Technical Memorandum

Lactobacillus rhamnosus HN001 – a probiotic with proven efficacy

DANISCO

INTRODUCTION

A growing awareness of the relationship between diet and health has led to an increasing demand for products that are able to enhance health beyond providing basic nutrition. Studies have shown that ingestion of probiotics – friendly bacteria – is beneficial in maintaining the body's delicate microbial balance. This balance is known to enhance intestinal health and the immune system in particular, not to mention other physiological functions. In this way, it is a critical factor for general human well-being.

> Probiotics are live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host.
>
> *FAO/WHO 2001 [1]*

Most probiotics are either lactobacilli or bifidobacteria, although some strains of other microbial genera are also thought to have probiotic properties. The beneficial effects of probiotics either involve reducing risk factors for a certain disease or improving some of the body's natural functions, thereby helping to maintain the health of the consumer. So far these effects have been documented primarily in two areas, which are also Danisco's main areas of probiotic study:

- gastrointestinal well-being
- beneficial modulation of the immune system

The suggested health benefits of probiotics are many, and some effects are better established than others. It should, however, be noted that each probiotic strain has its own specific health benefits, and no probiotic elicits all the health benefits proposed for probiotics in general. Furthermore, when one probiotic strain has a certain health benefit, it cannot be assumed that another strain, not even when of the same species, has similar properties. The origin of a bacterial strain, e.g. the human gastrointestinal tract, is no guarantee or precondition of its performance as a probiotic. For a probiotic strain to be successful, it has to fulfil certain requirements. These will improve its functionality in the intestine after consumption and enhance its survival in the product.

- The strain must be safe. For this the strain has to be identified by appropriate molecular techniques
- The strain must have clinically proven health benefits
- The strain should be able to resist acid and bile
- The strain should have good technological properties, such as the ability to survive in the final consumer product, whether food or dietary supplements, and either be neutral or contribute favourably to the flavour of the food product.

The only certain way to establish the true quality and value of a probiotic strain is by systematic *in vitro* and *in vivo* studies and, in particular, human clinical trials. *L. rhamnosus* HN001 has been subject to all these types of study. In several reviews the scientific evidence for this strain is highly rated. [2,3,4,5,6,7].

CHARACTERISTICS OF THE SPECIES

Lactobacillus rhamnosus is a Gram-positive, facultatively anaerobic, non-motile and non-spore-forming, rod-shaped microorganism.

L. rhamnosus was originally thought to be a sub-species of *L. casei*. Later genetic research found it to be a species in its own right. As a result, in 1989 its taxonomic name was changed from *L. casei* subsp. *rhamnosus* to *L. rhamnosus* [8].

Lactobacillus rhamnosus has been consumed in cheese for hundreds of years and is also one of the most common *Lactobacillus* species in breast-fed infants [9].

SELECTION AND TAXONOMY

L. rhamnosus HN001 was originally isolated from a cheddar cheese produced in New Zealand and has been consumed as part of dairy products for more than 20 years.

After 200 strains were screened by the New Zealand Dairy Research Institute, *L. rhamnosus* HN001 was identified as a potential probiotic strain and selected for further studies in animals and humans because of its ability to survive at low pH and relatively high bile concentrations [10].

These studies revealed that *L. rhamnosus* HN001 satisfies the most stringent requirements for probiotic properties. Great attention was paid to the strain's immune-enhancing properties, which have been documented in several studies.

L. rhamnosus HN001 has been identified using modern molecular biology methods, such as DNA/DNA-homology, SDS page analysis and species-specific PCR primers. DNA-based methods such as PFGE and RAPD have been used for differentiation of *L. rhamnosus* HN001 at strain level [10].

The strain has been deposited with the Australian Government Analytical Laboratories (AGAL) as deposit number NM97/09514.

SAFE FOR CONSUMPTION

Lactobacilli and bifidobacteria are generally regarded as safe and suitable for human consumption because of their long history of use in fermented foods and their ubiquitous presence in the human intestine and uro-genital tract. Very few instances of infection have been associated with these bacteria, and several published studies have addressed their safety [11,12,13].

