Lactobacillus rhamnosus 271

Consumption of live lactic acid bacteria

Consumption of live lactic acid bacteria (LAB), included in fermented foods, has been a regular part of the human food intake for a long time. In fact, there are archaeological signs that humankind has used this technique from the beginning of time; it was presumably invented 1.5 million years ago by the early humanoids (Leakey 1993; Leakey 1995). Thus, humans have in this way consumed large numbers of live LAB throughout their entire history.

Fermentation is the simplest and often the safest way to preserve food, and before the Industrial Revolution, fermentation was applied just as much in Europe as it still is in many rural areas of the World. Thus, it could very well be that the human digestive tract evolved to adapt to a more or less daily supply of live LAB. This supply of live LAB ceased in many industrialized countries during the twentieth century, which eventually may have led to increased frequency of gastro-intestinal (GI) and immunological dysfunctions in urbanised humans.

When beneficial effects of certain types of live bacteria have been discussed, these types of bacteria have been gradually called “probiotics”. The original concept of probiotics implies that the balance between beneficial and harmful bacteria in the microbiota of the GI-tract can be positively affected by eating the right type of live microorganisms (Parker 1974; Fuller 1989). However, the concept of probiotics is today used more generally for describing live bacteria that after ingestion, exercise health beneficial effects beyond conventional nutrition. It is presupposed that these health beneficial effects have been scientifically proved.
Functional groups and taxonomically based taxa

**LACTIC ACID BACTERIA**

The bacteria performing the conversion of carbohydrates to carboxylic acids, mainly lactic acid in traditional fermented foods, are called lactic acid bacteria (LAB). Food microbiologists used the term early, and 1919 the Danish bacteriologist Orla Jensen tried to define key features of LAB, unaware of the fact that LAB is not forming a systematically defined group based on evolutionary relationships; instead it can be regarded as a functional group used by food microbiologists, aiming at those bacteria that occur and multiply spontaneously in traditional lactic acid fermented foods. Furthermore, it is understood that LAB are harmless to human health. Already 2002, it was shown in meta-analyses of published clinical trials that different kind of LAB can be used to prevent antibiotic associated diarrhoea (D’Souza *et al.* 2002) and shorten the duration of acute diarrhoeal illness in children (Huang *et al.* 2002).

From the taxonomic point of view, LAB means a relatively wide variety of different taxonomically based groups (taxa). The only absolute condition for organisms involved in lactic acid fermentation of food must be that the bacteria mainly produce lactic acid and that they are harmless to consume in high numbers, even for consumers with underlying sicknesses that may have weaken their immunological defence. The different kind of lactic acid producing bacteria frequently occurring in high numbers in traditional, spontaneously fermented foods belong to genera as *Lactobacillus, Pediococcus, Weissella, Leuconostoc, Oenococcus, Lactococcus*, and the species *Streptococcus thermophilus* (and similar species).

The genera *Lactobacillus* and *Pediococcus* belong to the family *Lactobacillaceae* which also includes the relatively new genera *Paralactobacillus* and *Sharpea*. They can all be included in the trivial expression “lactobacilli”.

*Leuconostoc, Weissella* and *Oenococcus* belong to the family *Leuconostocaceae* together with the genus *Fructobacillus*.

*Lactococcus* and *S. thermophilus* have from the phylogenetic point of view relatively little in common with *Lactobacillaceae* and *Leuconostocaceae* even if they all are included in the order of *Lactobacillales.*
THE SPECIES LACTOBACILLUS RHAMNOSUS

*L. rhamnosus* is a bacterial species in the huge and relatively diverse genus of *Lactobacillus*, which comprises about 90 validly named species and subspecies. *L. rhamnosus* is a so-called facultatively heterofermentative *Lactobacillus*, i.e. *L. rhamnosus* ferments hexoses exclusively to lactic acid, but can also ferment pentoses and/or gluconate, and then producing lactic and acetic acid. The type strain of *L. rhamnosus* is ATCC 7469.

Striking characteristics of *L. rhamnosus* are that the species (i) grows rapidly in milk and (ii) often possess an ability to produce extra-cellular poly-saccharides (slime).

The species *L. rhamnosus* is frequently present on human gastrointestinal (GI) mucosa (Molin et al. 1993; Ahrné et al. 1998), and was the dominating *Lactobacillus* species in Swedish breastfed children (Ahrné et al. 2005).

