacteriumlactis (1×10^6 per gram) once daily	200g yogurt/ 2 weeks4 period follow up .	case group control group.	MS and LB counts in saliva(plate culturing)	No statistically significant reduction in MS and LB in case group, whereas significant reduction was found in control group between phases 1 and 4
DevasyaAshwin et al[5].,2015	60 childern (6-12 years) with zero DMFT	Bifidobacteriumlactis Bb-12 and Lactobacillus acidophilus La-5 (1×10^6 CFU)	Ice-cream/7 days	A: Ice-cream B:Icecream/probiotics	MS in saliva (plate culturing)	Significant reduction in the salivary levels of MS count after seven days and also after 30 days,but after six months the salivary levels of MS is similar to baseline.
Stenson et al[8] 2014	113 childern (9 years)	Lactobacillus reuteri,stain ATCC 55730 (10^8 CFU)	5 oil drops of oil containing probiotic to the mothers during last month of gestation and 1 yr children.	Placebo group Probiotic group	MS and LB counts in saliva (plate culturing)and plaque score	No statistically significant MS and lactobacilli , secretory IGA and plaque score differences among groups
Campus et al[9]., 2013	191 children (6–8 years) with 2- 3 carious lesions and salivary MS count $\geq 10^5$ CFU/ml	Lactobacillus brevis CD2 (2×10^9/g)	Lozenges twice a day/6 weeks and examined in period of o weeks,3 weeks and 6 weeks and 2 weeks after cessation of lozenges.	Probiotic group and plain group	MS in saliva and plaque pH (plate culturing) and bleeding on probing	Statistically significant decrease in salivary MS count in probiotic group and reduction inplaque p^H and bleeding on probing.
Taipaleet al[11], 2013	106 children(4 years)	Bifidobacterium animalis subsp. lactisBB-12 (10^{10} CFU/mL)	Tablets in slow-release pacifier or spoon twice daily/22–23 months	A: Probiotic B: Xylitol C: Sorbitol	MS in plaque (plate culturing)	No statistically significant MS differences among groups
Junejaet al[12], 2012	40 children (12–15 years)	Lactobacillus rhamnosushct 70 (2.34×109 CFU/day)	Milk twice daily/3 weeks	A: Milk B: Milk + Probiotic	MS in saliva (chair-side tests)	Statistically significant reduction in MS immediately after consumption and after 3 week follow-up in group A
Taipaleet al[13], 2012	106 infants (1 month)	Bifidobacterium animalis subsp. lactis BB-12 (1010 CFU/mL)	Tablets in slow-release pacifier or spoon twice daily/months	A: Probiotic B: Xylitol C: Sorbitol	MS in plaque and Lb and yeasts in mucosa/teeth (plate culturing)	MS colonization statistically significant differ, lactobacilli and yeasts not differ among groups
Singh et al[17], 2011 ,cross-over study	40 children (12–14 years)	Bifidobacteriumlactis Bb-12 ATCC27536 and Lactobacillus acidophilus La-5 (106 CFU/g)	Ice-cream/10 days And wash out periods -2 weeks	A: Ice-cream B:Icecream/probiotics	MS and Lb in saliva (chair-side tests)	Statistically significant reduction in MS in group B, but no significant effect on lactobacilli

