Dental Research Journal

Original Article

Effects of probiotic and fluoride mouthrinses on *Streptococcus mutans* in dental plaque around orthodontic brackets: A preliminary explorative randomized placebo-controlled clinical trial

Sepideh Dadgar[1,2], Amin Heydarian[3], Farhad Sobouti[1,2], Hamidreza Goli[4], Vahid Rakhshan[3], Mohadeseh Heidari[5]

[1]Dental Research Center, Mazandaran University of Medical Sciences, Sari, [2]Orthodontic Department, Dental Faculty, Mazandaran University of Medical Sciences, Sari, [3]Private Practice, Tehran, [4]Molecular And Cell Biology Research Center, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, [5]Dental Implant Research Center, Tehran University of Medical Science, Tehran, Iran

ABSTRACT

Background: Although it is shown that probiotic agents might reduce *Streptococcus mutans*, no study has evaluated this effect in the form of probiotic mouthrinse. The purpose of this study was to compare the effect of probiotic experimental mouthwash *Lactobacillus plantarum* versus sodium fluoride and placebo mouthwashes on the number of *S. mutans* present in dental plaque around orthodontic brackets in fixed orthodontic patients.

Materials and Methods: This study was a randomized clinical trial. The total of 38 patients participate consisting of 12 patients in the fluoride group, 13 in the probiotic, and 12 in the placebo group. They were given mouthwashes to use twice a day for 2 weeks. Plaque sampling was performed using the 4-pass technique in all three groups in two stages: before the intervention and after 2 weeks of using the mouthwash. The number of bacteria present in the dental plaque was then reported based on the number of colonies grown on agar medium. Data were analyzed using Kruskal–Wallis and Wilcoxon tests ($\alpha = 0.05$).

Results: Gender distribution, mean age, and protocol adherence were not significantly different among all three groups. After the intervention, the number of *S. mutans* present in the dental plaque followed an increasing manner in the placebo ($P = 0.005$) and probiotic ($P = 0.158$) groups and decreased in fluoride group ($P = 0.025$).

Conclusion: The *L. plantarum* probiotic mouthwash was ineffective in reduction of *S. mutans* in dental plaque. However, fluoride mouthwash is considerably effective against *S. mutans* and thus recommended.

Key Words: *Lactobacillus plantarum*, Mouthrinse, Probiotic, *Streptococcus mutans*

Received: 12-Apr-2020
Revised: 27-Jun-2020
Accepted: 17-Feb-2021
Published: 25-Sep-2021

Address for correspondence:
Dr. Mohadeseh Heidari, Dental Implant Research Center, Tehran University of Medical Science, Tehran, Iran.
E-mail: heidari_mohadeseh@yahoo.com

INTRODUCTION

Dental caries is the most common chronic disease of children worldwide.[1,2] It is a chronic bacterial disease, in which host factors, diet, and oral microbial flora are involved. These factors contribute to caries through dental demineralization.[3] *Streptococcus mutans* is a type of Gram-positive bacterium described by Clark in 1924. This bacterium plays a major role

Access this article online

Website: www.drj.ir
www.drjjournal.net
www.ncbi.nlm.nih.gov/pmc/journals/1480

How to cite this article: Dadgar S, Heydarian A, Sobouti F, Goli H, Rakhshan V, Heidari M. Effects of probiotic and fluoride mouthrinses on *Streptococcus mutans* in dental plaque around orthodontic brackets: A preliminary explorative randomized placebo-controlled clinical trial. Dent Res J 2021;18:74.

in tooth decay.[4] *Streptococcus* bacteria are initially replaced on the surface of the tooth and by preparing the environment for acidic conditions, also allow for the presence of other microorganisms.[5] *S. mutans* metabolizes sucrose to lactic acid and provides the basis for dental caries.[4,5] It has been suggested that therapies that interfere with the colonization of *S. mutans* can have a fundamental effect on reducing the incidence of caries in humans. Certain types of lactobacilli are also associated with caries. These species play a minimal role in the onset of caries but are believed to play a role in caries development.[6] However, not all types of lactobacilli are cariogenic and some are even known to be protective.[7]

