Acta Odontologica Scandinavica, 2009; 67: 19–24

informa
healthcare

ORIGINAL ARTICLE

Short-term effect of chewing gums containing probiotic *Lactobacillus reuteri* on the levels of inflammatory mediators in gingival crevicular fluid

SVANTE TWETMAN[1], BILAL DERAWI[1], METTE KELLER[1], KIM EKSTRAND[1], TÜLAY YUCEL-LINDBERG[2] & CHRISTINA STECKSÉN-BLICKS[3]

[1]*Department of Cariology and Endodontics, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark,* [2]*Department of Pediatric Dentistry, Institute of Odontology, Karolinska Institute, Huddinge, Sweden and* [3]*Department of Odontology, Pediatric Dentistry, Faculty of Medicine, Umeå University, Umeå, Sweden*

Abstract

Objective. To investigate the effect of a chewing gum containing probiotic bacteria on gingival inflammation and the levels of selected inflammatory mediators in gingival crevicular fluid (GCF). ***Material and Methods.*** Forty-two healthy adults with moderate levels of gingival inflammation entered a double-blind placebo-controlled study design. The subjects were randomly assigned to one of three parallel arms: Group A/P was given one active and one placebo gum daily, Group A/A received two active chewing gums, and Group P/P two placebo gums. The chewing gums contained two strains of *Lactobacillus reuteri*: ATCC 55730 and ATCC PTA 5289 (1×10^8 CFU/gum, respectively). The subjects were instructed to chew the gums for 10 min over the course of 2 weeks. Bleeding on probing (BOP) and GCF sampling were conducted at baseline and after 1, 2 and 4 weeks. The levels of IL-1β, TNF-α, IL-6, IL-8 and IL-10 were determined using luminex technology and multiplex immunoassay kits. ***Results.*** BOP improved and GCF volume decreased in all groups during the chewing period, but the results were statistically significant ($p < 0.05$) only in Groups A/P and A/A. The levels of TNF-α and IL-8 decreased significantly ($p < 0.05$) in Group A/A compared with baseline after 1 and 2 weeks, respectively. A non-significant decreasing tendency was also observed concerning IL-1β during the chewing period. The levels of IL-6 and IL-10 were unaffected in all groups after 1 and 2 weeks. ***Conclusions.*** The reduction of pro-inflammatory cytokines in GCF may be proof of principle for the probiotic approach combating inflammation in the oral cavity.

Key Words: *Cytokines, gingival inflammation, IL-8, probiotics, TNF-α*

Introduction

According to the World Health Organization, probiotic bacteria are defined as live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host [1]. The mechanisms of action are not fully known, but the background thinking is that relatively harmless micro-organisms compete with pathogens and exert health-promoting and therapeutic effects locally and systemically by influencing the immune system. While the beneficial impact of probiotic bacteria is well established for certain gastrointestinal diseases [2–4], its possible role in combating oral infections is sparsely investigated [5–7]. Previous studies *in vivo* have suggested that probiotic strains of lactobacilli and bifidobacteria can reduce the levels of caries-associated bacteria in saliva [8–10]. Concerning periodontal conditions, Teugels and co-workers [11] have shown that application of beneficial bacteria, as an adjunct to scaling and root planning, can inhibit re-colonization of pathogens in periodontal pockets and reduce bleeding on probing in dogs. Other clinical trials have demonstrated a reduced prevalence of moderate to severe gingival inflammation as well as improved plaque index and probing depth in adults after regular use of probiotic chewing gums or tablets [12,13]. However, the registrations were made by clinical-visual scoring. Since it is generally considered that the gingival crevicular fluid reflects the inflammatory reactions involved in periodontal disease with elevated concentrations of

Correspondence: Svante Twetman, Department of Cariology and Endodontics, Faculty of Health Sciences, University of Copenhagen, Nørre Allé 20, DK-2200 Copenhagen N, Denmark. Tel: +45 3532 6810. Fax: +45 3532 6505. E-mail: stw@odont.ku.dk

(Received 11 July 2008; accepted 30 September 2008)

ISSN 0001-6357 print/ISSN 1502-3850 online
DOI: 10.1080/00016350802516170

cytokines [14–16], it was of interest to perform a placebo-controlled trial in order to further study the effect of probiotics on the inflammatory reaction. The aim of the present study was therefore to investigate the effect of short-term use of probiotic chewing gums on gingival inflammation and the levels of selected pro- and anti-inflammatory cytokines in gingival crevicular fluid. The null hypothesis was that the levels of cytokines would not differ from baseline or from values obtained after use of a placebo chewing gum.

