



Research Article

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Evaluation of the Inhibitory Effect of Functional Fermented Milk Containing Different Probiotic Cultures on Salivary Mutans Streptococci Count in Experimental rats: A Randomized Control Animal Study

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ABSTRACT

Background: Probiotics are regarded as a part of the normal human microbiota. Previous studies have suggested that lactobacilli-derived probiotics in dairy products may reduce dental caries. Thus, the search for an effective caries preventing probiotic microorganisms appears to be a promising research avenue.

Objectives: The present study was carried out to investigate the inhibitory effect of feeding functional fermented milks containing five different probiotic strains on salivary mutans streptococci in the oral flora of rats.

*Materials and Methodology: Mutans streptococci were used in this study for infection the albino rats were isolated from Saudi child saliva, identified using Biolog system. The infected rats (n=42) were randomly divided according to the type of diet given into 7 groups (n=6). Group T1 received laboratory standard rat ration blended with milk without any probiotic strain. Groups T2, T3, T4, T5 and T6 received laboratory standard rat ration blended with functional fermented milk containing different probiotic strains *Lb acidophilus* NCTC12980R, *Lb plantarum* ATCC 14917, *Lb. reuteri* NCIMB1195, *Lb rhamnosus* ATCC7469 and *Bifi. bifidum* NCTC1300R respectively. While, rats received laboratory standard rat ration blended without milk were control group (Group C). Rats were housed up to five weeks where saliva samples were collected from rats' oral cavities at the end of each week to evaluate the inhibitory effect of different probiotics by counting the Mutans streptococci count.*

*Result: Mutans streptococci count decreased significantly in group T3, T4, T5, and T6 with varying degrees where group T4 showed the statistically significant lowest *S. mutans* mean count followed by T5 (P-value<0.001). Group C and T1 showed the highest significant mean Mutans streptococci count.*

*Conclusion and Recommendations: Functional fermented milks containing different Probiotic strains showed an inhibitory effect against Mutans streptococci count with varying degrees. Fermented milk containing *Lb. reuteri* NCIMB1195 could be one of the best dietary regimens for dental caries prevention.*

Keywords: Probiotic, mutans streptococci, lactobacilli, bifidobacteria, rats

INTRODUCTION

Dental caries is the one of most common universal oral disease which affects the majority of individuals in all age groups during their lifetime. It is regarded as an infectious disease in which changes in the oral environment lead to a pathological shift in the oral biofilm, which then results in localized destruction of the hard tooth tissues [1]. The principle causative agent is group of streptococcal species of which Mutans streptococci are the most important agent of dental caries. Though the surgical approach has been predominating for the past decades, nowadays, management of dental caries moved toward an antibiotic/antimicrobial model of diseases prevention. There has been a global development in using of commercially available dairy products as complementary foods and/or medicines, that may exert their prevention and therapeutic effect of dental caries [2].

The concept of probiotics evolved at the turn of 20-century. Probiotics have crop up as one of the positive effect on general health and managing several medical conditions. The word “probiotic” is Latin preposition “pro” for and Greek adjective “biotic” bios, life. It was first used by Lilly and Stillwell in 1955 [3]. Today, two principal descriptions are used according to (WHO/FAO., 2002) "Probiotics are defined as living microorganisms, which, when administered in adequate amounts, confer a health benefit on host". International Life Science Institute (ILSI) Europe suggests another description where probiotic is described as “alive microbial food ingredient that, when ingested in sufficient quantities, exerts health benefits on the consumer” [4]. The Lactobacillus strains are regarded as a component of the ecological community of typical human flora. The role of probiotics and their mechanism of action have been extensively studied regarding their effect on abdominal health and function [5-9]. Many studies have concluded beneficial effects of probiotics in improvement of oral health without negatively affecting the normal oral microbiota: reduction and inhibition in the number of mutans streptococci (MS), dental plaque and treatment of gingival inflammation [10-12]. Growing numbers of products containing probiotic bacteria is commercially available nowadays. Probiotic are provided in fermented food such as yoghurt drink, cheese, kefir, and can be inoculated into milk or dietary supplements. Since probiotics have been added to various foods because of their beneficial effects for human health, it is important to understand their effects in the oral environment. Furthermore, they need to be assessed individually for its favorable effect on oral health as positive or negative effect [13]. The most commonly used probiotic bacteria belongs to the genera lactobacilli and bifidobacteria, the genera that are also associated with dental caries [14]. Research in this area is ongoing but some controversies regarding the potent probiotic strains against SM, the proper delivery vehicle and the survival rate of these probiotic strains in the oral cavity are present [12, 15-21], thus the objective of this study is to investigate the inhibitory effect of functional fermented milks containing five different probiotic strains on the counts of salivary mutans streptococci aiming to provide enough information for future recommendation to be used in market to reduce dental caries in children.

