DOI: https://doi.org/10.2298/SARH201129001N
UDC: 616.314-002-084; 637.146.2.055:579.864

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The role of yogurt enriched with LGG culture (*Lactobacillus rhamnosus GG*) in dental caries prevention

Isidora Nešković[1,2], Aleksandra Maletin[1], Jelena Brakoč[1], Bojan Petrović[1,2], Sanja Vujkov[1,2], Tanja Veljović[1]

[1]University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia;
[2]Dentistry Clinic of Vojvodina, Novi Sad, Serbia

SUMMARY
Introduction/Objective Contemporary tendencies suggest that probiotics can significantly reduce the prevalence of caries in children, so it can be considered that they have a positive effect on general and oral health. *Lactobacillus rhamnosus GG* (LGG) is a probiotic culture of particular importance in preventive dentistry.
The aim was to assess the effects of consumption of probiotic strain *Lactobacillus rhamnosus GG* on the dental plaque accumulation in children with mixed dentition.
Methods Research included 90 children with mixed dentition (5–12 years old). The first study group consumed 200 ml of *Lactobacillus rhamnosus GG*-enriched yogurt (B-Activ LGG, Dukat) daily for a period of 14 days, while the remaining 30 formed the second study group who consumed 200 ml of yogurt (Jogurt 1.5% milk fat, Imlek) with manually added powder from probiotic capsule (Wayaforte LGG capsule, Medis) daily for 14 days. The control group consisted of 30 children who had regular diet during examination period. Silness–Löe plaque index and saliva pH (pH-Fix-0-14, Macherey-Nagel) were determined at baseline and also upon intervention completion.
Results An increase in pH values was observed in both study groups. In general sample, there is a significant decrease of mean plaque index values ($p < 0.001$). Both study groups had significant decrease of mean plaque index values on the baseline and after 14 days consumption of yogurt. In the control group the number of subjects with decrease plaque index values did not correlate and no association was found.
Conclusion Consistent consumption of LGG culture-enriched yogurt inhibits dental film accumulation and promotes saliva pH increase in children with mixed dentition.
Keywords: dental biofilm; probiotics; cariogenic bacterium

INTRODUCTION

The use of probiotics in a wide range of food products is attracting increasing interest due to their potential health benefits. The World Health Organization defines probiotics as "living microorganisms which when administered in adequate amounts confer a health benefit on the host" [1]. That microorganisms are usually part of the normal flora and this approach in therapy and prevention was first applied in the treatment of intestinal diseases. The general principle of bacteriotherapy or replacement therapy is to change the local micro-ecology, since the aim of treatment is to introduce and stimulate no pathogenic bacterial species [2]. Probiotic use is considered safe and significant, due to their positive effects, such as immunomodulation, hypocholesterolemic activity, protection against infections, and immune response normalization [3].

Most probiotics are Gram-positive bacteria that belong to the genera *Lactobacillus* or *Bifidobacterium* [4]. Studies based on the use of the intestinal probiotics *Lactobacillus rhamnosus* GG [5], *Lactobacillus reuteri*, and *Bifidobacterium* [6] have each reported achieving reduced levels of *Streptococcus mutans (S. mutans)*.

Probiotics can effectively prevent and treat some infectious diseases in the oral cavity, such as halitosis and periodontitis, and can reduce the development of dental caries and the concentration of harmful bacteria, according to clinical studies [7].

For decades, it has been known that the main cariogenic bacterium *S. mutans* is one of the dental biofilm constituents. It is the most important microorganism for the development of caries, both because of its rapid metabolism of sucrose, glucose and fructose, which lowers the pH, and due to the fact that it alters microbial homeostasis towards the caries-causing flora.

Increased, *S. mutans* count is associated with a higher risk of caries and its more rapid progression. Given the essential role of *S. mutans* in the development of caries, efforts have been made to influence its prevalence and cariogenic ability in the oral cavity.

Received • Примљено: November 29, 2020
Revised • Ревизија: December 14, 2021
Accepted • Прихваћено: December 16, 2021
Online first: January 11, 2022

Correspondence to:
Isidora NEŠKOVIĆ
Hajduk Veljkova 3
Novi Sad
Serbia
isidora.neskovic@mf.uns.ac.rs

After probiotics' use, there have been shown the suppressed growth of *S. mutans* and other oral streptococci with cariogenic potential [8, 9] has been demonstrated *in vitro*.

