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SHORT COMMUNICATION

Oral colonization by *Lactobacillus reuteri* ATCC 55730 after exposure to probiotics

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Objective. The aim of this study was to investigate whether *Lactobacillus reuteri* ATCC 55730 can be detected in the oral cavity after discontinuation of administration of a product prepared with this bacterium.

Materials and Methods. The study consisted of three 2-week periods: clearance period, intervention period, and post-treatment period. Twenty-five volunteers consumed a chewable tablet of *L. reuteri* ATCC 55730 (10^8 cfu/tablet) during a 14-day trial period. Saliva samples were collected and cultured onto MRS agar after a clearance period of 2 weeks and then daily after a 2-week intervention period for as long as *L. reuteri* was found. *Lactobacillus reuteri* colonies were analysed in saliva samples. The analysis was performed using selective media for *L. reuteri* followed by confirmation using the specific detection of reuterin produced by *L. reuteri.*

Results. The number of *L. reuteri* carriers decreased gradually, and after 1 week only 8% of the subjects harboured the bacterium. After 5 weeks, *L. reuteri* was not detected in any of the subjects.

Conclusion. Consuming *L. reuteri* for 2 weeks does not seem to be sufficient for permanent colonization of *L. reuteri* in the oral cavity.

Introduction

Probiotic bacteria are live microbial food supplements that may benefit the host by influencing the balance between the many species of the commensal flora both in oral cavity and the rest of the digestive system[1–6]. *Lactobacillus reuteri* is an obligate heterofermentative resident in the gastrointestinal tract in humans[7], and it is reported to produce antimicrobial substances with a broad spectrum activity (i.e. reuterin[8,9] and reutericyclin[10]).

The beneficial role that *L. reuteri* ATCC 55730 has in general health has been validated and described in a number of studies[11–14]. There are only few studies where the effect of *L. reuteri* in the oral cavity has been investigated. Consumption of a yoghurt containing *L. reuteri* SD 2112 (ATCC 55730) resulted in a significant growth inhibition of *Streptococcus mutans*, which was in contrast to other probiotic lactobacilli strains[15]. Çaglar *et al.*[16] investigated the effect of the probiotic bacterium *L. reuteri* ATCC 55730 on the levels of salivary mutans streptococci and lactobacilli in young adults when ingested by two different non-dairy delivery systems of straws and tablets. A significant reduction of the mutans streptococci levels was recorded after ingestion of probiotic bacteria via the straw and the tablets which was in contrast to placebo controls. Our research group evaluated the effect of probiotic chewing gums containing two strains of *L. reuteri* ATCC 55730/ATCC PTA 5289 on levels of salivary mutans streptococci and lactobacilli in young adults[17]. Daily chewing on gums containing probiotic bacteria reduced the levels of salivary mutans streptococci in a significant way. Recently, Çaglar *et al.*[18] investigated the effect of the probiotic *L. reuteri* ATCC 55730/ATCC PTA 5289 on the levels of salivary mutans streptococci and lactobacilli in young women with high mutans streptococci counts.

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Daily ingestion of lactobacilli-derived probiotics delivered by a new medical device containing probiotic lozenge reduced the levels of salivary mutans streptococci in certain amounts. Although its suppressive effect on cariogenic microflora growth is demonstrated, the survival of *L. reuteri* ATCC 55730 in oral cavity is still unknown.

The aim of this study was to investigate whether *L. reuteri* ATCC 55730 can be detected in the oral cavity after discontinuation of administration of a tablet prepared with this bacterium. Our research hypothesis was that the selected bacteria would colonize the oral cavity for an extended period of time.

Materials and methods

The study design was a short-term intervention trial. The material comprised of 25 healthy young adults (14 females, 11 males, aged 21–22) who volunteered after receiving verbal and written information. Subjects with a history of systemic antibiotic or topical fluoride treatments within 4 weeks prior to baseline were not invited, nor were individuals who consumed dairy probiotics and chewing gums. The subjects had good oral health, and none exhibited untreated active caries lesions or signs of either gingivitis or periodontal disease.