MATERIAL AND METHODS

Microorganisms and culture condition:

Mutans streptococci used in this study for infection of albino rats were isolated from Saudi child saliva attending the outpatient’s clinic, Dental department, Al-Qatif Central Hospital. Informed written consent was obtained from the patient prior to sampling. Criteria of inclusion were as follows: Healthy children aged 5-12 years old, free of any systemic diseases with no history of antibiotic treatment for the past two months, having active caries lesions. All saliva samples were transported to the laboratory of Department of Food Science and Technology, Faculty of Agriculture, Ain Shams University.

Mutans streptococci were identified using Biolog system[22]. The isolated mutans streptococci was grown in Trypticase Soy broth supplement with 0.5% yeast extract (TSBY) incubated at 37°C in anaerobic incubator with 5% CO₂. Cells were harvested during the exponential growth phase by centrifugation at 1000 RPM, washed twice with Phosphate buffer saline (PBS), re-suspended in the same buffer and subjected to hand shaking to disperse bacterial aggregates according to Nikawa et al. (2004) [10].

Five Probiotics strains provided by Quality Medical Sciences Co. Ltd, were used in the study include Lb. reuteri NCIMB1195, Lb rhamnosus ATCC7469, Lb acidophilus NCTC12980R, Lb plantarum ATCC 14917, and Bifi. bifidum NCTC1300R. Each strain was propagated in MRS broth media supplemented with 0.05% cystein

hydrochloride at 37°C for 24h. Stock cultures of probiotic strains were made by mixing a pure culture that had been grown over night with equal amount of solution and stored at -20°C until experimentally used.

Functional fermented milk preparation:

The samples of fermented milk were prepared in Food Science Department, Faculty of Agriculture, Ain Shams University. The bio-fermented milk was manufactured according to Farahat & El-Batawy (2013) [23] as follows: reconstituting 14% skim milk powder in water. The mix was heated to 85°C for 10 minutes, and then rapidly cooled to 37°C. The mother culture of probiotic strains (*Lb. reuteri* NCIMB1195, *Lb rhamnosus* ATCC7469, *Lb acidophilus* NCTC12980R, *Lb plantarum* ATCC 14917, and *Bifi. bifidium* NCTC1300R) were added at a rate of 3% (w/v). The inoculated mix was filled into 100 ml glass containers and incubated at 37°C. Incubation was terminated till pH 4.5[23]. At this point, the fermented milk was stored in a refrigerator (4±1°C) for 1 day, to be ready for feeding the rats.

Animal Study:

Total of forty-nine albino rats weaned at age of 19 days, were subjected to the same atmospheric condition at room temperature, time of feeding, handling, noise level and food and water were provided equally. All procedures were done at the Animals' house, (The food Technology Research Institute in Agriculture Research Centre, Cairo, Egypt.). The animals were placed under supervision of well-trained technician. The handling of the animals was carried out to a high standard regarding their cleanliness, cage washing, and ventilation and feeding. Oral swabs were taken from the rats' tongue, palatal, lingual and labial surfaces of teeth and streaked on mitis salivarius agar plus bacitracin for screening the indigenous mutans streptococci [24].

The rats at the age of 30- days were infected on three consecutive days with isolated mutans streptococci, by using a dropper containing suspension of the bacterial strain[24]. In addition, their drinking water contained 5% sucrose plus mutans streptococci to confirm the bacterial infection. At the age of 33 days, rats were randomly divided according to the type of diet, into seven groups (n=6). Group T1 received laboratory standard rat ration blended with milk without any probiotic strain. Groups T2, T3, T4, T5 and T6 received laboratory standard rat ration blended with functional fermented milk containing probiotic strains *Lb acidophilus* NCTC12980R, *Lb plantarum* ATCC 14917, *Lb. reuteri* NCIMB1195, *Lb rhamnosus* ATCC7469 and *Bifi. bifidium* NCTC1300R respectively. While, rats received laboratory standard rat ration blended without milk were control group (Group C). Randomization was done using SPSS software version 20.0 (Armonk, NY; IBM Corp.) for each cage (1-7) separately with a uniform random variable generation. Allocation concealment was assured by handling sequentially numbered opaque sealed envelopes to attending candidate. At time of intervention, the candidate opened each envelope and allocated the cage to written intervention inside that envelope. All rats were fed two meals per day with 5 ml of different fermented milks in addition to 5% sucrose solution for 5 weeks' duration according to the diet regimen specified for each group.