Clinical trials investigated the effect of probiotics on caries prevalence as a final goal in preschool and school children [8, 10, 11, 12].

In most of these cases, probiotics contained in milk, ice cream, yogurt, and other dairy products have been examined. Empirical evidence also shows that probiotic technology is a revolutionary approach to maintaining optimal oral cavity health [13].

Lactobacillus rhamnosus GG (LGG) is a probiotic with a very important role in preventive dentistry, as it is believed to reduce caries prevalence in children and was shown to confer the same benefits in adults when combined with fluoride [14, 15, 16].

This microorganism is capable of colonizing the oral cavity and thus replacing cariogenic streptococci bound to the tooth surface because the adherent ability of *Lactobacillus rhamnosus* for oral tissues is greater than the adherent ability of streptococci [15]. Short and long-term intake of probiotics could reduce the caries risk among children, decrease gum bleeding and reduce gingivitis, reduce the pocket depth and positively affect the gain of clinical attachment, as well as reduce the counts of *Candida albicans* in elderly [17].

Consequently, the aim of this study was to investigate the impact of consuming strain of *Lactobacillus rhamnosus GG* on dental plaque accumulation in preschool- and school-aged children in period of mixed dentition.

METHODS

The research presented here was conducted at the Dentistry Clinic of Vojvodina in Novi Sad, at the Department of Pediatric and Preventive Dentistry. This randomized double-blind study recruited 90 healthy children with the age range of 5–12 years old of both sexes (mean age = 7.86 ± 1.7 years). Participants had not received any products containing probiotic, xylitol, corticosteroids, systemic antibiotics, and local fluoride therapy at least four weeks before taking part in the study. The study had three parallel groups: 30 children in the control group and two study groups (each group consisting of 30 children). Children from the first study group consumed 200 ml of *Lactobacillus rhamnosus GG* enriched yogurt (B-Activ LGG, Dukat) daily for 14 days, this widely available yogurt includes *L. rhamnosus ATCC53103*. Other study group consumed 200 ml of yogurt without probiotic culture (Jogurt 1.5% milk fat, Imlek) with manually added powder from probiotic capsule (Waya forte LGG capsule, Medis) daily for 14 days, this product also contains the same strain *L. rhamnosus ATCC53103* with at least 10×10^9 colony forming units. Tooth brushing was not allowed at least an hour after eating probiotic yogurt. The control group consisted of children who had normal diet during examination period. Oral hygiene habits were not a factor for inclusion or exclusion from the study. The parents were asked not to change the children`s oral hygiene habits during the two-week period. To ensure the use of the probiotic yogurt participants were asked to fill out a table for two weeks each time they used the yogurt.

Prior to commencing the study, parents of the participating children were informed of all research procedures (Appendix 1: Informing parents / guardians about the study) and provided signed consent for the child's participation (Appendix 2: Parent's / guardian's consent to for their child's participation in the study).

Oral examinations were conducted by an experienced pediatric dentist, using mirrors and periodontal probes under focused flashlights in a conventional dental chair.

Silness–Löe plaque index and saliva pH (pH-Fix-0-14, Macherey-Nagel) were determined for all subjects on the first and last day of the study.

The Silness–Löe plaque index was measured by examining four tooth surfaces (vestibular, vestibulo-mesial, vestibulo-distal, and lingual) with a periodontal probe, and each of the surfaces was rated on a 0–3 scale (0 = no dental biofilm, 1 = small amount of biofilm not visible to the naked eye, 2 = greater amount of biofilm visible to the naked eye, 3 = abundance of dental biofilm). The plaque index was calculated by summing the scores pertaining to all four surfaces of all teeth and dividing by four (number of surfaces examined) and the number of teeth examined in both upper and lower jaw.

The saliva pH was determined using pH-Fix indicators, which measure pH in the range from 0 to 14.

The research was conducted in accordance with the Helsinki declaration and was approved by the Committee on Ethics of the Dentistry Clinic of Vojvodina in Novi Sad.

