ORIGINAL ARTICLE

Comparative Evaluation of Antibacterial Efficacy of Commercially Available Rhamnosus Derived Therapeutic Agent Against Cariogenic Microorganism

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Abstract

Aim: This study was designed to evaluate and compare the effectiveness of probiotics, synbiotics, and CHX 0.2%-against the Streptococcus mutans and *Streptococcus salivarius*, which are considered the most important targets in the pathways of causation that lead to dentistry-carious lesions. **Materials and Methods:** Pure cultures of S. mutans ATCC 25175 and *S. salivarius* ATCC 25975 were grown on Muller-Hinton agar and tested at 10^6 CFU/mL bacterial inoculum. Assessing anti-bacterial properties was done through the assessment of the zone of inhibition (ZOI). Tooth surface swabs samples were evaluated on Mutans Sanguis Agar, with confluent colonies counted after 24-48 hours for biofilm assessment. **Results:** The highest ZOI produced by the CHX 0.2% was for *S. mutans* (25 ± 1.8 mm) followed by probiotics (18 ± 1 mm) and synbiotics (12 ± 0 mm). *S. salivarius* showed corresponding values of ZOI 24.3 ± 2.1 mm for CHX 0.2% and 16.6 ± 1.2 mm for probiotics and 13 ± 1 mm for synbiotics. One-way ANOVA explained that there is a statistically significant difference among groups ($p < 0.05$). It was stated in the biofilm assay that no growth was recorded for CHX 0.2% and synbiotics, while growth was recorded from the probiotics measured at 10^8 CFU/mL. **Conclusion:** CHX 0.2% revealed the strongest antibioactivity example, but probiotics and synbiotics demonstrated mild effects. Therefore, future studies are needed to ascertain the applicability of probiotics and synbiotics as alternative agents for caries prevention, though thus far they have proven to be promising measures.

Key words: Dental caries, *Lactobacillus*, probiotics, *Streptococcus mutans*, synbiotics

INTRODUCTION

Dental caries is one of the most prevalent global health disorders. It is a disease that crosses the age barrier and affects all people. It is a multifactorial disease that is most often caused by demineralization of both enamel and dentin caused by cariogenic microorganisms such as *Streptococcus mutans* and *Streptococcus salivarius*.[1,2] Initially, caries management has been directed mechanically against plaque control, such as brushing with fluoridated toothpaste, and through chemical application by the likes of chlorhexidine (CHX) 0.2% effective, by its broad-spectrum bacteriostatic action.[3] However, they may not work well because of drawbacks such as tooth staining and a different taste. Probiotic formulations have shown potential *in vitro* against oral pathogens, bolstering the interest in functional probiotic foods for oral health.[4] For instance, *Lactobacillus rhamnosus* GG would make up live microorganisms that exhibit health benefits upon sufficient intake by the consumer through inhibition of pathogenic bacteria and modulation of healthy microbiota.[5] However, though limited, these bacteria may be delayed in the colonization of the oral biofilm.[6] Fructooligosaccharides are prebiotics that enhance the growth of beneficial bacteria such as *L. rhamnosus*, and those prebiotics can be derived

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from foods such as bananas and onions.[7] The combination of probiotics with prebiotics was given a name, termed synbiotics, which proved to be the future for managing caries by boosting the therapeutic potential of both components.[8]

In theory, probiotics, prebiotics, and synbiotics have great promise, but little has been said or done about their actual application in the prevention and management of caries. Preliminary studies have suggested that probiotics would inhibit the cariogenic bacteria by means of competitive receptor binding or antimicrobial production, while perhaps interfering with biofilm formation and the behavior of bacteria to enhance the clinical efficacy of synbiotics.[9] This *in vitro* study aims to assess the antibacterial efficacy of *L. rhamnosus*-based probiotics, along with a synbiotics formulation of *L. rhamnosus* with fructooligosaccharides, and CHX 0.2% against two important cariogenic pathogens, *Streptococcus mutans* and *S. salivarius*. The primary objectives are to measure the zone of inhibition (ZOI) and to evaluate biofilm formation, colony-forming units (CFUs), and persistence of bacteria. The study aims to evaluate probiotics and synbiotics as potential substitutes or adjunctive modalities to chemical agents in caries management.[10]

MATERIALS AND METHODS

The Ethical Approval Committee, Saveetha Dental College and Hospitals, SIMATS, approved the study before undertaking the research and gave it the review number: SRB/SDC/ENDO-2303/23/210.

