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Review Article

Modulation of Oral Biofilm and Immune Response Associated to Mucosa with Probiotic Bacteria as a Potential Approach in the Prevention of Dental Caries: A Systematic Review

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ABSTRACT

Background: A variety of approaches have been developed for the control of dental caries, a pathology with high incidence and prevalence worldwide. The use of probiotic strains for the modulation of dental biofilm in the prevention of caries has been studied, but the available evidence shows varied methodologies; and the strains tested differ from one study to another.

Objective: To analyse through a systematic review of clinical trials, the efficacy of using probiotic strains to prevent dental caries.

Methods: A search was made in the scientific bases PubMed, Cochrane and Science Direct, prioritizing randomized double and triple-blind clinical trials from 2010 to 2020, including a total of 20 studies to be analysed. The selection criteria were consistent with the Preferred Reporting Items for Systematic Reviews protocol.

Results: Within the studies, different types of probiotic bacteria were analysed, dominating *Lactobacillus Paracasei*. While most clinical trials show a favorable response in terms of a significant reduction of *Streptococcus mutans* in the oral microbiota, very few studies evaluated salivary pH and sIgA levels.

Conclusion: The heterogeneity of the studies analysed and the multifactorial nature of dental caries do not allow us to ensure that probiotic therapy is completely effective in preventing this pathology. Although probiotic therapy can help by regulating the microbiological factor, there are other determinants that can favor the development of caries and that are barely approached in their relationship with bacteriotherapy. Future studies that homogeneously evaluate the use of probiotics could give us a clearer idea of their effectiveness.

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Introduction

Dental caries is a chronic disease and a global public health problem, imposing a large economic and health burden internationally; a wide

variety of approaches to its control have been developed and applied, with varying degrees of success [1, 2]. According to Global Burden data on oral diseases, untreated caries in permanent teeth affects about 2.5 billion people around the world, making it the most prevalent oral pathology above others [3]. Caries also greatly affects primary teeth,

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with a prevalence of 17% in children with 2 years old, 48% at age 4 and rising to 70% in children aged 6 according to the World Health Organization (WHO). During childhood, this disease attacks more aggressively and can progress to tooth loss if is not treated in time [1, 4, 5]. Early childhood caries increases the risk of tooth decay in permanent teeth; it is also associated with a 3-7 month delay in the development of permanent teeth and its late maturation affects occlusion and can lead to problems with eating, speech, appearance, and behaviour [5-7]. This is a multifactorial disease; however, the main causal agent is represented by pathogenic acidic/aciduric microorganisms such as *Streptococcus mutans*, accompanied by dysbiosis in the oral cavity [8-10].

The negative effects of dental caries on health are cumulative, from childhood to adulthood, nevertheless, it is a preventable pathology [11, 12]. Proper oral hygiene is essential to prevent its development [13]. Nowadays the most commonly used prophylactic chemical agents inhibit bacterial growth and biofilm formation. However, these chemical agents also eliminate the oral microbiota, altering its balance and potentially affecting oral health [4, 14]. Although several medicines can inhibit the pathogenic microbiota, none has been able to successfully prevent the proliferation of residual strains, which is why the selective inhibition of pathogenic microorganisms through the use of probiotic strains has been studied recently [4]. The definition of "probiotics" has been adopted by the World Health Organization as: "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" [15]. These have been used historically for gastrointestinal and immunological treatments and are now widely studied as preventive therapy in oral health [16-18]. The main mechanism of action is based on enhancing the commensal microbiota and preventing colonization by true pathogenic microorganisms [16]. The present study aims to analyse, through a systematic review of different randomized and controlled clinical trials, the efficacy of probiotic bacteria for potential preventive therapy of dental caries by modulating biofilm and stimulating the immune response associated to mucosa.

Data collected from most studies show that probiotics produce a significant reduction in the colonization density (expressed by the count of colony forming units/ml/cm²) of *Streptococcus mutans* in short and medium term; other studies demonstrated an increase in salivary pH and secretory IgA levels (sIgA), which provide a positive effect in the prevention of dental caries. However, the evidence for the immunomodulatory effect of probiotic strains, measured by salivary pH and/or salivary buffer capacity, in addition to sIgA levels, is very poor. Therefore, more high-quality studies that can be reproduced are needed.

