Original Research
Pediatric Dentistry

Effects of probiotic fermented milk on biofilms, oral microbiota, and enamel

Carolina Simonetti LODI[a]
Lidiane Viana de OLIVEIRA[a]
Fernanda Lourenção BRIGHENTI[b]
Alberto Carlos Botazzo DELBEM[a]
Cleide Cristina Rodrigues MARTINHON[a]

[a]Universidade Estadual Paulista – UNESP, Faculdade de Odontologia de Araçatuba, Department of Pediatric and Social Dentistry, Araçatuba, SP, Brazil.

[b]Universidade Estadual Paulista – UNESP, Faculdade de Odontologia de Araraquara, Department of Orthodontics and Pediatric Dentistry, Araraquara, SP, Brazil.

Abstract: The aim of this study was to evaluate *in vitro* and *in vivo* the effects of 2 brands of probiotic fermented milk on biofilms, oral microbiota, and enamel. For the *in situ* experiment, ten volunteers wore palatine devices containing four blocks of bovine dental enamel over 3 phases, during which 20% sucrose solution, Yakult® (Treatment A), and Batavito® (Treatment B) were dropped on the enamel blocks. Salivary microbial counts were obtained and biofilm samples were analyzed after each phase. For the *in vivo* experiment, the same ten volunteers drunk Yakult® (Treatment C) and Batavito® (Treatment D) in two phases. Saliva samples were collected for microbial analysis after each phase. The *in situ* study showed that in comparison with Treatment A, Treatment B resulted in fewer total cultivable anaerobes and facultative microorganisms in biofilms, higher final microhardness, lower percentage change in surface hardness, and smaller integrated subsurface enamel hardness. In the *in vivo* study, Treatment D resulted in a reduction in the counts of all microorganisms. The results suggested that the probiotic fermented milk Batavito®, but not Yakult®, reduced the amount of oral microorganisms and mineral loss in bovine enamel.

Keywords: Probiotics; Tooth Demineralization; Dental Caries; Streptococcus Mutans; Lactobacillus.

Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

Corresponding Author:
Carolina Simonetti Lodi
E-mail: carol_lodi@yahoo.com.br

DOI: 10.1590/1807-3107BOR-2015.vol29.0033

Submitted: Mar 25, 2014
Accepted for publication: Nov 14, 2014
Last revision: Jan 06, 2015

Introduction

Research on probiotics has progressed over the last 20 years; significant advances have been made in the selection and characterization of specific probiotic cultures as well as in the substantiation of health claims related to their consumption.[1]

Although the mechanism underlying probiotic activity in the oral cavity is not fully understood, it is usually considered to be a combination of local and systemic immune responses as well as non-immunological defense mechanisms.[2] The principal health-promoting effects are ascribed to the ability of probiotics to enhance the intestinal mucosal immune defense and macrophage activity and elevate the numbers of killer cells, T cells, and interferons.[3] In order to show effectiveness against oral infections, probiotic bacteria need to adhere to the oral mucosa and dental tissues as part of the biofilm and compete with dental pathogens.[4] The most widely used species belong to the genera *Lactobacillus* and *Bifidobacterium*, because these organisms are already produced in the dairy industry and are very

rarely implicated in infections in humans,[1] although some species seem to play a role in the microbiology of dental caries.[5]

The archetypical probiotic food is yogurt, and daily consumption of dairy products seems to be the most natural way to ingest probiotic bacteria.[2] Another advantage is that milk products contain basic nutrients for growing children; they are also considered safe for the teeth and have possible beneficial effects on the salivary microbial composition and inhibition of caries development due to their natural casein, calcium, and phosphorous content.[6,7]

However, the optimal vehicle for probiotic delivery is yet to be determined. Çaglar *et al*.[8] examined the effects of probiotics ingested via 2 different non-dairy delivery systems on the levels of salivary mutans streptococci and lactobacilli. They found that the probiotic significantly reduced the streptococcal levels in comparison with the placebo regardless of the delivery system. Further, Montalto *et al*.[9] studied the effects of oral administration of lactobacilli on the salivary lactobacillus count and found that oral administration of probiotic lactobacilli, irrespective of the delivery system, significantly increased the salivary lactobacillus count.