The data obtained were analyzed using the statistical software *The jamovi project,* Jamovi (2020) (Version 1.6). The Shapiro–Wilk test was used to test normality, prior to selection of appropriate parametric/nonparametric test. The overall data were analyzed using Friedman nonparametric test and Durbin–Conover pairwise comparisons. For all analyses, $p < 0.05$ was considered statistically significant.

RESULTS

The first study group that consumed yogurt enriched with LGG included 30 children, 14 (46.6%) of whom were boys, and 16 (53.4%) were girls. The second study group that consumed yogurt with manually added probiotic powder incorporated 30 children, 12 (40%) of whom were boys and 18 (60%) were girls. The control group comprised 30 children, 15 (50%) of whom were boys and 15 (50%) were girls.

The distribution of the age of children was analyzed using the Friedman test, which indicates that the sample is uniformed since there is no statistically significant difference in all three examined groups ($p = 0.114$).

The mean pH value does not increase significantly in general sample ($p = 0.155$). Mean pH value did not increase significantly in the first study group which consumed

Table 1. Impact of probiotic treatment on mean pH value at baseline and after 14 days of consuming LGG probiotic culture

Parameters	Control group pH 1 (n = 30)	Control group pH 2 (n = 30)	Study group yogurt pH 1 (n = 30)	Study group yogurt pH 2 (n = 30)	Study group capsule pH 1 (n = 30)	Study group capsule pH 2 (n = 30)
Mean	6.43	6.43	6.70	6.90	6.63	6.60
Median	6.50	6.50	7.00	7.00	7.00	7.00
Standard deviation	0.626	0.626	0.702	0.662	0.490	0.498
Shapiro–Wilk W	0.742	0.742	0.781	0.794	0.612	0.624
Shapiro–Wilk p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

*pH 1 – pH value at the baseline
*pH 2 – pH value after 14 days

Table 2. Comparison of mean pH values of all three studied groups using Pairwise Comparison (Durbin–Conover) test

Groups		Statistic	p
Control g. pH 1	Control g. pH 2	0.0000	1.000
Control g. pH 1	Study g. yogurt pH 1	1.1122	0.268
Control g. pH 1	Study g. capsule pH 1	1.0232	0.308
Control g. pH 2	Study g. yogurt pH 2	2.4468	0.016
Control g. pH 2	Study g. capsule pH 2	0.7563	0.451
Study g. yogurt pH 1	Study g. yogurt pH 2	1.3346	0.184
Study g. yogurt pH 1	Study g. capsule pH 1	0.0890	0.929
Study g. yogurt pH 2	Study g. capsule pH 2	1.6905	0.093
Study g. capsule pH 1	Study g. capsule pH 2	0.2669	0.790

*pH 1 – mean pH value at baseline
*pH 2 – mean pH value after 14 days

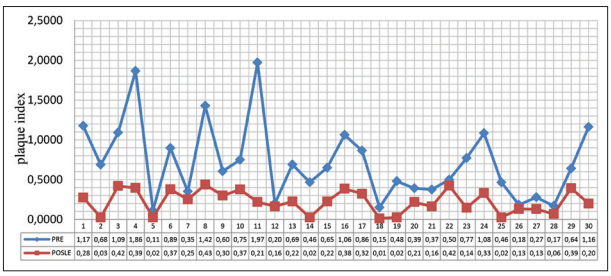

Figure 1. Plaque index values at the baseline and 14 days after intake of commercial yogurt

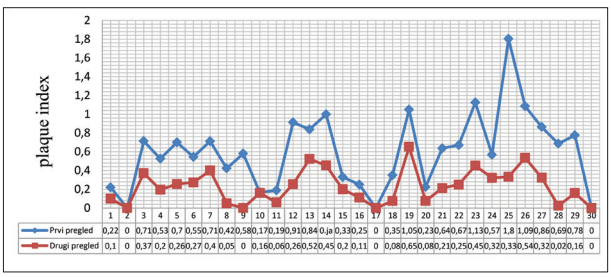

Figure 2. Plaque index values at the baseline and 14 days after intake of yogurt with manually added probiotic powder

commercial yogurt, at the baseline and after 14 days of consuming. The second study group which consumed yogurt with manually added probiotic powder also did not show significantly increase of mean pH value before and after consuming probiotic. The findings are reported in Tables 1 and 2.

