

ORIGINAL ARTICLE

## Salivary mutans streptococci and lactobacilli levels after ingestion of the probiotic bacterium *Lactobacillus reuteri* ATCC 55730 by straws or tablets

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### Abstract

**Objective.** Previous studies have suggested that lactobacilli-derived probiotics in dairy products may affect oral ecology, but the impact of different vehicles of ingestion has received little attention. The aim of the present study was to investigate the effect of the probiotic bacterium *Lactobacillus reuteri* ATCC 55730 on the levels of salivary mutans streptococci and lactobacilli in young adults when ingested by two different delivery systems. **Material and Methods.** The material comprised 120 healthy young adults (21–24 years) and a placebo-controlled study design with parallel arms was utilized. The subjects were randomly assigned to four equally sized groups: group A drank 200 ml of water through a prepared straw containing *L. reuteri* ATCC 55730 once daily for 3 weeks, while group B took 200 ml water through a placebo straw during the same period. Group C was given one tablet containing *L. reuteri* ATCC 55730 once daily for 3 weeks, while group D received placebo tablets without bacteria. Salivary mutans streptococci and lactobacilli were enumerated with chair-side kits at baseline and 1 day after the final ingestion. **Results.** A statistically significant reduction of the mutans streptococci levels was recorded after ingestion of the probiotic bacteria via the straw ( $p < 0.05$ ) and the tablets ( $p < 0.01$ ), which was in contrast to the placebo controls. A similar but non-significant trend was seen for lactobacilli. **Conclusions.** A short-term daily ingestion of lactobacilli-derived probiotics delivered by prepared straws or lozenges reduced the levels of salivary mutans streptococci in young adults.

**Key Words:** *Lactobacilli, mutans streptococci, probiotics, saliva, straw, tablet*

### Introduction

Probiotic bacteria are live microbial food supplements that may benefit the host by improving its intestinal balance [1]. Within dentistry, previous studies with probiotic strains such as *Lactobacillus rhamnosus* GG [2–4], *L. acidophilus* and *L. casei* [5], *L. rhamnosus* LC705 [4], *L. reuteri* [6], *Bifidobacterium* DN-173 010 [7], or a lactobacilli mix [8] have revealed mixed results on oral microorganisms, but a notable observation in half of the articles was that the recovery of oral mutans streptococci is hampered. In the studies mentioned above, dairy foods like milk, yogurt and cheese have been selected as vehicles for the selected bacteria. However, probiotics can also be delivered by vehicles such as

lozenges, powder, gelatine, straw, or tablets [9]. As stated by Meurman [10], one key question relating to the use of probiotics in oral medicine is the best way of administration. Montalto and co-workers [8] have recently demonstrated increased levels of salivary lactobacilli but not mutans streptococci when a probiotic lactobacilli mix was orally ingested in a liquid form or in capsules. Their findings indicate that there might be a systemic effect and that direct contact between the probiotic bacteria and the oral tissues may not be crucial. To our knowledge, the possible effects of various non-dairy consumer products intended for oral use has not been reported. The aim of the present study was therefore to examine whether or not a short-term consumption of water through specially prepared probiotic

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straws or tablets containing *Lactobacillus reuteri* could affect the levels of salivary mutans streptococci and lactobacilli in young adults. The null hypothesis was that the probiotic products would not alter the bacterial levels compared with placebo controls.

## Material and methods

### Study group

The material comprised 120 healthy young adults (71 M, 49 F; 21–24 years of age) who volunteered after receiving verbal and written information. Subjects with a history of systemic antibiotic or topical fluoride treatments within the 4 weeks prior to baseline were not invited, nor were individuals with a habitual use of dairy probiotics or xylitol chewing gums. The subjects had a non-compromised oral health and none exhibited untreated active caries lesions or signs of either gingivitis or periodontal disease.

### Study design

The protocol was approved by the School of Dentistry Ethics Committee at the University of Yeditepe, Istanbul, Turkey. The prospective investigation had a randomized placebo-controlled study design with four parallel arms and with an experimental period of 3 weeks. The subjects were randomly assigned to one of four equally sized groups ( $n=30$ ): Group A drank 200 ml water through a prepared straw containing probiotic bacteria once daily; group B drank 200 ml water through a placebo straw without bacteria once daily; group C ingested one sucking tablet with probiotic bacteria once daily; and group D ingested one sucking tablet without bacteria once daily.