The study protocol was in accordance with the Helsinki Declaration of Human Rights, and approved by the Ethical Committee at the School of Dentistry, University of Yeditepe, Istanbul. The study consisted of three 2-week periods: clearance period, intervention period, and post-treatment period. During the clearance and post-treatment periods, all probiotic products were prohibited. The tablet (BioGaia Probiotic chewable tablet, BioGaia, Stockholm, Sweden) contained *L. reuteri* ATCC 55730 (10^8 cfu/tablet). One chewing tablet was allowed to melt slowly in the mouth around noon (12:00–1:00 pm). Saliva samples from the participants were collected once after the clearance period and daily after the intervention period as long as *L. reuteri* was detected in the samples. One millilitre of paraffin-stimulated saliva was collected into a sterile plastic container and placed in 3 mL of VMG II transport fluid[19], which was tested and found to preserve *L. reuteri* counts for 48 h before the study had begun. The samples were transported at room temperature to the laboratory within 24 h. All samples were serially diluted with 0.9% saline solution and plated on De Man, Rogosa, Sharpe agar (MRS, Acumedia, Ljusne, Sweden) modified by addition of 2% sodium acetate and 50 mg/L vancomycin for the cultivation of *L. reuteri* (detection limit = 10 cfu/mL), and on Rogosa's agar (Merck KGaA, Darmstadt, Germany) for the total lactobacilli counts (detection limit = 10^2 cfu/mL). Plates were incubated anaerobically (AnaeroGen, Oxoid, Sollentuna, Sweden) at 37 °C for 48 h, after which colonies were confirmed as *L. reuteri* using a BioGaia AB proprietary method[20] based on reuterin production in the presence of glycerol. Five millilitres of soft agar (1% agar and 2% glycerol), which was melted and kept in water bath at 50 °C until ready for use, was poured directly onto the colonies on the MRS agar plates covering the entire surface and incubated aerobically at 37 °C. After 1 h, 5 mL 2,4-dinitrophenylhydrazine (DNPH) solution (0.1% DNPH, 1.7% HCl) was added for 3 min. This solution was discarded, and 5 mol/L potassium hydroxide was added for 30 s. A positive read-out was accepted as a reddish brown zone around the colonies, whereas no change in colour at all was accepted as negative (Fig. 1).

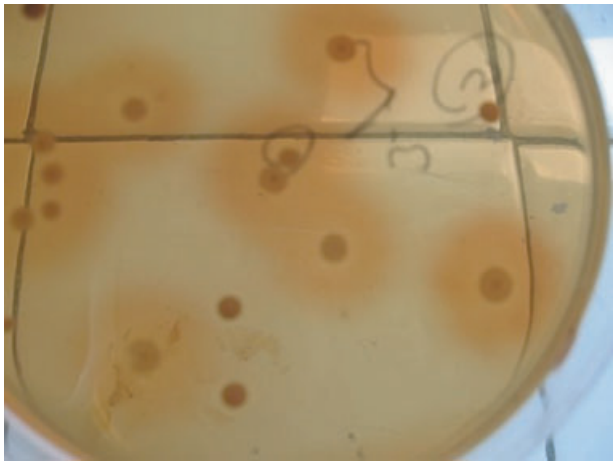

Fig. 1. Reddish brown zones around the *Lactobacillus reuteri* ATCC 55730 colonies after reuterin production in the presence of glycerol.

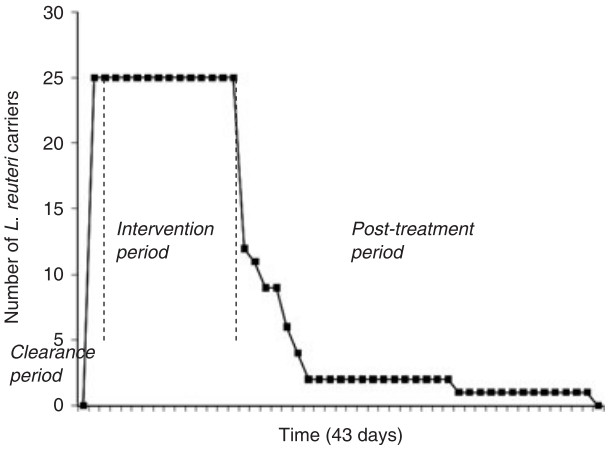

Fig. 2. Number of 25 individuals with detectable *Lactobacillus reuteri* during an observation period of 5 weeks after an exposure with a probiotic chewing tablet containing *L. reuteri* ATCC 55730 (10^8 cfu/tablet) for 2 weeks.

Results

None of the participants carried *L. reuteri* in saliva after the clearance period. On the first day after the intervention, 12 participants (48%) were positive for *L. reuteri*. The recovery of *L. reuteri* decreased gradually, and after 7 days two of the subjects harboured the bacterium for the next 2 weeks (Fig. 2). The occurrence of *L. reuteri* in the oral cavity decreased gradually, and none of the subjects were found to have detectable amounts of the bacteria on the 34th day after the intervention (Table 1). The compliance was high, and all the subjects completed the study.

Discussion

There are only a few studies on oral colonization of probiotic strains. *Lactobacillus rhamnosus* GG (LGG) has been shown to colonize in the oral cavity from 1–5 days up to a few weeks after consumption of a product containing this bacterium[21,22]. On the other hand, no lactobacilli were found in saliva samples from volunteers 1 week after consumption of a bioyoghurt containing two different strains of lactobacilli and a *Bifidobacterium bifidum* strain[23].