S. mutans can persist in an environment including mucosal surfaces exposed to salivary flow, by forming a colony or free living in saliva and proliferating; but other bacteria must attach to the mucosal surface.[8,9] *S. mutans* can be colonized in the mouth before tooth eruption and transiently infect, but for continuous colonization in the mouth, it depends on the presence of teeth. Therefore, its initial fixation occurs during the first 4–5 years of life, and its origin is usually the mother's saliva.[10,11]

Recently, the use of probiotics has been introduced as a method to reduce the amount of salivary *S. mutans* and subsequently dental plaque as the initiator of the cariogenesis. Probiotics are a dietary supplement made up of bacteria or potentially useful fungi.[4] According to the accepted definition of the World Health Organization and the US Food and Drug Administration, probiotics are "Living microorganisms that, if consumed in sufficient quantities, may have beneficial effects on maintaining their host health." These microorganisms, through a variety of mechanisms, create unfavorable conditions for the growth of harmful microorganisms and play a major role, especially in the prevention of gastrointestinal infections. Bifidobacteria and Lactobacillus are the most commonly used probiotics.[12-15] Previous studies have suggested that consumption of probiotic products may reduce dental caries. These studies have shown a decrease in the levels of some bacteria that are effective in causing caries such as *S. mutans*.[16-20] Probiotics or their products can have antimicrobial activity and resist the colonization of pathogens. Immunologically, they have an adjuvant effect and possibly stimulate the phagocytic process of blood leukocytes and increase IgA secretion. In addition, probiotics affect the production and activity of enzymes. They also have antigenic effects.[21] An *in vitro* study has shown that *Lactobacillus plantarum* probiotic, by reducing *S. mutans* bacteria in biofilms isolated from active caries children, can have a limiting effect on *S. mutans* growth.[22] In addition, the combination of probiotics with *L. plantarum*, *rhamnosus,* and *acidophilus* added to chocolate showed an inhibitory effect on *S. mutans* growth *in vitro*.[23]

Since there is no study on mouthrinses with such probiotic bacteria, we conducted this study for the first time in order to assess the efficacy of probiotic mouthwashes containing *L. plantarum* on *S. mutans* in comparison to placebo and fluoride.

MATERIALS AND METHODS

This study was a randomized clinical trial (IRCT20180901040922N1). The subjects were randomly selected from fixed orthodontic patients who referred to the orthodontic department in 2017.

Eligibility criteria

Inclusion criteria were systemic health; no systemic and local antibiotics as well as any antiinflammatory drugs within 4 weeks of starting the study; lack of fluoride therapy history within 4 weeks of starting the study; no use of any probiotic products and xylitol-incorporated chewing gums for 4 weeks before the study and during the study; and the habit of brushing twice a day. Absence of active carious lesions and periodontal disease; permanent dentition; fixed orthodontic applications on at least eight maxillary anterior teeth; at least 3 months passed from the start of fixed orthodontic treatment; no smoking; no sensitivity to probiotic, sodium fluoride, or placebo mouthwash (participants were asked about their sensitivity to probiotics and fluoride so far: no reports of sensitivity were reported during the study); no infectious disease; and being aged between 12 and 30 years.[20,24-27]

Sample size

The results of a previous study were used to determine the sample size.[24] The decrease in *S. mutans* was 100% in the intervention group and 18% in the control group. Therefore, with 95% confidence level and 90% test power using G-Power software (Axel Buchner, Universität Düsseldorf, Düsseldorf, Germany) and formula for comparison between two ratios, sample size was determined as 16 (eight in intervention group and eight in control group). As there were three

comparative groups, the sample size was adjusted to 36 (12 in each of the three groups) according to this formula:. It was also increased to 48 individuals (16 individuals in each of the three groups) with a 30% dropout in mind for the total sample size. After implementation of the design and losing a number of subjects, the final sample size was reduced to 38 (12 in the fluoride group and 13 in either the placebo and probiotic group). Patients' age ranged from 12 to 20 years. Of them, 14 were males and 24 were females.