More specifically, *L. rhamnosus* is listed in the *Inventory of Microorganisms With Documented History of Use in Human Food* [14]. The species is also included in the Qualified Presumption of Safety list of the European Food Safety Authority (EFSA) [15].

General safety

In order to assess the safety of *L. rhamnosus* HN001 further, several acute and chronic toxicity studies have been performed in mice, as well as *in vitro* studies on specific safety aspects.

In one study, mice were fed different doses of *L. rhamnosus* HN001 for 7 days (5x10E7, 10E9 or 5x10E10 cfu/mouse/day). No abnormal clinical signs were observed in any of the groups during the experiment. There were no significant differences in feed intake, water intake or live weight gain among mice fed the probiotic compared to a non-probiotic control group. No bacteria were detected in the spleen of any animals. Histological and haematological parameters also indicated that *L. rhamnosus* HN001 did not adversely affect mice health [16].

The general safety of *L. rhamnosus* HN001 was investigated in another feeding trial. Mice were administered the probiotic strain at 2.5x10E9, 5x10E10 or 2.5x10E12 cfu/kg body weight/day for 4 weeks. The results demonstrated that 4 weeks' consumption of *L. rhamnosus* HN001 had no adverse effects on the animals' general health status, haematology, blood biochemistry, gut mucosal histology parameters, or the incidence of bacterial translocation regardless of the dose [17].

Another study was performed to evaluate the acute oral toxicity of *L. rhamnosus* HN001 and investigate bacterial translocation and gut mucosal pathology in mice fed the strain for 8 days at a high dose of 10E11 cfu/mouse/day. Results showed that the strain had no adverse effect on general health status, feed intake, body weight gain and intestinal mucosal morphology. No viable bacteria were recovered from blood and tissue samples [18].

The results suggest that *L. rhamnosus* HN001 is non-pathogenic and non-toxic and has no adverse effect on the health of mice.

No risk for auto-immune disease

L. rhamnosus HN001 has been selected for its ability to modulate the immune system (see below). In the event that the immune system reacts to components that should not trigger an immune response, such as in the case of auto-immune diseases and allergies, the immune system should not be further stimulated. In an animal model for auto-immune disease, it has been shown that *L. rhamnosus* HN001 does not induce or enhance an auto-immune response. The strain can therefore be considered safe for subjects with or at risk of auto-immune disease [19].

No degradation of mucin

Additional investigations have demonstrated that *L. rhamnosus* HN001 does not degrade mucin *in vitro* [20]. Mucin is a mucus component that coats the inner surface of the gastrointestinal tract and acts as a protective physical barrier against bacterial invasion and mechanical and enzymatic damage. Any disturbance of this layer will compromise the host's mucosal defence function.

As *L. rhamnosus* HN001 is unable to degrade mucin, it is likely to be non-invasive and non-toxic at the mucosal interface. Indeed, in several animal studies the strain was not found to translocate to organs outside the intestine [16,17,18].

No induction of platelet aggregation

Further *in vitro* investigations have shown that *L. rhamnosus* HN001does not induce platelet aggregation, which is considered a risk factor for emboli formation and endocarditis (infection of heart valves). This finding further strengthens the safety record of the strain [21].

Resistance to antibiotics

Resistance to a limited number of specific antibiotics is a common property of bacteria, and probiotics are no exception. This is so-called intrinsic antibiotic resistance and is common for most strains of a given species. However, transferable antibiotic resistance is a concern as it may be transferred to potential pathogenic bacteria, leading to potentially untreatable infections.

L. rhamnosus HN001 has been found not to contain transferable antibiotic resistance and will therefore not contribute to the spread of antibiotic resistance among potential pathogenic microbes [22].

