

Comparative evaluation of the antimicrobial effects of probiotic milk and probiotic powder on the salivary *Streptococcus mutans* counts and the plaque scores in children aged 3–6 years: A randomized controlled trial

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Abstract

Background. Probiotics have been shown to have a positive influence on systemic and oral health. The prevention of dental caries and gingival diseases through the consumption of probiotics has been studied extensively.

Objectives. The aim of this research was to determine the effects of a short-term intake of probiotic milk and powder on the salivary levels of *Streptococcus mutans* (*S. mutans*) and the plaque scores in children.

Material and methods. In this short-term study, 34 healthy 3–6-year-old children were randomly assigned to group A (control), group B (enKor-D) or group C (Yakult). The probiotics were taken for 7 days. To screen for the amount of *S. mutans* measured in colony-forming units per milliliter of saliva (CFU/mL), unstimulated saliva samples were collected and cultured on Mitis Salivarius Agar plates before and after the intervention. The plaque scores were also recorded at pre- and post-intervention visits.

Results. A statistically significant reduction of salivary *S. mutans* was recorded after the consumption of probiotic milk (Yakult®) and powder (enKor®-D) ($p < 0.05$), with the decrease being greater for the enKor-D group. However, only the consumption of Yakult decreased the plaque scores significantly ($p < 0.05$).

Conclusions. A short-term use of Yakult and enKor-D can have a cariostatic effect by lowering oral microbial load in children with certain risk profiles. Further research is required to confirm this probiotic effect over a long-term period. Prior to prescribing or promoting Yakult or enKor-D as an adjunct caries prevention therapy for children, a thorough risk evaluation may be needed.

Keywords: probiotics, caries, dental plaque index, *Streptococcus mutans*

Cite as

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Introduction

Early childhood caries (ECC) is a distressingly widespread disease among children in both developed and developing countries, with *Streptococcus mutans* (*S. mutans*) considered to be the chief causative organism.¹ Despite the use of conventional caries treatment measures ranging from invasive approaches, such as extractions/restorations, to minimally invasive or non-invasive methods, such as the use of fluorides and remineralizing agents, the prevalence of ECC does not display a substantial decline.² This can be attributed to most individuals neglecting oral health care, either due to the high cost of treatment or because of the lack of awareness about simple preventive methods that can be incorporated in one's daily life. One of such upcoming preventive tools against the development of caries is the use of probiotics.

The concept of probiotics, which was introduced by Élie Metchnikoff,³ dates back to 1908. Originating from the Greek word meaning "for life", probiotics are defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host.⁴ Their effect on gut flora has been widely studied and it is believed that they have a similar effect in the oral cavity; they prohibit *S. mutans* from colonizing tooth surfaces, preventing the development of dental plaque and, as a result, decreasing the risk of dental caries.

A household name in the field of probiotics for over 50 years has been Yakult[®], a well-known probiotic product originating from Japan and Taiwan. Its constituents include artificially sweetened skimmed milk with a suspension of *Lactobacillus casei* strain Shirota (LcS). Studies have shown that Yakult can be consumed on a daily basis by all children for up to 55 months without the fear of developing adverse effects.⁵ Taking into account the high acceptability, minimal side effects and enhanced flavor of Yakult, it would be of great importance to determine the probiotic effects of LcS as a caries-preventing factor in people consuming this dairy beverage.

A newly emerging probiotic, enKor[®]-D, is an India-based product in the form of a sachet containing a blend of 2.7 billion colony-forming units (CFU) of different species of *Lactobacillus* bacteria. It is claimed that along with reducing the prevalence of caries, it can also alleviate gingivitis, periodontitis, halitosis, and a sore throat.⁶ As opposed to Yakult and other probiotics, enKor-D is in a powdered form; it is to be consumed by sprinkling the contents of the sachet onto the tongue and allowing the powder to dissolve. Having simple administration instructions for all age groups, this probiotic could be the "next Yakult" in the industry.

Although the correlation between probiotics and oral health is not a satisfactorily ventured avenue, it has been suggested that consuming these products from an early age promotes the development of healthy bacterial strains

in our bodies. The need to identify a suitable administration vehicle for these probiotic species is the first step. Currently, the globally fancied modes include milk, yogurt, ice cream, and cheese, but the ideal vehicle which could be widely accepted across age groups has not been confirmed yet. This study was conducted to determine the immediate short-term effects of probiotic milk and the newly emerging probiotic powder in a 3–6-year-old age group with the aim of comparing the salivary levels of *S. mutans* and the plaque scores before and after the administration of the probiotics.

