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Effect of LGG Yoghurt on *Streptococcus Mutans* and *Lactobacillus Spp*. Salivary Counts in Children

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A B S T R A C T

The aim of this study was to establish effect of 14 day consumption of commercially available yoghurt containing Lactobacillus rhamnosus *ATCC53103 – LGG (Bioaktiv LGG, Dukat, Croatia) on* Streptococcus mutans *and* Lactobacillus spp. *salivary counts in children. Twenty five patients, 6–10yr old participated in the study. At the inclusion in the study caries risk for every patient was evaluated. The saliva samples were tested with chair side kits for saliva buffer capacity (CRT buffer, Vivadent, Schaan, Liechtenstein),* S. Mutans *and* Lactobacillus *counts (CRT bacteria test, Vivadent, Schaan, Liechtenstein). Seven, 14 and 30d after yoghurt consumption saliva samples were tested again with CRT buffer and CRT bacteria tests. Obtained data were analyzed using* χ^2 *and Kruskal-Wallis tests. Results showed significant increase in saliva buffer capacity 30d after yoghurt consumption.* S. Mutans *salivary counts were significantly decreased after 30d. Significant differences in* Lactobacillus *counts were not observed. It could be concluded that daily consumption of yoghurt containing LGG have an inhibitory effect on oral pathogenic bacteria and may be beneficial in caries prevention.*

Key words: *caries, inhibition, mutans streptococci, lactobacilli, probiotic*

Introduction

Use of probiotic products in medicine has become very effective in the prevention and treatment of many diseases, mostly intestinal and urogenital[1,2]. In general terms the word pro-biotic means for life and the definition issued by the International Scientific Association for Probiotics and Prebiotic is live micro organisms administered in adequate amounts that confer a health benefit on the host[1]. That micro organisms 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 harmless (no pathogen) bacterial species[3–5].

The specific actions of the probiotic micro organisms involved are competition with pathogenic bacteria, influence on mucosa permeability and restitution of gut micro-ecology and influence on inflammation process. Generally speaking, the oral cavity is also part of the gastrointestinal system. The same mechanisms of action of probiotic bacteria can be adopted for caries prevention. In the oral cavity direct and indirect actions of probiotics can be observed: they directly influence binding of bacteria to proteins and other bacteria, influence bacterial metabolism and produce substances that inhibit cariogenic bacteria. Indirect actions are the same as in other parts of the intestinal system[6,7].

Most microorganisms that are considered to be probiotic are: *Lactobacillus spp.*, and *Bifidobacterium spp.* There are many others and genetical engineering is strongly involved in the fabrication of new species. *Lactobacillus rhamnosus* is today one of the most popular bac-

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terial species that is used as probiotic. It was isolated in 1985 by Gorbach and Goldin from the human intestine[8]. Laboratory research showed that it has inhibitory action on *Streptococcus mutans* and *Candida albicans*, it also has good adhesion to mucosal and dental tissues and it does not metabolise sucrose[9].

The aim of the present study was to test the effect of 14 day consumption of commercially available yoghurt containing *L. Rhamnosus* ATCC53103 – LGG on *S. Mutans* and *Lactobacillus spp*. salivary counts.

Materials and Methods

The sample size comprised 25 patients 6–10 yr old who voluntarily participated in the study. The study was explained to the parents and their children who accepted to participate in this study. They also received written explanation of the study, after which the parents signed the informed consent. The study protocol was in accordance with the Helsinki Declaration of Human Rights and was approved by the Ethical Committee at the School of Dental Medicine, University of Zagreb. All participants included in the study were healthy and had not used antibiotics or probiotics within the 2-weeks during the washout period prior to the study. Prior to commencing the study caries risk was assessed for each patient. Each patient consumed 200g of yoghurt daily, containing LGG bacteria (Bioaktiv LGG, Dukat, Croatia) during a continuous period of 14 days. After the washout period and before starting yoghurt consumption *S. Mutans*, *Lactobacillus* counts and saliva buffer capacity were established using CRT bacteria and CRT buffer tests (Vivadent, Schaan, Liechtenstein).

S. Mutans and *Lactobacillus* counts were also acquired 14 and 30 days after the study started. The obtained data were analyzed using χ^2 and Kruskal-Wallis tests.

Results

The results obtained in this study are shown in Tables 2–5. Statistically significant reduction of *S. Mutans* count was noted after 30 days of daily yoghurt consumption. At the start of the study ten patients displayed low caries risk, four patients had medium caries risk while eleven patients showed high caries risk (Table 1).

Results show that thirty days after yoghurt consumption percentage of the patients with high *S. Mutans* count dropped significantly from 80% to just 52% (Table 2). In the high caries activity group, high *S. Mutans* count dropped from 91% to 40%, which was highly significant as well (Table 3).