In the general sample, there is significant decrease in mean plaque index values ($p < 0.001$). In the first study group, there is statistically significant decrease of mean plaque index value on the baseline and after 14 days consumption of commercially available yogurt ($p < 0.01$). The baseline and after 14 days Silness–Löe plaque index values ranged from 0.113 to 1.972, and 0.013 to 0.437, respectively, as shown in Figure 1. It is evident from the graph that LGG yogurt consumption has led to marked plaque index reduction. The results of mean plaque index values in the second study group who consumed yogurt with probiotic powder from capsules, also showed a significant reduction ($p < 0.01$). In the second study group, the baseline and after 14 days Silness–Löe plaque index values ranged from 0. to 1.803, and 0 to 0.523, respectively, as shown in Figure 2. In the control group, the number of subjects with decrease plaque index values did not correlate and no association was found. Statistically significant difference is noticed between mean plaque index values (PI2 – after 14 days) of control group and both study groups mean values of plaque index after consumption of LGG culture ($p < 0.01$).

There is no significant difference in mean plaque index values between two study groups at the end of the observation period ($p > 0.05$). The findings are reported in Tables 3 and 4.

DISCUSSION

The aim of the present study was to demonstrate the effect of the use of *L. rhamnosus* enriched commercial yogurt and yogurt with manually added probiotic powder on the degree of dental plaque accumulation after two weeks of consumption. The results obtained by analyzing the saliva pH and plaque index in children measured before and after consuming yogurt indicated an increase in pH and a significant decrease in dental biofilm in all participants, from these facts we could indirectly infer a decreased presence of *S. mutans*. The mean saliva pH value at baseline in first study group was 6.70, increasing to 6.90 after two weeks of commercial yogurt intake. As expected [2, 4, 12], the amount of dental biofilm declined, as indicated by the mean plaque index of 0.717 and 0.224, before and after intervention, respectively. Although the second study group which consumed yogurt with manually added probiotic powder, did not show increase of mean pH value, the amount of dental biofilm declined, as indicated by the mean plaque index of 0.598 at the baseline, and 0.227 after 14 days.

In the majority of studies examining the effect of probiotics on the oral microflora, probiotics were consumed for up to 15 days, which is in line with the methodology adopted in the present investigation [6, 18–25]. The most

Table 3. Impact of probiotic treatment on plaque index value at baseline and after 14 days of consuming LGG probiotic culture

Parameters	Control group Plaque index 1	Control group Plaque index 2	Study group yogurt Plaque index 1	Study group yogurt Plaque index 2	Study group capsule Plaque index 1	Study group capsule Plaque index 2
Mean	0.668	0.622	0.717	0.224	0.598	0.227
Median	0.633	0.705	0.645	0.222	0.609	0.206
Standard deviation	0.428	0.361	0.477	0.142	0.400	0.180
Shapiro–Wilk W	0.938	0.954	0.915	0.921	0.948	0.943

*Plaque index 1 – mean value of Plaque index at baseline
*Plaque index 2 – mean value of Plaque index after 14 days

Table 4. Comparison of mean plaque index values of all three examined groups using Pairwise Comparison (Durbin–Conover) test

Group comparison	Statistic	p
Control group PI 1 – Control group PI 2	0.843	0.401
Control group PI 1 – Study group yogurt PI 1	0.295	0.768
Control group PI 1 – Study group capsule PI 1	0.674	0.501
Control group PI 2 – Study group yogurt PI 2	4.762	< 0.001
Control group PI 2 – Study group capsule PI 2	5.142	< 0.001
Study group yogurt PI 1 – Study group yogurt PI 2	5.310	< 0.001
Study group yogurt PI 1 – Study group capsule PI 1	0.379	0.705
Study group yogurt PI 2 – Study group capsule PI 2	0.379	0.705
Study group capsule PI 1 – Study group capsule PI 2	5.310	< 0.001

*PI 1 – mean value of Plaque index at baseline
*PI 2 – mean value of Plaque index after 14 days

commonly studied probiotics are those contained in fortified milk, ice cream, yogurt, and other dairy products. For example, Chinnappa et al. [24] observed a decrease in *S. mutans* count after a week-long daily consumption of ice cream and whey containing a probiotic. Similar results were obtained by Caglar et al. [6] with *Bifidobacterium lactis*, Jiang et al. [17] with the probiotic *L. rhamnosus*, Hedayati-Hajikand [11] with ProBiora3® blend of three strains of probiotic bacteria (*S. uberis* KJ2™, *S. oralis* KJ3™, *S. rattus* JH145™), and Burton et al. [26] using *S. salivarius M18*.

