

References relevant for probiotic use of *Lactobacillus reuteri*

This clinical study summary document prepared specifically for Dental Professionals

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References relevant for probiotic use of *Lactobacillus reuteri*

Lactobacillus reuteri Protectis is the human *Lactobacillus reuteri* strain used in BioGaia Probiotics for gut and immune health. BioGaia Probiotics for oral health contains *L. reuteri* Prodentis, which is a blend of two *L. reuteri* strains. Their benefits and safety in use for all ages is supported by the clinical studies summarized below.

To date 55 clinical studies on more than 4,100 individuals have been conducted, and the results are published in 28 articles in scientific journals. Another 13 studies are published as abstracts of presentations at scientific conferences.

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Oral health

Short-term effect of chewing gums containing probiotic *Lactobacillus reuteri* on the levels of inflammatory mediators in gingival crevicular fluid.

Twetman S, Derawi B, Keller M, Ekstrand K, Yucel-Lindberg T, Stecksén-Blicks C. (2008) Acta Odontol Scand 67:19-24.

42 healthy adults with moderate gingival inflammation were recruited into a double blind, placebo-controlled randomised study. The aim was to assess the dose-effects of a probiotic chewing gum containing *Lactobacillus reuteri* Prodentis (ATCC 55730 and ATCC PTA 5289, 1×10^8 CFU of each strain). Group A/P (n=13) received one active and one placebo chewing gum daily, group A/A received 2 active chewing gums daily and group P/P received 2 placebo chewing gums daily. Study treatment was administered for 2 weeks. The degree of inflammation was assessed both as Bleeding on Probing (BOP) and amount of gingival crevicular fluid (GCF). The GCF was also assessed for concentration of the inflammatory mediators IL-1, TNF- α , IL-6, IL-8 and IL-10. All assessments were carried out on day 0 before treatment, and after 1 and 2 weeks. Follow-up assessment was made at 4 weeks (i.e. 2 weeks after treatment was stopped). The BOP was improved and the GCF volume was decreased in all study groups, but the results were statistically significant only in the two groups (A/P and A/A) receiving *Lactobacillus reuteri* Prodentis. IL-6 and IL-10 remained unaffected during the 2-week treatment period in all groups. However, in the group receiving 2 active chewing gums per day, TNF- α and IL-8 decreased significantly and at 4-week follow up, IL-6 was also significantly decreased.

Conclusion: The study provides the first indication of a significant dose-dependent effect of *Lactobacillus reuteri* Prodentis on the oral immune response and the results support previous beneficial clinical results on gingival health.

Decreased gum bleeding and reduced gingivitis by the probiotic *Lactobacillus reuteri*.

Krassé P, Carlsson B, Dahl C, Paulsson A, Nilsson Å, Sinkiewicz G. (2006) Swed Dent J. 30:55-60.

A prospective, randomised, placebo-controlled trial to study the effect on gingivitis and dental plaque of a probiotic chewing gum and the occurrence of the probiotic in saliva. The 59 subjects suffering from moderate to severe gingivitis were randomly assigned to one of three different chewing gums, containing one of two *L. reuteri* strains, LR-1 and LR-2, respectively, or a placebo gum. On Day 0 the dentist cleaned all surfaces and the patients were instructed regarding daily oral hygiene and to use the chewing gum twice daily after brushing their teeth. After 14 days the subjects visited the dentist for outcome assessments. Gingival index fell significantly in all groups. *L. reuteri* group 1, but not group 2, improved significantly more than placebo ($p < 0.0001$). Plaque index fell significantly in both *L. reuteri* groups between day 0 and 14 while there was no significant change in the placebo group. Both probiotic strains colonized the saliva, LR-1 in 65% of the patients and LR-2 in 95% of the subjects.

Conclusion: *L. reuteri* was efficacious in reducing both gingivitis and dental plaque in patients with moderate to severe gingivitis.

A probiotic lozenge administered medical device and its effect on salivary mutans streptococci and lactobacilli.

