

ORIGINAL ARTICLE

Assessment of the Effect of Probiotic Yoghurt and Different Probiotic Strains on Salivary *Streptococcus Mutans* in Children: An In Vivo and an In Vitro Study

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ABSTRACT

Key words:

Probiotic yoghurt,
Streptococcus mutans,
Lactobacillus rhamnosus,
Bifidobacterium animalis

Introduction: *Streptococcus mutans* (*S. mutans*) is the major pathogen involved in caries development which is the most common childhood infection. *Lactobacilli* and *Bifidobacteria* are commonly used as probiotics and are added to fermented food such as yoghurt, and have been linked to reducing caries risk in children. **Objectives:** to investigate the effect of probiotic-containing yogurt on salivary levels of *S. mutans*, gingival inflammation and plaque accumulation in children and to evaluate the possible inhibitory effect of two probiotic bacterial strains (*Lactobacillus rhamnosus* and *Bifidobacterium animalis*) against *S. mutans* invitro. **Methodology:** This study was performed with 30 children; who were given yoghurt containing probiotic (group 1) or control yoghurt (group 2) daily for a period of 6 weeks; *S. mutans* count, gingival index (GI) and plaque index (PI) were assessed throughout the 6 weeks of intervention and 2 weeks post-intervention. *Lactobacillus rhamnosus* and *Bifidobacterium animalis* were tested against *S. mutans* invitro. **Results:** There was a statistically significant decrease ($p < 0.001$) in *S. mutans* count and GI in the intervention group as compared to the control group. The PI showed a statistically significant decrease in both groups 2 weeks after the intervention. *Lactobacillus rhamnosus* had an inhibitory effect whereas *B. animalis* had no inhibitory effect on the tested *S. mutans* strains invitro. **Conclusion:** The use of probiotic yoghurt decreases the salivary *S. mutans* count and improves oral dental health in children. Further research should assess the long term effect of plain and probiotic yoghurt on oral health conditions. The addition of alternate probiotic strains to yoghurt such as *Lactobacillus rhamnosus* should also be considered

INTRODUCTION

Dental caries is the most prevalent chronic disease in children¹. Children are specifically at risk of developing dental caries since the host defense systems are in the process of being developed, tooth surfaces are newly erupted and may be showing hypoplastic defects and they are experiencing the dietary transition between breast/bottle feeding and solid foods. The intensity of caries in preschool children is due to frequent sugar consumption² and the pathogenesis of the disease involves the interaction over time among cariogenic microorganisms, a diet rich in fermentable carbohydrates and host factors, like secretion rate and buffering capacity³.

Streptococcus mutans (*S. mutans*) is considered the major pathogen involved in caries development⁴.

Dental caries still continues to be the most prevalent oral infectious disease. Thus, there is a rising need for novel caries prevention strategies in addition to the existing conventional measures which are mainly dependant on physical and chemotherapeutic agents⁵.

Probiotic bacteria, are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host"⁶. *Lactobacilli* and *Bifidobacteria* are generally regarded as cariogenic^{7,8} so the idea of probiotics being beneficial from a dental point of view may appear controversial. The existing clinical studies, however, support the idea of beneficial rather than harmful effects on oral health. *Lactobacilli* administration appears to decrease salivary counts of *S. mutans* and its addition to milk has been shown to reduce caries risk in children^{9,10,11}.

Probiotics are commonly consumed as part of fermented foods with specially added active live cultures; such as in yoghurt or as dietary supplements.

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The main mechanism of probiotic action in caries prevention is to replace and displace cariogenic bacteria, mainly *S. mutans*, with non cariogenic bacteria resulting in control of plaque biofilm formation. The number of products containing *Probiotics* entering the market is increasing. These products usually contain *Lactobacilli* or *Bifidobacteria*^{12,13}.

The present study aimed to investigate the effect of probiotic-containing yogurt on salivary levels of *S. mutans*, gingival inflammation and plaque accumulation in children and to evaluate the possible inhibitory effect of two probiotic bacterial strains (*Lactobacillus rhamnosus* and *Bifidobacterium animalis*) against *S. mutans* *in vitro*.