Materials and methods

Subjects

The study group comprised 42 healthy adults who volunteered during March–June 2007 after verbal and written information had been given to them. The mean age was 24 years, with a slightly uneven sex distribution (16 F, 26 M). All subjects were outpatients at the dental school in Copenhagen, Denmark and had signed an informed consent form. The inclusion criteria were: (i) presence of at least two buccal marginal sites with moderate chronic gingival inflammation according to the Löe index [17] with a probing depth of <4 mm, (ii) no habitual use of probiotic food, and (iii) being a non-smoker. The exclusion criteria were (iv) pregnancy, (v) a history of antibiotics and/or anti-inflammatory drugs within 30 days prior to the baseline examination, (vi) a low stimulated saliva secretion (<0.8 ml/min), as well as (vii) rampant decay with excessive open caries lesions. Before the study, the subjects were given thorough instructions concerning oral hygiene, with special emphasis on daily toothbrushing with fluoride-containing toothpaste. Sample size was determined by a power analyse ($\alpha = 0.05$ and $\beta = 0.2$) based on cytokine mean values and standard deviations obtained previously [18].

Study design

The project was ethically approved by the Ethics Committee in the Capital Region of Denmark (H-KF-2007-0025). The prospective study was a double-blind placebo-controlled randomized trial with three parallel arms. After baseline examination and sampling, the subjects were randomly assigned to one of the study groups with the aid of computer-generated random numbers. Those in Group A/P ($n = 15$) were given one active chewing gum and one placebo gum, Group A/A ($n = 14$) received two active chewing gums, and Group P/P ($n = 13$) two placebo chewing gums. Follow-up registrations and samplings were conducted 1, 2, and 4 weeks after baseline. Four patients dropped out and were excluded during the study period since they failed to attend their recall visits.

Intervention

The probiotic chewing gums contained two strains of *Lactobacillus reuteri* (ATCC 55730 and ATCC PTA 5289) at a dose of 1×10^8 CFU/gum, respectively. The placebo gum was identical in size and composition but without the addition of the probiotic strains. Both gums were provided by BioGaia AB, Lund, Sweden. The participants were instructed to actively chew one of the assigned chewing gum pellets for 10 min twice daily – in the morning and in the evening and at least 1 h after any intake of food. The gums were packed in identical pots with a plastic screw cap and each of the participants was given two pots; one marked "morning", the other "evening". The three groups were separated by a colour code. All clinical registrations, as well as the laboratory analyses, were carried out without knowledge of the group assignment. The code was kept in a locker by an independent monitor at the University of Copenhagen and not unveiled until after the statistical analyses had been finalized.

Clinical procedures

Two contralateral buccal sites from the upper incisors (42%), canines (34%) or premolars (24%) were selected from each participant. The distribution of teeth did not differ significantly between the experimental groups. Visual supragingival plaque was carefully removed with a periodontal probe and cotton pellets. The sites were then gently dried in air and gingival crevicular fluid (GCF) was collected using periopaper strips (ProFlow, Amityville, N.Y., USA) gently inserted in the gingival sulcus for 20 s, as previously described [18]. The position of the strip was marked on a chart in an effort to standardize the samplings. In the event of blood contamination, the strip was discarded followed by a renewed collection after a few minutes. The volume of the collected GCF sample was immediately recorded using a Periotron 8000 (ProFlow) and expressed as μl. The strips were thereafter transferred to plastic tubes and stored frozen at –70°C until assayed. After the GCF samplings, an assessment of the bleeding upon probing [19] was carried out at four sites around each selected tooth.

Laboratory procedures

The absorbed GCF was eluated in neutral 120 μl PBS (phosphate-buffered saline) containing 0.05% Tween 20. The levels of IL-1β, TNF-α, IL-6, IL-8 and IL-10 were determined using luminex technology and multiplex immunoassay panels

(LINCOplex; LINCO Research, Inc., USA) in accordance with the manufacturer's manual. The concentrations of the cytokines were expressed as pg/ml.

Statistical methods

All data were processed with the SPSS software (v. 16.0; Chicago, Ill., USA). The follow-up values were compared to baseline within each regimen with the aid of the non-parametric Wilcoxon paired two-sided test; differences between the groups were subjected to the Wilcoxon unpaired test. A p-value <0.05 was considered as statistically significant. Three values considered as outliers (one baseline sample of IL-6 in Group A/P; two samples of IL-8 in Group P/P) were omitted from the statistical calculations since they differed more than 10-fold ($> \pm 3$ SD) from the mean value of the group.

