Oral Microbiology Immunology 2006: 21: 129–131
Printed in Singapore. All rights reserved

ORAL MICROBIOLOGY
AND IMMUNOLOGY

Short communication

Colonization of *Lactobacillus rhamnosus* GG in the oral cavity

H. Yli-Knuuttila, J. Snäll, K. Kari, J. H. Meurman
Institute of Dentistry, University of Helsinki, and Department of Oral and Maxillofacial Diseases, Helsinki University Central Hospital, Helsinki, Finland

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. © Blackwell Munksgaard, 2006.

Background/aims: *Lactobacillus rhamnosus* GG (LGG) is one of the most widely studied probiotic bacterial strain. The benefits of LGG treatment in gastrointestinal disorders are well documented. The aim of the present study was to investigate whether LGG can be detected in the oral cavity after discontinuation of administration of a product prepared with this bacterium.
Material and methods: 56 volunteers consumed Gefilus® juice (Valio Ltd, Helsinki, Finland) containing LGG during a 14-day trial period. Saliva samples were collected and cultured onto MRS agar after a clearance period and then daily after a 2-week intervention period for as long as LGG was found. LGG-like colonies were analyzed in saliva samples, identified by characteristic colony morphology, a lactose fermentation test, and PCR with specific primers.
Results: LGG was not able to colonize the oral cavity. It could only be temporarily detected. In one female subject, however, whose medical history revealed use of LGG in childhood, the bacterium was detected in all saliva samples taken up to 5 months. (She was excluded from the intervention trial).
Conclusion: Permanent colonization of LGG in the oral cavity is improbable but seems possible in individual cases.

Key words: colonization; lactobacilli; oral health; probiotics

H. Yli-Knuuttila, Institute of Dentistry, University of Helsinki, and Department of Oral and Maxillofacial Diseases, Helsinki University Central Hospital, PB 63, 00014 University of Helsinki, Helsinki, Finland
Tel.: +358 9 1912543;
fax: +358 9 19127517;
e-mail: heli.yli-knuuttila@helsinki.fi
Accepted for publication August 25, 2005

Probiotics are living microorganisms that belong to the natural flora and benefit the host by improving its intestinal microbial balance (3, 10). *Lactobacillus rhamnosus* GG, ATCC 53101 (LGG), is one of the most widely studied probiotic bacterium and it has been used in the treatment of gastrointestinal infections (5). Studies have also shown that LGG has beneficial effects on intestinal immunity and therefore is also effective in the prevention of early atopic disease in children (6, 7). The interest in use of probiotics is rapidly growing. The role of probiotics in the oral cavity is not widely studied and the knowledge of their possible probiotic action in the mouth is still lacking. There are only a few studies where the effect of LGG in the oral cavity has been investigated and in these studies the results were promising, indicating that LGG may prevent the growth of harmful microflora of the mouth. A 7-month placebo-controlled intervention study showed that the consumption of LGG reduced caries risk and initial caries development in children (9). In a short-term intervention study the combination of LGG and *L. rhamnosus* LC 705 reduced *Streptococcus mutans* in saliva of young adults (1). Probiotic intervention also reduced the prevalence of oral *Candida* in a randomized, double blind, placebo-controlled intervention study among the elderly (Hatakka et al., submitted). Further, LGG reduced the adhesion of *S. mutans* to saliva-coated hydroxyapatite beads, confirming its anticariogenic effect (12). The adherence of probiotic bacteria to oral surfaces is one of the essential aspects to be investigated when considering the use of probiotics in the prevention or treatment of diseases of the oral cavity.

