

LGG•Summatim

Lactobacillus GG and its health effects



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Second, updated edition

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Published by

Valio Ltd, R&D

P.O. Box 30,

FIN-00039

Helsinki, Finland

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Fax +358 10 381 3019

<http://www.valio.com>

Lay-out by

Imageneering

Printed in Finland by

Hämeen Kirjapaino Oy

2002

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Introduction

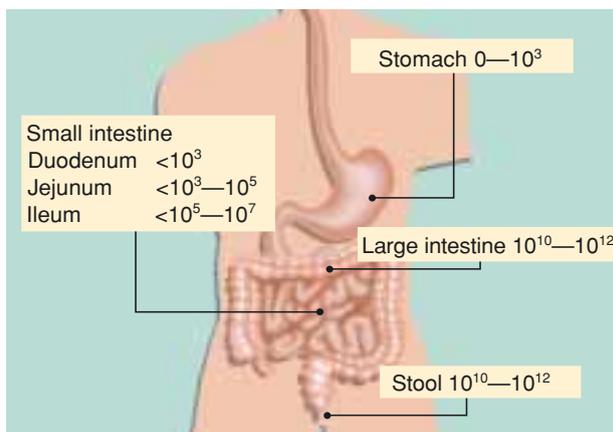


Figure 1. Bacterial levels in different sections of the gastrointestinal tract (cfu/g).

Bacterial concentrations at different sites of the gastrointestinal tract vary greatly (Fig. 1). The mucous membrane of the mouth and the surfaces of the teeth have high concentrations of bacteria, which pass, with saliva and chewed food, into the oesophagus and thereafter into the stomach, where the food is mixed with gastric juices and fluidised. The acidity of the gastric juice effectively destroys most of the bacteria that come into contact with it. Food stays in the stomach for around four hours and is gradually released into the small intestine.

The proximal part of the small intestine is also acidic due to the acid entering from the stomach. In addition, bile acids secreted into the proximal part of the small intestine destroy bacteria, so the bacteria level is relatively low. As acidity decreases and the bile acids are diluted, the bacteria level in the terminal part of the small intestine rises. The small intestine, several metres long, is densely proliferated with microvilli, which increase the internal surface area of the mucous membrane so much so that, if it were spread out, the small intestine would cover the area of a tennis court. The large surface area enables the efficient breakdown of food and the subsequent absorption of nutrients through the mucous membrane into

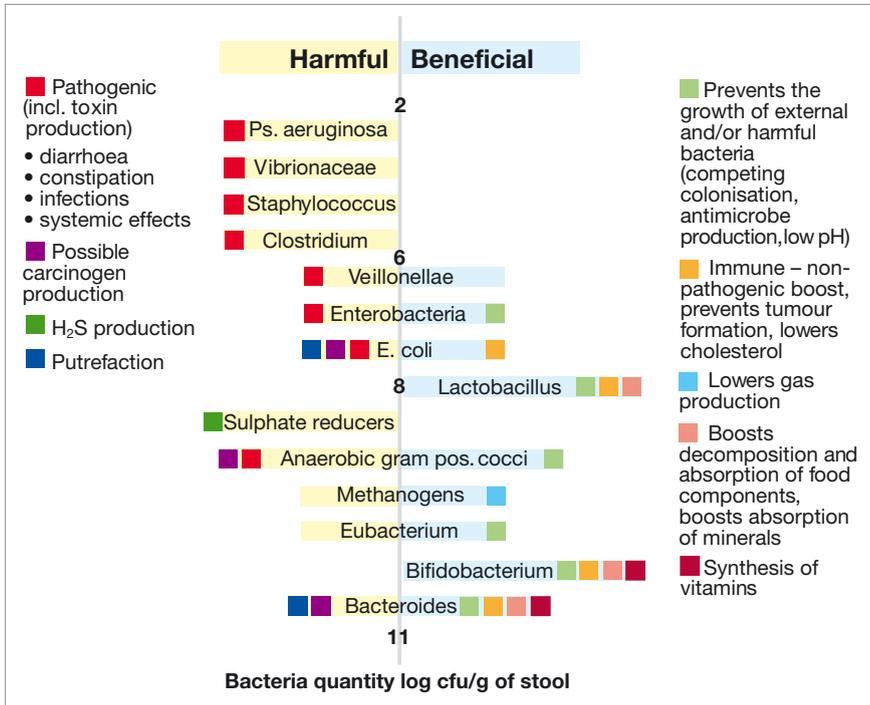


Figure 2. The most important microbe groups, their quantities, and rough division according to their potential for harmful and beneficial effects (1).

the blood stream. Most of the system's immunological tissue is connected with the small intestine and can be found immediately under the epithelial cells of the mucous membrane.

The digestive tract pushes food and chyme forward by powerful peristaltic contractions. Moving from the small intestine to the large intestine, peristalsis slows down and sodium and chloride ions are absorbed with water into the blood stream. As a result, the contents of the bowel become more solid. At the same time the bacteria level also rises very sharply. The large intestine has an extensive bacterial metabolism. Bacteria break down the nutrients remaining in the food, such as partially digested proteins and fibre components. Around half of the bulk of stools consists of bacterial mass. Between 400 and 500 species of bacteria have been rec-

Genus	Species	Strain
<i>Lactobacillus</i>	<i>L. rhamnosus</i>	<i>L. rhamnosus</i> GG
<ul style="list-style-type: none"> • Around 60 <i>Lactobacillus</i> species 	<ul style="list-style-type: none"> • Group of <i>L. rhamnosus</i> strains in whose total genome DNA-DNA homology is >70% 	<ul style="list-style-type: none"> • Can be distinguished from other strains of the same species by phenotype or genotype methods
Genus characteristics <ul style="list-style-type: none"> - gram-positive - rod - in chains - homo- or hetero-fermentative - catalase-negative - G+C% 33–55 	Species characteristics <ul style="list-style-type: none"> - common morphology - similar biochemical characteristics (within limits) - G+C% 45–47 	Strain characteristics <ul style="list-style-type: none"> - typical fermentation profile (API50CH) - no plasmids - genome analysis - probiotic characteristics: adhesion, colonisation, immunological effects etc.

G+C%= the proportion of guanine and cytosine in DNA

Table 1. The common and distinguishing characteristics of bacteria genera, species and strains using the *Lactobacillus rhamnosus* GG strain as an example. The lactic acid bacteria include 15 bacteria genera for which either homofermentative or heterofermentative lactic acid production is typical. They are gram-positive cocci, rods or coccobacilli which are non-spore forming and do not form catalase. *Lactobacillus* is one of the genera forming the lactic acid bacteria group.

ognised in the large intestine and in stools. Moreover, between 100 and 1,000 times more anaerobic bacteria are present than aerobic. Genome-based research methods have shown that human intestines have numerous, though as yet unidentified, species of bacteria, which do not grow in the culture media currently in use. Fig. 2 presents the most common bacteria genera or groups and their main influence on the bacterial metabolism of the bowel.

Lactobacilli are part of the normal intestinal flora. They can be found in the stomach and in the proximal part of the small intestine, because lactobacilli are species that tolerate acidity relatively well. The most common species recognised on the mucous membrane of the bowel are the *Lactobacillus acidophilus* group (*L. acidophilus*, *L. gasseri*, *L. jenseni*, *L. crispatus*), *L. casei*, *L. paracasei*, *L. rhamnosus*, *L. agilis*, *L. salivarius*, *L. plantarum*, *L. pseudoplantarum*, *L. buchneri* and *L. reuteri*. It has not been possible to identify all intestinal *Lactobacillus* species (2, 3). *Lactobacillus rhamnosus* GG (ATCC 53103), or more briefly *Lactobacillus* GG or

LGG, is a probiotic strain that has been isolated from a healthy human intestinal flora. Its probiotic effects on human well-being have been widely researched and documented in scientific journals. The term probiotic means live microorganisms which, when administered in adequate amounts, confer health benefits on the host (4). A probiotic must always be a certain bacterial strain (cf. Table 1) or a combination of known strains whose composition remains stable and whose effects have been demonstrated in studies performed on humans and documented in scientific journals. There is a great deal of research data about *Lactobacillus* GG in the care and preventive treatment of different intestinal symptoms. This summary describes the effects of *Lactobacillus* GG in healthy intestines, its known clinical uses, and the mechanisms underlying these effects.

LGG in healthy intestines

1.1 Colonises temporarily

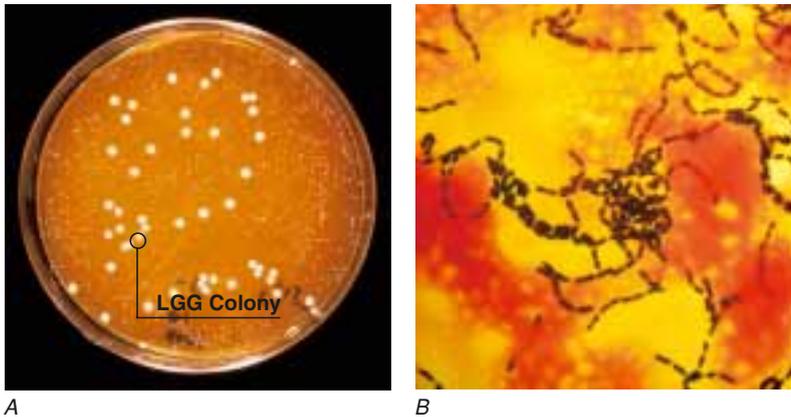


Figure 3. A) *Lactobacillus GG* colonies among other stool lactobacilli flora in an MRS-agar dish. **B)** *Lactobacillus GG* bacteria (light microscopy, gram staining).

In healthy intestines so-called colonisation resistance prevails. This naturally prevents exogenous microbes, both harmful and harmless to the intestine, from establishing themselves permanently in the digestive tract. Colonisation resistance depends on chemical (e.g. gastric acid, bile acids, enzymes), physical-biological (adhesion, prevention/elimination of harmful bacteria, peristalsis) and immunological factors. Anaerobic bacteria in particular are involved in maintaining colonisation resistance. Resistance easily breaks down, for example, as a consequence of antibiotic or other medical treatment, and this can cause diarrhoea and other intestinal disorders.