Colonization or rather temporary colonization is an important question for oral probiotics. It may be relevant to study both saliva, and supra- and subgingival occurrence of the exogenous bacteria. We have investigated the colonization with paraffin- stimulated saliva. Also, Motisuki *et al.*[24] reported that the most suitable method to detect the *Lactobacillus* spp. level in the oral cavity is the stimulated whole saliva.

Çaglar *et al.*[16] stated that chewing a *L. reuteri* ATCC 55730 tablet once daily for 3 weeks significantly prevents the growth of the cariogenic microflora in the mouth and that this effect seems to be closely related to the direct contact between the tablet and the oral biofilm. To be able to have probiotic effects in the mouth, a bacterium must adhere to oral surfaces and become part of the biofilm. Haukioja *et al.*[25] tested the binding of different lactobacillus strains to hydroxyapatite and microtitre wells coated with human saliva. *Lactobacillus reuteri* SD 2112 (ATCC 55730) was found to bind to a low degree to saliva-coated microtitre wells, hydroxyapatite beads, and BSA-coated hydroxyapatite beads. On the other hand, survival in saliva was high as no decrease in cfu/mL was detected after incubation in saliva for 24 h.

Table 1. Recovery of total lactobacilli and *Lactobacillus reuteri* in the saliva of 25 participants before and after *L. reuteri* ATCC 55730 supplementation for 2 weeks.

Period	After clearance *Lactobacillus* spp.*	After incubation									
		L. reuteri†									
Day			1	2	3	4	5	6	7	20	34
n‡	17	0	12	11	9	9	6	4	2	1	0
Mean (cfu/mL)	2.3×10^4	0	1.3×10^4	3.0×10^4	1.0×10^3	9.1×10^2	4.0×10^3	4.5×10^3	3.0×10^3	2.5×10^2	0
SD (cfu/mL)	3.5×10^4		2.9×10^4	7.8×10^4	1.9×10^3	1.2×10^3	7.8×10^3	5.2×10^3	2.8×10^3		

*Detection limit = 10^2 cfu/mL.
†Detection limit = 10 cfu/mL.
‡Number of samples where lactobacilli and *L. reuteri* was detected.

Dietary supplementation with the probiotic *L. reuteri* ATCC 55730 induces significant colonization of the stomach, duodenum, and ileum in healthy humans[14]. This study showed that it also induces colonization of *L. reuteri* in 48% of the subjects directly after the intervention period, whereas 4% harboured the bacterium for a month. The occurrence of *L. reuteri* in the oral cavity decreased gradually, indicating that no permanent colonization had occurred and that the oral persistence of *L. reuteri* was only temporary. Yli-Knuuttila *et al.*[22] notified that although had withdrawn to use LGG-containing products, one of their subjects who had received LGG milk at the age of 10 for 1 year was found LGG positive in her saliva, so that they speculated that the permanent colonization could be in childhood.

The present situation also indicates that constant uptake of *L. reuteri* is necessary to obtain beneficial effect. In conclusion, the results of the present intervention trial did not support the hypothesis that *L. reuteri* might easily colonize the oral cavity. A continuous administration will be attempted for permanent colonization.

What this paper adds

- Constant uptake of some probiotic strains is necessary to obtain permanent colonization and beneficial effect.

Why this paper is important to paediatric dentists

- There is a need for a more aggressive approach to prevention of oral disease to optimize clinical outcome. At this point paediatric dentists will further search for probiotics which could easily colonize in oral cavity of children.