The first phase involved assessing the antibacterial activity of CHX 0.2% (positive control), a synbiotics formulation, and a probiotic preparation against the two dental pathogens, *S. mutans* (ATCC® 25175) and *S. salivarius* (ATCC® 25975), by the agar well diffusion method on Mueller–Hinton Agar (MHA). Procurement of bacterial strains was done from the Department of Microbiology, Saveetha Dental College, Chennai, Tamil Nadu, India. The bacterial inoculum was standardized to 10^6 CFU/mL and uniformly spread onto the MHA plates. Subsequently, three 9-mm diameter wells are carefully punched into the agar and filled with the respective test samples for 2° diffusion in the surrounding medium.

The plates were subsequently exposed in a bacteriological incubator at 37°C for 24 h. After the incubation period, the diameter of the inhibition zone (ZOI) surrounding each well was measured with the HI Antibiotic Zone Scale™. The values were noted to be mean ± SD The samples were taken from 15 carious molars that were extracted for evaluating biofilm formation as well as CFUs of *S. mutans* in it. All experiments were done in triplicate so as to ensure reproducibility and accuracy of results. They were divided into three groups, consisting of five molars each. Each group was treated with one of the three test compounds: A probiotic preparation, a synbiotics formulation, or CHX 0.2%. The sample teeth were incubated in fresh broth with *S. mutans* suspension and, respectively, with one of the test compounds, allowing biofilm development for a period of 3 days. On the 3rd day, swabbing was done at the surfaces of each tooth, and lawn cultures were prepared by streaking this onto Mutans Sanguis Agar plates. These were incubated for 24–48 h at 37°C. Finally, the bacterial colonies were counted and expressed as CFU per milliliter (CFU/mL). This procedure was thus carried out in an organized manner to obtain a consistent assessment of biofilm growth and bacterial proliferation under the influence of each test compound.

RESULTS

The study results indicated that CHX 0.2% exhibited the highest antibacterial efficacy, as evidenced by the largest ZOI against *S. mutans* (25 ± 1.8 mm) and *S. salivarius* (24.3 ± 2.1 mm) [Figure 4 and Table 1]. The probiotic group demonstrated moderate antibacterial activity, achieving ZOI values of 18 ± 1 mm for *S. mutans* and 16.6 ± 1.2 mm for *S. salivarius* [Figure 4]. In comparison, the synbiotics group displayed the smallest ZOI, measuring 12 ± 0 mm for *S. mutans* and 13 ± 1 mm for *S. salivarius* [Figures 1 and 2, Figure 4 and Table 1].

When assessing biofilm growth, both CHX 0.2% and the synbiotics group effectively inhibited biofilm formation, with no detectable CFUs after 48 h [Figure 3]. On the other hand, the probiotic group failed to suppress biofilm growth, exhibiting consistent bacterial proliferation with CFU levels reaching 10^8 CFU/mL [Table 2].

Statistical analysis using one-way analysis of variance revealed significant differences among the three groups concerning ZOI values and biofilm inhibition ($P < 0.05$). However, no significant difference in ZOI measurements was observed between the two bacterial species. These findings underscore the superior antibacterial and biofilm-inhibitory properties of CHX 0.2%, along with the potential of synbiotics as an alternative strategy for managing dental caries. Conversely, the probiotic group showed limited effectiveness in controlling biofilm growth, suggesting the need for further investigation into its application.

DISCUSSION

The disease dental caries has long been defined as the mostcommon chronic disorder of all time around the world,being present even in history from several thousand yearsago and even earlier, in some respects.[2] For instance, the fossils of Microsyops latidens, discovered in 2021, an early member of primate fauna, manifested the earliest signs of tooth caries in ancestral species.[11] Breeding grounds of dysbiosis may influence the pathogenesis of caries; the close association has led to fruitful avenues of research, promoting

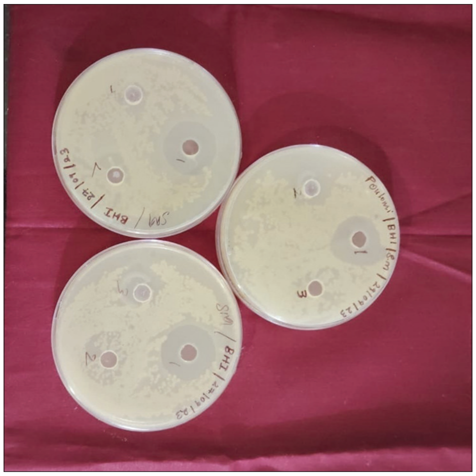

Figure 1: Zone of inhibition formed by *Streptococcus mutans* on (1) Chlorhexidine 0.2%, (2) Probiotic, (3) Synbiotics