Results

Clinical variables

The clinical variables on the various sampling occasions in each group are presented in Table I and II. There were slightly more sites with positive BOP in the placebo group at baseline compared with the two active groups, but the difference was not statistically different. The number of sites with BOP diminished during the experimental period in all groups and the decrease was statistically significant in Groups A/P and A/A after 2 weeks of chewing (Table I). There were no statistically significant differences in the amount of gingival crevicular fluid at baseline. The registered amounts tended to decrease by time in all groups and the difference compared with baseline was statistically significant in the two groups with active chewing gums at the 2-week follow-up (Table II). No harmful side effects or adverse events were reported during the intervention period.

Inflammatory mediators

The mean concentration of the selected inflammatory mediators is displayed in Table III. No statistically significant differences between the groups were recorded at baseline. The levels of TNF-α and IL-8 were significantly reduced compared with baseline in Group A/A after 1 and 2 weeks, respectively, while no significant alterations were seen in Groups A/P and P/P. A similar decreasing tendency was recorded for IL-1β in Group A/A during chewing, but the difference failed to reach statistical significance. The concentrations of IL-6 and IL-10 remained mainly unchanged in all groups during the 2-week chewing period. At the follow-up samplings 2 weeks after the chewing protocol (4 weeks after baseline), the recorded cytokine levels did not differ significantly from baseline in any of the study groups, with the exception of IL-6 in Group A/A.

Discussion

This study was undertaken to investigate the inflammatory response in the oral cavity after short-term exposure to probiotic bacteria, and this, to our knowledge, is the first *in vivo* report on the issue. The main finding was that statistically significant reductions of the pro-inflammatory cytokines TNF-α and IL-8 were demonstrated following the use of chewing gums containing *Lactobacillus reuteri*. Interestingly, a dose–response relationship, or a threshold level, seemed to appear, since no significant effects were disclosed in Group A/P with one active daily gum, but it would be premature to propose any clinical recommendations at this early stage.

Compliance with the study protocol was judged acceptable based on interviews and by picking up the distributed chewing gum pots. The improved gingival conditions displayed in all the groups were partly explained by the thorough oral hygiene instructions that preceded the project, although a pure placebo effect of participating in a trial cannot be excluded. Unfortunately, no attempt was made to monitor or estimate the amount of plaque accumulation during the intervention, nor was any cultivation of the supra- or subgingival microflora performed. It has to be stressed, however, that the selected teeth, as well as the teeth adjacent to the gingival sampling sites, were not subjected to mechanical forces in prosthetic constructions that otherwise could influence the cytokine levels [20]. The GCF sampling was standardized, but the volumes were generally small. This may have given rise to possible errors in the cytokine concentrations obtained, which

Table I. Distribution of bleeding on probing at baseline and designated time intervals during and after a 2-week use of chewing gums containing *Lactobacillus reuteri*. Values in table denote percentage positive sites.

Time	A/P (active+placebo) ($n=13$, 104 sites)	A/A (active+active) ($n=13$, 104 sites)	P/P (placebo+placebo) ($n=12$, 96 sites)
Baseline	54	58	68
1 week	23	23	58
2 weeks	8*	8*	45
4 weeks	23	15	64

*Statistically significant difference compared to baseline (Wilcoxon paired test, $p<0.05$).

Table II. Volume of gingival crevicular fluid (μl, mean (SD)) at baseline and at designated time intervals during and after use of chewing gums containing *Lactobacillus reuteri*.

Time	A/P (active+placebo) ($n=13$, 26 sites)	A/A (active+active) ($n=13$, 26 sites)	P/P (placebo+placebo) ($n=12$, 24 sites)
Baseline	0.30 (0.18)	0.30 (0.12)	0.38 (0.16)
1 week	0.19 (0.11)	0.19 (0.12)	0.32 (0.15)
2 weeks	0.17 (0.09*)	0.14 (0.10*)	0.27 (0.15)
4 weeks	0.18 (0.09*)	0.17 (0.07)	0.25 (0.13)

*Statistically significant difference compared to baseline ($p<0.05$, Wilcoxon signed rank test).

together with the individual variability resulted in considerable standard deviations in this material. For example, previous studies have shown that fluctuations in the host–response curve over time may have an influence on the recorded concentrations [21]. The expressed cytokine levels were not adjusted to the amount of GCF in each sample and expressed as "output", but it is very likely that reduced concentrations, together with a reduced flow, will enhance the cytokine alterations over a given time unit.