Materials and Methods

This section was planned following the criteria of the Preferred Reporting Items for Systematic Reviews and Meta-analyses system (PRISMA criteria) [19].

I Matter in Question

Probiotics prevent dental caries via modulation of the biofilm and mucosa associated immune response.

II Eligibility or Inclusion Criteria

Randomized, blinded, controlled clinical trials that evaluate the effect of probiotic strains on *Streptococcus mutans* count, salivary pH, salivary buffer capacity, and sIgA levels.

III Exclusion Criteria

Letters to the editor, review articles, association articles, *in vitro* experimental studies, unblinded randomized studies and articles that do not involve the subject matter.

IV Search Strategy

Three digital databases, PubMed, Science Direct and Cochrane, were selected for the collection of scientific articles published during the period 2010-2020. Four reviewers conducted the search strategy to identify eligible studies. MeSH terms in all fields were used for the search in order to maximize the search during the investigation [20]. The search strategies defined for the databases previously described were probiotics "AND" caries, and probiotics "AND" dental biofilm. Restrictions were imposed on publication data and access to the scientific article; only English language publications were considered. Articles available in more than one database were considered only once.

V Selection of Studies

Initially 27 randomized and controlled clinical trials were selected for the evaluation of salivary pH, CFU/ml values of *Streptococcus mutans* (Sm) and sIgA, however, in view of little evidence about prebiotics and their modulation of biofilm it was decided to exclude these studies. Finally, 20 articles were included for the systematic review. Figure 1 details the flowchart for the selection of studies.

VI Data Register

Microsoft Excel database was used for the processing of the data. The qualitative variables to be interpreted were: significant reduction (respect to the control group) in the Sm count, significant increase in the salivary sIgA level, significant increase of salivary pH, salivary buffer capacity; the age range is from 4 months to 73 years old, including both male and female individuals.

VII Results Prioritization

Data analysis was done through qualitative description.

VIII Bias Risk

Not assessed due to variability in methodology and plurality of objectives of each study. However, in order to increase the quality of evidence, of all articles related to the topic and obtained, we selected for full reading, only those that incorporated double and triple blinding. Discarding the clinical trials with simple blinding or without blinding.