Lactobacillus GG tolerates intestinal conditions, such as stomach acidity and bile acids, better than ordinary yoghurt bacteria (5). It adheres both to the intestinal

mucus (6-8) and to epithelial cells and tissues *in vitro* (9) and *ex vivo* (10, 11). *Lactobacillus* GG also produces antimicrobial material (12 - 14). Because of its typical colony morphology and other characteristic features, it is possible to analyse *Lactobacillus* GG from stool and biopsy samples, even though these contain a great many other lactobacilli (Fig. 3). In addition to the colony morphology genetic recognition of the strain is needed to confirm its identity (15). The capacity of *Lactobacillus* GG to stay alive within the digestive tract has been shown in many studies both in healthy people and in cases of illness (5, 16 - 19).

The attachment of *Lactobacillus* GG to the mucous membrane of the intestine has been shown by taking biopsy samples from the surface of the large intestine and by identifying *Lactobacillus* GG in them (20, 21). These studies demonstrated that the *Lactobacillus* GG strain adheres temporarily to the mucous membrane and stays there for about a week. The colonisation is not permanent because *Lactobacillus* GG triggers an immune response in the mucous membrane, which prevents permanent colonisation (22, 23). *Lactobacillus* GG, given immediately after birth, was still present in stools in approximately half of premature (24) and full-term (25) babies 2-4 weeks after the dosage ended. Administration of *Lactobacillus* GG to mothers at the time of delivery yielded a long-lasting colonisation (26). Thus, the permanent colonisation of the intestines of newly born babies may be possible.

1.2 Adapts to healthy intestinal flora

The composition of human intestinal microflora appears fairly constant. Conventional culture methods only measure bacterial groups or genera and it is evidently not yet possible to cultivate some of the intestinal bacteria. Changes in the composition of healthy intestinal microflora may occur on a species and strain level and cannot be measured by conventional culture methods (27).

Lactobacillus GG enhanced the adhesion of bifidobacteria *in vitro* (28). Milk products fermented with *Lactobacillus* GG, or *Lactobacillus* GG given in powder form, have been shown either to increase significantly the quantity of bifidobacteria and lactobacilli (29, 30) or else showed no changes in the composition of the flora (5, 31). However, in a recent study using the FISH method, the supple-

mentation of *Lactobacillus* GG capsules increased the level of total anaerobic flora, especially bifidobacteria, bacteroides and clostridia (32), but the level of lactobacilli/enterococci did not increase. Although *Lactobacillus* GG becomes part of the bowel's microbial flora, it does not displace all other lactobacilli. The relative proportion varies from individual to individual but it usually accounts for less than a quarter of the total quantity of lactobacilli (33, 34). However, the overall proportion of *Lactobacillus* GG may be greater on the mucous membrane of the intestine (20, 21).

1.3 Improves colonisation resistance

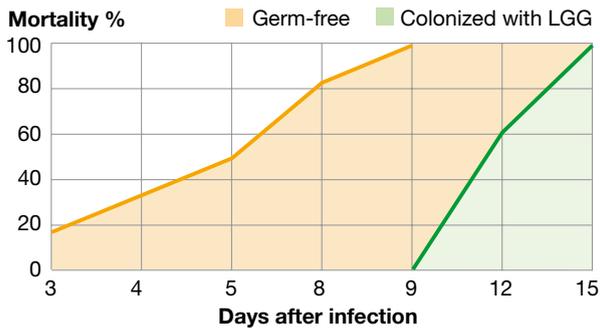


Figure 4. The mortality of mice with germ-free intestines vs. *Lactobacillus* GG colonised intestines following infection with *Salmonella typhimurium* (41).

In addition to the prevention of the adhesion and the colonisation of pathogens, colonisation resistance also means that intestinal bacteria are not translocated to the blood circulation and other sterile body sites. Animal experiments have shown that the addition of *Lactobacillus* GG to animal feed improves colonisation resistance and protects the intestine from harmful bacteria. *Salmonella* levels were considerably lower in the intestines of mice that received *Lactobacillus* GG than in the placebo group. Furthermore, the life spans of *Salmonella*-infected ex-germ-free mice were considerably extended by *Lactobacillus* GG (Fig. 4). *Lactobacillus* GG also protected the mice from *Candida albicans* infection, reduced the growth of

yeast and prolonged the life of the mice. The protective influence was based on both immunological and non-immunological factors (35, 36). *Lactobacillus* GG was also shown to prevent the attachment of *Clostridium difficile* onto the wall of hamsters' intestines and, in combination with xylitol, to protect hamsters from death caused by *C. difficile* (37).

Lethally irradiated mice died of bacteraemia of intestinal origin, but no cases of lactobacilli or *Lactobacillus* GG bacteraemia were observed. Rather, oral *Lactobacillus* GG intake was reported to prolong the survival of the mice (38). The influence of different probiotics on the extent of liver injury, bacterial translocation and intestinal flora in an acute liver injury model with rats was studied (39). The liver injury was induced by intraperitoneal injection of the rats with D-galactosamine. The bacteria were administered rectally eight days before the liver injury. *Lactobacillus* GG, which was one of the studied strains, reduced significantly the bacterial translocation to portal and arterial blood, and the liver and mesenteric lymph nodes. The liver injury, measured as alanine aminotransferase, was less serious in the *Lactobacillus* GG group compared to the control group (39). In another experimental study, liver injury was caused by chronic alcohol consumption in rats. The blood of the rats had a lower level of endotoxin and a less injured liver when they received *Lactobacillus* GG in their diet (40).

In a study with mice, the translocation rate of *Salmonella* to several organs was significantly reduced by the administration of *Lactobacillus* GG (41). In a study with neonatal rabbits, *Lactobacillus* GG was shown to significantly reduce small-bowel colonisation by *Escherichia coli*. It also reduced the frequency of intestinal bacterial translocation in the mesenteric lymph nodes and in the spleen (42).

In vitro studies support the theory of improvement of colonisation resistance by *Lactobacillus* GG. Although adhesion of *Salmonella typhimurium* on intestinal mucus was enhanced by *Lactobacillus* GG (43), the invasion to the cells was reduced (41). Furthermore, *Lactobacillus* GG reduced the adhesion of enteropathogenic *Escherichia coli* on intestinal mucus (43) and on intestinal cells (44). The translocation of *E. coli* through Caco-2 enterocyte monolayer was also reduced by pre-incubation of the monolayer with the probiotic (45). These results show that *Lactobacillus* GG is not an invasive organism. It strengthens the barrier mechanisms in the intestine either directly or via the modification of intestinal microecology.

1.4 Reduces harmful metabolism in the colon

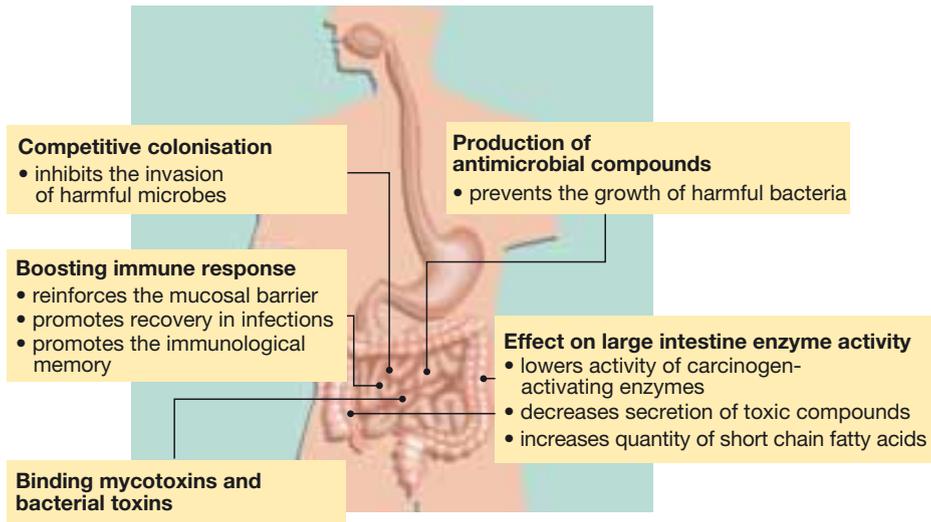


Figure 5. *The effects of Lactobacillus GG on intestinal microecology.*

Intestinal bacteria break down the components of food into a more easily digestible form, affect the local immune response of the mucous membrane and promote colonisation resistance against pathogens. The intestinal microflora have also been shown to participate in the metabolism of harmful compounds in the human diet and in breaking down drugs as well as toxins (46). The Western diet, with its high fat and low fibre content, supposedly increases the risk of colon cancer. Colonic microflora have been shown to be linked to this risk. The hydrolytic enzymes of the bacteria change pre-carcinogenic compounds in food into carcinogenic compounds. Epidemiological studies have demonstrated that a high consumption of fermented milk products may reduce the risk of colon cancer. Those in the risk group had less lactic acid bacteria in their intestines than those in the low-risk group (47). The mechanism may be the protective effect of calcium or conjugated linolic acid,

the low activity of hydrolytic enzymes in lactic acid bacteria (48), and the effect of lowering the pH of bowel contents. A diet containing *Lactobacillus* GG-fermented milk has been shown to lower the activity of hydrolytic enzymes (β -glucuronidase, glycocholic acid hydrolase, nitroreductase) and tryptic activity in the colon contents, and the urinary secretion of toxic compounds. Some of these studies have also found a lowering of the pH of stools and a decrease in the amount of ammonia (29, 30, 49-52). All these factors together (Fig. 5) suggest that *Lactobacillus* GG, and particularly the fermented milk products that contain it, change the bowel contents so as to lower the risk of tumour formation.

Further support for the idea has been obtained from experimental studies. In one study (53) intestinal tumours were chemically induced in rats that were being fed on a high-fat diet. When the rats' diet contained *Lactobacillus* GG, significantly fewer tumours formed in their large intestines, and the number of tumours per tumour-bearing rat was significantly lower than those in the placebo group (Fig. 6). This work showed that the initiation of tumour formation can be reduced or delayed by *Lactobacillus* GG, but that lactobacilli have no effect on the advance of tumours that have already begun (53).

