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ORIGINAL ARTICLE

Effect of yogurt with *Bifidobacterium* DN-173 010 on salivary mutans streptococci and lactobacilli in young adults

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Abstract
Probiotic bacteria are thought to reduce the risk of disease. Previous studies have suggested that lactobacilli-derived probiotics in dairy products may affect the oral ecology, but the effect of bifidobacteria has not previously been reported. The aim of the present study was to examine whether or not short-term consumption of yogurt containing bifidobacteria would affect the salivary levels of mutans streptococci and lactobacilli in young adults. A double-blind, randomized crossover study was performed and 21 healthy subjects were followed over four periods. During periods 2 and 4 (2 weeks each), they ingested 200 g yogurt containing *Bifidobacterium* DN-173 010 once daily or a control yogurt without viable bacteria. Periods 1 and 3 were run-in and washout periods, respectively. Salivary mutans streptococci and lactobacilli were enumerated with chair-side kits. A statistically significant reduction ($p < 0.05$) of salivary mutans streptococci was recorded after the probiotic yogurt consumption, which was in contrast to the controls. A similar trend was seen for lactobacilli, but this decrease failed to reach statistical significance. In conclusion, probiotic bifidobacteria in yogurt may reduce the levels of selected caries-associated microorganisms in saliva.

Key Words: *Bifidobacteria, caries prevention, lactobacilli, mutans streptococci, probiotics*

Introduction

Probiotic bacteria are live microbial food supplements that beneficially affect the host by improving intestinal balance [1]. The first species introduced into research were *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, and among a number of potential benefits that have been proposed are reduced susceptibility to infections, reductions in allergies and lactose intolerance, as well as lowered blood pressure and serum cholesterol values (for a review, see [2]). Within dentistry, previous studies with lactobacilli strains such as *L. rhamnosus* GG [3–5], *L. acidophilus* and *L. casei* [6], *L. rhamnosus LC705* [5], *L. reuteri* [7], or a lactobacilli mix [8] have revealed mixed results on oral microorganisms, as summarized in Table I. Among the significant findings, a reduced colonization of the caries-associated mutans streptococci has been suggested [4,5,7]. To our knowledge, the possible effect of bifidobacteria-derived probiotics on the oral microflora has not been reported. The aim of the present study was therefore to examine whether short-term consumption of yogurt containing bifidobacteria would affect the levels of salivary mutans streptococci and lactobacilli in young adults. The null hypothesis was that the probiotic yogurt would not alter the bacterial levels.

Material and methods

Study group

The study group comprised 26 healthy non-medicated subjects (aged 21–24 years; mean 22.1 ± 1.0 years) who volunteered after informed consent. Regular xylitol chewing gum consumers and subjects with systemic antibiotic or topical fluoride treatments within 4 weeks were not included. All subjects had good oral health with no open or untreated caries lesions and claimed daily toothbrushing habits. During

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Table I. Summary of recent clinical trials with lactobacilli-derived probiotics

First author, year [ref]	Design	*n*, age	Vehicle, time	Strain	Outcome in oral cavity
Meurman, 1994 [3]	No controls	9, 25 yr	Yogurt, 1 week	*L. rhamnosus GG*	Harboured LB GG up to 2 weeks after discontinuation
Buscher, 1999 [6]	No controls	14, 17–35 yr	Yogurt, 1 week	*L. acidophilus, L. casei, B. bifidum*	No installation of LB
Näse, 2001 [4]	RCT, DB	594, 1–6 yr	Milk, 7 m	*L. rhamnosus GG*	Decrease of MS
Ahola, 2002 [5]	RCT, DB	74, 18–35 yr	Cheese, 3 w	*L. rhamnosus GG, L. rhamnosus LC-705*	Decrease of MS
Nikawa, 2004 [7]	Crossover	40, 20 yr	Yogurt, 2 w	*L. reuteri*	Decrease of MS
Montalto, 2004 [8]	RCT, DB	35, 23–37 yr	Liquid/capsules, 45 d	*L. sporogens, bifidum L. bulgaricus, termophilus L. acidophilus, casei L. rhamnosus*	Increase of LB