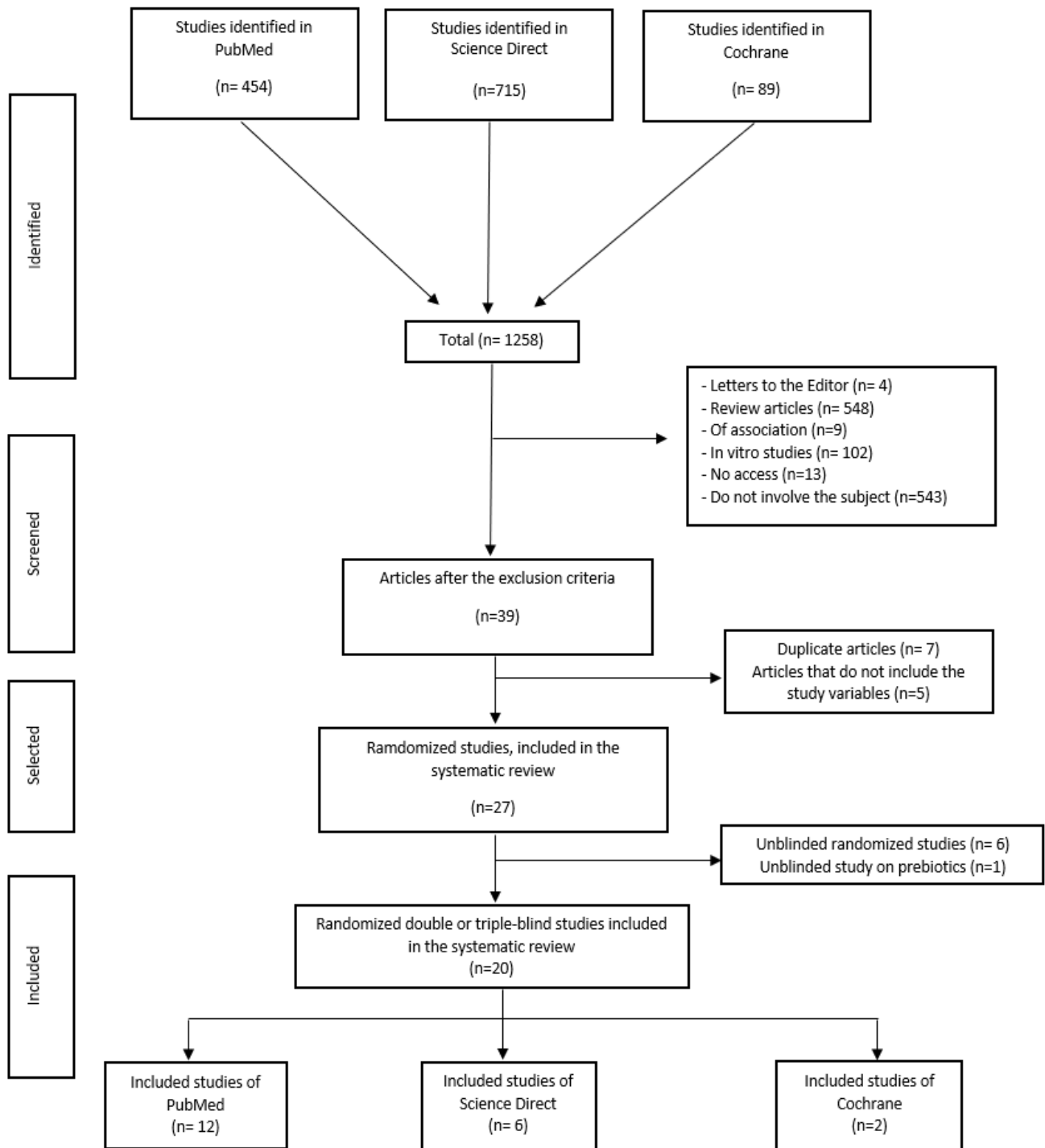


Figure 1: Flowchart that shows the methodology used in the selection of the studies that passed to the full analysis.

Results

The PubMed search provided 454 articles (2010 to 2020), ScienceDirect 715 articles (2010 to 2020) and Cochrane with 89 articles (2010 to 2020), with the keyword’s probiotics “AND” caries, and probiotics “AND” dental biofilm. After a thorough review in these databases, a total of 1258 articles were retrieved, of which 39 controlled and randomized studies were considered. Of these, 12 were excluded for not contributing with the variables chosen in this study and for being duplicated; an additional

filtering of the 27 studies was carried out after reading the abstract and methodology, which determined the exclusion of 7 articles that studied prebiotics and were not blinding. A total of 20 articles were finally included in the systematic review: PubMed provided with 12 articles, Science Direct with 6 and Cochrane with 2 articles. The total number of participants in the different randomized, double and triple-blind clinical trials analysed was 2037, the sample size of the total trial’s ranges from 19 to 321 participants with an average of 102. According to the gender of the participants, a total of 1013 were males representing 52.6% and

913 females representing 47.4% of all the studies; it is worth mentioning that three studies do not mention a gender-disaggregated registration, the total number of participants in these was 111. According to the age of the participants, the total range was from 4 months to 73 years, the average being 12.6 years.

In this review, different probiotic species were encompassed, of which the most predominant were: *Lactobacillus paracasei* with 30%, followed by *Lactobacillus rhamnosus* 20%, *Lactobacillus reuteri* 15%, combined probiotics 10%, *Bacillus coagulans* 10%, and the remaining 15% were found as probiotics: *Bifidobacterium longum*, *Lactobacillus acidophilus*, Bacteriocin, *Bifidobacterium lactis*, *Lactobacillus casei*, and *Streptococcus salivarius*. Our study determined that the treatment time range was 7 to 365 days, with an average treatment time of 89.32 days.