In another experimental study, bladder cancer cells were transferred to mice and the effect of oral administration of *Lactobacillus* GG on the development of tumour formations was studied (54). The administration of *Lactobacillus* GG, or saline as a placebo, was started immediately after implantation of the tumour cells or one week later. Early administration of *Lactobacillus* GG reduced the size of the tumours significantly or totally inhibited their formation. The levels of spleen T-lymphocytes (CD3, CD4, CD8a) and natural killer cells were significantly higher in the *Lactobacillus* GG group compared to the placebo group. The levels of lymphocytes and granulocytes were also higher in the tumours of the animals in the *Lactobacillus* GG group. The conclusion was that *Lactobacillus* GG may inhibit the growth of tumours via an immune response (54).

Aflatoxins (AF's) are a group of structurally similar toxins produced by the common moulds *Aspergillus flavus* and *A. nomius*. The toxins are potentially carcinogenic and harmful in food and feed. They can be produced in conditions conducive to the growth of the mould. The risk of the growth of fungus is higher in conditions with high relative humidity and temperature, and without competing

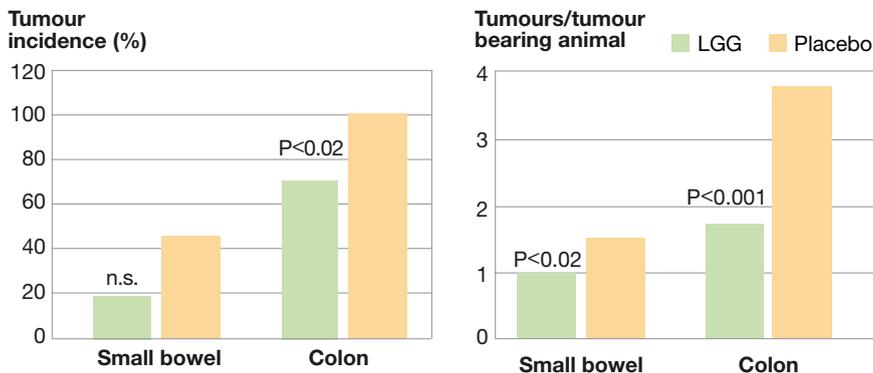


Figure 6. The effect of *Lactobacillus GG* on the formation of chemically induced tumours in rats (53).

microflora. In laboratory tests *Lactobacillus GG* has been shown to bind AFB₁ (55, 56) and AFM₁ (57). The binding of AF's seems to be mainly extra-cellular and stable, removing about 80% of the AFB₁ from a liquid growth medium (58, 59). AFM₁ is the main form found in the milk of lactating animals, indicating the contamination of feed by AFB₁. Pierides et al (57) demonstrated that lactobacilli and lactococci can potentially be used for the binding and removal of aflatoxin M₁ from milk.

In an *in vivo* study AFB₁ was injected with *Lactobacillus GG* bacteria into chicken duodena, and the concentration of AFB₁ in the luminal fluid and tissues of sacrificed animals was analysed after one minute. Half the toxin concentration was removed from the luminal fluid. The complex was stable under the luminal conditions for a one-hour test period and it reduced the uptake by the intestinal tissue by 74% (60). Based on these studies, it would seem possible to remove AF's from the intestine by *Lactobacillus GG* on a significant level and to reduce the toxic load of the intestine via excreted bacteria.

Most alcohol is metabolised in the liver but recently the role of oro-gastrointestinal bacteria has also been realised. Many intestinal facultative anaerobic and aerobic bacteria can oxidise ethanol to acetaldehyde, which itself is harmful to the mucous membrane. Nosova et al. (61) studied the capacity of intestinal bifidobacteria, lactobacilli and *Lactobacillus GG* to oxidise ethanol to acetaldehyde. In general, lactobacilli had weak oxidation potential, the most active being *Lactobacillus*

GG in anaerobic conditions. It also had the highest ability to degrade acetaldehyde to acetate. The degradation of acetaldehyde by bacteria was generally inhibited by ethanol, but *Lactobacillus* GG was not very sensitive to that chemical. The circumstances on the colon mucous membrane to oxidise acetaldehyde are favourable but the level of probiotics should be fairly high, and the relevance of the results remains to be shown in human trials.

1.5 Does it alleviate constipation?

Lactic acid bacteria are generally considered to alleviate constipation. However, there is little clinical proof of their efficacy in severe constipation, and many studies have been conducted without good research procedures or statistical analysis. *Lactobacillus* GG-fermented milk products have been seen to slightly increase the water content of stools but they have had no effect on the frequency in defecation of healthy volunteers (29, 51), nor did they have any effect on transit time in those suffering from constipation (62). However, *Lactobacillus* GG enhanced the laxative effects of rye fibre and had a tendency to reduce intestinal symptoms caused by the fibre (62). Suggestions of an increase in bowel activity were obtained in one *Lactobacillus* GG study with and without lactulose (63).

In Japanese studies, the daily consumption of an *Lactobacillus* GG-fermented milk product was shown to significantly increase the level and ratio of faecal bifidobacteria and lactobacilli and to reduce the level of lecithinase negative clostridia. The consumption of the product also increased significantly defecation frequency and relieved discomfort after the bowel movement. There was a tendency to increase the faecal moisture and decrease pH and ammonia content (30, 64). These results indicate that not all studies are necessarily applicable to other cultures with a totally different diet composition from that of the West.

LGG and immune response

The innate and adaptive immune systems are the two compartments traditionally described as important for the immune response. Macrophages, neutrophils, natural killer (NK) cells and a serum complement represent the main components of the innate system, in charge of the first line of defence against many microorganisms. However, there are many agents that this system is unable to recognize. The adaptive system (B and T cells) provides an additional means of defence, while cells of the innate system modulate the beginning and subsequent direction of adaptive immune responses. Several soluble compounds (cytokines, interleukins, interferons) are involved in the modulation of the immune system.

In vitro *Lactobacillus* GG induced the expression and production of the proinflammatory Th-1-type cytokines TNF- α , IL-1 β , IL-6 and IL-18 in peripheral blood mononuclear cells, but not the Th-2 type cytokine IL-4 and relatively little IL-10 (65, 66). *Lactobacillus* GG also activated the transcription factor NF- κ B, which is the central activator of innate immune response, and the Toll-like receptors TLR1 and TLR2, which mediate bacterial recognition and cellular signalling (67, 68). The results suggest that *Lactobacillus* GG is able to activate innate immune responses.

In an animal study, orally administered *Lactobacillus* GG bacteria had dose- and duration-dependent immunomodulatory effects on the proliferative activity of B and T murine spleen lymphocytes *ex vivo*. A dose relevant to human nutrition enhanced T-cell proliferation at the optimal concanavalin A concentration and B-cell proliferation at the optimal and supraoptimal concentrations of lipopolysaccharide (69). In a human intervention, *Lactobacillus* GG enhanced significantly the formation of the phagocytic receptors CR1, CR3, Fc γ RIII and Fc α R in neutrophil blood cells in healthy humans but suppressed the response of milk-hypersensitive human volunteers during a milk challenge. The conclusion was that probiotic bacteria appear to modulate the non-specific immune response differently in healthy subjects and hypersensitive subjects by immunostimulation in healthy and by down-regulation in hypersensitive ones (70).

Human administration of *Lactobacillus* GG combined with an oral rotavirus vaccine enhanced the formation of rotavirus specific IgM-secreting cells and rotavirus specific IgA in sera (71). There was also a trend towards a greater increase in antigen-specific IgA response when *Lactobacillus* GG was given with an oral *Salmonella typhi* Ty21 vaccine (72). In another study with *Salmonella typhi* vaccine, *Lactobacillus* GG enhanced significantly the IgG and IgA response to the vaccine (73). In children with rotavirus infection, *Lactobacillus* GG increased the formation of immunoglobulin secreting cells in all immunoglobulin classes and in rotavirus specific antibody-secreting IgA cells (74-76).

These studies show that *Lactobacillus* GG both activates the innate immune response and enhances adaptive immunity, especially during infections.

LGG and healthy children

3.1 Respiratory infections

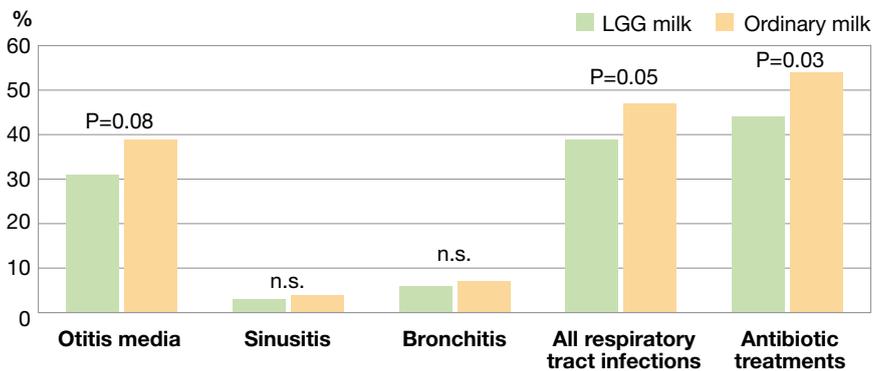


Figure 7. The effect of *Lactobacillus GG* on the prevalence of respiratory infections and frequency of antibiotic treatment in children. The children drank either LGG milk or ordinary milk during daily meals for a period of seven months (77).

Day care centres expose children to infections, especially of the upper respiratory tract. Overall, more than 90% of child absenteeism from day care is caused by infectious diseases. In addition to discomfort to the children and inconvenience to their families, illnesses are costly to society. The greatest costs result from the parents' absence from work because of a child's illness.

A long-term study was made to see if consumption of *Lactobacillus GG* had an effect on infections in children (77). A total of 571 children from 18 day care centres in Helsinki, Finland, participated in the study. During the seven-month research period, half the children were given pasteurised milk that contained *Lactobacillus GG* ($5\text{-}10 \times 10^5$ cfu/ml) to drink with all meals, and the other half were given ordinary milk. The average milk consumption was 260 ml/day. The children's health was carefully monitored: symptoms in the respiratory and digestive tract, as well as

absences from the day care centres, were recorded daily by the parents. Doctors' diagnoses and antibiotic treatments were also reported. Children in the *Lactobacillus* GG group had fewer days of absence from day care because of illness (4.9 vs. 5.8 days, $p=0.03$), an 11% difference. There was also a relative reduction of 17% in the number of children who suffered from respiratory tract infections with complications, especially ear infections, in the *Lactobacillus* GG group (Fig. 7). The number of children who received antibiotic treatment for respiratory infections was 19% lower in the *Lactobacillus* GG group than in the placebo group. The conclusion was that *Lactobacillus* GG may reduce children's respiratory infections and their severity.