METHODOLOGY

The present study was carried out in two phases in the period from July 2015 to October 2015. The first phase was *in vivo* in which the effect of probiotics added to yoghurt on the *S. mutans* count and the periodontal health was assessed. The second was *in vitro* in which the inhibitory effect of the probiotics *Lactobacillus rhamnosus* and *Bifidobacterium animalis* on the *S. mutans* was assessed using the disc diffusion method.

Subjects:

Thirty children were enrolled in the study; children from 4 to 6 years with no recent history of use of any drugs that may affect oral health were included in this study. Exclusion criteria included children with systemic conditions that could affect the oral cavity; children who were subjected to antibiotic treatment in the last two weeks before the examination; children using any oral hygiene aid other than routine teeth brushing; and children with known or suspected allergy to dairy products. The study sample was selected from an orphanage (Al Amal Orphanage in ElQobba, Cairo, Egypt) over the period of 8 weeks.

A written consent for the participation of the children in the study was given by the guardians.

1. Phase one (in vivo) phase

The study consisted of a 6 week intervention period and a 2 week post intervention period. The subjects were divided into two intervention groups; group I; included 15 children who received plain probiotic-containing yoghurt (Activia®) which contains *Bifidobacterium animalis* and group II which included 15 children who were given plain (control) yoghurt (Danone®). Each child was given 1 cup of assigned yoghurt daily and for 6 weeks. For each group, saliva samples were collected; microbiological assessment, plaque index (PI) and gingival index (GI) were measured at: Baseline before yoghurt administration, after 2 weeks of yoghurt administration, after 6 weeks of yoghurt administration. Administration of yoghurt

was then stopped and after 2 weeks the same variables were measured.

a. Saliva Sampling:

For collecting un-stimulated saliva, a sterile hard plastic container was given to each child and the child was asked to tilt his head slightly forward and spit the saliva into it. Saliva was collected over a period of 5 minutes by repeatedly spitting¹⁴.

b. Determination of *S. mutans* counts

Saliva samples were immediately transferred in their sterile plastic cups on ice to the Medical Microbiology Department laboratory at Ain Shams University for microbiological assessment. The selective medium Mitis Salivarius Bacitracin agar (MSB) was formulated by adding 20% sucrose and 1 ml of Chapman Tellurite solution (BBL™ Tellurite Solution 1%) and Bacitracin solution to the Mitis Salivarius Agar (Difco™ Mitis Salivarius Agar) medium¹⁵. Bacitracin solution was prepared by adding 2, 10 Units bacitracin disks (Bioanalyse™ Bacitracin 10 U) dissolved in 1 ml sterile water¹⁶. This method was modified so that the prepared bacitracin solution was added directly to the MSB instead of spreading the solution onto the surface of the medium. This modification of the medium yielded better results as regards the recovery of *S. mutans*.

Using a sterile disposable calibrated loop, 0.1 ml of saliva was spread onto modified MSB agar medium for selective recovery and enumeration of *S. mutans*. The plates were incubated at 37°C anaerobically in re-sealable plastic pouches¹⁶ added to it anaerobic gas generating sachets (Oxoid™ AnaeroGen™) for 72 hours. After anaerobic incubation, the colonies were counted manually to determine the number of bacteria (cfu) per mL whole saliva on each agar medium. Colonies suspected of being those of *S. mutans* were confirmed by examination for typical colonial morphology and Gram stain, fresh isolates had typically raised convex granular frosted appearance¹⁷ onto the Mitis Salivarius Bacitracin plates. Biochemical tests as positive fermentation of Mannitol were performed for verification of the colonies of *S. mutans*.