In the analysed studies, the count of colony forming units (CFU) of *S. mutans* per ml or cm² was made by cultures media; the salivary pH and the buffer capacity were measured with a digital laboratory pH meter or with pH test strips. On the other hand, the salivary levels of sIgA were determined by the ELISA method. Of the 20 studies collected, 19 (95%) evaluated the *S. mutans* count, 3 (15%) measured the sIgA levels and 6 (30%) evaluated the salivary pH and buffer capacity. Of the most found probiotic species, whose effect was evaluated in at least 3 of the 4 variables proposed in this analysis, were *L. rhamnosus* and *L. paracasei*. Meanwhile, *L. reuteri* was analysed in at least 2 of the 4 variables proposed in this review. Finally, the species *L. paracasei* was identified as the most effective in modulating the biofilm for caries prevention, because it significantly reduces the number of *S. mutans*, increases salivary pH and sIgA (Table 1).

Table 1: Distribution of probiotic species evaluated in terms of efficacy with respect to the control group, in all consensus studies.

Bacterial Species	Nº of studies (%)	Significant reduction in <i>Sm</i> (%)	Significant increase in salivary pH/ buffer capacity	Significant increase in salivary level of sIgA	Average treatment time in days
<i>L. rhamnosus</i>	5 (25%)	3 (60%)	2 (40%)	0	68,7 days
<i>L. paracasei</i>	6 (30%)	5 (83,3%)	1 (16,6%)	1 (16,6%)	128,5 days
<i>L. reuteri</i>	3 (15%)	1 (33,3%)	1 (33,3%)	0	147,6 days
<i>L. acidophilus</i>	2 (10%)	2 (100%)	0	0	22 days
<i>L. casei</i>	1 (5%)	1 (100%)	0	0	57 days
<i>L. sporogenes</i>	1 (5%)	1 (100%)	0	0	30 days
<i>Bacillus coagulans</i>	2 (10%)	1 (50%)	0	0	10,5 days
<i>Bifidobacterium lactis</i>	1 (5%)	1 (100%)	0	0	14 days
<i>Streptococcus salivarius</i>	1 (5%)	1 (100%)	0	0	91,2 days

Discussion

This study reviewed certain parameters that must be considered for approval of the use of probiotics in the prevention of dental caries, such as: 1) Reduction of key cariogenic bacteria such as *Streptococcus mutans*, 2) Increase in the pH and buffer capacity of saliva, and 3) Increase in the salivary level of secretory IgA (sIgA). The use of probiotics helps maintain health by providing balance to the gastrointestinal, genitourinary, and oral ecosystem; probiotics have been shown to have the ability to reduce the *S. mutans* count in saliva or dental plaque in the short term [21, 22]. In our study we found the same results, and we identified that *Lactobacillus rhamnosus*, *Lactobacillus reuteri* and *Lactobacillus paracasei* species were the most studied, being this last one the one with more positive effects in terms of significant decrease, relative to control group, in the number of CFU/ml/cm² of *S. mutans*, as well as, significant increase in sIgA and salivary pH levels. Summarizing the results at a global level for all the probiotic species evaluated, in 14 of the 20 selected articles a significant reduction in the *S. mutans* count was identified, in 4 articles a significant increase in salivary pH/buffer capacity was identified and one demonstrated a significant increase in sIgA levels.

On the other hand, we can mention that probiotics show other advantages for general health since they improve the immune system; in addition, the vehicles by which they can be administered are various, such as: pills, milk, cheese, yogurt, ice cream, drops, powder, mouthwash, chewing gums, among others [21, 23]. Chewing gums with probiotics and sugar-free have been shown to reduce the acidogenicity of plaque and increase

the remineralization of the enamel, improving salivary flow and oral health [22]. However, some disadvantages of probiotics have also been described, as they can cause adverse effects including upset stomach, diarrhea, headache, fever, and nausea or vomiting, according to the study by Teanpaisan *et al.* (2014) [24]. The main cause of dental caries is the accumulation of dental biofilm; in order to prevent this, it is important to eliminate bacterial plaque by mechanical removal through tooth brushing, however, there are chemical methods to control biofilm and as an alternative to these arise from the incorporation of probiotics that can be an excellent complement to brushing, and they are also considered safe for oral administration in humans [24-26].