Çaglar E, Kuscu OO, Kavaloglu SC, Kuvvetli SS, Sandalli N. (2008) Int J Pediatr Dent. 18:35-39.

This was a randomised, double blind placebo-controlled clinical study involving 20 women with high counts of salivary mutans streptococci, but otherwise healthy. During the study course over 10 days, the subjects either received one lozenge daily containing either 10^8 CFU of *Lactobacillus reuteri* Prodentis (n=10) or the corresponding placebo lozenge (n=10). Both study treatments were administered via a medical device, which released the contents of the lozenge over a 15-minute period. Salivary samples were collected immediately before treatment and then again after 10 days. The samples were analysed for load of mutans streptococci and lactobacilli. Counts of lactobacilli remained unchanged in both groups during the study period. However, mutans streptococci load was significantly decreased in the group receiving *Lactobacillus reuteri* Prodentis but not in the placebo group. The decrease was significant both versus the initial load and against the placebo group.

Conclusion: The probiotic lozenge containing *Lactobacillus reuteri* Prodentis significantly reduced salivary mutans streptococci.

Effect of chewing gums containing xylitol or probiotic bacteria on salivary mutans streptococci and lactobacilli.

Çaglar E, Kavaloglu SC, Kuscu OO, Sandalli N, Holgerson PL, Twetman S. (2007) Clin Oral Invest. 11:425-429.

This was a prospective, randomised placebo-controlled study aiming to evaluate the effect of xylitol and probiotics on salivary bacilli. 80 healthy young adults aged 21-24 years of age were included and divided into 4 groups (4x20). The study products were: One probiotic chewing gum containing 1×10^8 CFU of *Lactobacillus reuteri* Prodentis per piece three times daily (Group A); 2 pieces of xylitol chewing gum three times daily (Group B); 2 xylitol gums in the morning + 2 probiotic chewing gums mid-day + 2 xylitol chewing gums in the evening (Group C) and; 3 placebo chewing gums per day. Dosing continued for three weeks and salivary samples for *Streptococcus mutans* and lactobacilli assessment were collected on day 0 before treatment start and again after three weeks. There were no differences between the groups before and after treatment regarding lactobacilli load. For Groups C and D there were no changes in strep mutans score before and after treatment. However, the strep mutans score was significantly improved in both Group A and B.

Conclusion: The results suggest that a three-week consumption of either 3 *L. reuteri* Prodentis chewing gums or 6 xylitol chewing gums can reduce the load of mutans streptococci.

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

Caglar E, Cildir SK, Ergeneli S, Sandalli N, Twetman S. (2006) Acta Odontol Scand 64:314-318.

A prospective, randomised, placebo-controlled study with the aim to investigate the effect on mutans streptococci and lactobacilli of a probiotic delivered by two non-dairy delivery systems. The study included 120 young adults (21-24 years) with overall good health, including the oral cavity status. The study used parallel arms where the subjects were randomly assigned to four equally sized groups (n=30): group A drank 200 ml of water through a prepared straw containing *Lactobacillus reuteri* once daily for 3 weeks, while group B took 200 ml of water through a placebo straw during the same period. Group C was given one tablet containing the same probiotic 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. A statistically significant reduction of the mutans streptococci levels was recorded after ingestion of the probiotic bacteria via the straw and the tablet, compared to the placebo controls. A similar but non-significant trend was seen for lactobacilli.

Conclusion: A short-term daily ingestion of *Lactobacillus reuteri* delivered by drinking straw or tablets reduced the levels of salivary mutans streptococci in young adults.

Lactobacillus reuteri in fermented bovine milk decreases the oral carriage of mutans streptococci.

Nikawa H, Makihira S, Fukushima H, Nishimura H, Ozaki Y, Ishida K, Darmawan S, Hamada T, Hara K, Matsumoto A, Takemoto T, Objectivei R. (2004) Int J Food Microbiol 95:219-223.