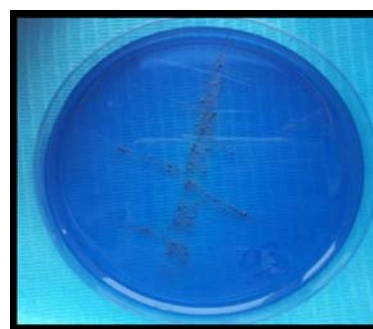


Fig. 1: *S. mutans* colonies growing on Mitis Salivarius Bacitracin agar

c. Determination of Plaque Index (PI) and Gingival Index (GI)

Children were seated upright and indices were measured using periodontal probe and results were scored and tabulated using the **Silness-Loe periodontal indices**:

d. Plaque Index

¹⁸Six teeth were examined and scored. Each of the four surfaces of the teeth (buccal, lingual, mesial and distal) was given a score from 0-3. The scores from the four areas of the tooth were added and divided by four in order to measure the PI for the tooth with the following scores and criteria; 0: No plaque, 1: A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen in situ only after application of disclosing solution or by using the probe on the tooth surface, 2: Moderate accumulation of soft deposits within the gingival pocket, or the tooth and gingival margin which can be seen with the naked eye and 3: Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin.

e. Gingival Index:

¹⁹ Six teeth were selected for examination; 0: No inflammation, 1: Mild inflammation (slight redness, edema, probing with a blunt probe does not induce bleeding), 2: Moderate (edema, redness, glazing, probing with a blunt probe induces bleeding and the marginal gingival is swollen). 3: Severe (marked redness, edema, spontaneous bleeding or ulceration).

2. Phase Two (in-vitro):

Antimicrobial effect of probiotics on *S. mutans*: 6 *S. mutans* strains that were previously isolated from the children were grown on MSB as before, then the strains were re-isolated on M17 broth (Conda Laboratories, Madrid)²⁰ to be used in the inhibition test. The probiotic strain *Lactobacillus rhamnosus* ATCC7469 was cultivated on the culture media MRS-Clindamycin [deMan, Rogosa and Sharpe agar (MRS; Oxoid, United Kingdom) + 0.5 ppm filter sterilized clindamycin]²¹. The probiotic strain *Bifidobacterium animalis* N 173010 was cultivated on LP-MRS [MRS agar supplemented with 2g/l lithium chloride and 3g/l sodium propionate]²². The inhibitory effects of tested probiotics on *S. mutans* was done using the "Disc Diffusion Assay"²³ as follows: Tryptone Soya

agar (TSA, Difco, Detroit, MI USA) was poured into sterile petri dishes (15ml each) and 50µl of Tryptone Soya broth containing *S. mutans* were dispersed on the surface of each agar plate. Sterile filter paper discs 6 mm in diameter were impregnated with the tested probiotic strains (*Lactobacillus rhamnosus* and *Bifidobacterium animalis*) and standard 6 mm discs containing saline (20µl/disc) were used as controls. Discs from each group were placed on the inoculated agar surface and the plates were incubated at 37°C for 24 h. At the end of the incubation period antimicrobial activity was evaluated by measuring the zones of inhibition²³.

3. Statistical Analysis:

Data were presented by mean, standard deviation (SD), Independent t test and its non parametric alternative (Mann Whitney) were used to compare between groups. Anova for repeated measures was used to compare between follow up periods within groups and for non parametric data Friedman test was used followed by Wilcoxon signed rank test with bonferioni correction. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

RESULTS

Analysis of data gathered for both the intervention (Group I) and the control (Group II) groups showed the following results:

I) *S. mutans* count:

At week 6 and week 8, a statistically significant decrease was found in the *S. mutans* count (cfu/ml) in Group I compared to Group II as shown in table 1 and Fig. 2.

Within Group I there was a statistically significant decrease in *S. mutans* count (cfu/ml) at week 2 and a statistically significant increase at week 6 and week 8. There was also a statistically significant decrease in *S. mutans* count between week 0 and week 6, 8. Group II showed a statistically significant decrease at week 2 and a statistically significant increase at week 6 and a statistically non significant decrease at week 8 (Table 1; Fig. 3&4)

Table 1: Comparison between *S. mutans* count between Group I and II and within each group

S. mutans count	Group I	Group II	p*
	Mean± SD	Mean± SD	
Week 0	563.3± 419.87 ^a	552.33± 377.98 ^a	0.884
Week 2	59.8± 86.03	103.66± 158.96	0.983
Week 6	194± 90.45 ^a	604.66± 172.7 ^a	<0.001
Week 8	330± 184.97 ^a	470±142.42 ^a	0.006
p#	<0.001	<0.001	