Very important factor is the use of different means of delivery such as dairy products, chewing gums and drops to transfer probiotics. The probiotic channels of supply are suitable for all ages, especially for young children [27]. In the presented study, yogurt is selected because it is safe, available and used routinely in Serbian children's diet. Unfortunately, the commercial yogurt, which is used in the study is not available in our country, although it is produced in Serbia. In conclusion, it appears that yogurt with manually added probiotic powder has shown same effect on the formation of dental plaque, even though commercial yogurt is easier to use.

The effects of *Lactobacillus acidophilus ATCC 4356* and *Bifidobacteriumbifidum ATCC 29521* changing *S. mutans* counts have been evaluated in study conducted by Ghasemi et al. [27], in both groups, S. mutans counts on the first day, second week, and fourth weeks after the intervention were significantly lower than baseline values.

L. rhamnosus is capable of colonizing the oral cavity and thus replacing cariogenic streptococci bound to the tooth surface because the adherent ability of *Lactobacillus rhamnosus* for oral tissues is greater than the adherent ability of streptococci. Hukioja et al. [28] noted that *L. rhamnosus GG* adheres well to hydroxyapatite, although there is a difference in the quality of adhesion between different individuals.

Despite the issue having been addressed in numerous studies and their findings indicated that the *Lactobacillus* probiotic plays a beneficial role in caries prevention, the exact mechanism of probiotic action has not been established [15, 16, 17, 19, 20, 22, 25, 26, 27].

Based on a review of the available literature, it can be presumed that the microflora in children is less stable and more susceptible to change compared to the microbial communities in adults. Consequently, probiotics may have a more lasting effect on the resident microbial population in children [16]. Owing to this disparity, the work presented here focused specifically on preschool- and school-aged children with mixed dentition.

Numerous strains of lactobacilli have been identified, but only a small subset of these strains promotes caries development. Available evidence indicates that *L. salivarius w24*, owing to its sucrose metabolism and pH-reducing capability, could act cariogenically. On the other hand, *L. rhamnosus*, *L. paracasei*, and *L. reuteri* can have a safe and positive effect on caries inhibition. Nonetheless, *in vitro* results preclude specific conclusions and recommendations. It can generally be stated that the effect of lactobacilli may be desirable in the case of carefully selected probiotic candidates [25].

In more recent literature, the role of prebiotics and symbiotics is increasingly being emphasized, in terms of promoting the growth of probiotic bacteria. Bijle at al. [29] demonstrated the usefulness and benign effects of a novel symbiotic with synergistic inhibitory effect on cariogenic bacterium *S. mutans*. Their results demonstrated that the use of L-arginine as a prebiotic enhanced the growth of the probiotic – *L. rhamnosus* GG, whose increase prevents adhesion of *S. mutans*. This observation and application of symbiotics and probiotics opens up opportunities for our new research.

It is important to emphasize that even strains of the same species have different characteristics and each should be individually investigated. It is possible that the same species is not optimal for all oral conditions, hence bacteriotherapy should be tailored to the oral health status of each individual.

CONCLUSION

Regular consumption of LGG enriched yogurt (*Lactobacillus rhamnosus GG*) has an inhibitory effect on the accumulation of dental biofilm and promotes saliva pH increase in children with mixed dentition. By using probiotic products, it is possible to modify dental biofilm composition and metabolism. Since microflora in children is more susceptible to change, it would be advantageous to include products such as LGG enriched yogurt in the regular diet of children of preschool and school age as a means of caries prevention.

Conflict of interest: None declared.