A study in several parts beginning with the effect of different probiotics on the growth of Streptococcus mutans (a bacterium correlated with the risk of caries). A laboratory screening of probiotic bacteria isolated from 18 different fermented dairy products available in Japan showed that L. reuteri was the only strain that inhibited the growth of S. mutans. A further laboratory study verified that L. reuteri had no harmful effect on dental enamel. A clinical study was also performed in which 40 subjects with healthy mouths took part. Half of them ingested 95 g daily of yoghurt containing L. reuteri with their lunch, while half ingested the same quantity of a placebo yoghurt. After 2 weeks the groups changed the study product and the subjects thus served as their own controls. Both groups showed a significant decrease in the number of S. mutans in the saliva during the 2-week period when ingesting L. reuteri. The group which started with L. reuteri yoghurt, also showed a significantly inhibiting effect on S. mutans in the two subsequent weeks when ingesting the placebo yoghurt.

Conclusion: The daily ingestion of yoghurt containing L. reuteri for a period of two weeks significantly reduced the number of Streptococcus mutans in the saliva of healthy subjects. This effect was consistent also for at least two weeks after ending the intake of the probiotic yoghurt. L. reuteri was the only bacterium in a laboratory screening test of probiotic bacteria from 18 fermented milk products that showed inhibition of S. mutans.

Safety aspects

Cow's milk allergic children can present sensitisation to probiotics.

Bruni FM, Piacentini GL, Peroni DG, Bodini A, Fasoli E, Boner AL. (2009) Acta Paediatr. 98: 321-323.

Study with the aim to evaluate sensitivity to probiotic products in children with cow's milk allergy. Eighty-five children with atopic dermatitis were screened for possible cow's milk allergy by skin prick test (SPT). 36/85 had a positive SPT (mean wheal diameter ≥ 3 mm) to cow's milk and were further skin prick tested with preparations of three probiotic products, in Italy sold under the brands of Fiorilac® (L. paracasei I 1688 and L. salivarius I 1794, sachet product), Dicoflor® (L. GG, sachet product) and Reuterin® (L. reuteri Protectis, oil suspension). Positive SPT was found in 26/36 subjects for Fiorilac, in two patients for Dicoflor, and in one child to Reuterin. In four children the mean diameter of the SPT wheal with Fiorilac was greater than the predictive cut-off index of positive response to oral challenge (> 6 mm for children less than 2 years old and > 8 mm for older patients). No child that was SPT-positive for Dicoflor or Reuterin had a mean wheal diameter > 6 mm. Oral challenge with the probiotic products was however not performed.

Conclusion: When considering the use of probiotic products with limited information on content of allergens, it is advisable to do a screening SPT in high risk, cow's milk allergic children to evaluate contamination with allergens.

Removal of antibiotic resistance plasmids from *Lactobacillus reuteri* ATCC 55730 and characterization of the resulting daughter strain *L. reuteri* DSM 17938.

Rosander A, Connolly E, Roos S. (2008) Appl Environ Microbiol. 74:6032-6040.

Two plasmids (= independent DNA fragments in bacteria) carrying the antibiotic resistance genes tet(W) for tetracycline and lnu(A) for lincosamide were removed from the *Lactobacillus reuteri* strain ATCC 55730 by methods that do not genetically modify the organism. The resulting daughter strain was designated *L. reuteri* DSM 17938. Direct comparison of the parent and daughter strains for a series of in vitro properties and in a human clinical trial were subsequently performed to assess the retention of probiotic properties in the daughter strain.

The clinical trial aimed to study gastrointestinal passage and safety, and included 16 healthy adult subjects. They were randomly assigned to ingest a placebo (n=4), an ATCC 55730 dose of 8×10^8 CFU/day (n=3), a DSM 17938 dose of 8×10^8 CFU/day (n=4) or a DSM 17938 high dose of 6.5×10^{10} CFU/day (n=5), for 28 days. Faecal samples were collected at baseline, on days 7, 14 and 28 and on days 42 and 56. *L. reuteri* specific clones were picked and analyzed for genetic content to identify them as *L. reuteri* ATCC 55730-like or the *L. reuteri* DSM 17938 strain. Fasting blood samples were collected at baseline and after 28 days. They were analyzed for the levels of major blood components, liver, kidney and immune functions, and major metabolic parameters. On day 28 a general bacterial analysis of the blood was also done, to detect the possible transfer of any bacteria into the blood. General health examinations revealed no changes in weight, pulse, blood pressure or body temperature in any group during the supplementation period. Blood safety and metabolic parameters were also unchanged in all groups. Blood samples taken directly after supplementation on day 28 were negative for bacteraemia.