3.2 Oral health

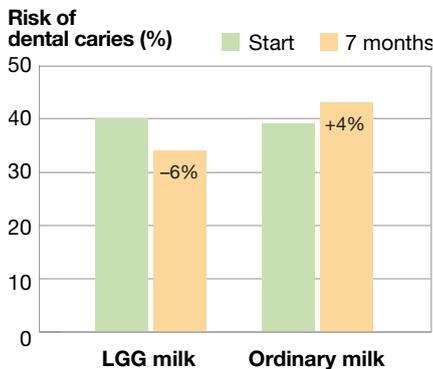


Figure 8. The effect of *Lactobacillus* GG on the risk of dental caries. The children drank either LGG milk or ordinary milk during daily meals for a period of seven months (79).

Teeth are in continuous interaction with the surrounding world, mainly saliva and whatever you put in your mouth. Milk provides calcium and phosphates in the mouth, which causes remineralisation of places demineralised by caries. Milk and dairy products are important elements in children's nutrition and dental health,

since teeth at this point are particularly vulnerable to attack from caries, having just begun the mineralisation process. Lactobacilli are common bacteria in the oral cavity, but they are generally regarded as potentially cariogenic, growing together with *streptococcus mutans*. However, in *in vitro* studies, *Lactobacillus* GG showed slow or no fermentation of sucrose and lactose (34), and suppressed the growth of the *mutans*-group streptococci, which are the indicator bacteria of dental caries (78).

The long-term effect of *Lactobacillus* GG on the risk of caries was studied in 18 day care centres in Helsinki, Finland (79). In a randomised, placebo-controlled study children were given pasteurised milk that contained *Lactobacillus* GG ($5-10 \times 10^5$ cfu/ml) or standard milk as a placebo, five days a week for seven months with their day care meals. The children's oral health was recorded at baseline and at the end, and *mutans*-group streptococci were cultivated from saliva-dental plaque samples. The risk was classified as high if the child had a score of decayed/missed/filled teeth (dmft) or initial caries of >0 and a *mutans* streptococci count $\geq 10^5$ cfu/ml, as moderate if either of these was detected, and as no risk if dmft was 0 and the *mutans* streptococci count $< 10^5$ cfu/ml. The results showed less dental caries in the *Lactobacillus* GG group at the end of the study and lower *mutans* streptococci counts. The risk of dental caries was 44% lower in the *Lactobacillus* GG compared to the placebo (OR=0.56, $p=0.01$; Fig. 8). The conclusion was that the milk containing the probiotic *Lactobacillus* GG bacteria may have beneficial effects on children's dental health beyond the effect of standard milk.

LGG and diarrhoea

4.

4.1 Preventive treatment

4.1.1 Acute diarrhoea in children

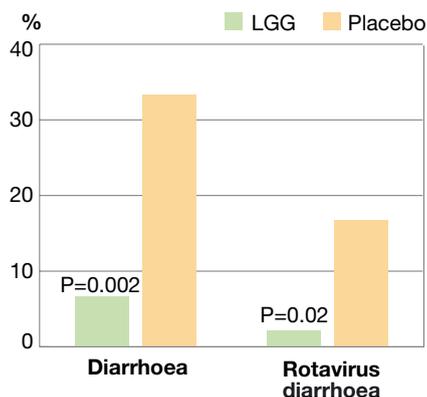


Figure 9. The effect of *Lactobacillus GG* on the occurrence of acute diarrhoea. Children hospitalised for non-diarrhoea reasons were given either *Lactobacillus GG* or placebo throughout their stay (81).

A fifteen-month study surveyed the incidence of diarrhoea among under-nourished Peruvian children living in poor conditions (80). One half of a group of 204 children received a *Lactobacillus GG* dose six times a week at home and the other half, a placebo. Altogether, 954 diarrhoea episodes were recorded and the infectious agent was determined in 58% of the cases. Pathogenic bacteria were isolated in about one half of the cases, parasites in one half, and viruses in one third. Mixed infections were therefore very common. The *Lactobacillus GG* group was found to have significantly fewer diarrhoea episodes caused by the adenovirus; no dif-

ference was found in the incidence of other pathogens. Looking at the data as a whole, the incidence of diarrhoea in the *Lactobacillus* GG group was 5.2 episodes per child per year, compared with 6.0 episodes in the placebo group ($p=0.028$). Diarrhoea prevention was most effective in children aged 18-29 months (4.9 episodes LGG vs. 6.2 episodes placebo, $p=0.004$) and was primarily of benefit to children who were not breastfed. *Lactobacillus* GG had no effect on the duration of diarrhoea in this study (80).

Another, short-term clinical study, to evaluate the reduction of the risk of diarrhoea by *Lactobacillus* GG, was made in a Polish hospital (81). Children hospitalised for reasons other than diarrhoea were given *Lactobacillus* GG or a placebo twice daily during their hospital stay. The risk of diarrhoea was reduced significantly in the *Lactobacillus* GG group compared to the placebo group (6.7% vs. 33.3%, RR 0.2, $p=0.002$). Surprisingly, there was an equal prevalence of rotavirus infection in both groups, but the administration of *Lactobacillus* GG significantly reduced the risk of rotavirus gastroenteritis (1/45 vs. 6/36, RR 0.13, $p=0.02$; Fig. 9). This result poses an interesting question as to the potential of *Lactobacillus* GG to protect against rotavirus after a non-diarrhoeal infection.

4.1.2 Antibiotic-associated side effects

Possibly the most common indication for the clinical use of probiotics is their ability to prevent the side effects of antibiotics, such as diarrhoea and abdominal pain. Antibiotics change the composition of the bowel microflora, allowing the possibility for opportunistic pathogens such as *Clostridium difficile* to proliferate. Antibiotics also interfere with the metabolism of the microflora, for instance, by impeding the formation of short-chain fatty acids in the colon. Probiotics are therefore well suited for maintaining or restoring the balance of the bacterial flora.

The effect of *Lactobacillus* GG taken in a capsule form has been proved to reduce the side effects of antibiotics in children. In a randomised, double-blind, placebo-controlled study, common acute infections in 188 children were treated by commonly used antibiotics, and under the care of family physicians (82). Half the patients received 1 - 2 *Lactobacillus* GG capsules (1×10^{10} cfu) once a day, the

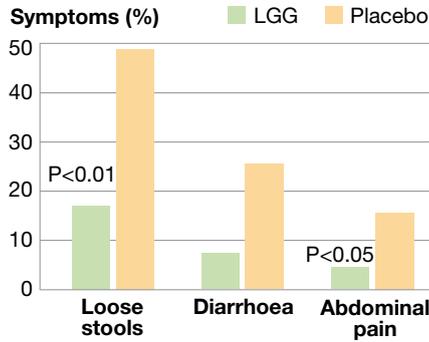


Figure 10. The effect of *Lactobacillus GG* on intestinal symptoms caused by antibiotics (82).

other half received identical placebo capsules without the bacteria (one capsule for children <12 kg, two capsules for those >12 kg). Any gastrointestinal complaints were monitored via telephone interviews. Significantly less diarrhoea and daily defecations were reported in the *Lactobacillus GG* group than in the control group. Furthermore, the stools were more solid and the study group had less abdominal pain than the placebo group (Fig. 10). *Lactobacillus GG* did not cause any side effects in this or in other studies.

Another study was conducted in Finland with children prescribed oral antibiotics for the treatment of acute respiratory infections (83). The children were randomised to receive either one placebo (n=58) or one *Lactobacillus GG* (n=61) capsule twice a day (2×10^{10} cfu). The parents kept a daily symptom diary at home and recorded stool frequency and consistency. In cases of diarrhoea, stool samples were analysed for adenovirus, rotavirus, calicivirus and astrovirus as well as for *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, *Clostridia difficile*, *Staphylococcus aureus* and yeasts. Within two weeks of antimicrobial treatment the incidence of diarrhoea was 5% in the *Lactobacillus GG* group and 16% in the placebo group (p=0.05). In diarrhoeal episodes two cases of *C. difficile* were found (one in each group) and three cases of Norwalk-like calicivirus were positive (one in the *Lactobacillus GG* group, two in the placebo group). No other pathogens were recovered (83).

In a small study with adult volunteers *Lactobacillus GG* reduced significantly

diarrhoea caused by erythromycin and somewhat reduced abdominal pain (84). In the study, volunteers took a *Lactobacillus* GG-fermented milk product or a placebo yoghurt (post-pasteurised yoghurt without the living bacteria) in the morning and evening, half an hour after they had taken an antibiotic.

Armuzzi et al. studied the effect of *Lactobacillus* GG on gastrointestinal discomfort caused by the antibiotic treatment of *Helicobacter pylori* (85, 86). In a pilot study (86) 120 asymptomatic volunteers carrying *H. pylori* were randomised to the eradication therapy with pantoprazole, clarithromycin and tinidazole for one week or the same regimen supplemented with *Lactobacillus* GG (6×10^9 cfu/sachet) for two weeks. *Lactobacillus* GG was taken 2 h after breakfast and dinner, mixed with water. Bloating, diarrhoea and taste disturbances were the most frequent side effects during the eradication week and were significantly reduced in the *Lactobacillus* GG group. The same pattern was observed throughout the follow-up period. The overall assessment of treatment tolerability showed a significant trend in favour of the *Lactobacillus* GG-supplemented group ($p=0.03$).

In another, double-blinded, placebo-controlled study, 60 healthy asymptomatic *H. pylori* positive volunteers were randomised to one week therapy with rebeprazole, clarithromycin, tinidazole and *Lactobacillus* GG (6×10^9 cfu/sachet) for two weeks, or to the same regimen with a placebo preparation (85). Again, diarrhoea, nausea and taste disturbances were significantly reduced in the *Lactobacillus* GG group compared to the placebo (RR=0.1, 0.3 and 0.5 respectively). An overall assessment of treatment tolerability showed a significant difference in favour of the *Lactobacillus* GG group ($p=0.04$). There was no difference between the groups in the success of the eradication of *H. pylori* (in both studies it was about 80%), but supplementation with *Lactobacillus* GG helped to improve the tolerability of the antibiotics.