Salivary samples were collected at baseline and 1 day after the final ingestion, as described below. During the experimental period, the subjects were encouraged to maintain their normal oral hygiene habits and to continue to brush their teeth twice a day.

### Study vehicles

The experimental probiotic straw (Life top straw, Biogaia, Stockholm, Sweden) consisted of a telescopic polypropylene membrane with an oil droplet containing *L. reuteri* ATCC 55730 (minimum  $10^8$  CFU/STRAW) attached to its inner part, while the placebo straw was identical in size and color but without the active live bacteria. The experimental tablet (Biogaia ProDenta, Biogaia, Stockholm, Sweden) consisted of *L. reuteri* ATCC 55730 ( $10^8$  CFU/TAB). The placebo tablets were identical in size, form, and taste, but without live bacteria. The straws were used to drink 200 ml of tap water slowly from standardized cups once daily at lunchtime. In the

tablet groups, one tablet was allowed to melt slowly in the mouth around noon (12:00–1:00 pm). The test and placebo products were provided by the manufacturer. No toothbrushing was allowed for at least 1 h after the probiotic ingestions.

### Saliva samples

Samplings of paraffin-stimulated whole saliva were carried out immediately before and after the trial. After a thorough rinse in water, the saliva was expectorated directly into a graded test tube for 5 min and the flow rate was calculated as ml/min. The counts of salivary mutans streptococci and lactobacilli were evaluated using Dentocult SM (Strip Mutans<sup>®</sup>) and Dentocult LB<sup>®</sup> chair-side kits (Orion Diagnostica, Espoo, Finland) as described earlier [11,12]. After cultivation at 37°C for 48 and 96 h, respectively, the colony forming units (cfu) were identified on the basis of morphology and counted in a stereomicroscope with 12–25 times magnification. The cfu were categorized in four scores as shown in the tables. The laboratory staff and the clinicians evaluating the test kits were blinded to the respective subject's group assignment.

### Statistical methods

The data were processed with the SPSS software (version 12.0, Chicago, Ill., USA). Post-treatment and pre-treatment values within each regimen were compared with a two-tailed marginal homogeneity test for categorical data. No comparisons were made between the different arms except at baseline. A  $p$ -value  $<0.05$  was considered statistically significant.

## Results

All subjects had a stimulated saliva secretion rate within normal limits (1.0–2.5 ml/min). The pre- and post-experimental levels of salivary mutans streptococci and lactobacilli are presented in Tables I and II, respectively.

### Probiotic and placebo straw

There were no differences between group A and group B at baseline concerning the bacterial levels. A statistically significant ( $p < 0.05$ ) reduction of salivary mutans streptococci was registered after the 3-week intake of water via the prepared probiotic straw, which was in clear contrast to the placebo straw. In group A, 18 subjects exhibited decreased scores, 10 had unchanged scores while 2 displayed a 1-step increase. The corresponding values in group B were 6, 20, and 4 subjects, respectively. Regarding the salivary lactobacilli, a similar tendency to reduced counts was seen after the 3-week period in

Table I. Distribution of salivary mutans streptococci counts before and after 3 weeks of daily ingestion of a probiotic strain (*Lactobacillus reuteri*) with straws or tablets. The values denote the number of subjects

Administration/time	n	Mutans streptococci score (cfu)				p
		0 No growth	1 ≤10 <sup>4</sup>	2 10 <sup>5</sup>	3 ≥10 <sup>6</sup>	
<b>Group A Probiotic straw</b>						
Baseline	30	6	4	5	15	<0.05
End	30	9	6	14	1	
<b>Group B Placebo straw</b>						
Baseline	30	6	9	9	6	NS
End	30	8	7	8	7	
<b>Group C Probiotic tablet</b>						
Baseline	30	9	5	5	11	<0.01
End	30	17	2	8	3	
<b>Group D Placebo tablet</b>						
Baseline	30	13	5	5	7	NS
End	30	12	6	6	6	

cfu = colony forming units; NS = not significant.

group A, but the difference was not statistically significant ( $p=0.07$ ). The post-experimental lactobacilli levels in group B did not differ from baseline.