Aminabadiet al[18], 2011	105 children (6–12 years)	Lactobacillus rhamnosus GG (2 × 108 CFU/mL)	Yogurt/3 weeks (chlorhexidinemouthrinse 2 weeks)	A: Chlorhexidine B: Probiotic C: Chlorhexidine, than probiotic	MS in saliva (plate culturing)	Statistically significant MS decrease immediately after probiotic use in group B; recolonization during the 5 consecutive weeks. In group C a statistically significant MS reduction that enhances during the 5 consecutive weeks
Jindal et al[19]., 2011	150 children (7–14 years)	Lactobacillus rhamnosus, Bifidobacteriumlongum, Saccharomyces cereviasae (1.25 billion) Bacillus coagulans (150 million)	Powders (dissolved in water and used as mouthrinse)/14 days	A: Placebo B: L. rhamnosus, B. longum and S. cereviasae C: B. coagulans	MS in saliva (plate culturing)	Statistically significant MS reduction in groups B and C
Stec s n-Blicks et al[24]., 2009	248 children (1–4 years)	Lactobacillus rhamnosus LB21 (107 CFU/mL)	Milk/21 months	A:Probiotic/fluoride B: Placebo	MS and Lb in plaque (plate culturing)	No statistically significant changes in MS and Lb
Näse et al[32]., 2001	594 children (1–6 years)	Lactobacillus rhamnosus GG, ATCC 53103 (5–10 × 105 CFU/mL)	Milk five daily/7 months	A: Milk/probiotic B: Milk	MS in plaque and saliva (chair-side tests)	Statistically significant MS reduction in group A

Table 3. Studies with caries risk factors as outcome in adults

Reference Study design	**Subjects Age**	**Strain (Concentration)**	**Delivery System/ Treatment Duration**	**Groups**	**Outcome(s)**	**Results**
Ahmad ZareJavid et al[2] 2015	66 students (18-30 years old)	10^6CFU/ml of B. lactis.	Yogurt 300g/day for 2 weeks	intervention group and control group	MS and Lb in saliva (plate culturing)	Statistically significant MS reduction in intervention group and no significant Lb alterations
Nishihara et al[6] 2014	64 adolescents (24 -35 years)	L. salivarius WB21, L. salivarius TI 2711, Ovalgen® DC xylitol.(2.0 × 10^9 colony forming units/day)	Tablets/2 weeks,	four groups L. salivarius WB21 L. salivarius TI 2711 Ovalgen® DC Xylitol	Levels of mutans streptococci and lactobacilli, amount of salivary flow, salivary pH, and salivary buffering capacity	- no significant differences between the groups -Lactobacilli levels significantly increased in the L. salivarius WB21 and TI 2711 groups -Salivary buffering capacity significantly increased in the L. salivarius TI 2711 group (P = 0.003) and Ovalgen® DC group -L. salivarius WB21-containing tablets significantly decreased the number of mutans streptococci.
G.S. Pinto et al[7] 2014	30 subjects	Bifidobacterium animalis subsp. lactis DN-173010	yogurt daily for 2 weeks	probiotic group yogurt group	MS and Lb in saliva (plate culturing)	no difference between the yogurt containing probiotic and the control yogurt
CaterinaHolz et al[10] 2013	60 adolescents	heat-killed L. paracasei DSMZ16671	sugar-free candies 4 times during 1.5 consecutive days	A: Placebo B:1 mg /candy C:2 mg /cany	MS in saliva (plate culturing)	the test groups' saliva had significantly reduced mutans streptococci as an immediate effect.