Saliva samples and microbiological analysis:

Saliva samples were collected weekly from the tongue, palatal, lingual and labial teeth's surfaces of each mouse using the using sterilize cotton swab. The tip inserted in a test tube with 0.95 ml of sterile physiological solution. Each flask containing the saliva sample was vortex for 1 minute to obtain a uniform suspension. Samples were diluted in a decimal series (10⁻⁵ to 10⁻⁶) in 0.05 M phosphate buffer. One ml of each dilution was inoculated in Mitis Salivarius agar (selective media for Mutans Streptococci). The plates were incubated at 37°C for 48 h. The counts of Mutans Streptococcus were performed by using the counting technique in each quadrant separately then summation of the number of colonies in the 4 quadrants. The results were expressed as log₁₀ (CFU/ml). The number of colonies per milliliter (CFU/ml) was determined by the following equation:

CFU/ml = $\frac{\text{Number of colonies} \times \text{dilution factor of sample}}{1\text{ml of sample}}$

1ml of sample

The microbiologist and the statistician were blinded to the treatment each group sample had received. Prior to carrying the microbial counting procedure, 3 microbiologists blindly and independently read 30 specimens. All the readings were compared statistically using Kappa test to evaluate the degree of agreement between the microbiologists.

Statistical Analysis:

Data were presented as mean and standard deviation (SD) values. A logarithmic transformation (log₁₀ transformation) of each CFU count was performed to normalize the data before statistical evaluation because of the high range of bacterial counts. One-way Analysis of Variance (ANOVA) was used for comparisons between the seven groups. Tukey's post-hoc test was used for pair-wise comparison between the groups. Paired t-test was used to compare between bacterial counts before and after application. The significance level was set at $P \leq 0.01$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

RESULTS

The study included 42 rats that were randomly divided according to the type of diet given into 7 groups (n=6). Rats housed up to five weeks where saliva samples were collected from rats' oral cavities at the end of each week to evaluate the inhibitory effect of the different probiotic strains and their survival rate. The mean and SD values of salivary mutans streptococci (log₁₀CFU/ ml) in study rats at baseline were 6.15±.018 (C), 6.17±.018 (T1), 6.15±.018(T2), 6.18±.017(T3), 6.16±.020(T4), 6.16 ±.012(T5) and 6.14±.020 (T6), with no statistical significant difference between the seven groups ($p = 0.054$) [Table 5.1, Fig 5.1]. By the end of the first week: no statistical significant difference was found between the seven groups (p -value =0.739) [Table 5.1, Fig 5.1]. By the end of second week; there was a statistical significant difference between group C and others groups ($p \leq 0.001$). However, no significant difference between group T1&T2 ($p=1.00$) and between T3, T4, T5 &T6 ($p \leq 1.00$) [Table 5.1, Fig 5.1]. There was a statistical significant difference between the seven groups by the end of the third week. ($p \leq 0.001$).

Group (T4) showed the statistically lowest mutans streptococci mean count 5.84 ± .026 (log₁₀ CFU/ml) followed by group (T5) 5.89 ±.028, (T6) group 5.92±.070 then (T3) 5.96±.036. By the end of the fourth week; the salivary mutans streptococcus mean count decrease in group T3, T4, T5&T6 while in group C, T1&T2 salivary mutans mean count increased. Group T4 & T5 showed the statistically lowest mutans mean count (5.71±.020, 5.75 ±.033) respectively, followed by group T6 (5.86 ± .030) then T3 (5.89 ± .020). Group C&T1 showed the statistically highest mutans mean count (6.84±.037, 6.82±.028). There was a statistical significant difference between the seven groups ($p \leq 0.001$) [Table 5.1, Fig 5.1].