Bacteria identification

To isolate and culture Lactobacillus, 1 ml of human saliva was dissolved in 9% sterile saline and diluted to concentrations of 1/10. 0.1 ml of appropriate concentration propagated on MRS agar plate (Merck, Germany). It was incubated in aerobic conditions for 48–72 h at 30°C. Catalase − Gr + colonies were identified as *Lactobacilli*. These colonies were stored in 15% MRS broth (v/v) glycerol stocks for subsequent study. In order to evaluate the probiotic effect of *Lactobacilli*, tests such as polymerase chain reaction, acid resistance, bile resistance, antibacterial properties, Bile-salt hydrolytic activity, NaCl resistance, lysozyme resistance, antibiotic resistance, and molecular detection of *Lactobacillus* species were performed. After diagnostic tests, it was determined that *L. plantarum* is our probiotic bacterium.[28]

Probiotic mouthwash

The mouthwash was initially made as powder and liquid in separate containers. Each powder container contained approximately 10^8 colony-forming unit (CFU) bacteria lyophilized and mixed with malt and dextrin, weighing 30 mg. The liquid container was handed to subjects as a 20 ml Falcon tube containing 5 ml phosphate-buffer saline (PBS). Each patient was told to mix the powder with the liquid for 30 s before use.

Placebo

Patients were given 30 mg of dextrose powder with 5 ml of distilled water to be mixed for placebo mouthwash.

Intervention

First, patients were examined and interviewed. Individuals were included in the study after completing the informed consent form and being properly briefed by the researchers. All patients were trained according to the standard protocol of hygiene compliance during orthodontic treatment.

These subjects, who were at least 3 months into their fixed orthodontic treatment, were randomly divided into three groups according to a random number table: group 1 (control) included 13 subjects receiving placebo mouthwash; Group 2 included 12 subjects receiving sodium fluoride mouthwash; and group 3 comprised 13 patients receiving probiotic mouthwash.

From all three randomized groups, plaque sampling was performed in two stages: The 1st day of study and before the intervention (T0) and 2 weeks after the mouthwash start (T1). During the mouthwash period, the patients brushed as usual and used the mouthwash twice a day after lunch and before bedtime. Each probiotic mouthwash served as a container containing powder (lyophilized and dextrose bacteria) and a container containing PBS to be mixed and homogenized before use. The placebo group used powder and liquid mouthwashes similar to the probiotic group, whereas in the liquid-containing container, distilled water, and in the powder container, only dextrose was free of lyophilized bacteria. The fluoride group was given only a 0.05% fluoride-containing container similar to that of the other groups, but the powdered container did not exist in this group. There was a leaflet in the patient package containing information on the patient's requirements including inclusion criteria and how to contact the project manager as well as checkboxes to be marked after each mouthrinsing. Each week, subjects were encouraged by a phone call to continue using mouthwash. All participants were able to contact the project manager during the project and inform him of any potential adverse effects. They also asked questions from the person in charge.

Plaque sampling

Plaque sampling was performed using the 4-pass technique recommended by Pellegrini *et al*.[29] In this method, a senior dental student collected plaque using a sterile plaque scaler on the labial surface of the maxillary lateral incisor, adjacent to the bracket sides, in four directions of mesial, distal, occlusal, and gingival.

The dental plaque was dissolved in 5 cc of PBS and stored in a refrigerator (4°C) and sent to the Microbiology Laboratory of the Medical School on the following day. The samples were diluted serially and cultured in Mitis salivarius sucrose bacitracin

agar medium under anaerobic conditions at 37°C for 24–48 h.[30] Then, the number of bacteria in the dental plaque was counted based on the number of colonies grown on agar medium, using the "CFU × Dilution factor × 1/aliquot" formula and with the CFU/4-pass technique.