Recently, we evaluated the properties of some fermented milk brands[10] and their effects on the inorganic composition of biofilms.[11] Although all the products were milk-based beverages and contained fluoride, calcium, and phosphate, they promoted caries in bovine enamel blocks.[10,11] In this context, the aim of the present study was to evaluate *in situ* and *in vivo* the effects of 2 brands of probiotic fermented milk on biofilm, oral microbiota, and enamel, and to evaluate the effect of short-term ingestion of these beverages on oral microbiota.

Methodology

Selection

The fermented milk brands used were Yakult® (Yakult S/A Indústria e Comércio, Lorena, Brazil) and Batavito® (BRF S.A., Carambeí, Brazil) (hereafter, fermented milk 1 and 2, respectively).

According to the manufacturers, Yakult® contains a single probiotic bacterial species, *Lactobacillus casei* Shirota, and Batavito® contains a combination of 3 probiotic bacteria (*Lactobacillus acidophilus*, *Bifidobacterium* sp., and *Lactobacillus paracasei*).

Total and Reducing Sugar Analyses of Fermented Milk

The levels of total and reducing sugars were determined using the methods described by Dubois *et al*.[12] and Somogyi-Nelson,[13] respectively.

Enamel Block Preparation and Analysis

Enamel bovine blocks were prepared, polished, and selected as described by Lodi *et al*.[11] The baseline (SMHi) and final (SMHf) enamel surface microhardness measurements was performed.[14] The percentage change in SMH (%SMH) was calculated as follows: %SMH = 100 (SMHf − SMHi)/SMHi. Cross-sectional microhardness was performed.[15]

In Situ Experiment

This experiment was approved by the local human ethics committee (FOA-UNESP, protocol #2008-01519). Ten healthy volunteers were selected on the basis of the criteria defined in previous studies.[11,16]

The *in situ* experiment involved a randomized double-blind crossover design performed in 3 phases with 20% sucrose solution (control treatment), fermented milk 1 (treatment A), and fermented milk 2 (treatment B). Each participant wore an acrylic palatal device.[17]

In each phase, participants applied 2 drops of 20% sucrose solution, fermented milk 1, or fermented milk 2 on the enamel blocks 8 times a day. Five minutes after the application, the device was reinserted into the mouth. After 14 days, biofilm samples were collected for microbial analysis. A washout period of 7 days was maintained between each phase. Only one dietary restriction was imposed on the patients: they were asked to avoid consuming other sources of probiotic bacteria. The participants were also instructed to remove the appliance while eating and performing oral hygiene procedures.[17] In addition, they were not allowed to use any antimicrobial or fluoride products during the experiment.

In Vivo Experiment

The same volunteers participated in the *in vivo* experiment. It involved a randomized single-blind crossover design performed in 2 phases, after the *in situ* study, with fermented milk 1 (treatment C) and fermented milk 2 (treatment D). Each participant drank 80 g of the fermented milk daily for 14 days. The dietary restrictions and conditions for oral hygiene were the same as those in the *in situ* study. Saliva samples[18] were collected at the beginning of the experiment and the end of each phase for microbial analysis.

Biofilm Analysis

At the end of the *in situ* experiment, the biofilm was harvested. Around 5 mg of each biofilm sample was resuspended in PBS (0.1 M, pH 7.2; 1 mL/mg biofilm).[19] The suspensions were serially diluted in PBS and plated on brain heart infusion agar (HiMedia Laboratories, Mumbai, India), mitis salivarius agar (HiMedia Laboratories), mitis salivarius sucrose bacitracin agar (Sigma-Aldrich Co., St. Louis, USA), and Rogosa agar (HiMedia Laboratories) to determine the total cultivable anaerobe and facultative (TCAF), total streptococcal, mutans streptococcal, and lactobacillus colonies, respectively. The plates for the TCAF, total streptococci, and mutans streptococci were incubated at 37°C for 48 h in an anaerobic chamber; the lactobacillus plates were incubated aerobically at 37°C for 72 h. Colonies were counted with a colony counter.

The remaining biofilm was dried with phosphorus pentoxide (Vetec Química Fina Ltda., Duque de Caxias, Brazil) for 12 h at room temperature. Insoluble extracellular polysaccharides (EPS) were extracted.[16,20] Carbohydrates were analyzed by using the phenol–sulfuric acid procedure.[12] The results are expressed as mg/g dry weight.