It has been shown that different strains of heat-killed, whole cells of *L. rhamnosus* were more efficient in triggering the production of the regulatory cytokine IL-10 in human blood mononuclear cells (monocytes), *in vitro*, than were cells of *Lactobacillus plantarum* or *Lactobacillus paracasei* (Hessle et al. 1999).

Different strains of *L. rhamnosus* has for long been used as probiotics in a wide range of different probiotic products, marketed in many countries. The most well known strain is *Lactobacillus rhamnosus* GG (Gorbach and Goldin 1992; Alander et al. 1999). This particular strain of *L. rhamnosus* has been proved to have several health beneficial effects. Most well documented is that *L. rhamnosus* GG shortened the duration of diarrhoea in acute rota virus gastroenteritis, probably by stabilising the intestinal mucosa and promoting immune response (Majamaa et al. 1995; Huang et al. 2002). Also well documented is that *L. rhamnosus* GG have been successfully used in the treatment of recurrent *Clostridium difficile* colitis by reducing the frequency of diarrhoea and preventing relapses (Bennet et al. 1996; D'Souza et al. 2002).

THE STRAIN, LACTOBACILLUS RHAMNOSUS 271

*L. rhamnosus* strain 271 (= DSM 6594) has been isolated from healthy human colonic mucosa (Molin et al. 1993).

*L. rhamnosus* 271 can be defined and identified by restriction endonuclease analysis (REA) of total chromosomal DNA by the use of relatively frequently cutting restriction enzymes such as *Eco*RI and *Cla*I, and traditional agarose gel electrophoresis (Johansson et al. 1995). This method was successfully used for strain-definition and re-isolation of *L. rhamnosus* 271 from mucosal biopsies obtained in an administration study in humans (Johansson et al. 1993). *L. rhamnosus* 271 could be re-isolated from intestinal biopsies after oral administration of the strains (Johansson et al 1993). In one individual *L. rhamnosus* 271 could even be found as a major part of the mucosal lactobacilli-flora 11 days after the end of administration (Johansson et al 1993).

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**Lactobacillus plantarum** 299v

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Irrespectively of what strain that is used as probiotics, a condition must be that the bacterium survives and remains active during the passage through the digestive tract. The ability of *L. rhamnosus* 271 when administrated in fermented milk products to survive the passage through the human digestive tract has been proved (Ahrné *et al.* 1995). In a few individuals, the strain can remain for a week or two after ended consumption (Johansson *et al.* 1993; Ahrné *et al.* 1995).

*L. rhamnosus* 271 has *in vitro* been shown to adhere to Caco-2 cells, survive a pH of 2.5 for 4h, and to tolerate 0.3% oxgall (Jacobsen *et al.* 1999).

*L. rhamnosus* 271 has been included in different types of yoghurts or similar products based on milk. The bacterium is added as probiotics but it is also growing in the product during manufacturing and by its activity improving taste and consistency of the product. *L. rhamnosus* 271 has been included in several commercial yoghurt products through the years [for example, Viktväktarnas yoghurt (Skånemejerier, Malmö, Sweden), and Fundo™ (Milka, Finland)]. The concentration of *L. rhamnosus* 271 in commercial products has usually been around $5 \times 10^7$ colony forming units [CFU] per ml.
Health effects

IN VITRO EFFECTS

*L. rhamnosus* 271 possesses antimicrobial activity *in vitro* against strains of potentially pathogenic species as *Listeria monocytogenes, Bacillus cereus, Escherichis coli, Shigella flexneri, Yersinia enterocolitica, Citrobacter freundii, Enterobacter cloacae* and *Enterococcus faecium* (Jacobsen *et al.* 1999).

ANIMAL MODEL

The translocation, i.e. the passage of viable bacteria through the epithelial mucosa into the lamina propria and then to the mesenteric lymph nodes and possibly other tissues (Berg and Garlington, 1979), can be slightly reduced in rats with acute liver injury (induced by D-galactoseamine; Kasravi *et al.* 1996a and Kasravi *et al.* 1996b) by the administration of *L. rhamnosus* 271 (Adawi *et al.* 1997). Daily rectal supplementation of *L. rhamnosus* 271 for 8 d before liver injury decreased the incidence of bacterial translocation to the arterial blood 24 h after liver injury (Adawi *et al.* 1997).

Reduced translocation was also seen in a methotrexate-induced enterocolitis model when the rats along with the methotrexate-treatment were given *L. rhamnosus* 271 (Mao *et al.* 1997). Supplementation of *L. rhamnosus* 271 significantly lowered the bacterial translocation to mesenteric lymph-nods, liver and aortic blood.