Conclusions: Removal of two plasmids from the *L. reuteri* ATCC 55730 strain resulted in a daughter strain designated *L. reuteri* DSM 17938. Strain comparisons by vitro tests and the clinical trial confirmed that DSM 17938 has retained the probiotic properties of ATCC 55730, is well tolerated and safe to ingest in doses up to 6.5×10^{10} CFU/day. *L. reuteri* DSM 17938 survives passage through the human GI tract to the same extent as ATCC 55730. After the 2-week washout period there was no detection of any *L. reuteri* strain in the supplemented individuals.

Safety and tolerance of a probiotic formula in early infancy comparing two probiotic agents: a pilot study.

Weizman Z, Alsheik A. (2006) J Am Coll Nutr 25:415-419.

This was a prospective, randomised, double blind and placebo-controlled study with the objective to evaluate the safety of two probiotic strains in healthy full-term infants. The 59 infants were 3-65 days old at enrolment and for four weeks they were given humanized cow's milk formula (control group, n=19), the same formula with *Bifidobacterium lactis* Bb-12 (n=20) or *L. reuteri* Protectis (n=20). Breast-feeding had ceased prior to inclusion in the study (parents' own decision). The mean daily intake of each of the probiotic strains was 1.2×10^9 CFU. Each participant underwent a physical examination at baseline and at four weeks. The parents filled out a daily questionnaire for seven days during the first and fourth week of the trial for documentation of feeding, behaviour and stools' characteristics. The parents were also instructed to report daily on every complaint or symptom. No clinical adverse effects were seen during the trial and the growth, feeding, behaviour and stools' characteristics were the same in the three groups.

Conclusion: Both probiotic strains were well tolerated during the four weeks study in these very young infants and no clinical adverse effects were seen.

Safety of D(-)-lactic acid producing bacteria in the human infant.

Connolly E, Abrahamsson T, Björkstén B. (2005) J Pediatr Gastroenterol Nutr 41:489-492.

Lactobacillus reuteri is one of many species of Lactobacillus known to produce both L(+)-lactic acid and D(-)-lactic acid through normal sugar fermentation. As part of a prospective study of decreasing the risk of allergy during the first years of life, a safety investigation was performed of the levels of D(-)-lactic acid in the blood at the age of 6 and 12 months. A sample group of 24 infants were randomly chosen from the total study population of 232 infants. They had been supplemented with L. reuteri Protectis or placebo for twelve months from birth. The daily dose of L. reuteri Protectis was 10^8 CFU, suspended in oil. 14 infants were supplemented with L. reuteri Protectis and 10 with placebo. All 24 infants had very low levels of D(-) lactic acid (range 0.020 – 0.130 mmol/L), and there was no difference between the infants ingesting L. reuteri Protectis and those receiving placebo. The highest level observed was well within the normal range seen in humans (0.020 – 0.250 mmol/L) and far below levels associated with D-lactic acidosis in humans (> 3 mmol/L). No symptoms were reported that would normally be associated with acidosis, and there were no safety problems in any of the participating children.

Conclusion: The daily supplementation of L. reuteri Protectis to healthy newborns during their first 12 months was safe, also in regard to the levels of D(-)-lactic acid in the blood.

Safety and possible antidiarrhoeal effect of the probiotic *Lactobacillus reuteri* after oral administration to neonates.

Karvonen A, Casas I, Vesikari T. (2001) Clin Nutr 20(suppl 3):63: abstract 216.