Saliva Analysis

Saliva was collected using PBS as oral rinse.[18] After 1 min of rinsing, the samples were collected and centrifuged for 10 min at 8,000 × *g*, the supernatant was discarded, and 2.5 mL PBS was added to the pellet. The suspensions were serially diluted in PBS and plated as described for the biofilm analysis. The results are expressed as CFU/mL saliva.

Statistical Analysis

Statistical analysis was carried out by using BioEstat Version 5.0 (*Instituto de Desenvolvimento Sustentável de Mamirauá*, Belém, Brazil). Data on CFUs were logarithmically transformed. The data were analyzed by using a single-factor ANOVA model. Multiple comparisons were conducted by using the Tukey or Bonferroni test when significant effects ($p < 0.05$) were detected. Data were analyzed by using Kruskal-Wallis one-way analysis when they were not normally distributed or when the variances were not equal. Data (means) from microorganisms in the saliva samples before and after each phase were compared with paired *t*-tests. The significance limit was set at 5%.

Results

Total and Reducing Sugar Content

The mean total sugar content of 20% sucrose, fermented milk 1, and fermented milk 2 was 155.67, 158.49, and 191.33 mg/mL, respectively. Further, the mean reducing sugar content was 0.15, 9.83, and 0.83 mg/mL, respectively. There were significant differences in the total and reducing sugar content of the three treatment agents.

Enamel Hardness

The means and SD values of the different enamel hardness variables are shown in Table 1. The SMHi was not significantly different among the treatment groups ($p = 0.853$). However, treatment B showed significant differences with respect to the SMHf, percentage of surface hardness change, and integrated loss of subsurface hardness ($p < 0.05$). No significant differences in these variables were observed between treatment A and the control treatment ($p > 0.05$).

In Situ Findings

Regarding the biofilm data, the TCAF levels were lower after treatment B than after treatment A and the control treatment, but no significant difference was observed between treatment B and the control treatment, and between treatment A and the control treatment. The treatment groups did not show significant differences in the total streptococcal levels and the levels of mutans

Table 1. Mean and standard deviation (SD) of the variables analyzed in the enamel surface.

Variable (n = 10)	20% Sucrose	Treatment A	Treatment B	Significance (p)
SMHi[1]	377.1[a] (0.7)	377.3[a] (0.4)	377.2[a] (0.4)	= 0.853
SMHf[2]	76.0[a] (66.8)	127.0[a] (74.8)	189.6[b] (67.0)	< 0.05
%SMH[3]	-79.8[a] (17.8)	-66.3[a] (19.8)	-49.7[b] (17.8)	< 0.05
ΔKHN[4]	9961.9[a] (1468.2)	8940.9[a] (2800.3)	4063.5[b] (1697.2)	< 0.05

[a,b]Means (SD) followed by distinct letters are significantly different for the variable.
[1]Initial microhardness (Knoop hardness).
[2]Final microhardness (Knoop hardness).
[3]Percentage of subsurface hardness change (Knoop hardness).
[4]Integrated loss of subsurface hardness (Knoop hardness).

streptococci and lactobacillus ($p > 0.05$; Table 2). Furthermore, the EPS concentration did not differ significantly among the treatments ($p > 0.05$). Both the control treatment and treatment A produced 13.84 mg/g dry biofilm, whereas treatment B produced 12.35 mg/g dry biofilm.

In the saliva analysis, no significant difference was noted between the baseline and the final microbial levels in each treatment (Table 3).

In Vivo Findings

Treatment C did not significantly alter the concentrations of any microorganisms from the baseline values. However, the levels of all analyzed microorganisms decreased significantly after treatment D (Table 4).

Discussion

In our *in vivo* experiment, commercially probiotic fermented milk samples were orally administered, enabling direct contact with oral tissues. Although fermented milk has not been developed for preventing dental caries by promoting changes in the oral microbiota, treatment D decreased the salivary counts of all investigated microorganisms after 2 weeks of ingestion. These results corroborate the findings of previous studies.[8,21,22,23]

Some studies of the effects of lactobacillus-based probiotics on mutans streptococci reported significant reductions in the levels of salivary mutans streptococci immediately after terminating daily intake.[8,21,22,23] The post-treatment reductions were not directly dependent on the delivery vehicles, which included milk, cheese, yogurt, lozenges, and straws prepared with freeze-dried strains. Çaglar *et al*.[8] investigated whether slowly melting tablets facilitate a more thorough contact between the probiotic and the oral environment compared with a direct swallowing pattern from a straw. Both methods of administration equally reduced the prevalence of salivary mutans streptococci after 2 weeks of use.