Statistical analysis

Descriptive statistics and 95% confidence intervals (CIs) were calculated for different variables. Data normality was assessed using Kolmogorov–Smirnov test which indicated nonnormal data. The Wilcoxon test was used to assess before-after changes of *S. mutans* in each group. Groups were compared using Kruskal–Wallis test of SPSS 22 (IBM, Armonk, NY, USA).

RESULTS

There were 4, 4, and 6 men in the groups probiotic, placebo, and fluoride (Fisher $P = 0.538$). The Kruskal–Wallis test showed that the three groups were similar in terms of age, brushing frequency, and the duration of brackets placed [$P > 0.05$, Table 1]. Furthermore, compliance with the protocol was similar between the three groups [Table 2].

In the probiotic group, nine individuals showed an increase in colony counts, while 3 and 1 showed reduction and no changes, respectively. In the placebo group, 11 patients showed increases in colony counts, and two people showed a reduction. In the fluoride group, only two people showed an increase in colony counts, and the rest (10 patients) showed a reduction. According to the Wilcoxon test, in the placebo group, the colony count increased after 2 weeks ($P = 0.005$). In the fluoride group, the colony count reduced significantly ($P = 0.025$). In the probiotic group, however, there was no significant change over time [$P = 0.158$, Table 3 and Figure 1]. There was not a significant difference among three groups in terms of either pretreatment colony counts or after-treatment colony counts [Table 3].

DISCUSSION

The findings of this study suggest that, unlike fluoride mouthrinse that can have antimicrobial effects, this particular type of probiotic bacteria could not help reduce *S. mutans*, when used in the form of mouthrinse. There have been earlier studies on the efficacy of various forms of probiotic products in reducing *S. mutans* and caries, and their controversial results indicate a great ambiguity in this area. In the present study, it was found that, in practice, the probiotic mouthwash could not have a significant effect on the amount of *S. mutans* bacteria and had almost the same effect as did the placebo group. This was in line with some studies (Montalto and Chuang) and in contrast with most others showing a decrease in the amount of *S. mutans* after consumption of probiotic-containing compounds.[18,20,30-33] These studies differ in the type of probiotic used: in the Nase and Caglar study, *lactobacillus rhamnosus* was evaluated.[18,31] The Nikawa *et al*. study showed the inhibitory effect of *Lactobacillus reuteri* on the growth of *S. mutans*.[34] In a study by Chuang *et al*., results showed that consumption of pills-containing *Lactobacillus paracasei* had no effect on reducing salivary *S. mutans*.[30] Caglar *et al*. studied the effect

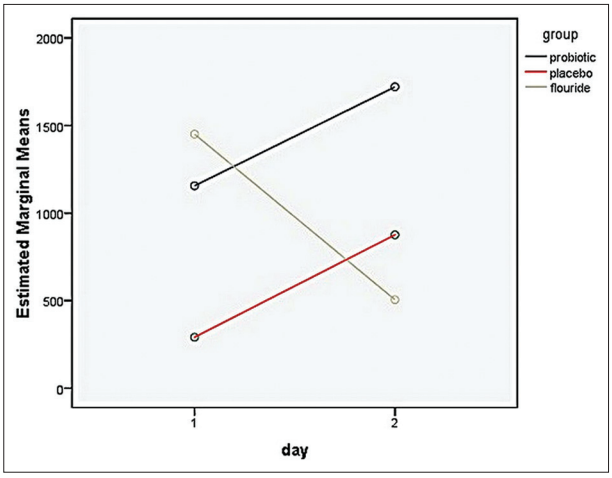

Figure 1: Estimated marginal means for colony counts.