A gingival infection is caused by a mix of Gram-positive and Gram-negative species and characterized by pronounced leukocyte infiltration and inflammatory exudation in the marginal area [22]. The mechanism of probiotic action in the oral cavity is not fully understood, but is commonly explained by a combination of local and systemic immunomodulation as well as non-immunologic defense mechanisms [23]. The principal health-promoting effects in the gastrointestinal tract are ascribed to enhancement of mucosal immune defense and macrophage activity as well as elevations of the numbers of killer cells, T-cells, and interferon levels [2]. In that perspective, our present results were not unexpected. The cytokines TNF-α and IL-1β, produced and secreted by antigen-presenting cells such as monocytes, macrophages, fibroblasts, and dendritic cells, are the central mediators of the pro-inflammatory cascade causing tissue damage [24,25]. IL-1β induces production of the cytokine IL-6 from Th-2 cells, so it was interesting to observe the "delayed" reduction of the latter cytokine in the present material. The levels of chemokine IL-8, produced mainly by macrophages and epithelial cells and with a mainly chemotactic function [26], were

Table III. Concentration of inflammatory mediators (pg/ml, mean (SD)) in gingival crevicular fluid at baseline and at designated time intervals during and after use of chewing gums containing *Lactobacillus reuteri*.

Variable/time	A/P (active+placebo) ($n=13$, 26 sites)	A/A (active+active) ($n=13$, 26 sites)	P/P (placebo+placebo) ($n=12$, 24 sites)
IL-1β			
Baseline	10.5 (10.5)	9.0 (7.6)	10.4 (12.3)
1 week	10.1 (9.8)	4.7 (6.9)	13.8 (14.0)
2 weeks	9.1 (8.9)	4.4 (4.7)	9.3 (10.5)
4 weeks	10.2 (11.6)	9.8 (11.1)	10.9 (13.3)
TNF-α			
Baseline	0.23 (0.24)	0.41 (0.29)	0.45 (0.52)
1 week	0.32 (0.31)	0.14 (0.15*)	0.43 (0.49)
2 weeks	0.41 (0.34)	0.24 (0.26)	0.39 (0.36)
4 weeks	0.22 (0.19)	0.33 (0.36)	0.40 (0.38)
IL-6			
Baseline	0.92 (1.67)	1.90 (3.28)	1.53 (2.48)
1 week	0.67 (0.68)	0.94 (1.70)	1.60 (2.49)
2 weeks	0.76 (1.23)	0.95 (1.69)	1.91 (2.50)
4 weeks	0.99 (1.20)	0.74 (1.56*)	1.03 (1.87)
IL-8			
Baseline	116.2 (51.8)	103.3 (36.8)	107.3 (69.9)
1 week	102.2 (43.7)	86.2 (54.4)	82.9 (32.9)
2 weeks	95.2 (60.2)	77.4 (31.8*)	85.8 (42.0)
4 weeks	97.7 (69.0)	105.8 (63.8)	106.7 (60.6)
IL-10			
Baseline	0.28 (0.27)	0.24 (0.38)	0.32 (0.44)
1 week	0.32 (0.38)	0.19 (0.24)	0.28 (0.31)
2 weeks	0.32 (0.38)	0.22 (0.37)	0.24 (0.26)
4 weeks	0.20 (0.23)	0.19 (0.20)	0.20 (0.20)

*Statistically significant difference compared to baseline ($p<0.05$, Wilcoxon signed rank test).

also reduced in the active gum group (A/A). The anti-inflammatory cytokine IL-10, which was unaffected in the present study, is produced by monocytes and mast cells and is thought to downregulate the expression of cytokines produced by Th-1 cells [27]. This cytokine is considered an essential immune-regulator in the intestinal tract, albeit its role in gingival inflammation is less explored [28]. Altogether, our results demonstrating that the levels of inflammatory cytokines TNF-α and IL-8 are significantly reduced by *Lactobacillus reuteri*, given in chewing gums, may to some extent explain the mechanism of probiotic action in the oral cavity.