#### Probiotic and placebo tablet

There was no difference in salivary mutans streptococci levels at baseline between groups C and D, but a slight difference was noted concerning the lactobacilli, since no subjects with the highest score were assigned to the latter group. A statistically significant ( $p < 0.01$ ) reduction of salivary mutans streptococci was registered after 3 weeks in group C compared with baseline, but this was not the case in group D. In group C, 14 subjects exhibited decreased scores, 15 had unchanged scores while one subject displayed a 1-step increase. The corresponding values for the placebo regime were 6, 21, and 5 subjects, respec-

tively. The post-experimental lactobacilli levels were unchanged in both tablets groups.

#### Discussion

The aim of this study was to investigate the effect of two different non-dairy delivery methods for probiotic bacteria on the levels of some selected oral bacteria associated with the caries process. Based on previous data [13], it was thought that the lozenges would allow a more direct and thorough contact with the oral mucosa and biofilm compared with intakes through the straw. It was stressed that the subjects should move the tablet around the mouth during the melting, in contrast to the straw users, who were instructed to swallow immediately. Compliance was excellent in all groups, with no drop-outs or reported side or adverse effects. The chair-side tests were considered as robust endpoints

Table II. Distribution of salivary lactobacilli counts before and after 3 weeks of daily ingestion of a probiotic strain (*Lactobacillus reuteri*) with straws or tablets. The values denote the number of subjects

Administration/time	n	Lactobacilli score (cfu)				p
		0 ≤10 <sup>3</sup>	1 10 <sup>4</sup>	2 10 <sup>5</sup>	3 ≥10 <sup>6</sup>	
<b>Group A Probiotic straw</b>						
Baseline	30	9	13	5	3	NS
End	30	15	9	3	3	
<b>Group B Placebo straw</b>						
Baseline	30	12	10	3	5	NS
End	30	15	5	7	3	
<b>Group C Probiotic tablet</b>						
Baseline	30	14	5	5	6	NS
End	30	18	4	7	1	
<b>Group D Placebo tablet</b>						
Baseline	30	19	3	8	—	NS
End	30	19	5	6	—	

cfu = colony forming unit; NS = not significant.

since they have been validated and compared with conventional cultivation methods on selective agar plates in previous studies [14,15].

*L. reuteri* has been shown to be an intestinal inhabitant of potential importance [16]. In particular, it secretes an antimicrobial compound, reuterin, which may partly be responsible for its positive effects [17]. The beneficial role that *L. reuteri* ATCC 55730 has in general health, immunomodulation, and disease protection has been validated and described in a number of studies [18–21]. The effect of *L. reuteri* on one of the caries pathogens has been evaluated *in vitro* by Nikawa and co-workers [6]. Consumption of a yogurt containing *L. reuteri* resulted in a significant growth inhibition of *S. mutans*, which was in contrast to other probiotic lactobacilli strains. To our knowledge, the present study is the first to examine the oral effects of *L. reuteri* through two non-dairy vehicles. A suppressive effect of salivary mutans streptococci was seen by both delivery methods, and the null hypothesis could be rejected. The results were mainly in agreement with previous findings with lactobacilli-derived probiotics [3,4,6]. The explanation for the findings, and the mechanism of action, is not fully clear, however. While it is relatively plausible that probiotics may competitively inhibit streptococci by replacement because of the direct contact with the oral tissues and biofilm, one may question how ingestions through a straw could have the same effect. From the existing literature, it is questionable whether probiotic bacteria can colonize on a permanent basis in the mouth and whether or not they have any residual effect after discontinuation of intake [2,5]. A systemic effect induced by the probiotic during periods of ingestion cannot be excluded, although this has not convincingly been evaluated in previous studies on oral ecology. Some additional information on this issue would possibly have been available if the salivary bacterial levels had been monitored more frequently during the intervention.

Modern molecular techniques have underlined the concept that mutans streptococci are associated with early enamel demineralization, while lactobacilli are pathogenic in deep caries lesions [22]. Therefore, a marked reduction of oral mutans streptococci over time would theoretically imply a reduced caries risk, at least for new lesions, but this must of course be verified in prospective clinical trials. The clinical significance of our findings is still unclear and any premature conclusion should be avoided at this stage. Nevertheless, the present observations merit further study in order to evaluate the possible effects of probiotics on oral ecology.

In conclusion, the results of this study indicate that daily short-term ingestion of a lactobacilli-derived probiotic bacterium through a prepared

straw or a lozenge tablet could reduce the levels of mutans streptococci in saliva.

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