Table 1: Descriptive statistics pertaining to group properties, and the results of the Kruskal-Wallis test

Group	Age			Brushing/day			Brackets in mouth (months)		
	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median
Probiotic	16.69	5.95	14	2.54	0.519	3	9.77	4.64	10
Placebo	18.62	8.02	16	2.31	0.48	2	10.00	4.16	12
Fluoride	19.17	6.15	18.5	2.25	0.45	2	11.25	6.86	11
P		0.510			0.290			0.780	

SD: Standard deviation

Table 2: Compliance with the protocol as the number of mouthrinse usage

Group	*n*	Mean	SD	95% CI	
				Lower	Upper
Probiotic	13	24.0769	4.05096	21.6290	26.5249
Placebo	13	25.3077	2.71982	23.6641	26.9513
Fluoride	12	24.1667	3.32575	22.0536	26.2797
Total	38	24.5263	3.36706	23.4196	25.6330

CI: Confidence interval; SD: Standard deviation

Table 3: Colony counts before and after the study period, and the result of Kruskal-Wallis test comparing the groups

Group	Baseline			After 2 weeks		
	Mean	SD	Median	Mean	SD	Median
Probiotic	1,155,770	1520	250,000	1,720,770	2126	1,000,000
Placebo	290,770	261,493	200,000	875,770	2001	300,000
Fluoride	1,450,830	2031	275,000	505,000	680,678	1,000,000
P		0.089			0.114	

SD: Standard deviation

of probiotic-containing ice cream in a group that had high levels of *S. mutans* at the beginning of the study.[18] However, in the present study, the variation in the baseline colony count was high (minimum: 10,000 maximum: 7,500,000). This scattering probably originated from a 4-pass technique. In the Näse *et al*. study, the effect of probiotic-containing milk in preschool children with a 7-month intervention was different from the age group in the present study, and the samples received a longer probiotic duration.[31] In a study of thirty children between 8 and 15 years old, Megha *et al*. studied the effect of probiotic yogurt on orthodontic children and found that the use of these probiotics can reduce oral microorganisms and subsequently reduce caries, which differed with our findings. The conditions and methodologies of the two studies differed and they had used a much higher but inaccurate concentration ($>10^9$).[35] Studies such as Iwasakir *et al*., Harini and Anegundi, and Kawai *et al*. have shown the effect of *L. plantarum* in reducing gingival disease.[36-38] There also appears to be a relationship between the reduction of periodontal microorganisms and the increase in *S. mutans*.[39,40] Consequently, it can be hypothesized that *L. plantarum* may provide the basis for increased *S. mutans* by removing periodontal pathogen bacteria.

There have been numerous *in vitro* studies on the efficacy of *L. plantarum* in inhibiting *S. mutans*. Ahn *et al*. reported that *L. plantarum* inhibits the formation of *S. mutans* biofilms.[41] An *in vitro* study also showed that *L. plantarum*, by reducing *S. mutans* bacteria in biofilms isolated from children with active caries, could have a restrictive effect on *S. mutans* growth.[22] Furthermore, the results of Khanafari *et al*. show that *L. plantarum* probiotic is effective in inhibiting growth of *S. mutans* and it may be useful to reduce the prevalence of caries.[23] The results of an *in vitro* study showed that the cell-free solution containing components of *L. plantarum* and *L. acidophilus* had significant inhibitory activity on the biofilm formation of cariogenic organisms. In addition, the interference (80% decrease) in glucose synthesis by *S. mutans* by these *lactobacilli* indicates their potential role in inhibiting glucosyltransferase (Gtf-I). As a result, *L. plantarum* can reduce the biofilm of this carcinogenic bacterium by suppressing *S. mutans* virulence genes.[42] Therefore, the result of the present study might root in methodological strategies. In the placebo group of the current study, there was an increase in the number of *S. mutans* after the intervention. One of the factors that can be effective in increasing the number of bacteria is the presence of dextrose in the placebo mouthwash formulation. This sugar can be used as a substrate for the metabolism of *S. mutans* present in plaque and increase the number of bacteria. On the other hand, probiotic mouthwash formulation also had dextrose and increased bacterial counts (not statistically significant), but this increase was lower than the placebo group, which may reflect the inhibitory effect of *L. plantarum* on *S. mutans*. The presence of dextrose is important for the growth and activation of lyophilized bacteria, and a higher percentage of lyophilized bacteria survive after activation in dextrose-containing medium.[43] Since not using dextrose may reduce the viability of probiotic bacteria, perhaps doses above 10^8 CFU, without dextrose, may work better in reducing *S. mutans*. For maximum efficacy, *L. plantarum* must be colonized in the oral environment. This colonization requires more time for bacterial activation as well as greater presence of bacteria in the mouth. Dissolving the lyophilized bacterial powder for 30 s is not enough to activate it. However, taking extra time of the patient at home naturally reduces the patient's cooperation and overshadows the clinical application of this mouthwash. The duration of the mouthwash usage is also important to provide the probiotic bacteria with the opportunity to colonize. The duration of mouthwash use cannot be exceeded by a certain limit, but other methods may help keep the