In light of the present findings, it is relevant to further speculate on the specific mechanisms of action ascribed to the probiotic strains incorporated in the chewing gums tested. *Lactobacillus reuteri* is a member of the beneficial and commensal microbiota in the mammalian oral cavity and gastrointestinal tract; ATCC 55730 is derived from mother's milk, while the PTA 5289 is an oral isolate. It has previously been demonstrated that a *Lactobacillus reuteri* strain can exhibit a powerful suppressive effect on TNF-α production by human monocytes and macrophages [29]. Furthermore, it has been proposed that *Lactobacillus reuteri* may produce an undefined immunomodulin capable of inhibiting TNF-α production by LPS-activated monocytoid cells [30]. A notable observation was that the effect of the putative immunomodulin, secreted from the cells, was not dependent on the presence of the entire bacteria [30]. It has also been postulated that the antibacterial agent reuterin, produced from glycerol by *Lactobacillus reuteri*, could play a role in the probiotic effects of *Lactobacillus reuteri* [31]. Furthermore, recent studies have shown that a wide spectrum of intestinal bacteria are very sensitive to reuterin [32,33], but whether or not this is the case concerning bacteria associated with periodontal diseases remains an open question. Although the complexity of the periodontal microbiota resembles that of the gastrointestinal tract, where infections are treatable via supplementation of probiotics, the composition of the bacterial communities inhabiting the mouth and the large intestines is quite different. Oral research on the potential beneficial effects of probiotic supplementation is still in its infancy, and one strain with a proven intestinal effect may not be the optimal strain in the oral environment [5,34,35]. Collectively, our *in vivo* data confirm previous observations concerning TNF-α, and the null hypothesis could be rejected for TNF-α and IL-8. It should be pointed out, however, that the number of patients was quite small and the follow-up time relatively short. Thus, the present report can be regarded as a pilot study that should be verified in further clinical trials. The outcome justifies screening for the most effective probiotic strain, or combination of strains, for various oral diseases.

In conclusion, this *in vivo* study provides the first indications of the significant dose-dependent modulating effect of a short-term intake of probiotic bacteria on the oral immune response, and the results reinforce previous beneficial clinical observations on gingival health.

Acknowledgments

The study was supported by grants from the Danish Dental Society, Tandlægernes Nye Landsforening, Västerbottens County Council (TUA) and BioGaia AB, Sweden.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Food and Agriculture Organization and World Health Organization Expert Consultation. Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria. 2001. Available at: http://www.who.int/food safety/fs_management/en/probiotic_guidelines.pdf)

[2] Fuller R, Gibson GR. Modification of the intestinal microflora using probiotics and prebiotics. Scand J Gastroenterol 1997;32 Suppl 222:28–31.

[3] Gill H, Prasad J. Probiotics, immunomodulation, and health benefits. Avd Exp Med Biol 2008;606:423–54.

[4] Reid G, Jass J, Sebulsky MT, McCormick JK. Potential uses of probiotics in clinical practice. Clin Microb Rev 2003;16:658–72.

[5] Meurman JH. Probiotics. Do they have a role in oral medicine and dentistry? Eur J Oral Sci 2005;113:188–96.

[6] Twetman S, Stecksen-Blicks C. Probiotics and oral health effects in children. Int J Paediatr Dent 2008;18:3–10.

[7] Teughels W, van Essche M, Sliepen I, Quirynen M. Probiotics and oral health care. Periodontology 2000 2008;48:111–47.

[8] Caglar E, Kavaloglu S, Ergeneli S, Sandalli N, Twetman S. Salivary mutans streptococci and lactobacilli levels after ingestion of the probiotic bacterium *Lactobacillus reuteri* ATCC 55730 by straws and tablets. Acta Odontol Scand 2006;64:314–18.

[9] Caglar E, Kavaloglu SU, Kuscu OO, Sandalli N, Lif Holgersson P, Twetman S. Effect of chewing gums containing xylitol and probiotic bacteria on salivary mutans streptococci and lactobacilli. Clin Oral Investig 2007;11:425–9.

[10] Çaglar E, Sandalli N, Twetman S, Kavaloglu SC, Ergeneli S, Selvi S. Consumption of yogurt with *Bifidobacterium* DN-173010 and its effect on dental caries risk factors. Acta Odontol Scand 2005;63:317–20.

[11] Teughels W, Newman MG, Coucke W, Haffajee AD, Van Der Mei HC, Haake SK, et al. Guiding periodontal pocket recolonization: a proof of concept. J Dent Res 2007;86:1078–82.

[12] Krasse P, Carlsson B, Dahl C, Paulsson A, Nilsson A, Sinkiewicz G. Decreased gum bleeding and reduced gingivitis by the probiotic *Lactobacillus reuteri*. Swed Dent J 2006;30:55–60.