Contrary findings were reported by Montalto *et al*.,[9] who evaluated the administration of probiotic lactobacilli in liquids and capsules to determine the role of direct contact of probiotics with the oral tissues. Interestingly, both means of administration significantly increased the salivary lactobacillus counts, whereas the mutans streptococci levels were not significantly altered. Some studies have indicated that direct contact with oral tissues is not a prerequisite for probiotics to have a

Table 2. Mean and standard deviation (SD) of microorganisms in the biofilm (log CFU/mg) after 14 days (in situ study).

Variable (n = 10)	20% Sucrose	Treatment A	Treatment B
TCAF[1]	6.87[a,b] (0.49)	7.04[a] (0.24)	6.66[b] (0.47)
Total streptococci	5.73[a] (0.53)	5.81[a] (0.87)	5.71[a] (0.65)
Mutans streptococci	3.24[a] (1.38)	2.99[a] (0.99)	3.14[a] (1.22)
Lactobacillus	5.19[a] (1.97)	5.55[a] (0.75)	5.18[a] (1.67)

[a,b]Means (SD) followed by distinct letters are significantly different for the variable.
[1]Total cultivable anaerobes and facultative microorganisms.

Table 3. Mean and standard deviation (SD) of microorganisms in saliva (log CFU/mL) at baseline and after 14 days (in situ study).

Variable (n = 10)	20% Sucrose		Treatment A		Treatment B	
	Baseline	14 days	Baseline	14 days	Baseline	14 days
TCAF[1]	6.80[a] (0.69)	6.42[a] (1.0)	6.96[a] (0.79)	6.43[a](0.71)	6.81[a] (1.15)	6.07[a] (1.17)
Total streptococci	6.20[a] (0.72)	5.82[a] (0.89)	6.38[a] (0.80)	5.85[a] (0.72)	6.17[a] (1.04)	5.59[a] (0.92)
Mutans streptococci	3.26[a] (1.08)	3.36[a] (0.91)	3.67[a] (1.60)	3.28[a] (1.03)	3.51[a](0.83)	2.72[a] (1.24)
Lactobacillus	4.06[a] (0.95)	3.90[a] (0.82)	4.09[a] (0.79)	3.54[a] (0.78)	3.64[a] (1.24)	3.05[a] (2.10)

[a,b]Means (SD) followed by distinct letters are significantly different for the variable.
[1]Total cultivable anaerobes and facultative microorganisms.

beneficial effect; purely systemic administration of a probiotic could enhance lactobacillus proliferation in the oral cavity.[8,9,22]

The conflicting results for probiotic efficacy among previous studies are probably attributable to differences among strains of the same species.[24] At present, most research is carried out with well-defined dairy-based live lactobacillus strains. In our study, fermented milk 1 contained a single probiotic bacterial species and fermented milk 2 contained a mix of 3 probiotic bacteria. The better results after the consumption of fermented milk 2 can be explained by the difference in their probiotic contents: simultaneous application of different probiotic bacteria can affect the balance of the oral ecosystem via possible additive, cumulative, or competitive modes of action.[3]

It should be noted that saliva samples may underestimate the true contents of oral biofilms.[25] Moreover, biofilms are difficult to collect *in vivo* because of the need for oral hygiene restrictions during experimentation. Therefore, we also performed an *in situ* experiment to investigate the effects of probiotic fermented milk on biofilms. In the *in situ* experiment, treatment B lowered the concentrations of TCAF in biofilm compared with the treatment A. However, the concentrations of total streptococci, mutans streptococci, and lactobacilli were not significantly different among the treatments. This condition can be explained by the fact that the optimal dose required for pathogenic bacterial suppression was not used during the experiment because the participants merely dripped the fermented milk onto the enamel blocks. Lee and Salminen[26] suggested an intake of 100 g containing 10^6-10^7 probiotic cells as the optimal dose for intestinal benefits; similarly, Çaglar *et al.*[27] showed a significant reduction in the number of mutans streptococci with a fixed amount of probiotic bacteria (53 g ice cream with 1×10^7 CFU/g ingested daily). Microorganism reduction was also achieved with 200 g of yogurt containing 2×10^8 CFU/g in their former study.[28]

To evaluate changes in the enamel surface and its demineralization, we used the microhardness test in the *in situ* experiment. Treatment B significantly differed from the other treatments with respect to the final microhardness, percentage of surface hardness change, and integrated loss of subsurface hardness. Both products used in this study were milk-based and contained fluoride, calcium, and phosphate.[10] Although these ions should confer a protective effect

Table 4. Mean and standard deviation (SD) of microorganisms in saliva (log CFU/mL) at baseline and after 14 days (in vivo study).