A randomised, double blind, placebo-controlled study on healthy newborn, full-term children. The aim was to study safety aspects following the daily intake of *L. reuteri* Protectis given from the day of birth and the following 28 days. Four groups of children were studied: *L. reuteri* Protectis in the doses 10^5 CFU/day (n=12), 10^7 CFU/day (n=25) or 10^9 CFU/day (n=25), and placebo (n=28). All dose levels of *L. reuteri* Protectis were well tolerated. The degree of *L. reuteri* Protectis colonization, measured as the number of living cells in stool samples, was related to the given dose. The occurrence of watery diarrhoea was significantly lower in children given *L. reuteri* Protectis.

Conclusion: *L. reuteri* Protectis was safe to consume for healthy full-term newborns during their first four weeks of life, in doses up to 10^9 CFU/day.

Safety and tolerance of *Lactobacillus reuteri* supplementation to a population infected with the Human Immunodeficiency Virus.

Wolf BW et al. (1998) Food Chem Toxicol. 36:1085-1094.

A prospective, double blind, placebo-controlled study in which 39 HIV-positive subjects (including 2 women), aged 23–50, consumed *L. reuteri* Protectis in a daily dose of 10^{10} CFU, or placebo, for 21 days. No significant differences could be seen in any of the parameters monitored (analyses of blood, serum and urine) or in regard to tolerance of *L. reuteri* Protectis intake. Colonization of *L. reuteri* Protectis in the active group was verified.

Conclusion: The daily consumption of *L. reuteri* Protectis at the dosage of 10^{10} CFU for 21 days was shown to be safe and without any side effects for adults infected with HIV.

Tolerance and fecal colonization with *Lactobacillus reuteri* in children fed a beverage with a mixture of *Lactobacillus* spp.

Ruiz-Palacios G et al. (1996) *Pediatr Res* 39(4) part 2:184A, abstract 1090.

A double blind, randomised and placebo-controlled safety study on 72 healthy children, aged 12-36 months, living in Mexico City. For three weeks *L. reuteri* Protectis was administered in a nutritional beverage containing a probiotic blend of three strains. The children were randomised into four groups: three groups given the probiotic beverage, which differed in the dosage of *L. reuteri* Protectis: 10^6 , 10^8 and 10^{10} CFU, respectively, and a control group consuming a placebo beverage. All dose levels of *L. reuteri* Protectis were well tolerated and no side effects were noted.

Conclusion: The daily consumption of *L. reuteri* Protectis, in doses up to 10^{10} CFU during three weeks, was shown to be safe for healthy children aged 12-36.

Safety and tolerance of *Lactobacillus reuteri* in healthy adult male subjects.

Wolf BW et al. (1995) Microb Ecol Health Dis. 8:41-50.

A prospective, double blind, placebo-controlled study in which 30 healthy males, aged 18–75, consumed *L. reuteri* Protectis in a daily dose of 10^{11} CFU, or placebo, for 21 days. The subjects made a daily note of any symptoms from the gastrointestinal tract, and samples were taken on days 0, 7, 14, 21 and 28 for analysis of serum, blood, urine and stools. *L. reuteri* Protectis colonized the subjects in the active group within one week, as shown by a significantly higher amount of *L. reuteri* Protectis in their stools compared with the control group. The colonization persisted for at least one week after intake had stopped, apart from in one individual, who remained colonized for up to 2 months after the end of the *L. reuteri* Protectis intake period. A tendency was observed for slightly increased, though transient, gas formation in a few subjects taking *L. reuteri* Protectis. No significant differences were shown in regard to blood and urine analyses or in regard to tolerance of bacterial intake.

Conclusion: The daily consumption of *L. reuteri* Protectis at the dosage of 10^{11} CFU for 21 days was shown to be safe and without any side effects for healthy male adults.

Colonization

Occurrence of *Lactobacillus reuteri* in human breast milk.

Sinkiewicz G, Ljunggren L. (2008) *Microb Ecol Health Dis.* 20:122-126.