A randomised, double-blinded, placebo-controlled study was performed with 267 initially hospitalised adult patients treated with intravenous or oral antibiotics for a presumed or proven infection (cellulites, pneumonia, urinary tract infection and pyelonephritis) (87). The main groups of antibiotics were β -lactams (cephalosporins 60%, penicillin 27%) and fluoroquinolones (39%). *Lactobacillus* GG (1×10^{10} cfu) or placebo capsules were given twice a day. The *Lactobacillus* GG intervention had no effect either on the incidence or on the duration of mild or severe diarrhoea.

Broad-spectrum antibiotics, especially for immunocompromised patients, can

Antibiotic	MIC $\mu\text{g/ml}$		
	Valio/Yhtyneet Laboratoriot Oy, E-test, AB Biodisc	Ref. 82	Ref. 90, MD plate for gram posit., Radiometer
Benzylpenicillin	0.19	1.0	0.25
Ciprofloxacin	2.0	0.2	>4.0
Gentamicin	24.0		>32.0
Ampicillin	0.5	0.5	1.0
Imipenem	2.0		2.0
Doxicycline	0.125		
Vancomycin	>258		>64
Cefotaxime	4.0	4.0	
Erythromycin	0.094	0.25	0.5
Amoxycillin / Clavulanate	0.5	0.5	
Cefalotin		16.0	4.0
Tetracyclin		2.0	<2.0
Trimethoprim / Sulphamethoxazole		76.0	>4.0/>76
Oxacillin			1.0
Clindamycin			0.5
Chloramphenicol			<4
Rifampin			<0.5

Table 2. The antibiotic sensitivity of *Lactobacillus* GG in MIC (minimum inhibitory concentration) values.

cause serious D-lactic acidosis due to the intestinal lactobacilli producing D-lactic acid. *Lactobacillus* GG produces L-lactic acid and has been successfully used to treat one such case (88).

The susceptibility of LGG to antibiotics

Although *Lactobacillus* GG is susceptible to the most common antibiotics (89, 90) (Table 2), it has been shown to survive in the intestines during antibiotic treatment in most test subjects. *Lactobacillus* GG was isolated in stools in 75, 76 and 57% of the test subjects being treated with erythromycin, ampicillin and penicillin respectively (5, 33, 84). The survival of *Lactobacillus* GG can be explained by the antibiotic and bacterial preparations being taken at different times, and possibly by the lower antibiotic level in the bowel than in the blood stream. Some species of lacto-

bacilli are naturally resistant to vancomycin, including all strains of the species *L. rhamnosus*, *L. casei*, *L. plantarum* and *L. reuteri*. It is also pertinent to ask whether the genes responsible for vancomycin resistance can be transferred to other bacteria. Vancomycin-resistance genes in *Lactobacillus* GG were shown to differ from the *van* genes in enterococci, and were not transferred to enterococci (90, 91). Antibiotic-resistance genes can sometimes be transferred via plasmids. *Lactobacillus* GG does not carry plasmids and is safe in that sense, too (91).

4.1.3 Traveller's diarrhoea

Intestinal troubles are a common complaint among those travelling from cold or cool climates to warm and tropical countries. Lactic acid bacteria are often used to prevent intestinal troubles while travelling, even though few studies have been conducted on their efficacy. The first *Lactobacillus* GG study was conducted on Finnish tourists (n=756) who visited two resorts in Turkey (92). An average of 43.8% of the travellers had diarrhoea. *Lactobacillus* GG taken twice a day significantly reduced the incidence of diarrhoea in those staying one week in one of the resorts but not in the other. No explanation for the difference in effectiveness between the resorts was found, but it is possible that the dose (1×10^9 cfu twice daily) of *Lactobacillus* GG used in the study was too low.

The second study was carried out with American tourists (n=245) whose destinations were primarily in Asia, East Africa, South America, India and Central America (93). One *Lactobacillus* GG capsule per day provided statistically significant protection. In the *Lactobacillus* GG group the average incidence of diarrhoea was 3.9%, whereas in the placebo group it was 7.4% ($p=0.05$), i.e. a protection factor of 47%. Travellers who had previously suffered from tourist diarrhoea benefited the most. The best protection against traveller's diarrhoea is still good personal hygiene such as hand washing, drinking bottled water and drinks without ice cubes, and the consumption of adequately cooked, hot food. However, *Lactobacillus* GG provides extra protection.

4.2 Treatment studies

4.2.1 Rotavirus diarrhoea

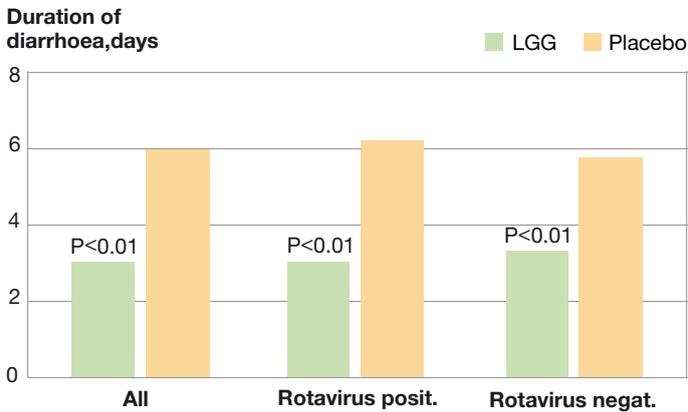


Figure 11. The effect of *Lactobacillus* GG on the duration of acute diarrhoea. Ambulatory children were given *Lactobacillus* GG mixed in milk or mother milk substitute for a maximum of five days (101).

Lactobacillus GG accelerates recovery in acute diarrhoea. Studies have been primarily conducted on children with rotavirus, which is the most common cause of diarrhoea in western countries. *Lactobacillus* GG accelerated by about one day the recovery of children hospitalised with acute diarrhoea (Table 3). The acceleration of recovery was generally noted on the second day: children treated with *Lactobacillus* GG defecated less often and their stools were more solid than those in the placebo group (94). Children treated at home, with *Lactobacillus* GG administration starting on the second day after the onset of diarrhoea, suffered symptoms for approximately half as long as the placebo group (Fig. 11). In addition, these children spread the virus for a shorter time than those in the placebo group, since after six days significantly fewer of them excreted rotavirus in their stools than in

Infection	n	Lactobacillus GG	Duration of diarrhoea days or hours (SD)		p	Ref.
			LGG group	Placebo		
82% rotavirus	71	Fermented milk Powder	1.4 (0.8)	2.4 (1.1)	<0.001	94
88% rotavirus	39	Fermented milk	1.1 (0.6)	2.5 (1.4)	0.001	74
100% rotavirus	42	Powder	1.5 (0.7)	2.3 (0.8)	0.002	105
100% rotavirus	49	Powder	1.8 (0.8)	2.8 (1.2) Lactophilus ¹ 2.6 (1.4) Yoghurt starter 2.6 (1.3) Placebo	0.04 ANOVA	76
100% rotavirus	26	Powder Inactivated powder	1.5 (0.1–2.2) 1.6 (1.1–2.3)		0.83	75
27% rotavirus 21% bacterial	123	Powder	2.7 (2.2)	3.8 (2.8)	0.02	102
Watery diarrhoea (subgroup)	26	Powder	1.9 (0.6)	3.3 (2.3)	<0.05	98
Total	39		No report	No report	n.s.	
Watery diarrhoea (subgroup)	32	Powder	Diarrhoea 31% after 2 days treatment	Diarrhoea 75% after 2 days treatment	<0.01	99
Total	38		No report	No report	n.s.	
61% rotavirus ²	100	Powder	3	6	<0.01	101
92% rotavirus	123	Within ORS: single or multiple dose, after ORS, or placebo	17.7 (12.2–25.6) h (within ORS)	30.4 (23.6–39.3) h (after ORS or placebo)	0.03 ANOVA	97
Total	287		58.3 (27.6) h	71.9 (35.8) h	<0.03	95
Rotavirus posit.	101	Within ORS + continuous	56.2 (16.9) h	76.6 (41.6) h	<0.008	
Invasive	53		73.3 (29.3) h	72.0 (32.4) h	n.s.	
Unknown	99		53.2 (32.4) h	64.2 (30.5) h	<0.05	

1) Other L.rhamnosus strain
2) Ambulatory

Table 3. *Lactobacillus GG* in the treatment of acute diarrhoea. Hospitalised patients were given LGG twice a day after oral rehydration (ORS), if not otherwise stated.

the placebo group. The effect of *Lactobacillus GG* in the treatment of rotavirus diarrhoea has been confirmed through a multi-centre study carried out by the ‘diarrhoea working group’ of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (95). In a recent systematic review of published studies, *Lactobacillus GG* was shown to be, so far, the only probiotic strain with a consistent effect on the duration of diarrhoea and on the risk of diarrhoea lasting >3 days (96).

The best recovery is obtained when *Lactobacillus GG* is administered as early as possible after the symptoms of diarrhoea have appeared. If rehydration is needed,

then *Lactobacillus* GG treatment is best started at the same time as oral rehydration (95, 97). The effect of the treatment was the same whether *Lactobacillus* GG was administered in powder form (capsule/opened) or in the form of a fermented dairy product (cf. Table 3). It is also worth mentioning that heat-inactivated *Lactobacillus* GG accelerated the recovery in acute diarrhoea as effectively as the living bacteria (75) but the immune effect differed.

4.2.2 Other types of acute diarrhoea

Studies carried out in Thailand and Pakistan using *Lactobacillus* GG in the treatment of acute diarrhoea showed that recovery from watery diarrhoea was accelerated, but not from diarrhoea with bloody stools (98, 99). Nor did *Lactobacillus* GG succeed in removing *Klebsiella oxytoca* from the intestines of premature babies (100). On the other hand, an Italian study (101) and the European multicenter study (95) showed a significant effect of *Lactobacillus* GG both in rotavirus infections and in cases where the cause of the diarrhoea was unknown. Similarly, in a study performed in Petroskoi (Russia), the difference was significantly in favour of the *Lactobacillus* GG group, even though only 27% of the patients had rotavirus diarrhoea. About a fifth had diarrhoea caused by known bacteria and in about half the cases the aetiology was unknown (102). Therefore, it seems that *Lactobacillus* GG is effective not only in rotavirus diarrhoea but also in some infections where the aetiology is unknown. If the mucous membrane is profoundly inflamed or even destroyed, the effectiveness of *Lactobacillus* GG remains unclear.