Marttinen et al[14]., 2012 Cross-over study	13 adults (mean 25 years)	Lactobacillus rhamnosus GG or Lactobacillus reuteri (196 million CFU/tablet)	Tablet twice a day/2 weeks	A: LGG B: L. reuteri	Plaque acidogenicity, MS and Lb in plaque (plate culturing)	No changes in plaque acidogenicity. MS remained stable, while Lb increased in the L. reuteri group, but not in the LGG group
Keller & Twetman[15], 2012 Cross-over study	25 adults (mean 26 years)	Lactobacillus reuteri (DSM 17938 and ATCC PTA 5289) (2 × 108 CFU/tablet)	Tablets three times a day/2 weeks	A: L. reuteri B: Placebo	MS and Lb in saliva (chair-side tests) Lactatic Acid production in plaque	No statistically significant MS change; Lb increased significantly in group A. No significant differences in Lactatic Acid production
Keller et al[16]., 2012	62 adults (mean 23 years)	Lactobacillus reuteri (DSM 17938 and ATCC PTA 5289) (2 × 108 CFU/tablet)	Tablets twice daily/6 weeks	A: Probiotics B: Placebo	Inhibiting regrowth of salivary MS after full-mouth disinfection (chair-side tests)	L. reuteri did not seem to affect or delay the regrowth of MS
Petersson et al[20]., 2011	160 adults (58–84 years)	Lactobacillus rhamnosus LB21 (107 CFU/mL)	Milk once daily/ 15 months	A: Placebo B: Fluoride/ probiotic C: Probiotic D: Fluoride	MS and Lb in saliva (chair-side tests) and plaque (plate culturing)	Lower prevalence of MS and Lb, but not statistically significant
Chuang et al[21]., 2011	80 adults (20–26 years)	Lactobacillus paracasei GMNL-33 (3 × 108 CFU/mL)	Tablets three times per day/2 weeks	A: Probiotics B: Xylitol	MS and Lb in saliva (chair-side tests) and buffer capacity (Dentobuff strip)	No statistically significant differences in MS and Lb and buffer capacity. MS reduction intra probiotics group
Lexner et al[22]., 2010	18 adolescents (13–17 years)	Lactobacillus rhamnosus LB21 (107 CFU/mL)	Milk once daily/2 weeks	A: Probiotic B: Placebo	MS and Lb in saliva (plate culturing)	No statistically significant MS reduction and Lb
Cildir et al[23]., 2009 cross-over study	24 adolescents with fixed orthodontics (12–16 years)	Bifidobacterium manimalis subsp. lactis DN 173010 (2 × 108 CFU/g)	Yogurt once daily/2 weeks	A: Probiotic B: Placebo	MS and Lb in saliva (chair-side tests)	Statistically significant MS reduction in group A and no sig
Caglar. et al[25]., 2008 Cross-over study	24 adults (mean 20 years)	Bifidobacterium lactis Bb-12 (107 CFU/g)	Ice-cream once daily/10 days	A: Probiotic B: Placebo	MS and Lb in saliva (chair-side tests)	Statistically significant MS reduction in group A; salivary Lb levels unaltered
Caglar et al[26]., 2008	20 women (mean 20 years)	Lactobacillus reuteri ATCC 55730: ATCC PTA 5289 10:1 (1.1 × 108 CFU)	Lozenge once daily/10 days	A: Probiotic B: Placebo	MS and Lb in saliva (chair-side tests)	Statistically significant MS reduction in group A; Lb unaltered
Caglar et al[27]., 2007	80 adults (21–24 years)	Lactobacilli reuteri ATCC and Lactobacilli reuteri ATCC PTA 5289 (108 CFU/gum)	chewing gums three times daily/ 3 weeks	A: Probiotics B: Xylitol C:Probiotics/ xylitol D: Placebo	MS and Lb in saliva (chair-side tests)	Statistically significant MS reduction in group A, B and C; Probiotic + xylitol not enhance the efficacy.
Caglar et al[28]., 2006	120 adults (21–24 years)	Lactobacillus reuteri ATCC 55730 (108 CFU/straw or tablet)	Water or tablet once daily/3 weeks	A:Water/probiotic B: Placebo water C:Tablet/probiotic D: Placebo tablet	MS and Lb in saliva (chair-side tests)	Statistically significant MS reduction in groups A and C; similar but non-significant trend for Lb

Caglar et al[29]., 2005 [Cross-over study	26 adults (21–24 years)	Bifidobacterium DN-173 010 (7 × 107 CFU/g)	Yogurt once daily/2 weeks	A: Probiotic B: Placebo	MS and Lb in saliva (chair-side tests)	Statistically significant MS reduction in group A; similar but non-significant trend for Lb
Montalto et al[30]., 2004	35 adults (23–37 years)	L. sporogens, L. bifidum, L. bulgaricus, L. termophilus, L. acidophilus, L. casei, L. rhamnosus (1.88 × 109 live cells/day)	Liquid and capsule/45 days	A: Probiotics capsules placebo in liquid B: Liquid probiotics placebo in capsules C: Placebo in both liquid and capsule	MS and Lb in saliva (chair-side tests)	Statistically significant Lb increase in groups A and B. MS not significantly modified.
Ahola et al.[31], 2002	74 young adults (18–35 years)	Lactobacillus rhamnosus GG ATCC 53103 (1.9 × 107 CFU/g) and Lactobacillus rhamnosus LC 705 (1.2 × 107 CFU/g)	Cheese five daily/ 3 weeks	A: Probiotics B: Placebo	MS, Lb and yeasts in saliva (chair-side tests) and buffer capacity (Dentobuff strip)	No statistically significant differences in MS and Lb after the intervention; during the post-treatment period (3 weeks) a significantly reduction of the two species in group A. No statistically significant differences in yeast and buffer capacity