Material and methods

This parallel, randomized controlled trial (RCT) was carried out after obtaining approval from the institutional review board (IHEC/SDC/PEDO-2001/21/24) at a dental university setting (Saveetha Dental College and Hospitals, Chennai, India). It was conducted over a 1-month period, from December 2020 to January 2021. Children aged 3–6 years who reported to the Department of Pediatric and Preventive Dentistry at Saveetha Dental College and Hospitals and had a deft (for primary teeth – decayed, extracted, filled teeth)/DMFT (for permanent teeth – decayed, missing, filled teeth) score <2 were considered to be eligible for the study. Children having a history of dental treatment in the past 6 months, including topical fluoride application, severe medical conditions, any allergy to dairy products, and those on any medications were excluded from the study.

A sample size of 27 was determined with an 80% power of study at a significance level of 0.05 to detect a discrepancy between the groups. To account for a potential loss to follow-up of up to 20%, 7 children were added, bringing the overall number of participants to 34. The 34 children were randomly allocated with a coin toss to the control group or either test group (another coin toss) after their dental status was checked with a mouth mirror and a probe under operative light in a conventional dental chair. Allocation was performed by one of the investigators (VR). The allocation process is depicted in a flow chart (Fig. 1).

The study was explained to the parents before data was collected, and their informed consent was received. The included subjects were randomly divided into 3 groups: group A (control); group B (enKor-D); and group C (Yakult). The single-blinding procedure was followed, in which the individuals examining the subjects and gathering data were unaware of the group the participant belonged to. Subjects in group A were not given anything to consume, whereas those in group B received 1 sachet of probiotic powder (enKor-D; Tenshi LifeCare, Bengaluru, India) and those in group C received 10 mL of probiotic milk (Yakult; Yakult Danone India, New Delhi, India) for 7 days. The parents were instructed about the mode

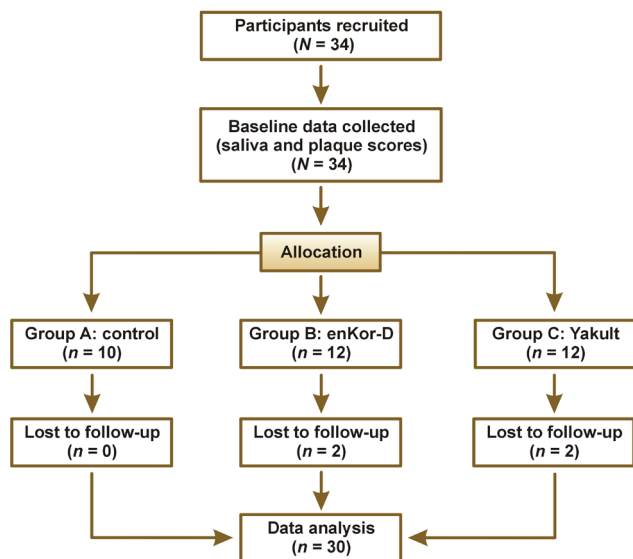


Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram

of administration of enKor-D, which is to be consumed by sprinkling the contents of the sachet onto the tongue and allowing the powder to dissolve. Rather than drinking Yakult directly, the subjects from group C were given similar orders, which included keeping the drink in their mouth for 1 min, and then swallowing it. This was done to ensure the topical effect of Yakult, similar to that of enKor-D. The parents were informed that their children should avoid rinsing their mouths and abstain from any liquid or solid intake for a period of 30 min after having the probiotic. They were asked to send the investigator a video of their child taking the probiotic every day. Common dietary recommendations and oral hygiene instructions were to be followed by all groups during the study period.

At the 1st visit, the collection of baseline unstimulated saliva into a 20-milliliter disposable sterile container was carried out. The salivary samples were immediately taken to the laboratory for culturing. These samples were inoculated for 48 h at 37°C on freshly prepared Mitis Salivarius Agar culture plates (Mitis Salivarius Agar Base; HiMedia Laboratories, Mumbai, India) and subjected to microbial analysis (Fig. 2). An automated colony counter (LT-37; Labtronics, Panchkula, India) was used to determine the amount of *S. mutans*, which was measured in CFU/mL of saliva. The Silness–Löe plaque index (PI) was used to record the plaque scores for all subjects. These findings were tabulated and used to establish baseline statistics.