* Mann whitney test

a. Friedman test followed by wilcoxon signed rank test with bonferioni correction

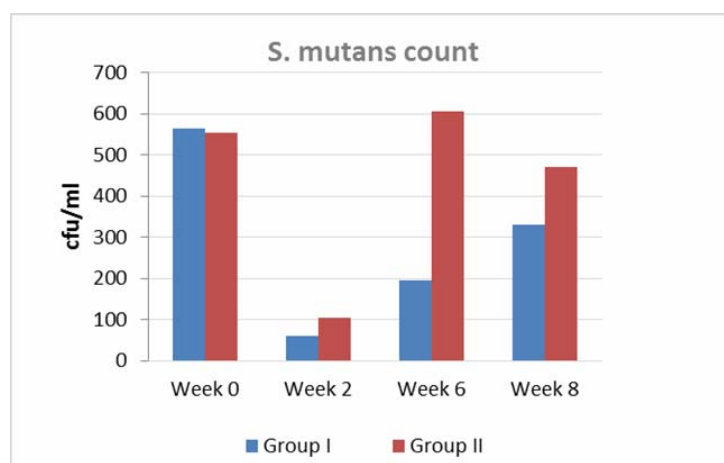


Fig. 2: Comparison between the *S. mutans* count between Group I and II

II) Gingival Index:

A higher value was found in the GI of Group I at week 0 which was statistically significant and at week 2 which was statistically non-significant. At week 6 and 8, Group I showed a lower value compared to Group II which was statistically non-significant as shown in table (2).

Group I showed a statistically significant decrease in the GI through the follow up periods. There was significant difference between week 0, week 6 and week 8 and between week 2 and week 8. Group II showed a statistically non-significant difference between the follow up periods (Table 2 Fig 3&4)

Table 2: Comparison between GI in Group I and II and within each group

Gingival Index	Group I	Group II	P*
	Mean ± SD	Mean ± SD	
Week 0	1.3± 0.21 ^a	0.95± 0.29	<0.001
Week 2	1.19± 0.26 ^{ac}	1.09± 0.22	0.267
Week 6	1.02± 0.28 ^{bc}	1.04± 0.29	0.849
Week 8	0.98± 0.28 ^b	1.05± 0.31	0.504
P#	<0.001	0.26	

*Independent t-test

Anova for repeated measures, similar superscript letters indicate no significant difference.

III. Plaque Index:

Group I showed a statistically non-significant higher value of PI in group I at week 0, week 2, week 6 and week 8 (table 3). Both Group I and Group II showed a statistically significant decrease in the PI between the follow up periods with significant difference between week 8 and the other follow up periods (Table 3 and Figure 3&4).

Table 3: Comparison between PI in Group I and II and within each group

Plaque Index	Group I	Group II	P*
	Mean ± SD	Mean ±SD	
Week 0	1.39± 0.24 ^a	1.29± 0.31 ^a	0.303
Week 2	1.66± 0.37 ^a	1.53± 0.35 ^a	0.341
Week 6	1.54± 0.39 ^a	1.42± 0.33 ^a	0.375
Week 8	1.02± 0.37	0.9± 0.29	0.332
P#	<0.001	<0.001	

*Independent t-test

Anova for repeated measures, similar superscript letters indicate no significant difference