The level of *Lactobacillus* count remained almost the same as at the start of the study. There was no significant difference after 14 days consumption of LGG yoghurt (Table 4). On the contrary, saliva buffer capacity differed significantly and was increased. Thirty days from the start of the study 86% of patients had high buffer capacity compared to 64% at the start. From 36% of patients

TABLE 1
CARIES RISK ASSESSMENT PRIOR THE STUDY

Gender	Caries risk			Total
	Low	Medium	High	
Male	5	3	7	15
Female	5	1	4	10
Total	10 (40%)	4 (16%)	11 (44%)	25

TABLE 2
S. MUTANS COUNTS AFTER 14D DAILY CONSUMPTION OF LGG YOGHURT

CRT bacteria	*Streptococcus Mutans* Count						χ^2 (p)
	Base		14 d		30 d		
	N	%	N	%	N	%	
$>10^5$	20	80	18	79	11	52	
$<10^5$	5	20	5	21	10	48	0.047
Total	25	100	23	100	21	100	

TABLE 3
S. MUTANS COUNTS IN HIGH CARIES RISK GROUP

Caries risk (high)		*S. Mutans* Base		*S. Mutans* 30 d		χ^2 (p)
		Low	High	Low	High	
N	11 (100%)	1 (9%)	10 (91%)	6 (60%)	4 (40%)	0.001

TABLE 4
LACTOBACILLUS SPP COUNTS AFTER 14D DAILY CONSUMPTION OF LGG YOGHURT

CRT bacteria	*Lactobacillus spp.* Count						χ^2 (p)
	Base		14 d		30 d		
	N	%	N	%	N	%	
>105	8	32	12	52	7	33	
<105	17	68	11	48	14	67	0.58
Total	25	100	23	100	21	100	

TABLE 5
SALIVA BUFFER CAPACITY AFTER 14D DAILY CONSUMPTION OF LGG YOGHURT

CRT buffer	Saliva buffer capacity						Kruskal Wallis (p)
	Base		14 d		30 d		
	N	%	N	%	N	%	
Low	0	0	1	4	0	0	
Medium	9	36	7	29	3	14	0.035
High	16	64	16	67	18	86	
Total	25	100	24	100	21	100	

with medium buffer capacity before yoghurt administration, medium buffer capacity dropped to only 14% of patients (Table 5).

Discussion

Products with probiotic effect comprise milk, cheese, yoghurt, juice, ice cream, lozenges etc.[2]. In order to be effective probiotic bacteria should adhere to the tooth surface, become part of the oral biofilm and compete with other existing and cariogenic bacterial species, reducing the level of their colonisation. Comelli et al. showed such an effect of *Lactoccocus lactis* on colonization of *S. Sobrinus*. They also noted that molecule of caseinglycomacropeptide (CGMP), component of milk, can have an inhibitory effect on attachment of *S. Mutans* and *S. Sobrinus* on hydroxyapatite[10]. It is suggested that the vehicle for administration of probiotics should be of milk origin due to contained casein phosphopeptides (CPPs) that have inhibitory effect on demineralization and promote the remineralization of dental enamel[11,12]. Guggenheim et al describe inhibitory effect of powdered milk micellar casein on colonization of *S. Sobrinus*, but the authors state that soluble form of caseinate does not retain such effect[13]. Also Reynolds and Johnson, and Reynolds and Black reports that caseins are caries preventive without affecting *S. Mutans*[14,15]. Bussher et al.[16] and Caglar et al.[17] used yoghurt as the vehiculum for *lactobacilli* and *Bifidobacterium bifidum*. In their study Caruana et al. showed that casein phosphopeptide-amorphous calcium phosphate (CPP–ACP) complex can reduce the fall of plaque pH following carbohydrate challenge[18].

Haukioja et al. noted that *Lactobacillus spp*. and Bifidobacteria can survive in saliva for at least 24 hours. They also showed that *Lactobacillus rhamnosus* GG adheres well to hydroxyapatite, although there is a difference in the quality of adhesion between different individuals. It seems that saliva components influence adhesion[19].