Salivary Mutans Streptococcus mean count by the end of fifth week decreased significantly in group T3, T4, T5 & T6 and increased significantly on group C, T1 & T2. Group T4 showed the statistically lowest mutans streptococci mean count 5.52 ± .020 (log₁₀ CFU/ml) followed by T5 (5.63 ±.016) (log₁₀ CFU/ml) and T6 (5.72 ± .020) then T3 (5.81 ± .012) ($p \leq 0.001$). Group C showed the statistically highest mutans streptococci mean count 7.12 ±0.021 ($p \leq 0.001$) [Table 5.1, Fig 5.1]

Table (1): Mean and SD of salivary mutans streptococci count in study groups received different probiotic strains during the experimental period

Treatment Group		Log ₁₀ CFU/ml count (week)						P-value [†]
		Baseline	1	2	3	4	5	
C	Mean	6.15	6.19	6.35	6.61 ^a	6.84 ^a	7.12	<0.001
	SD	±.018	±.044	±.045	±.028	±.037	±.021	
T1	Mean	6.17	6.18	6.24 ^a	6.54 ^a	6.82 ^a	6.98	< 0.001
	SD	±.018	±.014	±.030	±.031	±.028	±.046	
T2	Mean	6.15	6.17	6.25 ^a	6.56 ^a	6.71	6.84	< 0.001
	SD	±.018	±.021	±.026	±.031	±.028	±.028	
T3	Mean	6.18	6.19	6.05 ^b	5.96 ^b	5.89 ^b	5.81	< 0.001
	SD	±.017	±.028	±.032	±.036	±.020	±.012	
T4	Mean	6.16	6.18	6.00 ^b	5.84 ^b	5.71 ^c	5.52	< 0.001
	SD	±.020	±.025	±.020	±.026	±.020	±.020	
T5	Mean	6.14	6.17	6.04 ^b	5.89 ^b	5.75 ^c	5.63	< 0.001
	SD	±.012	±.023	±.030	±.028	±.033	±.016	
T6	Mean	6.17	6.19	6.10 ^b	5.92 ^b	5.86 ^b	5.72	< 0.001
	SD	±.020	±.033	±.031	±.070	±.030	±.020	
P-value ^{††}		0.054	0.739	0.000	0.000	0.000	0.000	

[†]Significant level is set at ≤ 0.01 between the groups using *F test*

^{††}Significant level is set at ≤ 0.01 within the group using *F test (pairwise comparisons)*

Group means sharing different letter between the groups are significantly different (*post hoc test*)

C: Rats received laboratory standard rat ration blended without milk, **T1:** Rats received laboratory standard rat ration blended with milk without any probiotic strain, **T2, T3, T4, T5** and **T6:** Rats received laboratory standard rat ration blended with functional fermented milk containing probiotic strains *Lb acidophilus*, *Lb plantarum*, *Lb. reuteri*, *Lb rhamnosus* and *Bifi. bifidium* respectively.