Safety aspects in human infants aged 0–2 years

As part of a double-blind placebo-controlled clinical trial of the effects of *L. rhamnosus* HN001 on infant eczema [40], data was collected on a range of safety outcomes. The objective was to examine whether infants at-risk of developing atopic dermatitis suffered any negative health effects due to long-term dietary supplementation with these probiotics.

Analysis of the results showed that daily consumption of the probiotic (daily dose 6x 10E9 cfu) from birth for two years had no effect on the general growth, health and tolerance of this sensitive group. The study concluded that *L. rhamnosus* HN001 was safe and well tolerated and did not affect normal growth or gut and immune development when given to infants from birth [23].

The strain has also been reviewed by many ethical committees for its use in human studies and has been approved, illustrating the confidence medical and scientific experts have in its safety.

From all the available evidence, there is no indication that the strain would not be safe for human consumption.

HEALTH RELATED PROPERTIES

L. rhamnosus HN001 has been extensively studied *in vitro* with a focus on characteristics that indicate beneficial effects.

In addition to the compelling *in vitro* evidence, strong probiotic benefits have been demonstrated in multiple animal trials and human studies.

These studies have provided extensive insight into the probiotic functionality of the strain. The main outcome of this research is summarised below.

BENEFITS TO INTESTINAL HEALTH AND WELL-BEING

The importance of the intestinal microbiota for health

The human gastrointestinal (GI) tract is an extremely complex ecosystem and represents the host's greatest area of contact with the environment.

This ecosystem comprises:

- the GI epithelium
- immune cells
- resident microbiota

The primary function of the human GI tract has long been considered to be the digestion and absorption of nutrients and excretion of waste end-products. In recent years, however it has become accepted that the gastrointestinal tract fulfils many other functions, which are essential to our well-being.

The human GI tract harbours a vast amount of microbial cells (10E14 cells), which is 10 times more than the number of human cells that comprise the human body. The intestinal microbiota as been estimated to consist of at least 1000 species, but only some 10 genera contribute 95-99% of all bacteria by number. Many members of the intestinal microbiota are beneficial, others potentially detrimental and, in other cases, their function is not known.

The resident microbes are involved in many metabolic processes, such as the fermentation of undigested carbohydrates into short-chain fatty acids, and also in lipid metabolism and vitamin synthesis.

Another important function of the intestinal microbiota is to stimulate the maturation of the immune system and provide protection against incoming, potentially pathogenic microbes.

A higher concentration of certain genera including *Lactobacillus* and *Bifidobacterium* in the intestine is generally considered to be associated with a healthier intestinal tract.

When the delicate ecological balance of this highly complex microbial community is disturbed by environmental or physiological factors, predisposition to infectious and immuno-inflammatory diseases is enhanced. It may then become necessary to re-establish a beneficial microbiota.

Research has shown that specific probiotic strains can be used to optimise the composition and activity of the intestinal microbiota and, thus, to treat or reduce the risk for a range of diseases or unfavourable conditions [24,25].

In vitro studies

Resistance to acid and bile

According to the generally accepted definition of a probiotic, the probiotic microorganism should be viable at the time of ingestion to confer a health benefit. This definition implies that a probiotic must survive GI tract passage and, according to some interpretations, transiently colonise the gut mucosa of the host.

A variety of traits are believed to be relevant for surviving passage through the GI tract, the most important of which is tolerance to both to the highly acidic conditions present in the stomach and to concentrations of bile salts found in the small intestine.

L. rhamnosus HN001 demonstrates high tolerance to low pH and high resistance to bile salts *in vitro* (figure 1) [10].

Adhesion to intestinal mucosa

While adhesion is not a pre-requisite for a strain to elicit probiotic properties, interaction with the intestinal mucosa is considered important for a number of reasons. Binding to the intestinal mucosa may prolong the time a probiotic strain can reside in the intestine. This interaction with the mucosa brings the probiotic in close contact with the intestinal immune system, giving it a better opportunity to modulate the immune