probiotic bacteria in the mouth for longer periods. The use of probiotic varnishes may help to resolve this problem.

Much research has been done on the use of fluoride-containing mouthwashes. In addition to its protective effect on enamel, fluoride also affects the process of decay by altering the invasion of bacteria and does so in two ways: 1 – change the ability of organisms to produce acid and 2 – by facilitating the growth of some of bacteria. Fluoride inhibits glucose transport due to its effect on enolase since phosphoenolpyruvate is essential for the phosphoenolpyruvate transferase system that is capable of forming glucose-1-phosphate. Increasing the concentration of hydrogen ions inside the cell by inhibiting glucose-hydrogen ion transfer prevents glucose movement. Fluoride also inhibits the membrane's ATPase action and removes hydrogen ions from bacterial cells by preventing glycolysis and reducing the hydrogen ion gradient on the other side of the cell wall. Therefore, the overall effect of fluoride will be to inhibit acid production and to disrupt cellular energy metabolism. It is important to note that the sensitivity of the bacteria to the effects of fluoride varies.[44] Most studies recommend a 0.2% sodium fluoride mouthwash once a week or a 0.05% mouthwash once a day. According to these studies, there is no doubt about the benefits of fluoride-containing mouthwashes in preventing tooth decay when used properly. There are other substances that have been tested for oral hygiene maintenance during orthodontic treatment. For example, Øgaard *et al*.[45] compared the effect of chlorhexidine-fluoride varnish with fluoride alone and with chlorhexidine in these patients. Chlorhexidine reduced the amount of *S. mutans* but had no effect on the white spots, compared with fluoride, but chlorhexidine and fluoride had the best results.[45] As a result, the simultaneous use of these two substances can have a favorable effect against dental caries.

CONCLUSION

The results of this study indicated that, compared to placebo, the experimental probiotic mouthrinse in use did not show any advantage in terms of controlling *S. mutans*. Therefore, our preliminary findings do not support the use of this particular dosage and type of probiotic as mouthrinse, for controlling *S. mutans* in the plaque around orthodontic brackets. However, fluoride mouthrinse proved effective against *S. mutans* in the plaque around the orthodontic bracket and is therefore recommended.

Financial support and sponsorship

Nil.

Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

REFERENCES

1. Ozdemir D. Dental caries: The most common disease worldwide and preventive strategies. Int J Biol 2013;5:55.
2. Selwitz RH, Ismail AI, Pitts NB. Dental caries. Lancet 2007;369:51-9.
3. Featherstone J. The continuum of dental caries – Evidence for a dynamic disease process. J Dent Res 2004;83:39-42.
4. Köhler B, Pettersson BM, Bratthall D. *Streptococcus mutans* in plaque and saliva and the development of caries. Scand J Dent Res 1981;89:19-25.
5. Oda Y, Hayashi F, Okada M. Longitudinal study of dental caries incidence associated with *Streptococcus mutans* and *Streptococcus sobrinus* in patients with intellectual disabilities. BMC Oral Health 2015;15:102.
6. Jenner F, Jaleel VA, Kulshrestha R, Maheswar G, Rao PK, Kranthi J. Evaluating the antimicrobial activity of commercially available herbal toothpastes on microorganisms associated with diabetes mellitus. J Contemp Dent Pract 2013;14:924-9.
7. Islam B, Khan SN, Khan AU. Dental caries: From infection to prevention. Med Sci Monit 2007;13:RA196-203.
8. Arshad MS, Hussain I, Mahmood MS, Khan MN. Evaluation of antimicrobial potential of *Acacia nilotica* (kikar) against oral pathogens associated with caries and periodontitis. Pak J Agric Sci 2017;54:423-30.
9. Dye BA, Afful J, Thornton-Evans G, Iafolla T. Overview and quality assurance for the oral health component of the National Health and Nutrition Examination Survey (NHANES), 2011-2014. BMC Oral Health 2019;19:95.
10. Pulliam L, Porschen RK, Hadley WK. Biochemical properties of CO2-dependent streptococci. J Clin Microbiol 1980;12:27-31.
11. Clara J, Bourgeois D, Muller-Bolla M. DMF from WHO basic methods to ICDAS II advanced methods: A systematic review of literature. Odontostomatol Trop 2012;35:5-11.
12. Björnstad L, Crossner CG. Stimulated salivary flow rate and buffer effect in schoolchildren from Greenland and Sweden: A comparative study. Acta Odontol Scand 2007;65:162-6.
13. Cummins RA. Cultural response bias. In: The Encyclopedia of Cross-Cultural Psychology. Wiley-Blackwell: Hoboken, NJ, USA; 2013. p. 330-3.
14. McClafferty H. Integrative Pediatrics: Art, Science, and Clinical Application. Location: London, United Kingdom: Routledge; 2017.
15. Moussa S, Gameil Gobran H, Salem M, Barkat I. Dental biofilm and saliva biochemical composition changes in young orthodontic patients. J Dent Oral Disord Ther 2017;5:1-5.