Abbreviations: *n* = number of subjects; RCT = randomized controlled trial; DB = double-blind; SB = single-blind; MS = mutans streptococci; LB = lactobacilli, w = weeks; m = months; d = days.

the experimental period, 5 participants were dropped due to systemic antibiotics ($n=3$) and violation of the study protocol ($n=2$). Thus, the final results were based on 21 young adults (10 F and 11 M).

Study design

The study had a double-blind, randomized crossover study design and the experimental period consisted of four consecutive time periods. During periods 2 and 4 the subjects consumed 200 g yogurt per day containing either probiotic bacteria or a control yogurt without bifidobacteria. Periods 1 and 3 were run-ins and washout periods of 1 and 4 weeks respectively, in which the subjects were told to refrain from all yogurt consumption. The subjects were encouraged to maintain their normal oral hygiene habits and to continue to brush their teeth twice a day. The study protocol was approved by the School of Dentistry Ethics Committee at the University of Yeditepe. The subjects received both oral and written information about the study and signed their informed consent.

Study yogurt

The experimental probiotic yogurt (Activia®; Danone, Istanbul, Turkey) contained *Bifidobacterium* DN-173 010 (7×10^7 cfu/g), while the control yogurt (Danone Natural®; Danone, Istanbul, Turkey) was without viable bacteria. The yogurts were specially made for the study and packed in special plastic cups, and neither the participants nor the examiners could identify test and control yogurts. The daily intake was 200 g at one sitting and the subjects were advised to eat the yogurt at noon. No toothbrushing was allowed for at least 1 h after eating the yogurt.

Saliva samples

Samplings of paraffin-stimulated whole saliva were carried out immediately before and after periods 2 and 4. After a thorough rinse with water, the saliva was collected (during the course of 5 min) directly into a graded test tube. The counts of salivary mutans streptococci and lactobacilli were evaluated using Dentocult SM (Strip Mutans®) and Dentocult LB® tests (Orion Diagnostica, Espoo, Finland) as described earlier [9,10].

Statistical methods

The data were processed with the SPSS software (SPSS Inc., v. 11.5, Chicago, Ill., USA). Post- and pretreatment values within each regimen were compared with a two-tailed marginal homogeneity test for categorical data. A *p*-value less than 0.05 was considered as statistically significant.

Results

The pre- and post-treatment levels of salivary mutans streptococci and lactobacilli are given in Tables II and III. All subjects but two had detectable levels of salivary mutans streptococci at baseline, while all exhibited growth of salivary lactobacilli. The initial median score was 2 for both bacterial groups. A statistically significant ($p<0.05$) reduction of salivary mutans streptococci was registered following the 2-week consumption of the test yogurt in contrast to the control yogurt. During the test periods with the probiotic test yogurt consumption, 8 subjects exhibited decreased scores (1–2 steps), 10 subjects had unchanged scores, while one displayed a 1-step increased score. The

Table II. Distribution of salivary mutans streptococci at baseline and after 2-weeks' consumption of probiotic and control yogurt ($n=21$). Values denote number of subjects

	Mutans streptococci score, cfu			
Time	No growth	$<10^5$	10^5–10^6	$>10^6$
Probiotic yogurt				
Baseline (pre-treatment)	2	6	10	3
2-weeks (post-treatment)*	6	4	10	1
Control yogurt				
Baseline (pre-treatment)	1	6	11	3
2-weeks (post treatment)	2	9	7	3

*Post-treatment values statistically different from baseline ($p<0.05$), two-tailed marginal homogeneity test for categorical data.