The most widely used chemical solution for biofilm control is chlorhexidine, but its use may present some drawbacks such as dental pigmentation, taste alteration, hypersensitivity and stenosis of the parotid duct; that is why the use of probiotics could represent an advantage against weaknesses of certain chemical products [26, 27]. The antiplaque activity of probiotics is achieved by reducing bacterial adhesion to the tooth surface, inhibiting the growth and proliferation of pathogenic microorganisms, avoiding the formation of intercellular matrix and modifying the biochemistry of plaque to reduce the formation of cytotoxic products, obtaining a less pathogenic microbiota [25, 26, 28]. Jothika *et al.* (2015) mention that chlorhexidine varnish, gel and mouthwash reduce the level of *S. mutans* in saliva, inhibit plaque formation and increase salivary pH values, however, it can produce dental pigmentation when used for a long time [25, 29]. In the study by Shah *et al.* (2019), the group treated with probiotic mouthwash and the group with chlorhexidine obtained significantly reduced plaque index,

compared to the control group, however, substantial improvement was observed in the gingival index than in the plaque indices with better results in the probiotic group [26].

Stensson *et al.* (2014), did not detect statistically significant differences for the plaque index between the control group and the probiotic group when using oral drops of *Lactobacillus reuteri* during the first year of life, however, 9-year-old children who had received perinatal supplements of *L. reuteri* since birth and during their first year were almost caries-free and had a lower prevalence of proximal caries [28, 30]. Matuq *et al.* (2020), report that probiotic tablets with *L. reuteri* and chlorhexidine (CHX) mouthwash significantly reduce the accumulation of plaque and *S. mutans* count and increase salivary pH values [25]. Statistically similar effects on plaque control were found in the trial by Jothika *et al.* (2015), however, their study used *Lactobacillus acidophilus* as a probiotic in mouthwash and was compared with CHX and sodium fluoride mouthwashes [29]. The antiplaque effect of *L. reuteri* strains in probiotic tablets can be attributed to their ability to prevent microorganisms from adhering and growing on the tooth surface, in addition to modifying the biochemistry of the biofilm [25, 28].

Regarding *Lactobacillus acidophilus*, Tahmourespour *et al.* (2011) and Jothika *et al.* (2015), clarify that its presence can cause a reduction in the adherence of streptococcal strains, mainly *S. mutans* [29, 31]. In our review, *L. paracasei* was the most widely used probiotic; and in the trial by Manmontri *et al.* (2020), this probiotic species reduces the number of cariogenic bacteria such as *S. mutans* in both saliva and dental plaque, and its reduction effect persists for at least 6 months after the discontinuation of the probiotic, this may be a consequence of the constant microbial modulation of *L. paracasei* SD1 and its ability to adhere to the epithelial cells of the oral mucosa [10]. In addition, other studies attribute the highest antimicrobial activity to *L. paracasei*, *Lactobacillus plantarum*, *L. rhamnosus* and *L. salivarius*, with *L. paracasei* being one of the commensal species of the genus *Lactobacillus* that has shown maximum interference activity against *S. mutans*, even *in vitro* [15].

In the study by Nishihara *et al.* (2014), 2 different strains of *Lactobacillus* were compared against an antibody obtained from the IgY-type egg yolk (Ovalgen DC), and Xylitol. In these groups, the changes in the density of colonization by *Streptococcus mutans* and anticariogenic commensal bacteria, increased salivary pH and buffer capacity were evaluated. Both, probiotic treatment and immunotherapy with Ovalgen DC were shown to reduce *S. mutans* levels, and increase salivary flow and pH, but without significant differences compared to xylitol treatment. However, of the 3 approaches, only the probiotic treatment demonstrated superiority in increasing colonization by commensal *Lactobacillus* species, and together with immunotherapy they demonstrated an increase in salivary buffer capacity, with significant differences over the group treated with xylitol. The study concluded that certain probiotic strains are more effective in preventing tooth decay than chemical plaque or biofilm control methods [32].