After a 7-day period of probiotic consumption (i.e., on day 8), unstimulated saliva was collected again into a different sterile container and incubated, after which the colonies were counted. The PI scores were also recorded. The data was tabulated in the same Microsoft Office Excel spreadsheet (v. 2010; Microsoft, Redmond, USA) as the baseline data.



Fig. 2. Salivary samples inoculated on Mitis Salivarius Agar culture plates Intervention – the consumption of the probiotic; left side – pre-intervention; right side – post-intervention (in group A, no intervention was employed).

Statistical analysis

The data was exported and subjected to statistical analysis with the use of the IBM SPSS Statistics for Windows software, v. 23.0 (IBM Corp., Armonk, USA).

Descriptive statistics were employed with regard to the plaque scores and the *S. mutans* counts. Parametric tests were used to determine statistically significant differences in both the microbial counts and the plaque scores. The intergroup comparisons (between the 3 groups) of the mean PI scores and *S. mutans* counts at both time points (before and after) were performed using the analysis of variance (ANOVA). Tukey's post hoc honestly significant difference (HSD) test was carried out for multiple comparisons. The intragroup comparisons of the mean PI scores and *S. mutans* counts in all 3 groups were made using the paired *t* test. For all analyses, $p < 0.05$ was considered statistically significant.

Results

Thirty-four children aged 3–6 years were recruited for this study. Four children dropped out after the 1st appointment; 2 were from the enKor-D group and 2 were from the Yakult group.

According to the findings of this research, all groups showed a decrease in the PI scores after 7 days as compared to baseline, but only the participants who consumed Yakult showed a statistically significant reduction ($p = 0.016$) (Table 1). The intergroup comparisons of the PI scores revealed no statistically significant differences between the groups (Table 1). A reduction in the *S. mutans* counts was also observed in all groups, with the control group showing the smallest decline (Table 2). This reduction was statistically significant after the consumption

of both probiotics, with enKor-D providing a more pronounced decrease from 560.3 CFU/mL to 340.1 CFU/mL (Table 2).

These comparisons indicate that during the study period, both probiotics decreased the salivary *S. mutans* levels, but only the consumption of Yakult decreased the plaque scores significantly ($p < 0.05$). No side effects were reported in any of the groups.

Discussion

Early childhood caries is a common disease globally, showing a varied prevalence, with less developed countries reporting an incidence of up to 70%.⁷ Not only does ECC have a long-term negative effect on dentition, but it may also have an effect on a child's general health, cognitive abilities and overall quality of life; dental caries pain has a detrimental effect on a child's physical well-being, sleep habits, and desire to understand and practice daily activities.⁷ These findings highlight the urgency of researching novel self-administered prevention approaches to supplement the current evidence-based guidelines for ECC regulation. As a result, the use of a number of anti-plaque products has been attempted to enhance oral hygiene, including local and systemic fluorides, pit and fissure sealants, and newer options, including xylitol, triclosan and probiotics.⁸

Bacterial therapy, also known as a replacement therapy, is a form of treatment that uses harmless bacteria rather than pathogenic microorganisms to cure diseases. Due to their beneficial effects in treating caries, gingivitis and

halitosis, probiotics have been added to toothpastes and mouthwashes. They present no chronic toxicity and have beneficial systemic effects when ingested. As a result, probiotics are truly novel, cutting-edge agents that can be used to treat dental caries, including ECC, with minimal side effects.⁹ According to Hedayati-Hajikand et al., a long-term use of dairy products containing probiotic strains may aid in the prevention and treatment of childhood caries.¹⁰

Poureslami et al. studied the effects of a dairy product of a probiotic nature (Espar) on the salivary calcium and *S. mutans* levels.¹¹ They concluded that increasing the calcium content of dental plaque, which can be successfully achieved by eating dairy products, can prevent enamel demineralization.¹¹ In the present study, milk was selected for analysis in one test group, since it possesses anticariogenic properties due to the casein, calcium and phosphorus content. Above all, milk is popular and commonly liked. The ideal vehicle for the delivery of probiotics has not been established yet; enKor-D, being a new probiotic, was included in this study, as it uses a new delivery system for administering probiotics.