Table 4. Studies with caries lesion development as outcome.

Reference Study design	**Subjects Age**	**Strain (Concentration)**	**Delivery System/ Treatment Duration**	**Groups**	**Outcome(s)**	**Results**
Stenson et al[8] 2014	113 childern (9 years)	Lactobacillus reuteri,stain ATCC 55730 (10^8 CFU)	5 drops to mothers before birth and 5 drops to child till 9 years in form of 3 parts containing coconut oil, peanut oil, cryoprotective	Placebo group Probiotic group	Visual and Radiographic examination of caries lesion	Caries Prevalence was lower in probiotic group. No statistical differences with respect to caries prevalence
Taipale et al[11]., 2013	106 children (4 years)	Bifidobacteriumanimalis subsp. lactis BB-12 (1010 CFU/mL)	Tablets in slow-release pacifier or spoon twice daily/ 22–23 months	A: Probiotic B: Xylitol C: Sorbitol	Caries increment (ICDAS index)	No differences in the occurrence of enamel caries
Petersson et al[20]., 2011	160 adults (58–84 years)	Lactobacillus rhamnosus LB21 (107 CFU/mL)	Milk once daily/ 15 months	A: Placebo B:Fluoride/ probiotic C: Probiotic D: Fluoride	Root Caries Index (RCI) and Electric Resistance Measurements (ERM)	Higher numbers of RCI reversals in groups B, C and D. Mean ECM values increased significantly in groups A, B and C
Stec s n-Blicks et al[24]., 2009	248 children (1–4 years)	Lactobacillus rhamnosus LB21 (107 CFU/mL)	Milk once daily/21 months	A:Probiotic / fluoride B: Placebo	Caries increment (dmfs index)	Statistically significant difference in caries increment in group A

Table 5: Bias assessment for the studies

Study	**Randomization**	**Concealment of randomization sequence**	**Blinding**	**Proper reporting of incomplete outcomes(dropouts)**	**Free of bias for selective outcomes**	**Free of other source of bias**
Bhalla et al[1] 2015	Yes	Yes	No	Yes	Yes	Yes
Ahmad ZareJavid et al[2] 2015	Yes	Yes	Yes	Yes	Yes	No
Sidhu GK et al[3] 2015	No	Yes	No	Yes	Yes	Yes
NozariA.,et al[4]. 2015	Yes	Yes	Yes	Yes	Yes	Yes
DevasyaAshwin et al[5].,2015	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Nishihara et al[6] 2014	Yes	Yes	No	Yes	Yes	No
G.S. Pinto et al[7] 2014	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Stenson et al[8]2014	Yes	Yes	Yes	Yes	Yes	No