[13] Shimauchi H, Mayanagi G, Hakaya S, Minamibuchi M, Ito Y, Yamaki K, et al. Improvement of periodontal condition by probiotics with *Lactobacillus salivarius* WB21: a randomized, double-blind, placebo-controlled study. J Clin Periodontol 2008. doi: 10.1111/j.1600-051X.2008.01306.x.

[14] Lamster IB. The host response in gingival crevicular fluid: potential applications in perodontitis clinical trials. J Periodontol 1992;63:1117–23.

[15] McCulloch CA. Host enzymes in gingival crevicular fluid as diagnostic indicators of periodontitis. J Clin Periodontol 1994;21:497–506.

[16] Seymour GJ, Gemmel E. Cytokines in periodontal diseases: where to from here? Acta Odontol Scand 2001;51:167–73.

[17] Löe H. The gingival index, plaque index and the retention index system. J Periodontol 1967;38:610–16.

[18] Yucel-Lindberg T, Twetman S, Sköld-Larsson K, Modéer T. Effect of an antibacterial varnish on the levels of prostanoids, leukotriene B_4 and interleukin 1-β in gingival crevicular fluid. Acta Odontol Scand 1999;57:23–7.

[19] Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J 1975;25:229–35.

[20] Giannopoulou C, Kamma JJ, Mombelli A. Effect of inflammation, smoking and stress on gingival crevicular fluid cytokine level. J Clin Periodontol 2003;30:145–53.

[21] Champagne CME, Buchanan W, Reddy MS, Preisser JS, Beck JD, Offenbacher S. Potential for gingival crevice fluid measures as predictors of risk for periodontal diseases. Periodontology 2000;31:167–80.

[22] Scannapieco FA. Periodontal inflammation: from gingivitis to systemic disease? Compend Contin Educ Dent 2004;25 Suppl 1:16–25.

[23] Meurman JH, Stamatova I. Probiotics: contributions to oral health. Oral Dis 2007;13:443–51.

[24] Tokoro Y, Yammamoto T, Hara K. IL-1 beta mRNA as the predominant inflammatory cytokine transcript: correlation with inflammatory cell infiltration into human gingiva. J Oral Pathol Med 1996;25:225–31.

[25] Wang PL, Ohura K. Porphyromonas gingivalis lipopolysaccharide signaling in gingival fibroblasts-CD14 and Toll-like receptors. Crit Rev Oral Biol Med 2002;13:132–42.

[26] Bickel M. The role of interleukin-8 in inflammation and mechanisms of regulation. J Periodontol 1993;64 Suppl 5:456–60.

[27] Berglundh T, Donati M. Aspects of adaptive host response in periodontitis. J Clin Periodontol 2005;32 Suppl 6: 87–107.

[28] De Nardin E. The role of inflammatory and immunological mediators in periodontitis and cardiovascular disease. Ann Periodontol 2001;6:30–40.

[29] Lin YP, Thibodeaux CH, Peña JA, Ferry GD, Versalovic J. Probiotic *Lactobacillus reuteri* suppress proinflammatory cytokines via c-Jun. Inflamm Bowel Dis 2008;14:1068–83.

[30] von Bodman SB, Willey JM, Diggle SP. Cell-cell communication in bacteria: united we stand. J Bacteriol 2008;190:4377–91.

[31] Axelsson LT, Chung TC, Dobrogosz WJ, Lindgren SE. Production of a broad spectrum antimicrobial substance by *Lactobacillus reuteri*. Microb Ecol Health Dis 1989;2:131–6.

[32] Cleusix V, Lacroix C, Vollenweider S, Duboux M, Le Blay G. Inhibitory activity spectrum of reuterin poduced by *Lactobacillus reuteri* against intestinal bacteria. BMC Microbiology 2007;7:101. doi: 10.1 186/1471-2180-7-101.

[33] Spinler JK, Taweechotipatr M, Rognerud CL, Ou CN, Tumwasorn S, Versalovic J. Human-derived probiotic *Lactobacillus reuteri* demonstrate antimicrobial activities targeting diverse enteric bacterial pathogens. Anaerobe 2008;14: 166–71.

[34] Comelli EM, Guggenheim B, Stingele F, Neeser JR. Selection of dairy bacterial strains as probiotics for oral health. Eur J Oral Sci 2002;110:218–24.

[35] Kõll P, Mändar R, Marcotte H, Leibur E, Mikelsaar M, Hammarström L. Characterisation of oral lactobacilli as potential probiotics for oral health. Oral Microbiol Immunol 2008;23:139–47.