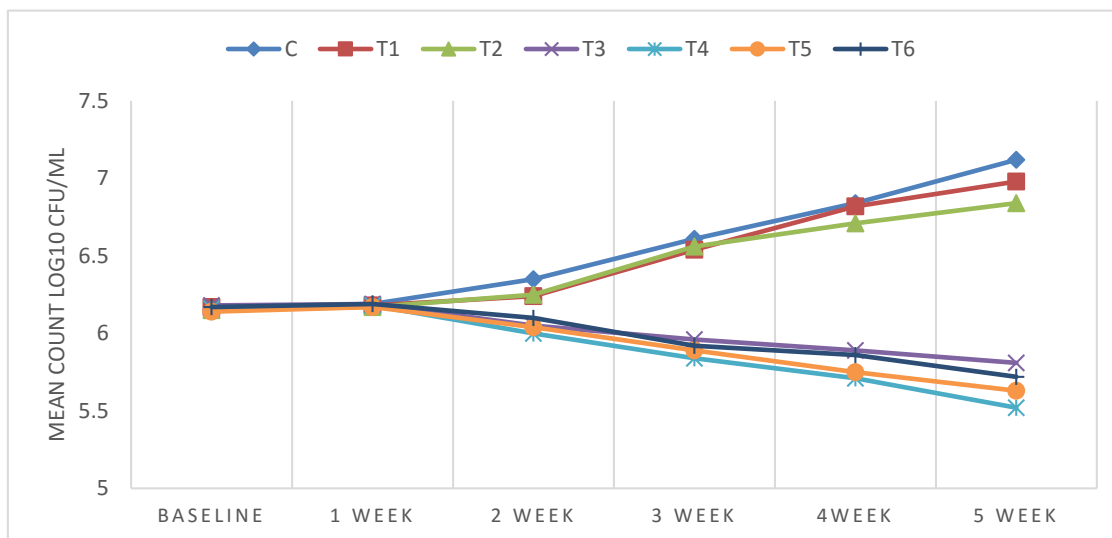


Figure (1): Mean value of salivary mutans streptococci count in study groups received different probiotic strains during the experimental period.

Percent change in bacterial count between groups:

The results showed that there was a statistically significant increase in mean change of mutans streptococci count in group C, T1 and T2 during follow up with the highest mean percent increase in group C (-15.7±0.44). There was statistically significant decrease was noted in Group T3, T4, T5 and T6. The statistically significant lowest mean percent was in T4 (10.3±0.14) [Table 2 and Fig 2].

Table (2): Mean, SD values, and percent change of salivary mutans streptococci count in study groups received different probiotic strains before and after treatment

	C	T1	T2	T3	T4	T5	T6	P-value [†]
	Mean	Mean	Mean	Mean	Mean	Mean	Mean	
	SD	SD	SD	SD	SD	SD	SD	
Pre-Treatment	6.15 ±.018	6.17 ±.018	6.15 ±.018	6.18 ±.017	6.16 ±.020	6.14 ±.012	6.17 ±.020	.054
Post Treatment	7.12 ±.021	6.98 ±.046	6.84 ±.028	5.81 ±.012	5.52 ±.020	5.63 ±.016	5.72 ±.020	<.001
% change	-15.7 ±.48	-13.1 ±.63	-11.2 ±.71	5.98 ±.40	10.3 ±.14	8.60 ±.41	7.29 ±.52	<.001
P-value ^{††}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	

[†]Significant level is set at ≤ 0.01 between the groups using *F test*

^{††}Significant level is set at ≤ 0.01 within the group using *t- test*

A positive sign indicates a decrease in CFU while a negative sign indicates an increase in CFU

C: Rats received laboratory standard rat ration blended without milk, **T1:** Rats received laboratory standard rat ration blended with milk without any probiotic strain. **T2, T3, T4, T5** and **T6:** Rats received laboratory standard rat ration blended with functional fermented milk containing probiotic strains *Lb acidophilus*, *Lb plantarum*, *Lb. reuteri*, *Lb rhamnosus* and *Bifi. bifidium* respectively.