4.2.3 Are all lactobacilli effective?

Lactobacillus GG was compared with another *L. rhamnosus* strain (Lactophilus®, Laboratoires Lyocentre, France), traditionally used in the prevention and treatment of children's diarrhoea in Finland, and with a common yoghurt starter culture powder (76). Only *Lactobacillus* GG was found to accelerate recovery from diarrhoea. This suggests that different bacterial strains within the same species have significant dif-

rial treatment gives additional protection against re-infection. This and later studies (74-76) show that the influence of *Lactobacillus* GG is specifically mediated through an enhanced immune response. *Lactobacillus* GG has also been found to induce an enhanced response with an oral rotavirus vaccine (71). Not all lactobacilli, however, increase the immune response, which in part explains the differences in their effects (76). The effect of *Lactobacillus* GG on innate defence systems might also contribute to the accelerated recovery from diarrhoea, e.g. enhanced production of induced nitric oxide (103), mucin production (44) and increased rate of enterocyte proliferation (104).

Another possible contributing factor in shortening the duration of diarrhoea is the balancing of intestinal microflora. Acute osmotic diarrhoea may be followed by bacterial imbalance and the overgrowth of specifically urease-producing bacteria. These may release ammonia, which is toxic to the intestinal mucous membrane. However, urease activity was not elevated in those subjects treated with *Lactobacillus* GG (105). *Lactobacillus* GG adheres to intestinal mucus (106) and is able to survive in the bowel even during acute diarrhoea, making it suitable for balancing intestinal microflora (19, 105).

4.3.2 Antibiotics and balancing intestinal flora

Data has been obtained on the efficacy of antimicrobial medication and *Lactobacillus* GG in the treatment of shigellosis and on their influence on the composition of bowel microflora (107). After ten days of treatment, 79% of the children had recovered in the group that received *Lactobacillus* GG and 67% in the group that only received medicinal treatment ($p < 0.05$). Due to the paucity of the material ($n = 31$) no far-reaching conclusions can be drawn about the clinical significance of the treatment.

At the start of treatment, the subjects' intestinal microflora was completely unbalanced, i.e. the quantity of aerobic bacteria was greater than that of anaerobic bacteria (Fig. 13). Furthermore, there were hardly any lactobacilli at all and the relative proportion of subordinate bacteria had risen considerably. After five days of treatment, the quantity of lactobacilli had increased and the microflora had partially normalised in both groups that received *Lactobacillus* GG. After ten days of

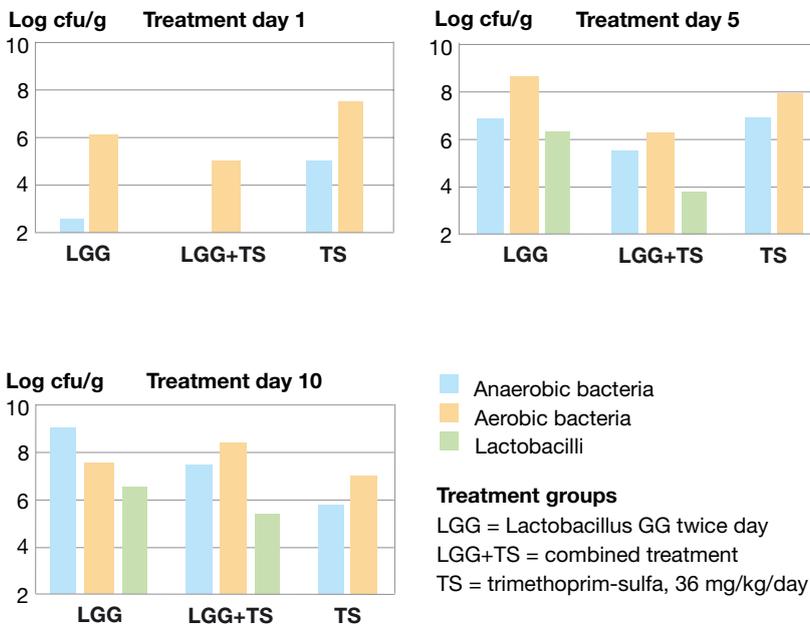


Figure 13. The effect of *Lactobacillus* GG on the quantity of faecal aerobic and anaerobic bacteria as well as total lactobacilli during the antimicrobial treatment of shigellosis (108).

treatment, the level of anaerobes was normal in the *Lactobacillus* GG group, was slightly normalised in those who received the combined lactobacilli and medicinal treatment, and was still low in those who had only received the medicinal treatment. Moreover, lactobacilli were still absent from the intestines of those who had only received the medicinal treatment (Fig.13). An intestinal microflora imbalance, and particularly a deficiency of anaerobic bacteria, increases the translocation of intestinal bacteria from the lumen to the tissues and increases the risk of infections and bacteraemia (37). *Lactobacillus* GG is resistant to trimethoprim-sulfamethoxazole, so it is well able to balance the intestinal flora during the treatment.

Changes in the intestinal microbe population can also be measured as changes in its metabolic activity. Bacterial metabolism produces short-chain fatty acids from carbohydrates and proteins, particularly acetate, propionate and butyrate. Most of

these are absorbed by the mucous membrane as an energy source for colonocytes. Short-chain fatty acids lower the pH of the bowel contents, and butyrate in particular is considered to have a protective influence on the mucous membrane (108). In premature babies who received antibiotic treatment, *Lactobacillus* GG did not cause any significant changes in the production of short-chain fatty acids (109). However, with medicinal treatment against *Salmonella* and *Shigella*, *Lactobacillus* GG normalised the production of short-chain fatty acids, which points to a normalisation of intestinal microflora (110).

4.4 Indications in *Clostridium difficile* treatment

C. difficile is an opportunistic pathogen which can also be found in normal human microflora. It does not usually cause any symptoms. However, when the microflora balance is disturbed - for example, as a consequence of antibiotic treatment - the *C. difficile* population can increase considerably and the toxin it produces can cause varying degrees of chronic diarrhoea and even pseudomembraneous colitis. *C. difficile* diarrhoea recurs in about 10-20% of subjects treated with antibiotics (vancomycin or metronidazole), and more effective treatments are scarce. The use of *Lactobacillus* GG in the treatment of recurrent *C. difficile* diarrhoea has been reported in around 40 subjects (111-113). A positive treatment response was achieved with a single treatment in 84% of the cases, and with repeat treatment in 94%. In the preliminary results from a placebo-controlled pilot study, a significant effect was obtained in those who had *C. difficile* colitis for the first time, but not in cases which had recurred often (114, 115). Further studies are underway.

The histopathology of *C. difficile* colitis and its effect on the intestinal microflora has been studied in an animal experiment (37). *C. difficile* combined with an antibiotic (ampicillin) is inevitably fatal in hamsters. As it was known that *Lactobacillus* GG maintains normal intestinal microflora and that xylitol prevents the adhesion of *C. difficile*, the effectiveness of the combination treatment was tested in hamsters. It was found that this could prevent both the development of enterocolitis in animals

(in 4 animals out of 5) and their death. Animals not undergoing the combination treatment died within 2.5 days. In the hamsters with enterocolitis, the anaerobic microflora of the epithelium of the bowel was almost completely destroyed and coliform, facultative bacteria had become the dominant microflora in the contents of the bowel. In those hamsters that survived without enterocolitis, the dominant microflora were anaerobic bacteria, and *C. difficile* was found in only low concentrations in the bowel lumen of two animals (37).

LGG and the permeability of the mucous membrane

5.

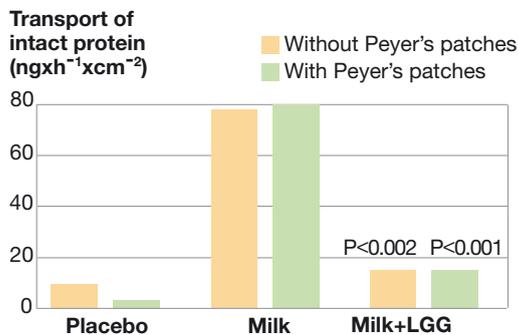


Figure 14. The effect of *Lactobacillus* GG on mucosal permeability. Fourteen-day old rats were gavaged daily with cow milk +/- *Lactobacillus* GG, and the jejunal permeability was analysed when the rats were twenty-one days old (118).

When intestinal inflammation and microflora imbalance occur, the permeability of the mucous membrane increases, and large antigen molecules (116) and intestinal bacteria (37) can migrate across the mucous membrane into the system. Similarly, it has been shown that sensitivity to food antigens increases after acute diarrhoea, because antigens are abnormally transported across the intestinal mucous membrane (117). Furthermore, experimental studies with rat pups show that both foreign antigens in the diet or rotavirus infection increase the permeability of the immature mucous membrane, with no antigen-specific local response. When test animals received *Lactobacillus* GG in their diet, the maturation of the mucous membrane occurred normally: the transport of antigens was strongly reduced and occurred in a controlled route via Peyer's patches (Fig. 14). The result was an

enhancement of a local, antigen-specific IgA response (116, 118). It has also been shown in humans that *Lactobacillus* GG enhances a local, antigen-specific IgA response to food antigens (31). Such an enhanced response is important as regards the tolerance of food antigens.

Chronic non-steroidal anti-inflammatory drugs destroy gastrointestinal mucosa, leading to ulceration. The protective effect of fermented milk drinks on indometacin-induced alterations of mucosal permeability has been studied (119). The fermented milk drinks contained active or heat-inactivated strains of *Lactobacillus* GG, *L. helveticus* and *L. acidophilus* ($>10^7$ cfu/g each). Four gastrointestinal permeability tests were carried out in randomized order on 16 healthy adults: 1) basal, 2) after indometacin, 3) after indometacin when the fermented milk drink with living bacteria was consumed for five days, 4) after indometacin when the fermented milk drink with heat-inactivated bacteria was consumed for five days. Gastric permeability was measured by sucrose urinary excretion, and intestinal permeability by lactulose/mannitol excretion. Indometacin significantly increased both gastric and intestinal permeability. The fermented milk with living bacteria significantly reduced abnormal gastric permeability, but not the intestinal permeability induced by indometacin. The drink with the heat-inactivated bacteria had no effect.

LGG and allergy

6.