Figure 2: Zone of inhibition formed by *Streptococcus salivarius* on (1) Chlorhexidine 0.2%, (2) Probiotic, (3)Synbiotic

probiotic therapy. CHX has long been hailed as the gold standard for dental plaque management and caries bacteria growth prevention.[13] Its disadvantages, however, are black staining, the modified sense of taste, and dysbiosis in the oral microbiome.[14] *In vitro* data indicate that probiotics can be as effective as chemical agents in inhibiting some specific microorganisms, such as *Candida albicans*, through a few conditions. The activity spectrum of probiotics extends beyond the previously mentioned pathogens, other than bacteria. Some manufacturers have performed experimentation on formulation variations of probiotics and synbiotics. Certain strains of probiotics have been significantly demonstrated they modulate the oral microflora by inducing beneficial bacteria and limiting the growth of many dangerous bacteria, such as *L. rhamnosus*.[15] Combined strains effectively compete for adhesion to the tooth surface and thereby boost the secretion of lactic acids, which in turn curtail the plaque buildup and caries.[16] The newer combination of agents–synbiotics-significantly combined both the prebiotic and the probiotic for augmented growth activities in beneficial bacteria and prevention of the growth of the non-beneficial

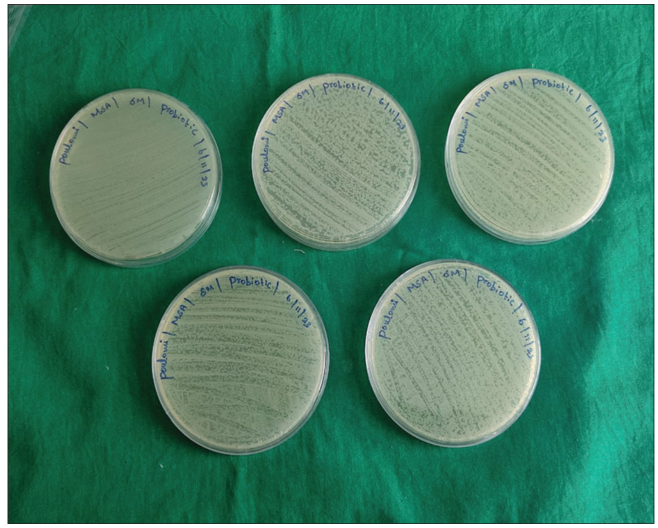

Figure 3: *Streptococcus mutans* biofilm growth on probiotic (after 48 h incubation period)

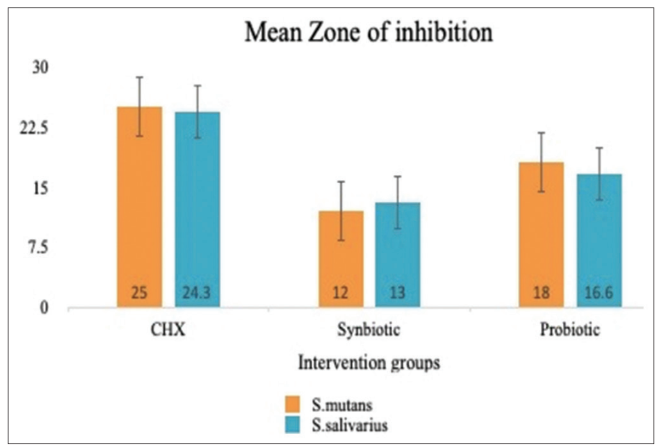

Figure 4: Graph depicting zone of inhibition of the three comparator groups against *Streptococcus mutans* and *Streptococcus salivarius*

Table 1: ZOI measurements (Mean±SD, mm)

Group	*Streptococcus mutans*	*Streptococcus salivarius*
CHX 0.2%	25±1.8	24.3±2.1
Probiotic	18±1.0	16.6±1.2
synbiotics	12±0.0	13±1.0

ZOI: Zone of inhibition, CHX: Chlorhexidine

microorganisms in the oral milieu.[17] The combination of *L. rhamnosus* synbiotics with fructooligosaccharides has brought down the CFU count of *S. mutans* in some other recent reports, indicating that synbiotics, as opposed to CHX, may be the long-lasting solution for managing caries.[18] Scientific literature provides supporting evidence of the potency of *L. rhamnosus* in halting the progression of dental caries; for instance, using ethanol extract of *L. rhamnosus* inhibiting streptococci caused verified caries. A study by Bijle *et al*. in 2023 reported that a combination of *L. rhamnosus* GG,

Table 2: Biofilm growth assay of three different comparator groups on *Streptococcus mutans*

Sample	Tooth 1	Tooth 2	Tooth 3	Tooth 4	Tooth 5
CHX 0.2%	No growth	No growth	No growth	No growth	No growth
Probiotic	10^8	10^8	10^8	10^8	10^8
Synbiotics	No growth	No growth	No growth	No growth	No growth

CHX: Chlorhexidine

arginine, and sodium fluoride has significantly shown a reduction in the number of bacteria present in the oral cavity when viewed under a confocal planar![19] In the present study, the synbiotics turf exploits reduced *S. mutans* CFU, a significant marker of action against caries, and hydrodynamic therapy. Hence, we could propose that the lesions showing biofilms of *S. mutans* over extracted infected teeth might have been due to overgrowth of the strain presented in the catalase test due to natural xenobiotic stressors. The variety of strains with improved proficiencies should be selected to mitigate these drawbacks.