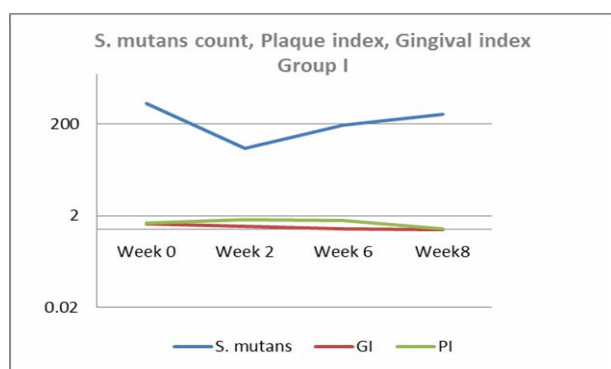


Fig. 3: Logarithmic scale comparing *S. mutans*, PI and GI in Group I

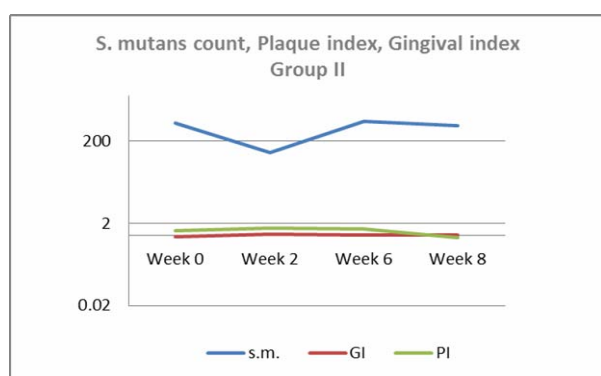


Fig. 4: Logarithmic scale comparing *S. mutans*, PI and GI in group II

Disk diffusion method results:

The probiotic strain *L. rhamnosus* had a clear inhibitory effect on the test strain *S. mutans* showing an inhibition zone of 10-12 mm (mean 10.83 ± 0.9) in diameter in the tested strains. *S. mutans* was resistant to the probiotic strain *B. animalis* (Figure 5a&b)

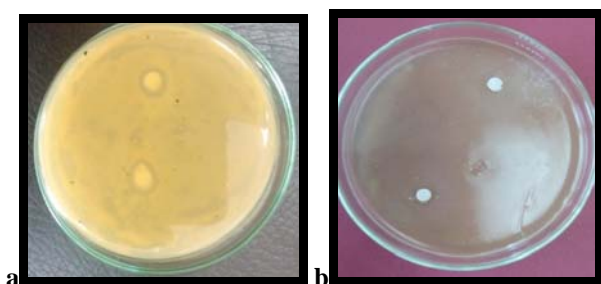


Fig. 5: Antimicrobial activity of probiotic strain *Lactobacillus rhamnosus* **a:** and probiotic *Bifidobacterium animalis*, **b:** by Agar Disc Diffusion method

DISCUSSION

The use of probiotics in dentistry added to the improvement of many aspects of oral health as supported by many studies including anti-cariogenic effect, the treatment of periodontal disease, a reduced crevicular fluid volume and cytokine content. Probiotics

are also used in the treatment of halitosis and *Candida albicans*²⁴. Many vehicles have been proposed for the delivery of probiotics. The use of dairy products particularly is desirable from a cariological point of view due to their high natural contents of calcium and phosphate that readily decrease the “critical pH” for enamel dissolution and enhance remineralization. It is also a more economical alternative for the average consumer than tablets or capsules¹³.

As regards the in vivo phase of the study, the current study showed statistically significant ($p < 0.001$) decrease in the count of *S. mutans* at week 2 in both groups decrease. However, at the end of yoghurt administration (week 6) and 2 weeks post-intervention (week 8), there was a statistically significant decrease ($p < 0.001$) in the *S. mutans* count in the intervention group as compared to the control group.

Decrease in the *S. mutans* counts was consistent with studies that used probiotic curd²⁵ and probiotic milk²⁶, and fermented milk containing *Bifidobacterium* probiotics which is the same probiotic strain used in our study²⁷. The decrease in the *S. mutans* counts in both groups after 2 weeks was consistent with other studies that assessed the effect of plain yoghurt on microbial count.^{28,29} The ability of plain yogurt to decrease the amount of *S. mutans* count may be attributed to the presence of some anti microbial products found in the yoghurt that might be responsible for the inhibition of *S. mutans* proliferation²⁹. The effect of plain yoghurt may also be explained by the ability of casein and casein derivatives to inhibit bacterial adherence and/or the bactericidal activity of bacteriocins with selective activity against *S. mutans* that are present in dairy products³⁰.