Two of the most commonly used probiotic bacterial genera are *Bifidobacterium* and *Lactobacillus*.¹² The *Lactobacillus* species have been found to prevent the development of the *S. mutans* colonies when the probiotics are applied during oral biofilm creation.¹³ The significant drop in the salivary levels of *S. mutans* found in this study may be explained by the potential mechanisms of action of probiotics, which include the inhibition of bacterial adhesion, preventing the colonization of the oral cavity early on, interspecies association, and immunomodulation.¹⁴

Table 1. Comparison of the plaque index (PI) scores within and between the study groups

Time point	Group A (control)	Group B (enKor-D)	Group C (Yakult)	Intergroup comparison <i>p</i> -value (ANOVA)
Baseline/Pre-intervention	0.53 ± 0.15	0.47 ± 0.19	0.60 ± 0.16	0.243
Post-intervention	0.44 ± 0.15	0.37 ± 0.21	0.41 ± 0.17	0.678
Intragroup comparison <i>p</i> -value (paired <i>t</i> test)	0.089	0.097	0.016*	–

Data presented as mean ± standard deviation ($M \pm SD$). * statistically significant.

Table 2. Comparison of the *Streptococcus mutans* (*S. mutans*) counts [CFU/mL] within and between the study groups

Time point	Group A (control)	Group B (enKor-D)	Group C (Yakult)	Intergroup comparison <i>p</i> -value (ANOVA)
Baseline/Pre-intervention	468.1 ± 169.3	560.3 ± 121.6	566.5 ± 109.6	0.212
Post-intervention	452.7 ± 172.4	340.1 ± 111.4	395.4 ± 112.0	0.195
Intragroup comparison <i>p</i> -value (paired <i>t</i> test)	0.092	0.003*	0.005*	–

Data presented as $M \pm SD$. * statistically significant.

As opposed to this study, previous studies showed that probiotics containing *Lactobacillus reuteri* (*L. reuteri*) led to a substantial decrease in the plaque scores due to their anti-inflammatory action.^{15–17} The current findings show that a significant decrease in the plaque scores was observed only with the consumption of Yakult, which does not contain that particular species of *Lactobacillus*. On the other hand, the use of enKor-D did not result in a significant decrease in the plaque scores, even though it contains *L. reuteri*. The different tooth brushing habits of children may have played a role in lowering the plaque scores in the Yakult group more than in the enKor-D group.

As shown in Table 2, the salivary levels of *S. mutans* recorded after 7 days of probiotic consumption were statistically significantly lower as compared to baseline ($p < 0.05$). The findings of this research match those of Chinnappa et al., who observed differences after 1 h, but they became statistically significant after 7 days.¹⁸ Our analysis showed a statistically significant decrease in salivary *S. mutans* after the intake of probiotic milk and powder, which is consistent with previous research by Jindal et al.¹⁹ Chuang et al. presented results that were contrasting to ours; they showed no statistically significant differences between the control and probiotic groups.²⁰

The beneficial effects of probiotics are transient and they do not persist for long after the discontinuation of consumption.²¹ Depending on the vehicle utilized for the probiotic, different studies suggest varying washout times. In a study by Mahantesha et al., the participants consuming probiotic ice cream (Amul) showed reduced *S. mutans* levels when compared to a probiotic drink (Yakult) after a 90-day washout period.²² The participants in a study by Manoharan et al. were given homemade probiotic curd to consume and the gradual recolonization of the studied organisms was observed after a washout period of 14 days.²³ Hence, further studies would be required to assess the long-term efficacy of probiotics, based on their washout periods.

Limitations

Even though only short-term probiotic administration was evaluated in this study, a substantial decrease in the *S. mutans* cariogenic bacterial count was noticed. It is possible that taking probiotics for a longer period of time would help deter caries from developing. Long-term research on the effects of probiotics in reducing bacterial counts would be beneficial. Another limitation of this study is a relatively small sample size, suggesting that the study could be underpowered. On the other hand, the major effects observed in the respondent sample suggest that the possible impact merits further study.

Conclusions

Probiotic milk (Yakult) was found to be as effective as probiotic powder (enKor-D) in decreasing the salivary *S. mutans* levels. Yakult was more effective in reducing the plaque scores over a 7-day period. Since they are ingestible and have limited systemic toxic effects, probiotics can be used as an alternative to other preventive measures for dental caries, especially in children above the age of 3 years. The use of such agents for an extended period of time, however, should be analyzed in further studies.

Ethics approval and consent to participate

This study was carried out after obtaining approval from the institutional review board (IHEC/SDC/PEDO-2001/21/24) at Saveetha Dental College and Hospitals, Chennai, India. The participants' parents provided written informed consent prior to the investigation.

Data availability


The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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