The decrease in translocation was accompanied by a reduction in the total load of bacteria and in the number of *Enterobacteriaceae* in the ileal and cecal content. The gut mucosal protein, DNA and RNA content were significantly increased by the supplementation with *L. rhamnosus* 271 (Mao *et al.* 1997).

AN UNPUBLISHED TRIAL ON IRRITABLE BOWEL SYNDROME (IBS)

The effect of a yoghurt-like product, containing *L. rhamnosus* 271, on the experienced abdominal pain, flatulence and stool function of patients with irritable bowel syndrome (IBS) was evaluated in a placebo controlled, double blinded study (Nobaek *et al.* 2000; the study was run 1998 but has never been published). Patients were divided into two treatment groups and a placebo group. One treatment group was administered *L. rhamnosus* 271 together with *Lactobacillus acidophilus* SKB3 (a relatively low concentration) in milk fermented with *Streptococcus thermophilus* SKB3 (RA-group), and the other treatment group was given *L. rhamnosus* 271 in fermented milk (R-group). The placebo group (P-group) was given the *Str. thermophilus* fermented milk. Patients consumed the different products for 28 d, 400 ml per day. RA and R products contained 5x10^7 colony forming units (CFU) of *L. rhamnosus* 271. RA-group also contained 1x10^5
CFU *L. acidophilus* SKB3 per ml, i.e. >100 times lower than the dose of *L. rhamnosus* 271.

A general problem with the study (Nobaek *et al.* 2000) was that there were patients with positive discoveries of *L. rhamnosus* 271 that was not supposed to harbour *L. rhamnosus* 271. *L. rhamnosus* 271 was found in three persons before treatment (one person in the RA-group; two persons in the R-group) and in 10 persons consuming placebo. These persons were excluded from the final evaluation as they, against their instructions, obviously had been consuming a commercially available product containing *L. rhamnosus* 271 (*L. rhamnosus* 271 was at that time included in a yoghurt with the brand name Prima Liv, marketed by Skånemjierier). This commercial product was unfortunately, most probably, known to the participants. Thus, the efficacy evaluation included 32 persons in the RA-group, 32 persons in the R-group and 22 persons in the P-group. When the study was sent to a scientific journal for publication, this exclusion of participants was the main criticism of the study and presumably the main reason for rejection.

*L. rhamnosus* 271 was found in faeces and in rectal mucosa of most patients in the RA and R groups. The viable count of lactobacilli in faeces was constant in all three groups throughout the study. There was a trend in all groups that the viable count of *Enterobacteriaceae* from the rectal mucosa was higher 15 d after the end of the administration period than before, statistical significance being reached in the RA-group. The viable count of Gram-negative anaerobes and sulphite reducing clostridia showed a tendency to increase by the administration, statistically significant for the R-group and RA-group, respectively. The concentration of succinic acid in faeces significantly increased in the treatment groups, while isovaleric acid increased in the P-group. No changes were shown in total concentration of carboxylic acids. Significant improvements in the stool function and flatulence were seen in all groups, but the decrease in flatulence was more pronounced in the RA and R groups. Abdominal pain was significantly reduced in the RA and R groups while the decrease in mean value for the placebo group failed to reach statistical significance. It is suggested that *L. rhamnosus* 271 counteract pain in individuals with IBS by affecting the activity or composition of the intestinal bacterial flora (Nobaek *et al.* 2000).

**CELL-MEDIATED IMMUNITY IN HEALTHY HUMAN VOLUNTEERS**

In a blind placebo-controlled study, the effect of a daily intake for 5 weeks of *L. rhamnosus* 271 on the innate and acquired immune system was investigated in vivo (Rask *et al.* 2013). Blood lymphocyte subsets were quantified by flow cytometry (FACS) and the expression of activation and memory markers was determined. The strain was also examined for its capacity to be phagocytosed by human peripheral blood mononuclear cells (PBMCs). Intake of *L. rhamnosus* 271 significantly decreased the expression of CD25 and CD45RO within the CD4+ 16 cell population (Rask *et al.* 2013).
Safety

*L. rhamnosus* 271 has been evaluated in the EU funded PROSAFE project (Vankerckhoven *et al.* 2008). The identity of the strain was confirmed and no acquired antibiotic resistance could be detected (PRO SAFE report on strain *Lactobacillus rhamnosus* 271).
References


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