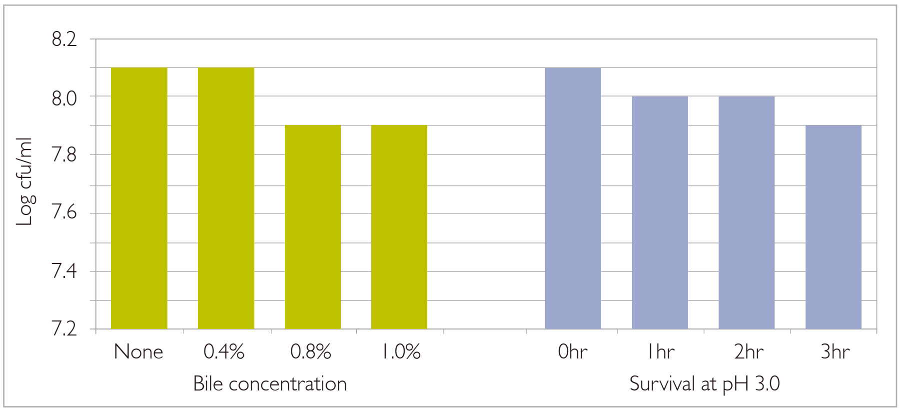

Figure 1. Effect of low pH (pH 3.0) and high bile concentration on the survival of *L. rhamnosus* HN001. [10]

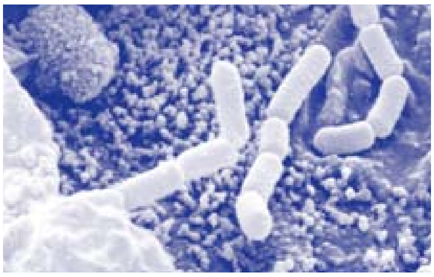

Figure 2. *L. rhamnosus* HN001 adhering to a monolayer of Caco-2 cells [26].

Bacterial strain	HT-29	Caco-2	HT-29 MTX
Lactobacillus rhamnosus HN001	161	219	410
L. rhamnosus	105	145	257
L. johnsonii	121	155	360
L. bulgaricus	0	1	2

Table 1. Adhesion index of different strains to human intestinal epithelial cells *in vitro* [26].

response. It may also protect against enteric pathogens by limiting their ability to colonise the intestine.

Currently, adherence is measured using *in vitro* cell lines, mainly Caco-2 and HT-29 (figure 2). While this is not a thorough test of the ability of probiotics to adhere to intestinal mucosa in the body, attachment to these cell lines is considered a good indicator of their potential to attach.

L. rhamnosus HN001 showed strong adhesion to the human epithelial cell lines applied in *in vitro* studies (table 1) compared with a positive (recognised probiotic *Lactobacillus* strain) and negative control (non-probiotic *L. bulgaricus*) [26].

Inhibition of pathogens

The protective role of probiotic bacteria against gastrointestinal pathogens is highly important to therapeutic modulation of the enteric microbiota. Probiotics are able to inhibit, displace and compete with pathogens, although these abilities are strain-dependent.

The probiotic strains' putative mechanisms of action against pathogenic microorganisms include the production of inhibitory compounds, competition with pathogens for adhesion sites or nutritional sources, inhibition of the production or action of bacterial toxins, ability to coaggregate with pathogens, and the stimulation of the immune system.

In the above-mentioned study the inhibitory effect of *L. rhamnosus* HN001 against the intestinal cell monolayer of the enterotoxic *Escherichia coli* O157:H7 was also investigated.

Pre-treatment of *E. coli* with a cell-free culture supernatant of *L. rhamnosus* HN001 reduced the number of culturable *E. coli* along with the invasive ability and cell association characteristics of the toxic strain [26].

Human studies

Survival in intestinal passage

In order to elicit their health benefits, probiotics must generally be able to survive and be active in the GI tract. As discussed above, *in vitro* studies have shown that *L. rhamnosus* HN001 is able to resist low pH conditions similar to those in the stomach. The strain is also able to survive the presence of bile at concentrations present in the duodenum [10].