This study investigated the presence of total lactobacilli and *Lactobacillus reuteri* in milk from 220 lactating mothers, 6-32 days after delivery. The women were living in urban or rural areas in Sweden, Denmark, Israel, South Africa, South Korea, Japan and Peru. In all, 50% of mothers from rural areas in Japan and Sweden were *L. reuteri*-positive, whereas mothers from urban areas in South Africa, Israel and Denmark had very low or non-detectable levels. Overall, 15% of mothers had detectable *L. reuteri* in their milk. There were no significant differences in the prevalence of total *Lactobacillus* or *L. reuteri* in women from rural and urban habitats in the participating countries.

Conclusion: This study suggests that *L. reuteri* is a natural component of human milk. It was found in about one in seven of nursing mothers living in countries geographically widely apart.

Intestinal microbiota in infants supplemented with the probiotic bacterium *Lactobacillus reuteri*.

Abrahamsson T, Jakobsson T, Sinkiewicz G, Fredriksson M, Björkstén B (2005) J Pediatr Gastroenterol Nutr 40(5):692, abstract PN 1-17.

As part of a prospective study of decreasing the risk of allergy during the first years of life, the colonization rate of *Lactobacillus reuteri* in the enrolled infants was also investigated. 232 mothers and their infants were recruited in the main study. The mothers were randomised to ingest *Lactobacillus reuteri* (1×10^8 CFU) or placebo during 4 weeks until delivery. After delivery their babies ingested the supplement in the same dose up to 12 months of age. At day 5-6, 80% of the infants were colonized in the *L. reuteri* group compared to 19% in the placebo group, measured as live cells in the stools. At 12 months of age, 63% and 23% of the infants, *L. reuteri* vs. placebo, respectively, were colonized. The first expressed breast milk (colostrum) was positive for *L. reuteri* in 12% and 2% of the mothers in the *L. reuteri* and placebo group, respectively.

Conclusion: The gastrointestinal tracts of infants from birth up to one year of age, were colonized in a high degree by *L. reuteri* after long-term supplementation.

The Lactobacillus and Bifidobacterium microflora of the human intestine: composition and succession.

Reuter G. (2001) Curr Issues Intest Microbiol. 2:43-53.

An article describing how *L. reuteri* has been isolated in living form from every part of the digestive tract: the oral cavity, the stomach, the small intestine, the colon and from faeces. *L. reuteri* has also been isolated from the vagina. Professor Reuter puts forward evidence that *L. reuteri* is a bacterium that belongs to the indigenous intestinal flora, i.e. has the digestive tract as its natural ecological niche and establishes itself naturally in the newborn child. Only a few other probiotic bacteria can be described as belonging to the natural, indigenous intestinal flora.

Colonization of the human gastrointestinal tract by the lactic acid bacteria *Lactobacillus reuteri*.

Björkman P. (1999) M.Sc. thesis, Dept. of Food Technology, University of Helsinki, Finland.

This is an open study with the objective to study the colonization of the colon by *Lactobacillus reuteri*, in ten voluntary adult patients undergoing a colonoscopy examination. *L. reuteri* was ingested at a dosage of 10^9 CFU/day, as yoghurt (5 patients) or as probiotic fruit juice (5 patients) for 12 days before the colonoscopy. Faecal samples were analyzed before and after 12 days of consumption of the probiotic foods. Biopsies from all three parts of the colon were sampled on day 12. Identification methods for *L. reuteri* were based on biochemical properties and a gene based method (RiboPrint system). No subject was colonized with *L. reuteri* before the start of the study. Live *L. reuteri* cells were found on day 12 in faecal samples from 3/5 subjects in the yoghurt group and in all five in the juice group. *L. reuteri* was identified at the strain level in the colon biopsies from one subject.

Conclusion: The daily consumption of *L. reuteri* in yoghurt or fruit juice for 12 days resulted in successful colonization of 80% of the study subjects, measured as live bacteria cells in faecal samples. One of the subjects also had the administered *L. reuteri* strain cultivated from the colon biopsies taken.