Campus et al[9]., 2013	No	Yes	No	Yes	Yes	Yes
CaterinaHolz et al[10] 2013	Yes	Yes	Yes	Yes	Yes	No
Taipale et al[11], 2013	Yes	Yes	Yes	Yes	Yes	No
Juneja et al[12], 2012	No	Yes	No	Yes	Yes	Yes
Taipale et al[13], 2012	Yes	Yes	Yes	Yes	Yes	No
Marttinen etal[14].2012	No	Yes	Yes	Yes	Yes	Yes
Keller & Twetman[15], 2012	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Keller et al[16]., 2012	Yes	Yes	Yes	Yes	Yes	No
Singh et al[17], 2011	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Aminabadi et al[18], 2011	Yes	No	No	Yes	Yes	Yes
Jindal et al[19]., 2011	Yes	Yes	No	Yes	Yes	Yes
Petersson et al[20]., 2011	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Chuang et al[21]., 2011	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Lexner et al[22]., 2010	Yes	Yes	Yes	Yes	Yes	No
Cildir et al[23]., 2009	Yes	Yes	Yes	Yes	Yes	No
Stec s n-Blicks et al[24]., 2009	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Caglar. et al[25]., 2008 Cross-over study	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Caglar et al[26]., 2008	Yes	Yes	Yes	Yes	Yes	Yes
Caglar et al[27]., 2007	Yes	Yes	No	Yes	Yes	Yes
Caglar et al[28]., 2006	Yes	Yes	No	Yes	Yes	Yes
Caglar et al[29]., 2005	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Montalto et al[30]., 2004	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Ahola et al.[31], 2002	Yes	Yes	Yes	Yes	Yes	No
Näse et al[32]., 2001	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**

Risk of bias of included studies: The included studies were subjected to critical analysis following the Cochrane Collaboration tool for evaluating the risk of bias, and we classified 23 articles as having a low risk of bias and 9 articles as having a high risk of bias. The domain in which the trails were judged to have the high risk of bias was improper blinding and improper randomization [3,9,12,18], improper randomization [14] , improper blinding[1,6,19,27,28]. Among 32 included studies 10 studies were funded by private laboratories. (Table 5)
No differences were observed between the two main databases used. Selected papers were divided between those performed on children and those on adults. They were also divided based on the outcome variables like caries risk factors and caries lesion development. All studies utilized parallel arms with intervention and a placebo/control or a crossover design. The sample sizes were generally small or medium, and the majority of them (80%) were short-term interventions (between 10 and 42 days). Different vehicles for the administration and different dosage of probiotics were used.

Studies focussed on caries prevention as outcome in children:

Fourteen studies were evaluated [1,3-5,8,9,12,13,17-19,24,32]. Only one study was performed to verify the effect of the early administration of probiotics (*Bifidobacteriumanimalis*subsp. *lactis*BB-12) on the oral colonization of mutans streptococci (MS) in 106 infants from a low-caries population [13]. Subjects received probiotic bacteria, xylitol or sorbitol (polyol 100–300 mg) from the age of 1–2 months to the age of 2 years, twice a day. The MS concentration in plaque of the mothers at the start of the study was high and similar in all

subjects, without significant differences. At the end of the study, children showed a rather low MS colonization percentage, with a statistically significant difference among groups(13). At the age of 4 years, the same children were re-evaluated to assess the MS level in plaque and the occurrence of dental caries in deciduous teeth [11]. No differences were observed for both parameters among the three groups.

Otherwise, thirteen studies were carried out to verify the effect of probiotics strains on MS levels in saliva and/or dental plaque, using different vehicle [1,3-5,8,9,12,17-19,24,32]. Only two studies did not demonstrate any change in SM level [3,4].

The effect of milk containing *L. rhamnosus*on MS counts was evaluated in three papers (one short and two long-term studies). In the short-term study [12], the effect of milk containing *Lactobacillus rhamnosus*(hct70) for three weeks was registered in small groups of children. The difference in post treatment regarding MS count between test and control group was not statistically significant, while the difference in follow-up was highly significant [12]. In the long-term studies [24,32], *L. rhamnosus*was administered for several months (7 and 21 months respectively). Statistically significant reductions were recorded with *Lactobacillus rhamnosus*GG, ATCC use [32], while no statistically significant changes were observed in SM counts in subjects receiving *Lactobacillus rhamnosus*LB21 [24].