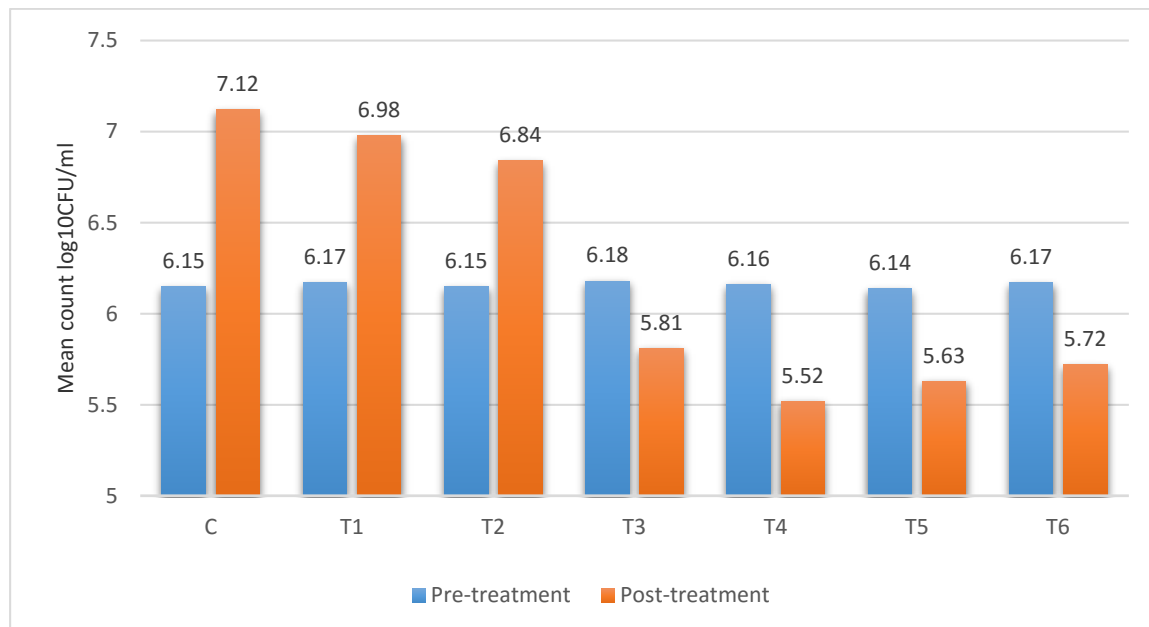


Figure (2): Mean of salivary mutans streptococci count in study groups received different probiotic strains before and after treatment

DISCUSSION

Probiotics have been supplemented to several foods because of their valuable effects on human health. They are mostly taken as a part of fermented foods with specially added active live cultures; such as in milk, yogurt or as dietary food. The inhibitory effect of five different probiotic bacterial strains against salivary mutans streptococci (log₁₀ CFU/ml) was investigated among the different experimental groups. There is a significant increase in mean log₁₀ salivary mutans streptococci mean count in rat ration containing *Lb acidophilus* NCTC12980R [T2] as shown in [Table1]. This increase might be due to the presence of interaction between *Lb acidophilus* NCTC12980R and salivary mutans streptococcus. The results came in agreement with Hasslöf et al. (2010) who tested *Lactobacillus acidophilus* La5 against mutans streptococci and found that low concentrations of *L. acidophilus* La5 (103 CFU/ml) did not affect MS growth [17].

The results disagree with Tahmourespour and Kermanshahi (2011) who reported that the presence of *Lactobacilli acidophilus* DSM 20079 resulted in a significant reduction in adherence streptococcal strains [25]. This study was conducted in an in-vitro environment, and our study more oriented toward biofilm studies and the ecological niche of the oral cavity is complicated. Bacteria in a biofilm express different genes and response differently to antimicrobial substances. The result also disagrees with Singh et al. (2011) where they found that the ice cream containing *Bifidobacterium lactis* Bb-12, ATCC27536 and *Lactobacillus acidophilus* La-5 reduced salivary mutans streptococci level[26]. This disagreement may be due to a combination of two probiotics together with the significant difference between groups at baseline scores of salivary mutans streptococci and lactobacilli in saliva. Besides, some of the subjects were prescribed antibiotics during Stage II and failed to report it to the investigator during the study. Though the reduction of mutans streptococci levels in consumption of probiotic ice-cream were observed, some children, especially with high scores of mutans at baseline, did not exhibit any change .

The results of present study showed a significant decrease in mean log₁₀ salivary mutans streptococci count in rat ration containing *Lb. plantarum* ATCC 14917 [T3] as shown in [Table 1, Fig 1]. The results are in agreement with Simark-Mattsson et al. (2007), Yang et al. (2008), Hasslöf et al. (2010), Söderling et al. (2011) and Hasslöf (2013) [17, 27-30]. The present study showed a significant reduction in mean log₁₀ salivary mutans streptococcus count in Group [T4] consuming *Lb. reuteri* NCIMB1195 as a part of their diet which may be attributed to the antimicrobial effect of *Lb. reuteri* on mutans streptococcus [Table 1, Fig 1]. This is in agreement with some in vivo studies (Caglar et al., 2006, Caglar et al., 2007, Çaglar et al., 2008a, Stensson et al., 2013) who concluded that ingestion of *Lactobacillus reuteri* ATCC 55730 delivered by prepared straws, lozenges, chewing gum, via medical device containing lozenges or drops reduced the level of salivary mutans streptococci significantly [15, 31-33]. On the other hand, the results disagreed with Söderling et al. (2011) who showed the weakness of *Lactobacillus reuteri* PTA 5289 when compared to *Lactobacillus reuteri* SD2112 in inhibition of the biofilm formation of the strains of mutans streptococci [29]. This might be due to the antimicrobial activity against *S. mutans* which was pH-dependent. The results also disagree with Cildir et al. (2012), where they concluded that the novel drop containing *L. reuteri* could not decrease the salivary mutans streptococci and lactobacilli levels in children aged 4-12 years with cleft lip/palate. It must be figured out that the effect of probiotics and their actions on the oral microflora of patients with cleft palate might be complicated [34]. Possible explanation is the complex condition in the oral cavity, with hundreds of species competing for nutrients, food intake lowering the pH, and saliva always flushing and altering the conditions for bacterial growth. It is possible that the biofilm in children is less mature and consequently more easily modified. The different intervals might explain these conflicting findings between the administration of probiotics and the oral microbiota analyses.