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Улога јогурта обогаћеног пробиотском културум *Lactobacillus rhamnosus GG* у превенцији каријеса

Исидора Нешковић[1,2], Александра Малетин[1], Јелена Бракоч[1], Бојан Петровић[1,2], Сања Вујков[1,2], Тања Вељовић[1]

[1]Универзитет у Новом Саду, Медицински факултет, Нови Сад, Србија;
[2]Клиника за стоматологију Војводине, Нови Сад, Србија

САЖЕТАК

Увод/Циљ Савремени трендови говоре у прилог томе да пробитици могу значајано да утичу на смањење преваленце каријеса у дечјем узрасту, те се сматра да позитивно утичу на опште и орално здравље. Пробиотска култура која је у области превентивне стоматологије показала значајне резултате је *Lactobacillus rhamnosus GG* (ЛГГ).
Циљ рада је испитати утицај употребе соја ЛГГ на акумулацију денталног плака код деце са мешовитом дентицијом.

Методе У истраживању је учествовало укупно 90 деце у периоду мешовите дентиције (узраста 5–12 година). Прву екперименталну групу чинило је 30 испитаника који су током 14 дана конзумирали једном дневно по 200 *ml* јогурта са додатком ЛГГ (*B-Activ LGG*, Дукат), другу групу чинило је 30 деце који су користили једном дневно прах из пробиотске капсуле (*Waya forte* ЛГГ капсуле, Медис) растворен у 200 *ml* јогурта који раније није садржао пробиотске културе (јогурт 1,5% млечне масти, Имлек) током 14 дана. Контролну групу чинило је 30 испитаника који су се уобичајено хранили. Плак индекс по Силнесу и Лоу и *pH* вредност пљувачке (*pH-Fix-0-14*, *Macherey-Nagel*) одређивани су првог и последњег дана истраживања.

Резултати У обе експерименталне групе уочен је пораст средњих *pH* вредности. У целокупном узорку дошло је до смањења средњих вредности плак индекса ($p < 0,01$). У обе експерименталне групе дошло је до значајног смањења средњих вредности плак индекса на почетку истраживања и након 14-дневног конзумирања јогурта обогаћеног ЛГГ културом. У контролној групи није забележена промена средњих вредности плак индекса.

Закључак Редовна употреба јогурта обогаћеног ЛГГ културом делује инхибиторно на акумулацију денталног биофилма и подстиче пораст *pH* вредности пљувачке код деце са мешовитом дентицијом.

Кључне речи: дентални биофилм; пробиотици; кариогене бактерије

APPENDIX 1

Informing parents / guardians about the study titled:

The role of probiotic culture *Lactobacillus rhamnosus GG* in caries prevention

Dear parents,

This scientific study aims to demonstrate that the consumption of LGG culture (*Lactobacillus rhamnosus*) reduces the microbial flora of the oral cavity and plays a significant role in the prevention of caries.

The study involves a two-week consumption of LGG yogurt (b-Activ LGG, Dukat) or LGG probiotic capsules, before and after which a dental examination will be performed, including assessment of the plaque deposits on teeth and measuring the acidity of saliva.

The study poses no risk to the physical or mental health of your child. It is conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Dentistry Clinic of Vojvodina.

Your child's participation in this research is voluntary and yields no material gain to you or your child. You can withdraw your child from the investigation at any time without any consequences. The data obtained will be treated as confidential and will be used only for the purposes of this research.

If you agree with your child's participation in this scientific research, please sign the form "Parent's / guardian's consent for their child's participation in the study."

Thank you for your cooperation.

APPENDIX 2

Parent's / guardian's consent for their child's participation in the study titled:

The role of probiotic culture *Lactobacillus rhamnosus GG* in caries prevention

I am fully informed of the details of the scientific research entitled "The role of probiotic culture *Lactobacillus rhamnosus GG* in caries prevention". I have read the information related to the planned investigation, and was given the opportunity to ask further questions about this study, whereby all my queries have been addressed to my satisfaction.

I consent to my child's participation in the aforementioned research study and am aware that I can withdraw my consent at any time without any consequences.

Child's first name and surname: _____

Child's date of birth: _____

Parent's/guardian's first name and surname: _____

Date: _____

Parent's/guardian's signature: _____

Researcher's signature: _____