The ability to modulate the populations or activity of the human intestinal microbiota is considered an important probiotic characteristic. Further studies have been conducted to determine whether the strain is able to colonise the gut *in vivo*. The impact on resident gut microbiota was also assessed.

In a long-term human study, the effect of consuming *L. rhamnosus* HN001 on the micro ecology of the human GI tract was studied. The subjects consumed 1.6x10E9 cfu *L. rhamnosus* HN001 a day in a milk product (6-month control period, 6-month test period, 3-month post-test period). The composition of the faecal lactobacillus population of each subject was analysed by pulsed-field gel electrophoresis of bacterial DNA digests in order to differentiate between *L. rhamnosus* HN001 and other strains of the same species.

The results showed that both the frequency of detection and concentration of lactobacilli (cfu/g) in the faeces of volunteers increased during the period when *L. rhamnosus* HN001 was administered, but declined to baseline soon after administration ceased (figure 3). The majority of the isolates belonged to the *L. casei* group (at the time including *L. casei*, *L. paracasei, L. rhamnosus*, and *L. zeae*).

Enterococci also increased both with regard to frequency of detection and concentration in the faeces. During the period of ingestion, *L. rhamnosus* HN001 was detected at varying levels in the faeces of all the subjects, but was not detected after administration ceased. This study concluded that *L. rhamnosus* HN001 survives passage through the digestive tract, but that relatively long-term administration of the strain does not alter the biochemistry or permanent bacteriology of the faecal microbiota in humans [27].

BENEFICIAL MODULATION OF THE IMMUNE SYSTEM

The probiotic concept & the immune system

The human immune system is a highly efficient and complex system for defending the body against foreign infectious agents (bacteria, viruses and parasites) as well as from malignant cells and other noxious agents.

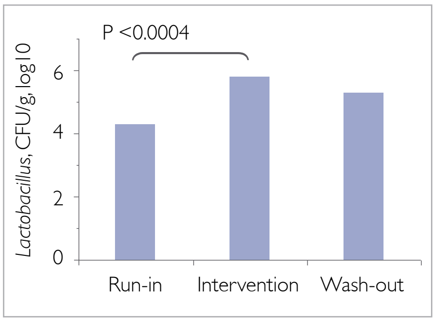

Figure 3. Supplementation with *L. rhamnosus* HN001 increases faecal lactobacilli count [27].

An immune system that functions optimally is an important safeguard against infectious and non-infectious diseases. The gastrointestinal tract is the body's largest immune organ, containing an estimated 80% of all antibody-producing cells. The intestinal microbiota represents one of the key elements in the body's immune defence system [28].

The immune system of a newborn is functionally immature. Exposure to antigens during early life is essential to drive the development of the gut mucosal immune system and to maintain immune homeostasis. Microbial antigens derived from the intestinal microbiota and the environment play a crucial role in the maturation of gut-associated lymphoid tissue (GALT) and normal resistance to disease. This has been demonstrated in studies on germ-free mice. Germ-free animals have a poorly developed immune system with fewer IgA plasma cells and intraepithelial lymphocytes in the intestinal mucosa, and lower levels of immunoglobulins. Compared to conventionally reared animals, they exhibit increased susceptibility to disease. Reduced microbial exposure in Western societies has also been associated with an increased incidence of atopic and autoimmune disorders [29].

There is a significant amount of evidence to suggest that specific probiotics strains are able to stimulate and regulate several aspects of natural and acquired immune responses. This could either be through stimulation of the gut immune system, or modulation of immune cell production and function [2,29].

Probiotic bacteria with the ability to modulate certain immune functions may improve the response to oral vaccination, shorten the duration or reduce the risk of certain types of infection, or reduce the risk of or alleviate the symptoms of allergy and other immune-based conditions [2,29].

Animal studies

Enhancement of natural and acquired immunity

Modulation of the immune system is an area of intense study in relation to the Danisco probiotic range. The goal is to understand how each strain contributes to the maintenance and balance of optimal immune function.