This study showed a statistically significant ($P < 0.001$) decrease in the GI after the end of intervention and two weeks post-intervention in the probiotic yoghurt group. Similar results were reported by Toiviainen et al.³¹. On the other hand, the PI showed a statistically significant decrease in both groups 2 weeks after the intervention. The decrease in the intervention group is similar to a study evaluating the efficacy of probiotics in plaque reduction and gingival health maintenance among school children³². The reduction in the PI in the control group could also be explained by the ability of plain yoghurt and its content of casein and casein derivatives to inhibit bacterial adherence³⁰.

Toiviainen et al.³¹ also examined the effect of orally administered lozenges containing *Bifidobacterium animalis* and *Lactobacillus rhamnosus* on adults. He assessed the *S. mutans* count, PI and GI for only 4 weeks. The probiotic lozenge decreased both PI and GI ($p < 0.05$), however, no changes were observed in the control group and no changes were found in *S. mutans* count. This could be explained by a potentiating effect of naturally found probiotic bacteria in yoghurt on the added probiotics as multi-strain probiotics appear to

show greater efficacy than single strains. However, whether this is due to synergistic interactions between strains or a consequence of the higher probiotic dose is unclear³³. In contrast, a study was done by Pinto et al.³⁴ to assess how consumption of yoghurt containing *Bifidobacterium animalis subsp. lactis* probiotic for a period of 2 weeks affects salivary and dental plaque levels of *mutans Streptococci* and *Lactobacilli* in patients undergoing orthodontic treatment. The trial was performed with 26 volunteers. There was no difference between the yoghurt containing probiotic and the control yoghurt for any of the studied variables. A reduction in counts of total cultivable microorganisms was observed in dental plaque samples after ingestion of either yoghurts but not in saliva. The difference between the result of this study and the current study may be due to the longer duration of the current study and may also be due to difference in the target population.

As regards the in vitro part of this study, the probiotic strains chosen were *B. animalis* which is the same strain found in the probiotic yogurt used in the in vivo part of the current study and *L. rhamnosus* as one of the most commonly studied *Lactobacillus* probiotic strains. *Lactobacillus rhamnosus* had an inhibitory effect on the tested *S. mutans* strains while *B. animalis* had no inhibitory effect on *S. mutans* in accordance to a study testing the effect of five probiotic lactobacillus strains on *S. mutans*³⁵, and other studies that tested the effect of *L. rhamnosus* on *S. mutans*^{36,23}. One study tested the effect of *B. adolescentis* on *S. mutans* by plate count technique and showed an inhibitory effect³⁷. A study examined the effect of *Bifidobacterium* on periodontal pathogens and showed no inhibitory effect on *Streptococcus sanguis*³⁸. The ability of the probiotic bacteria *B. animalis* to exert an inhibitory effect on the *S. mutans* in vivo when added to the yogurt but not when used alone in vitro may be explained by the potentiating effect of plain yogurt bacteria and the synergistic effect of multiple probiotic strains^{33,30}.

CONCLUSION

The use of probiotic yoghurt decreases the salivary *S. mutans* counts and improves oral dental health in children. Further research should assess the long term effect of plain and probiotic yoghurt on oral health conditions on larger population samples using different vehicles as other dairy products, tablets or lozenges. The addition of alternate probiotic strains to yoghurt such as *Lactobacillus rhamnosus* should also be considered.