Variables (n = 10)	Treatment C		Treatment D	
	Baseline	14 days	Baseline	14 days
TCAF[1]	6.74[a] (1.25)	6.70[a] (1.15)	6.96[a] (0.79)	6.05[b] (1.28)
Total streptococci	6.04[a] (0.97)	6.19[a] (1.10)	6.48[a] (0.83)	5.41[b] (0.94)
Mutans streptococci	3.48[a] (0.94)	3.74[a] (1.21)	4.17[a] (0.62)	3.18[b] (1.17)
Lactobacillus	3.11[a] (0.79)	3.57[a] (1.49)	4.01[a] (0.93)	3.02[b] (0.77)

[a,b]Means (SD) followed by distinct letters are significantly different for the variable.
[1]Total cultivable anaerobes and facultative microorganisms.

on enamel, enamel demineralization occurred in both treatments, possibly because of the presence of sucrose.

Sucrose is considered the most cariogenic factor present in human diets because of its fermentability and ability to act as a substrate for the synthesis of polysaccharides in biofilm.[29] To interpret the aforementioned results, we investigated the amounts of total and reducing sugars in both brands of fermented milk and the EPS concentration in biofilm. Both brands contained sugars; moreover, EPS was detected in biofilm, which could explain the observed demineralization. Fermented milk 1 contained a lower amount of total sugars and higher amount of reducing sugars than fermented milk 2, which caused less mineral loss after treatment B. This could indicate that the probiotic, in this experimental model, exhibited some protective effect against enamel demineralization.

Conclusion

Under the conditions of this study, the findings demonstrate that fermented milk 2 (Batavito®) can reduce the amount of microorganisms in the oral cavity and mineral loss in bovine enamel. More systematic studies and randomized controlled trials are needed to determine the optimal probiotic strains, daily doses, and vehicles for safely improving oral health.