Several immune assays have been used to explore the efficacy of *L. rhamnosus* HN001 with regard to immune-enhancing effects.

The mammalian immune system is generally considered to consist of two major parts: The natural or innate immune system and the acquired or adaptive immune system. To explore whether *L. rhamnosus* HN001 could provide a health benefit through immune enhancement, both the natural and acquired parts of the immune system were tested.

A study was conducted to evaluate the effect of orally administered *L. rhamnosus* HN001 on various indices of the natural and acquired immunity of healthy mice. Supplementation with *L. rhamnosus* HN001 resulted, for example, in a significant increase in the phagocytic activity of peripheral blood leucocytes and peritoneal macrophages. Moreover the serum antibody responses to orally and systemically administered antigens were significantly enhanced. The findings of this study suggest that *L. rhamnosus* HN001 is able to enhance several indices of natural and acquired immunity in mice [30].

Cytokines are hormone-like proteins made by cells that affect the behaviour of other cells and, thereby, play an important role in the regulation of immune system functions.

One study investigated the ability of *L. rhamnosus* HN001 to modulate cytokine production in mice expressing an on-going Th2-type immune response. In this study *L. rhamnosus* HN001 showed the ability to simultaneously increase Th1 and Th2 cytokine production. It is suggested that the balance between these two cytokines may determine the character of the immunoregulatory properties of the strain [31].

A further study investigated whether the immune modulating effect of *L. rhamnosus* HN001 was retained in different delivery substrates. The strain was delivered to mice in different milk-based substrates: whole milk supplemented with *L. rhamnosus* HN001, fermented milk supplemented with *L. rhamnosus* HN001 and whole milk which had been part fermented by *L. rhamnosus* HN001. With all three substrates the strain was shown to enhance the phagocytic activity of blood cells to a similar degree. These results have further confirmed the immune stimulatory properties of HN001. These findings are also relevant to the use of the strain in probiotic foods [32].

The aim of another study was two-fold: first to determine the effects of bacterial cell viability on immune enhancement and second to determine the dose-response of immune enhancement conferred by *L. rhamnosus* HN001 in viable form.

Both live and heat-killed preparations of *L. rhamnosus* HN001 were shown to enhance the phagocytic activity of blood and peritoneal leucocytes in mice, at a dose of 10E9 micro-organisms daily. In contrast, only live cells enhanced gut mucosal antibody responses to cholera toxin vaccine.

In the dose–response study it was shown that feeding mice with 10E7 cfu viable *L. rhamnosus* HN001 for 14 days enhanced the phagocytic capacity of blood leucocytes, with incremental enhancement observed at 10E9 and 10E11 cfu/day. In contrast, a minimum dose of 10E9 cfu viable HN001/d was required to enhance the phagocytic activity of peritoneal leucocytes and no further increment was observed with 10E11 cfu daily.

This study demonstrates that *L. rhamnosus* HN001 exhibits dose-dependent effects on the phagocytic defence system of mice. It also suggests that while the innate cellular immune system is responsive to killed forms of bacteria, specific gut mucosal immunity may only be stimulated by live cells [33].

Increased resistance to infections

Increased resistance to infections and disease as a benefit of immune stimulation can be demonstrated via *in vivo* models and infection-challenge studies. *L. rhamnosus* HN001 has been shown to be effective against common pathogens in several animal models (see below).

<u>Protection from experimental *Salmonella* infection</u>

In order to investigate whether this immune enhancement actually results in improved resistance to pathogenic bacteria a study examined the ability of *L. rhamnosus* HN001 to protect mice against *Salmonella typhimurium* a common gastro-intestinal infective agent.

A set of experiments was designed to mimic both a single exposure to high pathogen levels (as might be encountered after consumption of heavily contaminated food) and chronic exposure to pathogens in 2 different doses over a period of time, either after pre-treatment with the probiotic strain or simultaneously. [34].