6.1 Speeds recovery in allergy

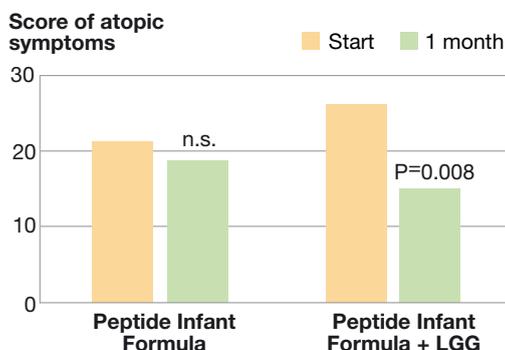


Figure 15. The effect of *Lactobacillus* GG on the atopic dermatitis of milk allergic children, during a milk elimination diet (121).

Allergies have increased and are still increasing in western countries. In Finland approximately 2.5% of small children suffer from allergy caused by cow's-milk protein. In recent years there has been intensive research into how this trend could be altered through bacterial treatment. Studies on the treatment of atopic and food allergies have suggested that by restoring the permeability of the intestinal mucous membrane, by modulating the local immune response and by using bacteria that suitably alter the food antigens, an immune response that has gone awry can be guided back in the right direction (120).

A randomised, placebo-controlled study on children with an atopic eczema with allergy to milk showed that the intensity and extension of the rash and subjective symptoms decreased significantly faster when their milk elimination diet contained *Lactobacillus* GG (Fig. 15). The intestinal inflammation was measured using the cytokine content of their stools. Tumour necrosis factor- α was found to fall signifi-

cantly more rapidly in the *Lactobacillus* GG group compared to the placebo, indicating a faster recovery from inflammation. *Lactobacillus* GG also helped those children who were only fed on mother's milk and where the bacteria were administered to the mothers (121).

In another clinical study, *Lactobacillus* GG was given to infants who manifested atopic eczema during exclusive breastfeeding, and who had no exposure to any infant food or substitute formula (122). They were weaned to a probiotic (*Lactobacillus* GG or bifidobacteria) -supplemented extensively hydrolysed whey protein formula or to the same formula without probiotics. The skin condition, the growth and concentrations of circulating cytokines and chemokines as well as soluble cell surface adhesion molecules in serum and methyl-histamine and eosinophilic protein X in the urine were determined. According to results after two months, the atopic eczema was significantly improved in the probiotic groups compared to the placebo. The median score of atopic dermatitis during breastfeeding was 16 (7-25) and decreased in the *Lactobacillus* GG group to 1 (0.1-8.7), vs. 13.4 (4.5-18.2) in the placebo group ($p=0.01$). The concentrations of serum soluble CD4 decreased in the same period in the probiotic groups but not in the placebo group, and the serum tumour growth factor- β tended to increase in the *Lactobacillus* GG group. Before intervention, the urine eosinophilic protein X correlated significantly with the clinical score of atopic symptoms. Its concentration decreased significantly in the *Lactobacillus* GG group during supplementation, which supports the clinical results. In conclusion, the data confirmed that *Lactobacillus* GG supplementation during the weaning period counteracted inflammatory responses and helped to tolerate new dietary antigens.

6.2 Prevents the risk of allergy in infancy

An interesting question is whether the development of allergic diseases can be prevented in early infancy by modulating the intestinal microflora with probiotic bacteria. To evaluate this, a group of families at high risk of allergy was selected (123). The only inclusion criterion was a family history of atopic disease: one or more family members with atopic eczema, allergic rhinitis or asthma. In all, 159

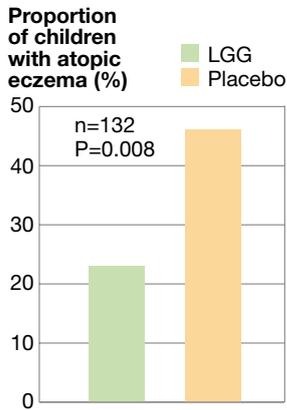


Figure 16. The effect of *Lactobacillus GG* on the incidence of atopic eczema in infants. Pregnant mothers took *Lactobacillus GG* or identical placebo capsules daily for 2–4 weeks before the delivery. After the birth it was given to the newborn baby or alternatively to the breast-feeding mother (123).

mothers were randomised to receive two *Lactobacillus GG* (10^{10} cfu) or placebo capsules daily for 2–4 weeks before the expected date of the birth. After the birth, either the breastfeeding mother or the infant consumed the bacteria for six months. The children were clinically examined until they were two years old and the incidence of atopic diseases calculated. Parents reported any symptoms observed in their children which might be related to atopic disease. Sensitisation to common dietary and respiratory antigens was measured by the skin prick test and total and antigen-specific IgE assays. Altogether, 132 families with atopic diseases completed the study. Atopic eczema was found in 46 out of 132 children (35%) at the age of two years, asthma in six and allergic rhinitis in one child. Almost every other baby in the placebo group developed an atopic disease, but only one in four in the *Lactobacillus GG* group (Fig. 16). The mean duration of breastfeeding was as long in both the atopic (7 mo) and the non-atopic (6.7 mo) group. Surprisingly, there was no difference in the effect, no matter whether *Lactobacillus GG* was given directly to the infant or to the breast-feeding mothers. Concentration of total IgE as well as frequencies of increased antigen-specific IgE concentrations and of positive skin-prick tests were similar between the *Lactobacillus GG* group and the placebo group.

It is possible that the risk of allergy in infants can be reduced by maintaining a good bacterial balance in pregnant mothers. The addition of probiotics to the diet of the nursing mothers enhanced the protective effect of breast milk. In a randomised, placebo-controlled study (124) with 62 mother-child pairs, *Lactobacil-*

lus GG increased the level of anti-inflammatory TGF- β 2 in breast milk significantly, compared to the placebo group. The risk of developing atopic eczema during the first two years of life of the infants was significantly reduced in the probiotic group compared to the placebo group (15% vs. 47%; relative risk 0.32, $p=0.0098$).

6.3 Mechanisms behind the effects

The mechanisms by which probiotics have an effect in the prevention and alleviation of allergy are not yet fully understood but many factors have been found (125). Microbial flora has an effect on the development of immune response and the balance of T-helper cell types (Th1/Th2). The balance in turn determines the development of oral tolerance. Th-2 type immune cells produce interleukin (IL)-4, which is essential for B-cell differentiation into IgE-producing cells, and IL-5, which is important for the activity of eosinophil lymphocytes. Intestinal permeability also is disturbed, allowing the absorption of antigenic macromolecules (126).

Food antigens, like caseins, enhanced the mitogen-induced proliferation of lymphocytes of atopic children, but caseins degraded by *Lactobacillus* GG had a moderating effect (127). Caseins degraded by *Lactobacillus* GG also down-regulated the IL-4 production of lymphocytes compared to the control (128, 129). T-cell activation was suppressed *in vitro* by *Lactobacillus* GG-degraded caseins, production of IL-2 mRNA was suppressed and the production of IL-2 protein reduced. At the same time, the levels of IL-4 and IFN- γ were reduced. The mechanism was based on the inhibition of the translocation of protein kinase C (one of the markers of cell activation) in the peripheral blood mononuclear cells of healthy children (130). Oral administration of *Lactobacillus* GG reduced the soluble CD4+, a marker of T-cell activation (122) and the secretion of IL-10, which is associated with the Th1/Th2 balance in a concentration-dependent manner (130). Not only the degraded caseins but also the cell-free homogenates of probiotic bacteria are shown to affect cell proliferation (131), indicating that the degradation component of bacteria may possibly play a role in the modification of immune response. Since it degrades milk proteins, *Lactobacillus* GG may also form bioactive peptides, which may in turn have an influence on the digestive tract (132).

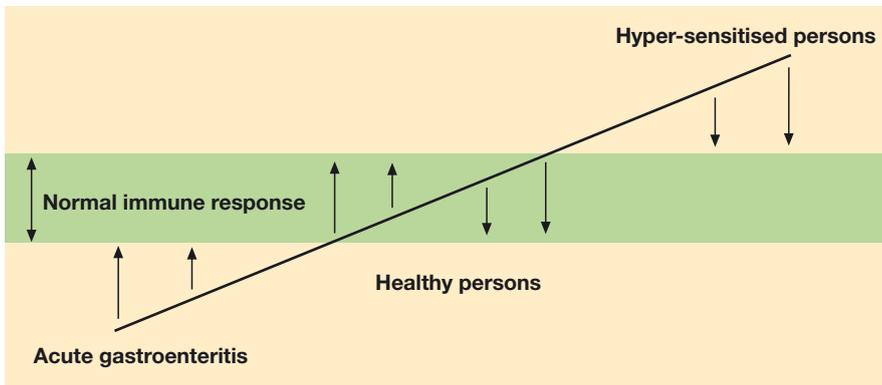


Figure 17. The effect of *Lactobacillus* GG on immune response of healthy persons, during gastrointestinal infection and on milk-hypersensitised persons.

Milk allergy is widely believed to be exclusive to young children. However, the latest studies have shown that a clear immune response can be observed in lactose-tolerant adults who show or feel symptoms during exposure to milk (133). This manifested itself as the boosting of a non-specific immune response (increasing of phagocyte receptors and boosting of phagocytoses). *Lactobacillus* GG administered in conjunction with milk exposure reduced the inflammation response significantly. In the healthy control group, milk did not cause a phagocyte response; milk with *Lactobacillus* GG, however, increased the non-specific immune response instead of lowering it (70). This reflects the balancing effect of *Lactobacillus* GG with regard to immune responses. On one hand, it increases immunological defences and boosts immune responses in healthy subjects and in those with infections (see chapter 2); and on the other, it reduces the hyperactive immune response in allergies (Fig. 17).

The bacteria are transferred from a mother to her child at birth. There are indications that the intestinal flora of atopic infants differs from the flora of healthy infants. At three weeks of age infants who later developed an atopic disease had a lower level of intestinal bifidobacteria than non-atopic ones (134). *Lactobacillus* GG has been shown to enhance the growth of bifidobacteria in newborn babies (25) and in milk-hypersensitive adults (32).