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References

1 Çaglar E, Kargul B, Tanboga I. Bacteriotherapy and probiotics' possible role on oral health. *Oral Dis* 2005; **11**: 131–137.
2 Çaglar E, Sandalli N, Twetman S, Kavaloglu S, Ergeneli S, Selvi S. Consumption of yogurt with *Bifidobacterium* DN–173 010 and its effect on dental caries risk factors. *Acta Odontol Scand* 2005; **63**: 317–320.
3 Lenoir-Wijnkoop I, Sanders ME, Cabana MD, *et al*. Probiotic and prebiotic influence beyond the intestinal tract. *Nutr Rev* 2007; **65**: 469–489.
4 Caglar E, Kuscu OO, Cildir SK, Kuvvetli SS, Sandalli N, Twetman S. Effect of an ice-cream containing *Bifidobacterium lactis* Bb-12 on salivary mutans streptococci and lactobacilli. *Acta Odontol Scand* 2008; **66**: 154–158.
5 Cildir SK, Germec D, Sandalli N, *et al*. Reduction of salivary mutans streptococci in orthodontic patients during daily consumption of yoghurt containing probiotic bacteria. *Eur J Orthod* 2009. DOI 10.1093/ejo/cjn108.
6 Twetman S, Stecksén-Blicks C. Probiotics and oral health effects in children. *Int J Paediatr Dent* 2008; **18**: 3–10.
7 Itsaranuwat P, Al-Haddad KSH, Robinson RK. The potential therapeutic benefits of consuming 'health promoting' fermented dairy products: a brief update. *Int J Dairy Technol* 2003; **56**: 203–210.
8 Speck ML, Dobrogosz WJ, Casas IA. *Lactobacillus reuteri* in food supplementation. *Food Technol* 1993; **47**: 90–94.
9 Talarico TL, Casas IA, Chung TC, Dobrogosz WJ. Production and isolation of reuterin, a growth inhibitor produced by *Lactobacillus reuteri*. *Antimicrob Agents Chemother* 1988; **32**: 1854–1858.
10 Ganzle MG, Holtzel A, Walter J, Jung G, Hammes WP. Characterization of reutericyclin produced by *Lactobacillus reuteri* LTH2584. *Appl Environ Microbiol* 2000; **66**: 4325–4333.
11 Weizman Z, Asli G, Alsheikh A. Effect of a probiotic infant formula on infections in child care centers: comparison of two probiotic agents. *Pediatrics* 2005; **115**: 5–9.
12 Casas IA, Dobrogosz WJ. Validation of the probiotic concept: *Lactobacillus reuteri* confers broad-spectrum protection against disease in humans and animals. *Microb Ecol Health Dis* 2000; **12**: 247–285.
13 Connolly E. *Lactobacillus reuteri* ATCC 55730: a clinically proven probiotic. *Nutrafoods* 2004; **3**: 15–22.
14 Valeur N, Engel P, Carbajal N, Connolly E, Ladefoged K. Colonisation and immunomodulation by *Lactobacillus reuteri* ATCC 55730 in the human gastrointestinal tract. *Appl Environ Microbiol* 2004; **70**: 1176–1181.
15 Nikawa H, Makihira S, Fukushima H, Nishimura H, Ozaki Y, Ishida K. *Lactobacillus reuteri* in bovine

milk fermented decreases the oral carriage of mutans streptococci. *Int J Food Microbiol* 2004; **95**: 219–223.
16 Çaglar E, Kavaloglu S, Ergeneli S, Sandalli N, Twetman S. Salivary mutans streptococci and lactobacilli levels after ingestion of the probiotic bacterium *Lactobacillus reuteri* ATCC 55730 by straws or tablets. *Acta Odontol Scand* 2006; **64**: 314–318.
17 Çaglar E, Kavaloglu SC, Kuscu OO, Sandalli N, Holgerson PL, Twetman S. Effect of chewing gums containing xylitol or probiotic bacteria on salivary mutans streptococci and lactobacilli. *Clin Oral Invest* 2007; **11**: 425–429.
18 Çaglar E, Kuscu OO, Kavaloglu SC, Kuvvetli SS, Sandalli N. Dissolution of probiotics from a lozenge administered medical device and their possible effects on salivary mutans streptococci and lactobacilli. *Int J Pediatr Dent* 2008; **18**: 35–39.
19 Dahlén G, Pipattanagovit P, Rosling B, Möller AJ. A comparison of two transport media for saliva and subgingival samples. *Oral Microbiol Immunol* 1993; **8**: 375–382.
20 Dobrogosz WJ, Lindgren SE, inventors; BioGaia AB, assignee. Method of determining the presence of an antibiotic produced by *Lactobacillus reuteri*. US patent 5352586. 4 October 1994.
21 Meurman JH, Antila H, Salminen S. Recovery of *Lactobacillus* strain GG (ATCC 53103) from saliva of healthy volunteers after consumption of yoghurt prepared with the bacterium. *Microb Ecol Health Dis* 1994; **7**: 295–298.
22 Yli-Knuuttila H, Snäll J, Kari K, Meurman JH. Colonization of *Lactobacillus rhamnosus* GG in the oral cavity. *Oral Microbiol Immunol* 2006; **21**: 129–131.
23 Busscher HJ, Mulder AF, van der Mei HC. *In vitro* adhesion to enamel and *in vivo* colonization of tooth surfaces by lactobacilli from a bio-yoghurt. *Caries Res* 1999; **33**: 403–404.
24 Motisuki C, Lima LM, Spolidorio DM, Santos-Pinto L. Influence of sample type and collection method on *Streptococcus mutans* and *Lactobacillus* spp. counts in the oral cavity. *Arch Oral Biol* 2005; **50**: 341–345.
25 Haukioja A, Yli-Knuuttila H, Loimaranta V, Kari K, Ouwehand AC, Meurman JH. Oral adhesion and survival of probiotic and other lactobacilli and bifidobacteria *in vitro*. *Oral Microbiol Immunol* 2006; **21**: 326–332.