This study was made to assess whether LGG can colonize the oral cavity. The study design was a short-term intervention trial. A total of 57 healthy students and staff members of the University of Helsinki participated in the intervention study. The study protocol was approved by the Ethical Committee of the Helsinki University Central Hospital. Before the study the volunteers filled in a questionnaire about their general health, medication, oral health care habits, and use of probiotic products. All participants included were healthy and had not used antibiotics within the 2 weeks prior to the study. Seven

percent of the subjects ($n = 4$) had regularly used LGG-containing products and 7% ($n = 4$) were daily smokers. Two of the subjects received antibiotic medication during the intervention. The study consisted of three 2-week periods: clearance period, intervention period, and post-treatment period. During the clearance and post-treatment periods all products containing LGG were prohibited. One participant who carried LGG in saliva after the clearance-period was excluded from the actual trial. Thus the remaining number of volunteers was 56 (43 women, 13 men, mean age ± SD 25.8 ± 10.5). During the intervention period the subjects used 200 ml LGG containing 5×10^6 CFU/ml LGG Gefilus® juice (Valio Ltd, Helsinki, Finland) three times daily. All xylitol products were forbidden during the intervention and post-treatment periods. The participants kept a diary of smoking, medication and use of probiotic and xylitol products during the study. Saliva samples from the participants were collected once after the clearance period and daily after the intervention period as long as LGG was detected in the samples.

Aliquots (100 µl) of nondiluted saliva samples were cultured on MRS agar (Laboratory M, Lancashire, UK) containing 50 mg/l vancomycin and incubated overnight at 37°C in 5% CO_2 atmosphere. The agar plates were viewed by stereomicroscope to detect the colonies with morphology typical of LGG (white, smooth colonies). Further identification was based on the fact that LGG is unable to ferment lactose. A couple of presumptive LGG colonies from each agar plate were inoculated onto indicator-dye-containing (bromocresol purple, 0.04 g/l) MRS agar where lactose was the only carbon source. Bacteria were also investigated by phase contrast microscopy to ascertain that the cells had cell shapes typical of LGG (rods in chains). All these potential LGG isolates were further identified by polymerase chain reaction (PCR) using LGG-specific primers developed by Valio Ltd. (4). Samples for PCR were prepared by picking adding a few colonies to 200 µl of distilled water and were kept in −75°C until analyzed.

The one subject (female, 19 years) who was found LGG positive after the clearance period and was therefore excluded from the intervention trial was, however, followed up to 5 months with repeated saliva samplings. She withdrew from using LGG-containing products throughout the observation. This follow-up sampling was thought interesting because the subject had received LGG milk starting at the age of 10 for 1 year as a supportive treatment for atopic dermatitis. Thereafter she reported not having used LGG-containing products but, as it appeared, she probably had been permanently colonized in childhood.

In the intervention trial, the compliance was high and all the subjects completed the study. The following day after the intervention, 37 participants (66%) were positive for LGG. The occurrence of LGG decreased gradually and after 7 days only 3.6% of the subjects harbored the bacterium. The results are given in Fig. 1. Antibiotics did not seem to influence the results because the two subjects who had received such medication during the trial still harbored LGG after intervention. The female subject who was excluded from the trial but was still followed up for 5 months with repeated saliva samplings was positive for LGG in all the samples analyzed.

In the trial, the occurrence of LGG in the oral cavity decreased gradually, indicating that no permanent colonization had occurred and that the oral persistence of LGG was only temporary. In our pilot study (8) the oral colonization of LGG extended over 2 weeks. This over-optimistic result could now be explained by inadequate methods of analyses by the early 1990s when, for example, selective MRS agar plates had not been used and the PCR primers for LGG verification of the present study were not available.

To be able to have probiotic effects in the mouth, a bacterium must adhere to oral surfaces and become part of the biofilm (12). Probiotic lactobacilli have been demonstrated to persist in fecal samples for at most a few weeks after their administration has ended (11). However, in a later study LGG was shown to persist in the colonic mucosa even after its disappearance from fecal samples, indicating that studying feces alone may underestimate the colonization (2). In the oral cavity the situation may be the same as saliva sampling only indirectly measures the true situation in the oral biofilm. Prolonged adhesion and persistence of probiotic bacteria on mucosal surfaces could support probiotic effects. The results of the present intervention trial did not support the concept that LGG might easily colonize the oral cavity. Thus a probiotic effect would call for a continuous administration. However, as our one (excluded) case showed, permanent LGG colonization still seems possible.