References

1. Teughels W, Van Essche M, Sliepen I, Quirynen M. Probiotics and oral healthcare. Periodontol 2000. 2008 Aug;48:111-47.
2. Çaglar E, Kargul B, Tanboga I. Bacteriotherapy and probiotics' role on oral health. Oral Dis. 2005 May;11(3):131-7.
3. Fuller R, Gibson GR. Modification of the intestinal microflora using probiotics and prebiotics. Scand J Gastroenterol Suppl. 1997 Jan;222:28-31.
4. Comelli EM, Guggenheim B, Stingele F, Neeser JR. Selection of dairy bacterial strains as probiotics for oral health. Eur J Oral Sci. 2002 Jun;110(3):218-24.
5. Beighton D. The complex oral microflora of high-risk individuals and groups and its role in the caries process. Community Dent Oral Epidemiol. 2005 Aug;33(4):248-55.
6. Levine RS. Milk, flavoured milk products and caries. Br Dent J. 2001 Jul;191(1):20.
7. Petti S, Tarsitani G, D'Arca AS. A randomized clinical trial of the effect of yoghurt on the human salivary microflora. Arch Oral Biol. 2001 Aug;46(8):705-12.
8. Çaglar E, Cildir SK, Ergeneli S, Sandalli N, Twetman S. Salivary mutans streptococci and lactobacilli levels after ingestion of the probiotic bacterium Lactobacillus reuteri ATCC 55730 by straws or tablets. Acta Odontol Scand. 2006 Oct;64(5):314-8.
9. Montalto M, Vastola M, Marigo L, Covino M, Graziosetto R, Curigliano V, et al. Probiotic treatment increases salivary counts of lactobacilli: a double-blind, randomized, controlled study. Digestion. 2004 Jan;69(1):53-6.
10. Lodi CS, Sassaki KT, Fraiz FC, Delbem AC, Martinhon CC. Evaluation of some properties of fermented milk beverages that affect the demineralization of dental enamel. Braz Oral Res. 2010 Jan-Mar;24(1):95-101.
11. Lodi CS, Manarelli MM, Sassaki KT, Fraiz FC, Delbem AC, Martinhon CC. Evaluation of fermented milk containing probiotic on dental enamel and biofilm: in situ study. Arch Oral Biol. 2010 Jan;55(1):29-33.
12. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem. 1956 Mar;28(3):350-6.
13. Nelson N. A photometric adaptation of the Somogyi method for the determination of glucose. J Biol Chem. 1944 Jan;153(1):375-80.
14. Vieira AE, Delbem AC, Sassaki KT, Rodrigues E, Cury JA, Cunha RF. Fluoride dose response in pH-cycling models using bovine enamel. Caries Res. 2005 Nov-Dec;39(6):514-20.
15. Spiguel MH, Tovo MF, Kramer PF, Franco KS, Alves KM, Delbem AC. Evaluation of laser fluorescence in the monitoring of the initial stage of the de-/remineralization process: an in vitro and in situ study. Caries Res. 2009 Jul;43(4):302-7.
16. Ccahuana-Vásquez RA, Tabchoury CP, Tenuta LM, Del Bel Cury AA, Vale GC, Cury JA. Effect of frequency of sucrose exposure on dental biofilm composition and enamel demineralization in the presence of fluoride. Caries Res. 2007 Dec;41(1):9-15.
17. Cury JA, Rebelo MA, Del Bel Cury AA, Derbyshire MT, Tabchoury CP. Biochemical composition and cariogenicity of dental plaque formed in the presence of sucrose or glucose and fructose. Caries Res. 2000 Nov-Dec;34(6):491-7.
18. Samaranayake LP, MacFarlane TW, Lamey PJ, Ferguson MM. A comparison of oral rinse and imprint sampling techniques for the detection of yeast, coliform and Staphylococcus aureus carriage in the oral cavity. J Oral Pathol. 1986 Aug;15(7):386-8.
19. Bowen WH. Interpretation and use of microbiological findings in dental caries. Oral Microbiol Immunol. 1986 Nov;1(1):82-6.
20. Santos MN, Santos LM, Francisco SB, Cury JA. Relationship among dental plaque composition, daily sugar exposure

and caries in the primary dentition. Caries Res. 2002 Sep-Oct;36(5):347-52.

21. Näse L, Hatakka K, Savilahti E, Saxelin M, Pönkä A, Poussa T, et al. Effect of long-term consumption of a probiotic bacterium, Lactobacillus rhamnosus GG, in milk on dental caries and caries risk in children. Caries Res. 2001 Nov-Dec;35(6):412-20.
22. Ahola AJ, Yli-Knuuttila H, Suomalainen T, Poussa T, Ahlström A, Meurman JH, et al. Short-term consumption of probiotic-containing cheese and its effect on dental caries risk factors. Arch Oral Biol. 2002 Nov;47(11):799-804.
23. Nikawa H, Makihira S, Fukushima H, Nishimura H, Ozaki Y, Ishida K, et al. Lactobacillus reuteri in bovine milk fermented decreases the oral carriage of mutans streptococci. Int J Food Microbiol. 2004 Sep 1;95(2):219-23.
24. Twetman S, Stecksén-Blicks C. Probiotics and oral health effects in children. Int J Paediatr Dent. 2008 Jan;18(1):3-10.
25. Meurman JH. Probiotics: do they have a role in oral medicine and dentistry? Eur J Oral Sci. 2005 Jun;113(3):188-96.
26. Lee YK, Salminen S. The coming age of probiotics. Trends Food Sci Technol. 1995 Jul;6(7):241-5.
27. Çaglar E, Kuscu OO, Selvi Kuvvetli S, Kavaloglu Cildir S, Sandalli N, Twetman S. Short-term effect of ice-cream containing Bifidobacterium lactis Bb-12 on the number of salivary mutans streptococci and lactobacilli. Acta Odontol Scand. 2008 Jun;66(3):154-8.
28. Çaglar E, Sandalli N, Twetman S, Kavaloglu S, Ergeneli S, Selvi S. Effect of yogurt with Bifidobacterium DN-173 010 on salivary mutans streptococci and lactobacilli in young adults. Acta Odontol Scand. 2005 Nov;63(6):317-20.
29. Bowen WH. Do we need to be concerned about dental caries in the coming millennium?. Crit Rev Oral Biol Med. 2002 Mar;13(2):126-31.