Other probiotic strains, such as *Lactobacillus paracasei* and *Lactobacillus fermentum*, also demonstrate antimicrobial activity against both *S. mutans* and planktonic cells.[20] Aside from probiotics and synbiotics, newly developed antimicrobial agents that have shown considerable antimicrobial action against oral pathogens include zinc oxide nanoparticles synthesized by herbal formulations such as *Ocimum tenuiflorum* and *Ocimum gratissimum*. This indicates a potential complementary or alternative mode for prevention and managing caries.[21] Such green synthesized nanoparticles use the antimicrobial properties of both the metal oxide and herbal bioactive compounds to enhance efficacy while reducing side effects associated with chemical antimicrobials.[22]

Currently developing in caries control, great success has been met with combinations such as fructo-oligosaccharides and *Lactobacillus acidophilus*. Clinical trials with synbiotics such as arginine and probiotics show a reduction in caries-associated bacteria with long-term use. In the current study, the combination of the synbiotics *L. rhamnosus* and fructooligosaccharides greatly reduced *S. mutans* growth, suggesting that synbiotics may work as a useful adjunct or alternative to CHX. More clinical and *in vivo* studies are needed to provide evidence of the benefits of *L. rhamnosus* and fructooligosaccharides in the long term and the best formulation. The best part of using *L. rhamnosus* for caries management is its safety and natural origin. It has no disadvantages chemical-wise compared to CHX, a synthetic agent.[23] *L. rhamnosus* is accepted as having excellent safety for prolonged use among the wide array of patients, including children and those with sensitive oral mucosa. Moreover, it is thought to facilitate remineralization processes of incipient enamel lesions, an important step in preventing caries. In addition, the capacity of *L. rhamnosus* to reset the balance of oral microbiota profiles provides the major basis for its use in the prevention of caries and other pathologies of oral environments, such as periodontal disease and oral candidiasis.[24] Other probiotics, such as *Limosilactobacillus reuteri* and Bifidobacterium dentium, have shown significant reduction in *S. mutans* levels, thus underscoring the extensive antibacterial potential of probiotics.[25] Synbiotics, being a combination of bifidobacteria and inulin, have also enhanced the prevention of caries by protecting the beneficial population.[26] CHX remains an effective acute antibacterial; however, its shortcomings in long-term modulation of the microbiome stand in sharp contrast to the holistic benefits of probiotics and synbiotics. This data, in particular, does not, in general, suggest that micro biome-friendly approaches present effective alternatives or adjuncts in caries management.[27] Aside from conventional agents such as CHX, probiotics can be good substitutes for modulating the oral microbiota, inhibiting *S. mutans* and producing acids, and promoting beneficial bacterial growth. Among these probiotics, *L. rhamnosus* stands tall. Synbiotics may also further enable this as they integrate prebiotics such as fructooligosaccharides with probiotics to augment the effect, much more concerning long-term microbiota balance. An effective formulation would include isolated proven probiotic strains, prebiotics that are compatible, and delivery systems such as lozenges or mouth rinses as vehicles to ensure bacterial viability. Clinical evidence will have demonstrated the efficacy of these products in decreasing cariogenic bacteria and promoting enamel remineralization, thus offering a safe, microbiome-friendly approach to sustainable prevention of caries and oral health maintenance. Moreover, this *in vitro* study does not reflect the multitude of complexities involved in the oral environment, such as biofilm dynamics, saliva flow, and immune responses. The study's findings are also strain-specific: *S. mutans* and *S. salivarius* only. It does not encompass other important interactions between different microbes, nor does it include long-term evaluation or clinical trials, hence limiting the real-world implications of this study. In addition, no comparison against standard anti-cariogenic therapies such as fluoride was made. Hence, there is a need to address these limitations in future studies for practical clinical applications.

CONCLUSION

Probiotics and synbiotics, particularly *L. rhamnosus*, show promise as alternatives or adjuncts to CHX in managing dental caries. These bacteria play significant roles in regulating the oral microbiome, and one of their most important actions is the reduction of *S. mutans* and promotion of remineralization, thus helping with caries prevention. Indeed, even with the effectiveness of CHX, some adverse effects are associated with it, leading to probiotics and synbiotics being a more natural long-term alternative. More research is warranted to optimize formulations and effectiveness against long-term utilization for holistic oral health care.

DECLARATION

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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