LGG and promising research areas

7.1 Rheumatoid arthritis

In children with chronic arthritis, *Lactobacillus* GG has been proved to enhance the IgA class local immune response, increase the specific IgA response to food antigens, and normalise high urease enzyme activity in stools. High urease activity indicates an imbalance in the intestinal microflora. All changes were transient and related to the short-term (10 days) use of *Lactobacillus* GG (135, 136). These results suggest that *Lactobacillus* GG has the ability to strengthen the intestinal immune barrier of the mucous membrane in chronic arthritis. In a double-blind, placebo-controlled, randomised study *Lactobacillus* GG or placebo capsules were taken by 21 patients with rheumatoid arthritis (137). Clinical examinations were made and blood samples taken five times during the one-year study. The activity of the arthritis was evaluated by laboratory tests, functioning ability, the number of swollen and tender joints, a physician's assessment and subjective evaluation by the patient. At the end of the study the number of swollen and tender joints tended to be reduced in the *Lactobacillus* GG group compared to the placebo group. The activity of the arthritis tended to decrease more in the *Lactobacillus* GG group compared to placebo, and the patients in the *Lactobacillus* GG group also needed less medication for rheumatoid arthritis. Due to the limited number of patients, the results were not statistically significant but the tendency towards a beneficial effect was clear (137).

7.2 Inflammatory bowel diseases

There are several chronic intestinal diseases without known aetiology, such as Crohn's disease, ulcerative colitis and pouchitis. They are collectively called inflammatory bowel diseases (IBD). In addition to the genetic background and autoimmune

nature of the disease, the role of intestinal microflora in the development of these diseases is also speculated (138). IBD is thought to be caused by an aggressive immune response to luminal bacteria and is characterised by a Th-1 type cytokine pattern.

Crohn's disease can appear in any section of the digestive tract but is most often found in the bowel. The clinical description includes increased permeability of the intestinal mucous membrane and disturbed processing and transport of food antigens. Because *Lactobacillus* GG is known to restore the permeability of the mucous membrane, its effect was studied in patients with Crohn's disease. The study confirmed that *Lactobacillus* GG increased local, antigen-specific immune response in the mucous membrane and in this way corrected the permeability disturbance of the membrane (135, 136). In a small, open-label pilot study *Lactobacillus* GG was given in enterocoated tablets to four children with mildly to moderately active Crohn's disease for six months. The results showed a significant improvement in clinical activity and improved intestinal permeability (139). There is still a dearth of randomised, double-blind, placebo-controlled trials.

Human *in vivo* administration of *Lactobacillus* GG led to a decrease in the initially strong proliferative response of peripheral blood CD4⁺T-lymphocytes towards foreign intestinal flora and their bacterial components (*Bacteroides fragilis* and *E. coli*). The secretion of IL-10 (Th-2 type cytokine) by peripheral blood CD4⁺T-lymphocytes increased and the level of IFN- γ and TNF- α (Th-1 type cytokines) was reduced (140, 141). These results indicate that adjunct administration of *Lactobacillus* GG might have a beneficial effect in the treatment of IBD.

Preliminary results from an open-label pilot study in the treatment of refractory "pouchitis" with capsules filled with *Lactobacillus* GG and fructooligosaccharide report a beneficial effect as an adjunct therapy to antibiotics (142). Placebo-controlled studies are in progress.

To study experimentally the potential effect of *Lactobacillus* GG on colon inflammation, this was given to rats with acetic acid-induced colitis, without significant health improvement. The need of host-specific lactobacilli strains to protect the colon is still an open question, since another, rat-specific lactobacilli strain had beneficial effects (143). Theoretically, *Lactobacillus* GG might suppress inflammation via the induction of nitrogen oxide (NO) production in enterocytes (103). NO is an important part of the defence system in the enterocytes of the mucosa.

Compounds that induce the epithelial cells to produce NO are known to help the epithelial cell defence systems and to suppress inflammation. However, they have short-term effects, and if NO is induced by intestinal flora, the effect might be more long-term and might support the normal cell functions and defences. NO also enhances mucin formation, which characteristic has also been demonstrated to take place with *Lactobacillus* GG (44).

7.3 Irritable bowel syndrome

Irritable bowel syndrome (IBS) is a widespread functional disorder of the digestive tract. Among the symptoms are bloating, abdominal pain, constipation, faecal urgency and diarrhoea. Its aetiology is unknown and therapeutic options are limited. There are only a few trials, which have studied the potential benefits of probiotics in improving the symptoms caused by IBS. A pilot study was made with enterocoated *Lactobacillus* GG tablets (10^{10} cfu). The study was a randomised double-blinded placebo-controlled and crossover setting with 24 volunteers. The intervention was a two-week run-in with the placebo, followed by 8-wk interventions with *Lactobacillus* GG or placebo, a two-week wash-out, and an 8-wk cross-over, changing the products. IBS medication (used by 83%) was discontinued at the beginning of the trial. Symptoms were recorded in daily diaries and by periodic questionnaires. The efficacy of the placebo (during the run-in period) varied from 0% (nausea) to 29% (constipation and bloating). *Lactobacillus* GG intake did not have any significant effects on the symptoms. The study group consisted of patients with bloating as the main symptom. It was noted, however, that there tended to be a reduction in the number of unformed bowel motions with *Lactobacillus* GG treatment for patients with diarrhoea (144).

In preliminary open-label studies the capsules filled with *Lactobacillus* GG and fructooligosaccharides relieved the gas-production in patients with IBS (145) and lactose malabsorption (146). Placebo-controlled studies are in progress.

7.4 Cystic fibrosis

One interesting area of application for bacterial therapy is in the treatment of cystic fibrosis. In a preliminary report, an Italian research group (147) has shown that taking *Lactobacillus* GG bacteria daily for six months significantly reduced the number of pulmonary infections and abdominal pains, and particularly improved weight gain in children suffering from *Pseudomonas* infection. Further study confirmed the benefits for *Pseudomonas*-infected patients. The incidence and duration of their infections were significantly reduced, pulmonary function improved and weight gain increased compared to the placebo group (148). Final reports of the results are still missing.

LGG and functional foods

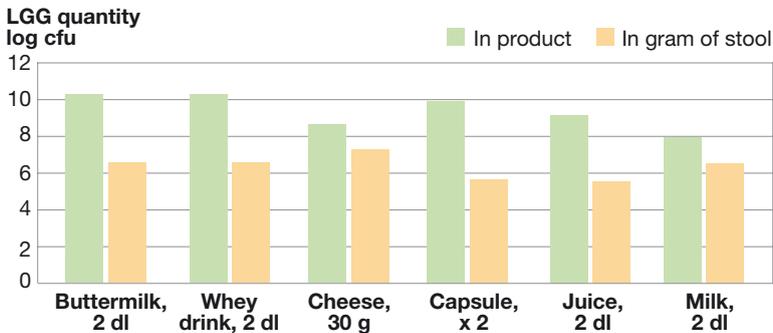


Figure 18. *Lactobacillus* GG doses obtained from Gefilus® products and *Lactobacillus* GG levels in stools, when the products are taken daily.

We are traditionally accustomed to thinking that food is food and medicine is medicine with no overlap between the two. At the end of the 1980s and particularly during the 1990s interest in this 'grey area' increased greatly. Nowadays such products are termed functional, i.e. foods that have an effect on health beyond their nutritional value. Their development has aroused wide interest and there are already hundreds of foods on the market that, in addition to nutrition, also have health-maintaining or even therapeutic effects. The efficacy of the active ingredient used in a functional food or of a product that contains it has to be demonstrated in humans. There has to be a sufficient quantity of the active ingredient in the food.

The quantity of *Lactobacillus* GG varies according to the type of product and the manufacturer. Finnish *Lactobacillus* GG products (Gefilus®) have been shown to contain sufficient *Lactobacillus* GG to colonise the bowel (16-18, 33, 77)(Fig. 18). It has been observed that milk and apparently other protective compounds in food improve

the survival of *Lactobacillus* GG through the stomach, i.e. there is a buffering effect. Consequently the quantity of *Lactobacillus* GG in powder form or in capsules has to be greater ($\sim 10^{10}$ cfu/day) than in milk-based products ($10^8 - 10^9$ cfu/day).

The lowest dose, with which the clinical efficacy of *Lactobacillus* GG in powder form has been documented, was 3×10^9 cfu twice a day, in the prevention and treatment of acute diarrhoea (81, 101). On the other hand, clinical efficacy was achieved with dairy products, which had a corresponding quantity of *Lactobacillus* GG (see Table 3). In healthy children even a lower level ($\sim 10^8$ cfu/day) of *Lactobacillus* GG in milk reduced the risk of respiratory infection (77) and dental caries (79). It is a matter of individual preference whether one chooses to consume probiotic bacteria in everyday food or in a more pharmaceutical form.

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Abbreviations in the text

cfu = colony forming units

log = logarithm

n.s. = not significant

n = quantity

SD = standard deviation

P = statistical significance

Products containing LGG

around the world, spring 2002

Country	Brand	Products
Europe		
Bosnia-Herzegovina	Dukat BioAktiv	Dairy products
Croatia	Dukat BioAktiv	Dairy products
Estonia	Valio Gefilus	Dairy products, juices, capsules
Finland	Valio Gefilus	Dairy products, juices, capsules
France	Ergyphilus plus	Capsules
Germany	Emmifit	Dairy products
	Infectodiarrstop, LGG	Powders, capsules
Iceland and Greenland	LGG+, PLUS+	Dairy products
Ireland	Yoplait everybody	Dairy products
Italy	Dicoflor, Floridral, Giflorex	Powders
	Vivi Vivo	Dairy products
Lithuania	Valio Gefilus	Capsules
The Netherlands	Vifit Vitamel	Dairy products
Norway	Tine Biola	Dairy products
Portugal	Emmifit	Dairy products
Slovenia	Dukat BioAktiv	Dairy products
Spain	Kaiku Actif	Dairy products
Sweden	Valio Gefilac	Dairy products, juices
Switzerland	Emmi Aktifit Plus, 4PLUS	Dairy products
Middle East		
United Arab Emirates	Al Ain Laban	Dairy products
Israel	Tnuva LGG1	Dairy products
Asia		
Indonesia	Vaalia	Dairy products
Japan	Onaka-He-GG, LGG Milk	Dairy products
Korea Republic	Maeil GG	Dairy products
Papua-New Guinea	Vaalia	Dairy products
Taiwan	Beautiful Day LGG	Dairy products
Oceania		
Australia	Vaalia	Dairy products
Latin America		
Chile and Bolivia	Uno al Dia	Dairy products
Ecuador	Toni	Dairy products
North America		
USA	Culturelle	Capsules