Investigating the inhibitory effect of *Lb. rhamnosus* ATCC7469 [T5], the results showed a significant reduction in mean log₁₀ salivary mutans streptococci count [Table 1 Fig 1]. This result agreed with in the vitro study of Söderling et al. (2011) concluded that *Lactobacillus rhamnosus* GG interfered with mutans streptococci biofilm formation in vitro [29]. Inhibitory effect of *Lb. rhamnosus* against mutans streptococci reported in this study is also supported by several in vivo studies, Diab et al., 2007, Simark-Mattsson et al. (2009) and Glavina et al. (2012) [35-37].

The results of this study proved a significant decrease in mean log₁₀ salivary mutans streptococci count in rat ration containing *Bifi. bifidum* NCTC1300R [T6] [Table 1, Fig 1]. This goes in agreement with Caglar et al., 2005a, Caglar et al., 2008b, Cildir et al., 2009, Singh et al., 2011 and Plonka et al., 2012 where Bifidobacteria strains significantly inhibited mutans streptococci [11, 16, 26, 38, 39].

In the present study, there was no statistically significant difference in the mutans mean count between the groups at the beginning of the study, which was considered an important factor regarding standardization of the experiment. The inhibitory effect of different probiotic strains against salivary mutans streptococci counts among the control groups (C, T1) and experimental groups (T2, T3, T4, T5 & T6) before and after the intervention are shown in [Table 1, Fig 1]. All groups had a detectable level of salivary mutans streptococci at baseline with no statistical significant difference ($p=0.054$). After one week interventions, no significant different ($p=0.73$) was reported. An absolute decline in salivary mutans streptococci counts was evident after intake the probiotics bacteria by the end of the second week resulting in a statistically significant difference between group [C] and others groups since the ($p < 0.001$). However, group [T1 & T2] showed no statistically significant difference with [T3, T4, T5 & T6] ($p \leq 1.00$). By the end of the experimental period, the results showed that there was a statistical significant decrease in mean change in salivary mutans streptococcus count in group [T4] during the follow-up period which showed the statistically significant lowest mutans mean count, followed by group [T5] then group [T6] & [T3]. On the other hand, there was a statistical significant increase on mean salivary mutans streptococci count on group [T2], [T1] and [C] (Negative control) which showed the statistical significant highest mutans mean count.

The present findings should be interpreted with caution for a couple of reasons. First, the length of the intervention was short. In the present study, only the effect of short-term administration of probiotics was investigated. It seems credible that prolong administration of probiotic might have a preventive effect against development caries which urge the need of long terms studies for evaluation of their permanent colonization. Additionally, more studies involving different strains of probiotics are necessary. Moreover, combinations of probiotic strains may be more

efficient than a single probiotic. The interest in oral probiotics has been growing during the last decades. It is important to recognize that the proposed health benefits of each probiotic bacterial strain must be studied individually. The current study compared between 5 different probiotic strains showing their behavior against mutans streptococci over time in vivo. The results showed that Probiotic strains have an inhibitory effect against MS count with varying degrees. The *Lb. reuteri* NCIMB1195 showed the most potent effect against MS followed by *Lb rhamnosus* ATCC7469 while the *Lb acidophilus* NCTC12980R showed no effect on growth of MS overtime.

CONCLUSIONS:

Within the limitation of the present study, it could be reported that, the reduction of *S. mutans* count in rats' saliva is associated with the application of bio-fermented milk with different probiotic bacteria. Different Probiotic strains showed an inhibitory effect on MS count with varying degree. Milk containing *Lb. reuteri* NCIMB1195 has the most potent effect against mutans streptococcus. More researches are needed to detect the effect of other Probiotic bacterial strains against salivary mutans streptococci, and to decide the best and appropriate vehicle for probiotics administration into the oral cavity. Further studies on the combination effect of different probiotics applied simultaneously to test the possible additive, cumulative, or competitive modes of action in the oral environment are still needed.

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