Table III. Distribution of salivary lactobacilli at baseline and after 2-weeks' consumption of probiotic and control yogurt ($n=21$). Values denote the number of subjects

	lactobacilli, cfu/ml			
Time	$\leqslant 10^3$	10^4	10^5	$\geqslant 10^6$
Probiotic yogurt				
Baseline (pre-treatment)	5	6	4	6
2-weeks (post-treatment)	9	4	3	5
Control yogurt				
Baseline (pre-treatment)	7	6	2	6
2-weeks (post treatment)	7	7	5	2

corresponding values during the control regimen were 7, 11, and 3 subjects, respectively.

Regarding salivary lactobacilli, a similar trend towards reduced bacterial counts was seen after the 2-week period of probiotic yogurt, but this failed to reach statistical significance ($p=0.09$). After the test yogurt, decreased lactobacilli levels (1 score) were found in 7 subjects, while 2 subjects displayed increased scores. After the control regimen, the majority had unchanged scores.

Discussion

Bacteriotherapy, or replacement therapy, is an alternative way to combat infections by using harmless bacteria to displace pathogenic microorganisms. Since the late 1980s, a range of dairy products containing bifidobacteria have been marketed in a number of countries worldwide, and studies have been performed to validate the survival and positive effects of *Bifidobacterium* DN-173 010 within the gastrointestinal tract [11,12]. Bifidobacteria are the predominant anaerobic bacteria naturally occurring within the intestinal lumen and play a critical role in maintaining the equilibrium among normal intestinal flora [13]. To our knowledge, the present study is the first to examine possible effects of *Bifidobacterium* DN-173 010 on caries-associated microorganisms in the oral cavity. It should be noted, however, that the material was limited in size and consisted of healthy young adults with no dental caries problems. Moreover, the short intervention time and the semi-quantitative assessment of salivary counts are shortcomings that need to be taken into consideration. Yogurt was chosen since it may be considered as an archetypical probiotic food [14]. The product was well accepted by the participants, which was expected since yogurt-eating is a life-long tradition in Turkey. During the experimental periods, no yogurt intake except for the scheduled was allowed. The compliance was checked and was excellent in those subjects that remained in the study.

Today, research on probiotics is concentrated essentially on lactobacilli and bifidobacteria. The novel observation revealed here was a significant diminishing effect of the bifidobacteria-containing yogurt on salivary mutans streptococci, and for this microorganism the null hypothesis was rejected. This finding is in agreement with several previous findings with lactobacilli-derived probiotics [4,5,7], while it contrasts with another [8]. Whether the present reduction was due to competitive inhibition, similar to demonstrated events in the vaginal and intestinal mucosa [15], is not clear. We did not attempt to analyse oral colonization of the probiotic test strain, and from the existing literature it may be questioned if this occurs and whether or not they have any residual effect after discontinuation of intake [3,6]. Bifidobacteria, as well as mutans streptococci and lactobacilli, are Gram-positive lactic acid-producing bacteria commonly found in the oral biofilm. Modern molecular techniques have underlined the concept that mutans streptococci are associated with early enamel demineralization, lactobacilli with carious dentine, while bifidobacteria may be a major pathogen in deep caries and in progression of caries [16]. Theoretically, a long-term reduction of mutans streptococci could thus imply a reduced risk for initial lesions but this thinking has not always been verified for other antibacterial regimens [17]. On the other hand, as could be seem from Table II, it appeared that the majority of the subjects with high mutans streptococci counts remained high irrespective of intervention. Nevertheless, the present pilot observation merits further study to evaluate the optimal dosage and possible effects of bifidobacteria on oral ecology. Furthermore, some probiotic strains of lactobacilli, such as *L. rhamnosus* GG, may be of particular interest in caries prevention as they are incapable of fermenting sucrose [3,18].

In conclusion, the results of the present study indicate that daily short-term consumption of yogurt containing *Bifidobacterium* DN-173 010 may affect the levels of mutans streptococci in saliva. The clinical significance of the present findings remains unclear, but the potential influence of probiotic dairy products on the complex oral microflora justifies further and extended studies with upgraded technology.

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