REFERENCES

1. Rao SK, Bhat GS, Aradhya S *et al.* Study of the efficacy of toothpaste containing casein phosphopeptide in the prevention of dental caries: a randomized controlled trial in 12- to 15-year-old high caries risk children in Bangalore, India. *Caries Res.* 2009; 43(6), 430-435.
2. Harris R, Nicoll AD, Adair PM, and Pine CM. Risk factors for dental caries in young children: a systematic review of the literature. *Community Dental Health.* 2004;21(1 Suppl):71-85.
3. Selwitz RH, Ismail AI., and Pitts NB. Dental caries. *Lancet.* 2007; 369:51-59. doi: 10.1016/S0140-6736(07)60031-2.
4. Takahashi N and Nyvad B. The role of bacteria in the caries process: Ecological perspectives. *J. Dent. Res.* 2011, 90, 294-303.
5. Gupta S and Marwah N. 'Use a Thorn to Draw Thorn' Replacement Therapy for Prevention of Dental Caries. *Int J Clin Pediatr Dent.* 2010; 3(3), 125-137.
6. World Health Organization. Available online: http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf (accessed on 9 March 2016).
7. Beighton D. The complex oral microflora of high-risk individuals and groups and its role in the caries process. *Community Dent Oral Epidemiol* 2005; 33:248-255.
8. Haukioja A, So"derling E, and Tenovuo J (2008): Acid production from sugars and sugar alcohols by probiotic lactobacilli and bifidobacteria in vitro. *Caries Res* 42:449-453.
9. Hatakka K and Saxelin M: Probiotics in intestinal and non intestinal infectious diseases—clinical evidence. *Curr Pharm Des* 2008; 14:1351-1367.
10. Na'se L, Hatakka K, Savilahti E, Saxelin M, Po"nka" A, Poussa T, and Korpela R, Meurman JH: 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' 35:412-420.
11. Steckslen-Blicks C, Sjo"stro"m I, and Twetman S: Effect of long term consumption of milk supplemented with probiotic lactobacilli and fluoride on dental caries and general health in preschool children: a cluster-randomized study. *Caries Res* 2009; 43:374-381.
12. Sheikh S, Pallagatti S, Kalucha A *et al*: Probiotics, going on the natural way. *J Clin Exp Dent* 2011; 3(2), 150-154.
13. Twetman S: Are we ready for caries prevention through bacteriotherapy? *Brazilian Oral Research* 2012;26:64-70.
14. Dawes C: Physiological factors affecting salivary flow rate, oral sugar clearance, and the sensation of dry mouth in man. *Journal of Dental Research* 1987; 66 Spec No:648-53.
15. Gold OG, Jordan H V, Houte JV AN: A selective medium for *Streptococcus mutans*. *Archives of Oral Biology* 1973;18:1357-64.
16. Edelstein B: Screening preschool children for dental caries using a microbial test. *Pediatric Dentistry* 1989;11(2):129-32.
17. Coykendall AL: Proposal to Elevate the Subspecies of *Streptococcus mutans* to Species Status, Based on