16. Simark-Mattsson C, Emilson CG, Håkansson EG, Jacobsson C, Roos K, Holm S. Lactobacillus-mediated interference of mutans streptococci in caries-free vs. caries-active subjects. Eur J Oral Sci 2007;115:308-14.
17. Söderling EM, Marttinen AM, Haukioja AL. Probiotic lactobacilli interfere with *Streptococcus mutans* biofilm formation *in vitro*. Curr Microbiol 2011;62:618-22.
18. Caglar 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;66:154-8.
19. de Vrese M, Schrezenmeir J. Probiotics, prebiotics, and synbiotics. Adv Biochem Eng Biotechnol 2008;111:1-66.
20. Cildir SK, Germec D, Sandalli N, Ozdemir FI, Arun T, Twetman S, *et al*. Reduction of salivary mutans streptococci in orthodontic patients during daily consumption of yoghurt containing probiotic bacteria. Eur J Orthod 2009;31:407-11.
21. Vilahur G, López-Bernal S, Camino S, Mendieta G, Padró T, Badimon L. Lactobacillus plantarum CECT 7315/7316 intake modulates the acute and chronic innate inflammatory response. Eur J Nutr 2015;54:1161-71.
22. Lin X, Chen X, Tu Y, Wang S, Chen H. Effect of probiotic lactobacilli on the growth of *Streptococcus mutans* and multispecies biofilms isolated from children with active caries. Med Sci Monit 2017;23:4175-81.
23. Khanafari A, Porgham SH, Ebrahimi MT. Investigation of probiotic chocolate effect on *Streptococcus mutans* growth inhibition. Jundishapur J Microbiol 2012;5:590.
24. Jose JE, Padmanabhan S, Chitharanjan AB. Systemic consumption of probiotic curd and use of probiotic toothpaste to reduce *Streptococcus mutans* in plaque around orthodontic brackets. Am J Orthod Dentofacial Orthop 2013;144:67-72.
25. Lesan S, Rahbar M, Valaei N, Abbasi Vardoogh S, Abbasi Vardoogh M. Effect of probiotic yoghurt on salivary *Streptococcus mutans* and *Lactobacillus*. Pajouhesh Dar Pezeshki [Research in Medicine] 2013;37:151-7.
26. Gizani S, Petsi G, Twetman S, Caroni C, Makou M, Papagianoulis L. Effect of the probiotic bacterium *Lactobacillus reuteri* on white spot lesion development in orthodontic patients. Eur J Orthod 2016;38:85-9.
27. Keller M, Hasslöf P, Dahlén G, Stecksén-Blicks C, Twetman S. Probiotic supplements (*Lactobacillus reuteri* DSM 17938 and ATCC PTA 5289) do not affect regrowth of mutans streptococci after full-mouth disinfection with chlorhexidine: A randomized controlled multicenter trial. Caries Res 2012;46:140-6.
28. Zhang ZY, Liu C, Zhu YZ, Wei YX, Tian F, Zhao GP, *et al*. Safety assessment of *Lactobacillus plantarum* JDM1 based on the complete genome. Int J Food Microbiol 2012;153:166-70.
29. Pellegrini P, Sauerwein R, Finlayson T, McLeod J, Covell DA Jr., Maier T, *et al*. Plaque retention by self-ligating vs elastomeric orthodontic brackets: Quantitative comparison of oral bacteria and detection with adenosine triphosphate-driven bioluminescence. Am J Orthod Dentofacial Orthop 2009;135:426-7.
30. Chuang LC, Huang CS, Ou-Yang LW, Lin SY. Probiotic *Lactobacillus paracasei* effect on cariogenic bacterial flora. Clin Oral Investig 2011;15:471-6.
31. 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;35:412-20.
32. Sudhir R, Praveen P, Anantharaj A, Venkataraghavan K. Assessment of the effect of probiotic curd consumption on salivary pH and *Streptococcus mutans* counts. Niger Med J 2012;53:135-9.
33. 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;69:53-6.
34. 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;95:219-23.
35. Megha S, Shalini G, Varsha SA, Abhishek D, Neetu J. Effect of short-term placebo-controlled consumption of probiotic yoghurt and Indian curd on the *Streptococcus mutans* level in children undergoing fixed interceptive orthodontic therapy. Turk J Orthod 2019;32:16-21.
36. Kawai T, Ohshima T, Shin R, Ikawa S, Maeda N. Determination of the antibacterial constituents produced by lactobacilli against a periodontal pathogen: Sodium lactate and a low molecular weight substance. J Probl Health 2016;4:2.
37. Iwasaki K, Maeda K, Hidaka K, Nemoto K, Hirose Y, Deguchi S. Daily intake of heat-killed *Lactobacillus plantarum* L-137 decreases the probing depth in patients undergoing supportive periodontal therapy. Oral Health Prev Dent 2016;14:207-14.
38. Harini PM, Anegundi RT. Efficacy of a probiotic and chlorhexidine mouth rinses: A short-term clinical study. J Indian Soc Pedod Prev Dent 2010;28:179-82.
39. De Soete M, Dekeyser C, Pauwels M, Teughels W, van Steenberghe D, Quirynen M. Increase in cariogenic bacteria after initial periodontal therapy. J Dent Res 2005;84:48-53.
40. Quirynen M, Gizani S, Mongardini C, Declerck D, Vinckier F, Van Steenberghe D. The effect of periodontal therapy on the number of cariogenic bacteria in different intra-oral niches. J Clin Periodontol 1999;26:322-7.
41. Ahn KB, Baik JE, Park OJ, Yun CH, Han SH. *Lactobacillus plantarum* lipoteichoic acid inhibits biofilm formation of *Streptococcus mutans*. PLoS One 2018;13:e0192694.
42. Raj A, Bhati P, Bhadekar R. Effect of lactic acid bacteria on biofilm formation by Streptococcus mutans: An *in vitro* study. Int J Pharm 2017;8:2533-8.
43. Staab JA, Ely JK. Viability of lyophilized anaerobes in two media. Cryobiology 1987;24:174-8.
44. ten Cate JM. Current concepts on the theories of the mechanism of action of fluoride. Acta Odontol Scand 1999;57:325-9.
45. Øgaard B, Larsson E, Henriksson T, Birkhed D, Bishara SE. Effects of combined application of antimicrobial and fluoride varnishes in orthodontic patients. Am J Orthod Dentofacial Orthop 2001;120:28-35.