Two studies were performed with yogurt as probiotics vehicle [4,18]. The effect of administration of yogurt containing *Bifidobacteriumlactis*for 2 weeks in 24 children was evaluated with no significant difference in SM count whereas there was significant difference in SM count in 25 children who consumed normal yogurt for 2 weeks [4].The effect of the administration of yogurt containing *Lactobacillus rhamnosus*GG for three weeks in 105 children was evaluated with a significant decrease in SM count immediately after probiotics use alone, but recolonization was described during the five consecutive weeks [18]. Pre-treatment with chlorhexidine produced a statistically significant reduction in salivary SM counts that enhances during the five consecutive weeks.

Two studies used ice-cream as probiotic vehicle [5,17]. The effect of Probiotic ice-cream containing *Bifidobacteriumlactis*Bb-12 and *Lactobacillus acidophilus* La-5 in 60 children aged between 6 to 12 years for one month was evaluated.There was statistically significant reduction in the salivary levels of MS count after seven days and also after 30 days, but after six months the salivary levels of MS is similar to baseline [5]. The effect of Probiotic ice-cream containingBifidobacteriumlactis Bb-12 ATCC27536 and Lactobacillus acidophilus La-5in 40 children for 10 days was evaluated. There was statistically significant reduction in salivary MS scores was reported after consumption of the probiotic compared to baseline..

Two studies used curd as probiotic vehicle [1,3].The effect of probiotic curd containing B.lactis 12 in 30 children for 1 week was evaluated. There was statistically significant reduction in MS immediately after 1 hr and after 1 week among probiotic group(1). The effect of probiotic curd containing B.lactis 12 in 20children for 1 year was evaluated. In experimental group, 87% children showed decrease and 7% showed increase in *S. mutans* count ($P = 0.83$).(3)

One study was performed using lozenges as probiotic vehicle. The effect of lozenges containing *Lactobacillus brevis*CD2 administered for six weeks was evaluated in 191 high caries risk children [9]. A statistically significant reduction of the cariogenic microorganism was recorded.

One study used two powders as probiotic vehicle in 150 children aged 7–14 years, containing the first *Lactobacillus rhamnosus*, *Bifidobacteriumlongum*and *Saccharomyces cereviasae*and the second*Bacillus coagulans*, and compared them to a placebo powder [19]. Powders dissolved in 20 mL of water were used as a mouth rinse for one minute for 14 consecutive days. Data analysis showed a statistically significant reduction in MS counts in both probiotics groups.

Six studies of the fourteen reported above, investigated the effect of the probiotics strain on Lb level also [3,4,8,13,17,24]. In all studies, a statistically significant change in Lb counts in saliva and/or plaque was not observed. Moreover, one study evaluated the effect of the probiotic on oral yeasts, failing to prove any statistically effect [13]. The effect of probiotics on plaque pH modification after a rinse with a 10% sugared solution was investigated and plaque acidogenicity resulted significantly lower in subjects that have used probiotic lozenges [9]. Two studies evaluated the probiotic effect on caries lesion development [8,11.24]. A statistically significant difference in caries increment was recorded only in one paper in subjects who received probiotic and fluoride compared to subjects who received placebo milk [24].

Studies focussed on caries prevention as outcome in adults:

Eighteen studies were selected [2,6,7,10,14-16,20-23,25-31]: all of them investigated the effects of probiotic administration on MS counts in plaque and/or saliva and ten demonstrated a MS reduction.

Caglar and co-workers [25-29], performed several studies on the change of salivary MS concentration after the use of several probiotics (*Bifidobacteriumlactis*Bb-12, *Lactobacillus reuteri*ATCC 55730 and ATCC PTA 5289, *Bifidobacterium*DN-173 010) using different vehicles (ice-cream, chewing-gum, water, yogurt and tablets). MS concentrations decrease significantly in all studies.

Two studies were performed with yogurt as probiotics vehicle [2,23]. The effect of the administration of yogurt containing B.lactis for two weeks in 66 adolescents was evaluated with a significant decrease in SM count.A double-blind, crossover study was carried out on 24 healthy adolescents, undergoing orthodontic treatment, with the aim to assess the effect of yogurt containing *Bifidobacterium animalis* subsp. *lactis* DN-173010 administered once daily [23]. Statistically significant reduction of MS was recorded after probiotic yogurt consumption.