- Their Molecular Composition. *International Journal of Systematic Bacteriology* 1977; 27(1):26–30.
18. Silness J and Loe H: Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontologica Scandinavica* 1964; 22:121–35.
 19. Loe H and Silness: Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontologica Scandinavica* 1963; 21:533–51.
 20. Zimbro MJ, Power DA, Miller SM, E. G, and Johnson JA: M17 agar. *Difco and BBL manual* 2011; 33(2):33–5.
 21. Van de Castele S, Vanheuverzwijn T, Ruysen T, Van Assche P, Swings J, and Huys G: Evaluation of culture media for selective enumeration of probiotic strains of lactobacilli and bifidobacteria in combination with yoghurt or cheese starters. *International Dairy Journal* 2006; 16(12):1470–6.
 22. Lima KG de C, Kruger MF, Behrens J, Destro MT, Landgraf M, and Gombossy de Melo Franco BD: Evaluation of culture media for enumeration of *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium animalis* in the presence of *Lactobacillus delbrueckii* subsp *bulgaricus* and *Streptococcus thermophilus*. *LWT - Food Science and Technology* 2009; 42(2):491–5.
 23. Elgamily HM, Nagi SM, Kassem A A, Nour K A, Zaazou MH, and Mehanna NS: In-Vitro comparative Study of Antimicrobial Activity of Two Plant Extracts and Probiotic Strain against Isolated oral Cariogenic Pathogen. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 2015;6(3):1705–9.
 24. Flichy-Fernández A-J, Alegre-Domingo T, Peñarrocha-Oltra D, and Peñarrocha-Diago M: Probiotic treatment in the oral cavity: an update. *Medicina Oral, Patología Oral y Cirugía Bucal* 2010; 15(5):e677–80.
 25. Bhalla M, Ingle N, Kaur N, and Yadav P: Mutans streptococci estimation in saliva before and after consumption of probiotic curd among school children. *Journal of International Society of Preventive and Community Dentistry* 2015; 5(1):31.
 26. Wipapun Ritthagol and Chontira Saetang RT: Effect of probiotics containing *Lactobacillus paracasei* SD1 on salivary Mutans Streptococci and Lactobacilli in orthodontic cleft patients: A double-blinded, randomized, placebo-controlled study. *The Cleft Palate-Craniofacial Journal* 2014; 51(3):257–63.
 27. Lodi CS, Oliveira LV, Brighenti FL, Delbem ACB, and Martinhon CCR: Effects of probiotic fermented milk on biofilms, oral microbiota, and enamel. *Brazilian Oral Research* 2015; 29(1):1–7.
 28. Ferrazzano GF, Cantile T, Sangianantoni G, Amato I, and Ingenito A: The effects of short-term consumption of commercial yogurt on salivary mutans streptococci and lactobacilli counts: an in vivo investigation. *European Journal of Clinical Nutrition* 2011; 65(10):1170–2.
 29. Nozari A, Motamedifar M, Seifi N, Hatamizargaran Z, and Ali M: The Effect of Iranian Customary Used Probiotic Yogurt on the Children ' s Salivary Cariogenic Microflora. *Journal of Dentistry (Shiraz, Iran)*. 2015;16(2):81–6.
 30. Petti S, Tarsitani G, and Simonetti D'Arca A: Antibacterial activity of yoghurt against viridans streptococci in vitro. *Archives of Oral Biology* 2008; 53(10):985–90.
 31. Toiviainen A, Jalasvuori H, Lahti E, Gursoy U, Salminen S, Fontana M, Flannagan S, Eckert G, Kokaras A, Paster B, and Söderling E: Impact of orally administered lozenges with *Lactobacillus rhamnosus* GG and *Bifidobacterium animalis* subsp. *lactis* BB-12 on the number of salivary mutans streptococci, amount of plaque, gingival inflammation and the oral microbiome in healthy adults. *Clinical Oral Investigations* 2015; 19(1):77–83.
 32. Karuppaiah RM, Shankar S, Raj SK, Ramesh K, Prakash R, and Kruthika M: Evaluation of the efficacy of probiotics in plaque reduction and gingival health maintenance among school children - A Randomized Control Trial. *Journal of International Oral Health: JIOH* 2013; 5(5):33–7.
 33. Chapman CMC, Gibson GR, and Rowland I: Health benefits of probiotics: are mixtures more effective than single strains? *European Journal of Nutrition* 2011; 50(1):1–17.
 34. Pinto GS, Cenci MS, Azevedo MS, Epifanio M, and Jones MH: Effect of yogurt containing *Bifidobacterium animalis* subsp . *lactis* DN-173010 probiotic on dental plaque and saliva in orthodontic patients. *Caries research* 2014; 48(1):63–8.
 35. Hasslöf P, Hedberg M, Twetman S, and Stecksén-Blicks C: Growth inhibition of oral mutans streptococci and candida by commercial probiotic lactobacilli--an in vitro study. *BMC Oral Health* 2010; 10(18).
 36. Khanafari A, Porgham SH, and Ebrahimi MT: Investigation of probiotic chocolate effect on *Streptococcus mutans* growth inhibition. *Jundishapur Journal of Microbiology* 2012; 5(4):590–7.
 37. Lee DK, Park SY, An HM, Kim JR, Kim MJ, Lee SW, Cha MK, Kim SA, Chung MJ, Lee KO, and Ha NJ: Antimicrobial activity of *Bifidobacterium* spp. isolated from healthy adult Koreans against cariogenic microflora. *Archives of Oral Biology* 2011; 56(10):1047–54.
 38. Zhu Y, Xiao L, Shen D, and Hao Y: Competition between yogurt probiotics and periodontal pathogens in vitro. *Acta Odontologica Scandinavica* 2011; 68(14):261–8.