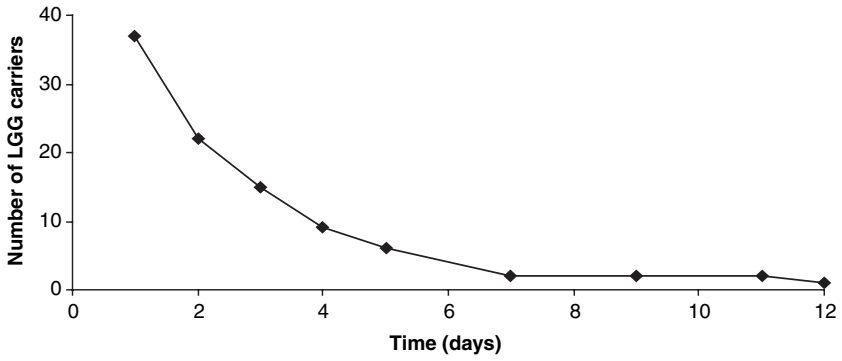

Fig. 1. Numbers of *Lactobacillus rhamnosus* GG (LGG) carriers found daily after intervention.

Acknowledgments

We thank Soile Tynkkynen from Valio Ltd. for providing LGG-specific primers. The Juho Vainio Foundation, Helsinki, Finland, is thanked for financial support for the study.

References

1. Ahola AJ, Yli-Knuuttila H, Suomalainen T, Poussa T, Ahlstrom A, Meurman JH, et al. Short-term consumption of probiotic-containing cheese and its effect on dental caries risk factors. Arch Oral Biol 2002: **47**: 799–804.
2. Alander M, Satokari R, Korpela R, Saxelin M, Vilpponen-Salmela T, Mattila-Sandholm T, et al. Persistence of colonization of human colonic mucosa by a probiotic strain, *Lactobacillus rhamnosus* GG, after oral consumption. Appl Environ Microbiol 1999: **65**: 351–354.
3. Fuller R. Probiotics in human medicine. Gut 1991: **32**: 439–442.
4. Halme T, Suoniemi A, Tynkkynen S. Strain specific identification of *Lactobacillus rhamnosus* GG and LC705 from faecal samples [abstract]. FEMS Seventh Symposium on Lactic Acid Bacteria – Genetics, Metabolism and Applications, 2002: B41.
5. Isolauri E, Kirjavainen PV, Salminen S. Probiotics – a role in the treatment of intestinal infection and inflammation. Gut 2002: **50** (Suppl. 3): III54–59.
6. Kalliomäki M, Kirjavainen P, Eerola E, Kero P, Salminen S, Isolauri E. Distinct

patterns of neonatal gut microflora in infants in whom atopy was and was not developing. J Allergy Clin Immunol 2001: **107**: 129–134.
7. Kalliomäki M, Salminen S, Poussa T, Arvilommi H, Isolauri E. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. Lancet 2003: **31**: 1869–1871.
8. Meurman J, 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.
9. Nase L, Hatakka K, Savilahti E, Saxelin M, Ponka 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**: 412–420.
10. Salminen S, Bouley C, Boutron-Ruault M-C, Cummings JH, Franck A, Gibson GR, et al. Functional food science and gastrointestinal physiology and function. Br J Nutr 1998: **80**: 147–171.
11. Saxelin M. *Lactobacillus* GG – a human probiotic strain with thorough clinical documentation. Food Rev Int 1997: **13**: 293–313.
12. Wei H, Loimaranta V, Tenovuo J, Rokka S, Syväoja E-L, Korhonen H, et al. Stability and activity of specific antibodies against *Streptococcus mutans* and *Streptococcus sobrinus* in bovine milk fermented with *Lactobacillus rhamnosus* strain GG or treated at ultra-high temperature. Oral Microbiol Immunol 2002: **17**: 9–15.