Short-term administration trial was conducted using L. salivarius WB21-containing tablets in 64 adolescents for 2 weeks which significantly decreased the number of mutans streptococci. (6).One study was conducted using sugar-free candies4 times during 1.5 consecutive days containing heat-killed L. paracasei DSMZ16671 as a probiotic vehicle in 60 adolescents There was significant reduction in salivary levels of mutans streptococci as an immediate effect.(10)

No statistically significant differences in MS counts were recorded immediately after consumption of cheese containing *Lactobacillus rhamnosus*GG and *Lactobacillus rhamnosus*LC 705, but a significant reduction was reported three weeks after the experimental period [31].

Conversely, the other eight of the eighteen studies did not reveal an effect of probiotics administration on MS counts [6,7,14-16,20-22,30]. Four short-term studies were performed using tablets containing *Lactobacillus rhamnosus*or *Lactobacllusreuteri*; MS counts remained stable after the administration of both probiotics twice a day for two weeks in 13 adults [14]. No significant differences were also observed after the use for two weeks of *Lactobacillus reuteri*on MS counts in 18 adults [15], and using the same strains after full mouth disinfection with chlorhexidineon 62 adults on regrow of MS [16]. Tablets containing *Lactobacillus paracasei*GMNL-33 were unsuccessfully administered to 80 young adults [21]. One short term study evaluated the effect of Lactobacillus rhamnosus LB21delivered in milk on MS count in saliva in 18 older adults for 2 weeks (22). No statistically significant reduction in MS count was registered. One long-term study evaluated the effect of *Lactobacillus rhamnosus*LB21 delivered in milk on MS count in saliva and supra-gingival plaque in 160 older adults for 15 months [20]. No statistically significant reduction in MS count was registered. Results from a study utilizing several strains of *Lactobacillus* spp. in liquid and capsules form in 35 adults revealed no significantly MS count reduction [30].One study was conducted using L. salivarius WB21, L. salivarius TI 2711, Ovalgen® DC (antibody against glucosyltransferase from Streptococcus mutans), or xylitol containing tablets in 64 adolescents for 2 weeks which significantly decreased the number of mutans streptococci (6).The levels of mutans streptococci seemed to decrease in the L. salivarius WB21, TI 2711, and Ovalgen® DC groups compared to the xylitol group, with no significant differences between the groups.

Moreover, sixteen studies of the eighteen reported above, investigated the effect of the probiotics strain on Lb level in saliva and/or plaque [2,6,7,14,15,20-23,25-31]. Twelve studies failed to prove any effect on Lb counts and four studies demonstrated a statistically significant change in Lb counts [6,14,15,30]. Two studies evaluated also the effect of probiotics on plaque acidogenicity, but no significant changes were found [14,15]. Two studies investigated the effect on buffer capacity failing to demonstratea statistically significant difference on it [21,31].But one study showed the significant increase in Salivary buffering capacity significantly in the L. salivarius TI 2711 group ($P = 0.003$) and Ovalgen® DC group (6). One of these did not demonstrate an effect on oral yeast yet [31].

IV. Discussion

The aim of this review was to evaluate the effect of probiotics in the prevention and treatment of dental caries. Results described by various research groups were encouraging [9, 12, 13, 17-20, 23, 25, 29, 31, 32], but the scientific evidence is still unclear and often not very high. The main goal for the use of probiotics in caries prevention is to replace and displace cariogenic bacteria, mainly mutans streptococci, with noncariogenic bacteria [33].