Results obtained in this study confirm the results of Nase et al. who found a significant decrease in *S. Mutans* count after LGG milk consumption[20]. This finding was observed in a population of 3–4 year-old children. Also, Ahola et al. noted a 20% decrease of *S. Mutans* count after consumption of cheese with addition of LGG[21]. Decrease in *S. Mutans* count was observed in the post-treatment period, while during the treatment the values were insignificant. The results of this study are in agreement with Ahola's study, a significant decrease in *S. Mutans* count was observed 30 days after yoghurt consumption. Wei et al. demonstrated a reduction in adherence of *S. Mutans* to enamel hydroxyapatite by 40% when LGG was added to milk without antibodies. However, reduction in adherence of *S. Mutans* increases to 70% in the presence of antibodies in LGG fermented milk[22]. According to Meurman et al. manifestation of the preventive effect of *Lactobacillus rhamnosus* GG is not only in competition for adherence sites with *S. Mutans*, but also in metabolism, because of the fact that LGG does not ferment sucrose[23]. Sucrose serves not only as a metabolic substrate for cariogenic bacteria, but also has a cariogenic effect per se. A review by Paes Leme et al. showed that the role of sucrose is not yet completely elucidated[24]. Sucrose is important as a substrate for extracellular (EPS) and intracellular polysaccharides (IPS) formation. EPS promote bacterial adherence to the tooth surface and contribute to the structural integrity of dental biofilm. Also, sucrose reduces concentration of Ca, inorganic phosphorus (P_i) and F in the dental plaque. Ions of Ca, (P_i) and F are important in maintaining equilibrium between the tooth and the oral environment in view of that fact that reduced ion availability may increase the cariogenic potential of the biofilm[24].

Significant differences in *Lactobacilli* count between caries-free and caries-active individuals were observed in some previous studies. Simark-Mattsson et al. in their *in vitro* study showed that only 31% of the study subjects with low caries risk harboured *S. Mutans* compared to 71% and 89% in groups with moderate and high caries activity respectively. *Lactobacilli* in a group without caries activity repressed the growth of their autologous *S. Mutans*. Species with most effective interference capacity were *L. Paracasei*, *L. Plantarum* and *L. Rhamnosus*[25]. These results are in accordance with a review by van Palenstein Helderman et al. showing that *S. Mutans* is ubiquitous in children aged 7 years and older in Africa, Europe and North America, regardless of diet[26].

Besides LGG, it was noted that some other probiotic bacterial strains can be effective in decreasing *S. Mutans* counts. Such properties show genetically engineered *S. Mutans* JH 1000, *S. Equi* subspecies *zooepidemicus*, *S. Salivarius* TOVE-R, *E. Faecalis*, *Bifidobacterium* DN-173, *Lactobacillus spp*[27,28].

Caglar et al. showed significant reduction in *S. Mutans* count in study subjects (aged 20 years) sucking a medical device containing a probiotic lozenge with *L. Reuteri*[29].

Concerning saliva buffer capacity, it was observed that this value did not drop from high to low or medium in none of the cases. On the contrary, in all patients, the buffer capacity increased from low and medium to high. Thus, most of the patients with medium buffer capacity increased their buffer capacity, although all the patients with high buffer capacity retained their high values. This observation confirms the assumption that casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) complex can influence pH or probiotics application can have preventive effects per se, and can lead to the reduction of bacterial acid production. This is matter of further investigation.

Conclusions

The results of this study show that daily consumption of LGG yoghurt can have an inhibitory effect on oral pathogenic microflora. Yoghurt also significantly increases saliva buffer capacity 30 days after consumption. The obtained results allow the conclusion that daily consumption of LGG yoghurt can be recommended as a beneficial procedure in caries prevention.

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UČINAK LGG JOGURTA NA SALIVARNU RAZINU *S. MUTANS*-a I *LAKTOBACILLUS SPP.* U DJECE

SAŽETAK

Svrha ovog istraživanja bila je ustanoviti učinak 14-dnevnog uzimanja jogurta s dodatkom probiotika *Lactobacillus rhamnosus* ATCC53103- LGG (Bioaktiv LGG, Dukat, Hrvatska) na salivarnu razinu *Streptococcus mutans*-a i *Lactobacillus spp*. u djece. Istraživanje je provedeno na 25 djece, 6–10 godina starosti. Prilikom uključivanja u istraživanje svakom pacijentu je ustanovljen karijes rizik. Testiran je također puferski kapacitet sline (CRT buffer, Vivadent, Schaan, Liechtenstein) te je ustanovljena razina *S. Mutansa i Lactobacila* (CRT bacteria test, Vivadent, Schaan, Liechtenstein). Uzorci sline su testirani ponovo 7, 14 i 30 dana nakon uzimanja jogurta. Dobiveni podaci su analizirani neparametrijskim statističkim testovima χ^2 i Kruskal-Wallis. Rezultati su pokazali značajan porast puferskog kapaciteta sline 30 dana nakon uzimanja probiotičkog jogurta. Razina *S. Mutansa* u slini 30 dana nakon uzimanja jogurta bila je značajno snižena. Statistički značajna razlika u razini Laktobacila nije ustanovljena. Moguće je zaključiti da svakodnevno uzimanje jogurta s dodatkom LGG ima inhibitorno djelovanje na oralne patološke bakterije i može biti učinkovito u prevenciji karijesa.