Reduction in the level of *Streptococcus mutans* colonization can be achieved by a vaccine, however, only few controlled and double-blind clinical studies have been published to evaluate active immunization strategies; and only open clinical studies have been conducted to

evaluate the efficacy of passive immunization [33-36]. In the passive vaccines, antibodies are administered that distribute a specific protection, this significantly reduces the colonization of *Streptococcus mutans*, but they cannot be maintained, therefore they are less effective and require multiple applications to maintain the immunization [34, 37]. The results of controlled clinical trials on the efficacy of active immunization in the prevention of dental caries have been encouraging; however, the salivary responses were variable, transitory and low magnitude [38-42].

Randomized, double-blind phase 1 clinical trials conducted by Childers group demonstrate that active immunization with an antigenic preparation enriched in *Streptococcus mutans* glycosyltransferase (GSF) and administered by nasal route with liposomal coating is more effective than tonsillar/buccal administration and without liposomal coating, to generate a significant local immune response both salivary and nasal based on sIgA-anti-GSF [43, 44]. These findings are encouraging; however, the sample size of the study by Childers and collaborators is 21 healthy volunteers. It would be necessary to evaluate the efficacy of active immunization with *Streptococcus mutans* GSF in a larger group of patients, and to compare the nasal route of administration with the subcutaneous route directly in salivary glands [34, 37, 43, 44].

Among the limitations found in the sample of the present study (20 studies), there is the evaluation of the effect in a short time, since only one article evaluated the effect at 3, 6 and 9 years. Furthermore, the lack of homogeneity in the methodologies, the absence of studies reporting information on CFU/ml/cm², pH level and salivary buffer capacity, as well as the measurement of the level of salivary sIgA; the lack of a uniform distribution among the experimental branches and the little existence of studies within the Latin American population, do not allow formulating robust conclusions on the efficacy of probiotic systems in the modulation of the oral biofilm and the immune response associated to mucosa to prevent dental caries.

Conclusion

Given the wide methodological heterogeneity of the studies processed in this review, we cannot assure that the use of certain probiotic bacteria constitutes effective systems in the prevention of dental caries, by means of biofilm modulation and mucosal associated immune response. However, based on our analysis we can report:

- i. *Lactobacillus paracasei* was the most prevalent probiotic in the present review, showing promising results that should be evaluated on a larger scale and in different ethnic groups, in future studies.
- ii. The regular use of probiotics reduces the level of oral colonization of *Streptococcus mutans*, but its protective antiplaque effect would be preserved only for 6 months, after the suspension of the product. We need more evidence on the effect of these probiotic systems on the stimulation of the mucosal associated immune response, and to clarify the scope of this prevention approach.
- iii. Probiotics appear to be superior to chemical agents in controlling the cariogenic biofilm, stimulating beneficial commensal species, and increasing salivary buffer capacity.

- iv. Certain probiotic strains show comparable benefit to immunotherapy with heterologous antibodies (chicken IgY) in modulating oral biofilm and increasing salivary buffer capacity.

Recommendations

The limited evidence for the importance of probiotics in oral health may be explained by the recent interest, from the scientific community, in this topic. More clinical trials using similar methodology and larger sample sizes are needed to compare different types of probiotic strains and their long-term efficacy. Additionally, new studies should evaluate other variables such as modulation in salivary pH and sIgA levels that play an important role in oral homeostasis. Another recommendation for future double-blind, placebo-controlled studies may be to study the efficacy of using probiotics alone versus probiotics in combination with chicken IgY-based immunotherapy (Ovalgen DC). Indeed, the latter approach has emerged as an innovative product with limited evidence, which has shown encouraging results in preventing dental caries.

Conflicts of Interest

None.

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