Most clinical trials reviewed had a small sample size and reported caries risk factors as intermediate or surrogate endpoints, which limited the conclusions about the real efficacy of probiotics administration in caries lesion prevention. From the analysis of the RCTs selected, it is reasonable to affirm that probiotic strains may play a role as antagonistic agent on cariogenic bacteria. In the two-thirds of the selected papers, probiotics have demonstrated the capacity to reduce MS counts in saliva and/or plaque regardless of the product or strain used. However, this effect is variable and probably short-lasting. In addition, MS are no longer considered the main cariogenic bacteria involved in the caries progress, since the important role of non-mutans acidogenic and aciduric bacteria was clarified [34]. Different results are reported on the effect of probiotics on lactobacilli counts. From the sixteen studies that evaluated the changes of this interim outcome, just four reported a positive result [6, 4, 5, 30] The other interim outcomes considered (yeasts and plaque acidogenicity) were investigated in few studies and the results are unclear. Only three selected papers [11,20,24], two performed on children and another one on adults/elderly samples, had caries lesion development as outcome; two studies reported a statistically significant difference in caries increment after 15/21 months of probiotics use [20,24].

Several mechanisms of action for probiotic are described in literature, some of them still not fully understood. Several local and systemic effects are describing, including adhesion, co-aggregation, competitive inhibition, production of organic acids and bacteriocin-like compounds and immune-modulation [35]. However, probiotic bacteria are not able to colonize oral cavity permanently [25], so a continuous regular, almost daily intake is required. This may be a compliance aspect to be considered.

In thirteen selected papers, a dairy product (milk, cheese, yogurt and ice-cream) was used as delivery vehicle for probiotics [2,5,7,12,17,18,20,22-24,29,31,32]. These non-sweetened products are known to possess caries preventive effects related to a natural high contents in calcium and phosphate that enhance remineralisation of hard oral tissues and contrast acids produced by cariogenic bacteria after sugared foods and drinks intake [33]. Only one selected paper used chewing gums as delivery vehicle [27]. The use of non-sugared chewing gum has been considered useful for dental health, since it reduces plaque acidogenicity and increases enamel remineralisation, enhancing salivary flow rate [36]. The remaining thirteen papers used as probiotic vehicle products (lozenges, tablets, powders) without any reported preventive effects themselves [6,9-11,13-16,19,21,25,28,30].

One study evaluated the combined effect of probiotics and fluoride on cariogenic bacteria and caries lesion increment. No statistically significant differences were recorded between the group using probiotics alone and those using probiotic and fluoride together [20]. Another paper studied the combined effect of probiotics and low dosage of xylitol on cariogenic microorganisms [27], but no statistically significant differences were noted compared to probiotics alone. Finally only one study has investigated the effect of probiotic on MS counts after chlorhexidine mouthwash disinfection [18]. Pre-treatment with chlorhexidine produced a long-lasting reduction in salivary SM compared to probiotics alone. It is interesting to note that up to date, none products have successfully approved by the European Food Safety Authority (EFSA) [37, 38].

A theoretical risk of the probiotic assumption is the increase of caries risk due to the capacity of probiotic strains to form biofilm and produce acids, but this aspect was not taken into consideration by any papers. One approach have been used to assess the quality of RCTs in the present review ie. the Cochrane collaboration tool. These tool takes into account 25 important methodological items, providing an accurate evaluation of the methodological correctness with which the study was planned and carried out. From the analysis of the checklists of the selected papers, the main deficiencies observed were the lack of information on methods to define the hypothesis, the sample size calculation, the absence of data on the results of estimated effects size and their precision. These methodological weaknesses reduce the validity of studies and the interpretation of the results may lead to biased findings. Moreover, few studies reported correctly the results of the RCTs not taking into account other sources of bias. In general, the quality of reporting of RCTs was quite low, with half of all studies scoring as poor with the exception of three studies that were scored as excellent. These results are similar to those reported of other systematic review [33], but it is possible to observe a progressive improvement in the scientific evidence of the effect of probiotic on caries prevention.

V. Conclusion

The use of probiotic strains for caries prevention showed promising results even if only few studies have demonstrated clear clinical outcomes. Therefore, the scientific evidence is still poor. A continuous regular almost daily intake is probably required; this maybe a compliance aspect to be considered. However, for all products effective in caries prevention (*i.e.*, fluoride and chlorhexidine) a frequent intake is required, so a possible way of administration could be to insert probiotic in other daily preventive products like toothpaste.

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