In mice challenged with a single oral dose of *S. typhimurium* the general health scores, as well as feed and water intake and weight gain, were significantly higher among the mice receiving probiotics, and mortality was much lower. Only 2 of the 29 control mice survived to the end of the study, while 27 of 30 mice fed *L. rhamnosus* HN001 survived (figure 4). The anti-*S. typhimurium* antibody titers were significantly higher in blood and intestinal fluid samples taken from the probiotic-fed mice.

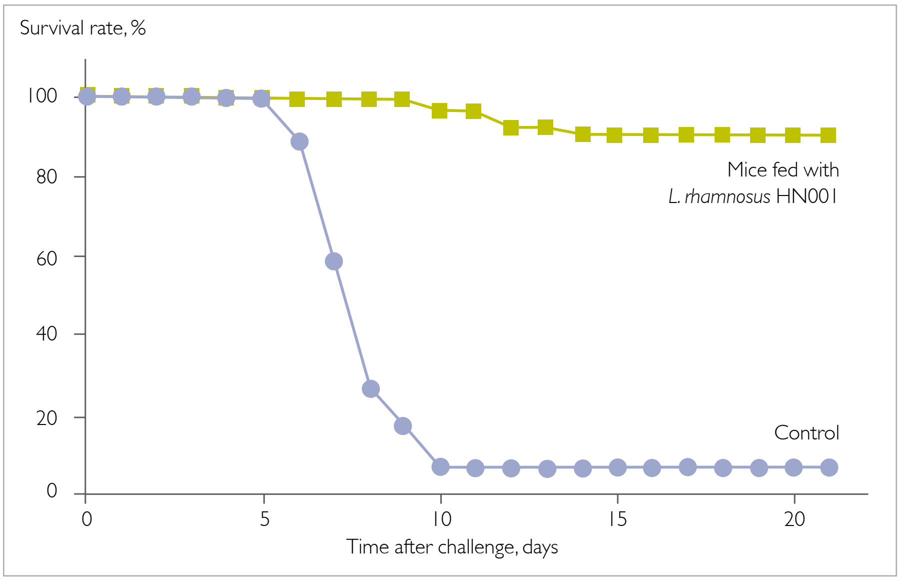

Figure 4. Survival of Salmonella-infected mice with and without supplementation with *L. rhamnosus* HN001 [34].

Results from the other sub-sets of experiments showed significantly higher counts of *Salmonella* in the liver and spleen of the control mice than the probiotic-fed mice, and the phagocytosis responses of the blood and peritoneal leucocytes were significantly higher among the *L. rhamnosus* HN001-fed mice than the controls.

These experiments demonstrated that ingestion of *L. rhamnosus* HN001 significantly reduces the ability of *S. typhimurium*, a highly infective species, to translocate to the visceral organs. This effect was seen with both high and low *Salmonella* doses. Further, this probiotic enhanced the animals' innate immune response to the pathogenic challenge among different host cells and at different somatic sites, neutrophils in blood and macrophages in peritoneal leucocytes [34].

<u>Reduction of *E. coli* infection in mice</u>

In a similar study, where the challenge applied was *Escherichia coli* O157:H7, lower morbidity and translocation of *E. coli* was observed among mice fed with *L. rhamnosus* HN001 than for the control group. The probiotic-fed mice also showed significantly higher intestinal anti-*E. coli* IgA responses and blood leucocyte phagocytic activity [35].

<u>Reduction of blood glucose levels in diabetic rats</u>

Diabetes mellitus, often referred to as type 1 diabetes, is a metabolic disorder characterised by abnormally high blood sugar (hyperglycaemia) due to insufficient levels and/or action of the hormone insulin. In an animal model of chemically-induced type 1 diabetes, it was shown that treatment with L *rhamnosus* HN001, in combination with B. lactis HN019 and an L. acidophilus strain, reduced the elevated blood glucose level in diabetic rats by up to 50%, most likely due to an insulin-independent mechanism. No effect was observed on the blood glucose level of healthy rats. These results suggest that certain strains of probiotics may be a beneficial supplement to standard diabetes treatment [36].

Human studies

Enhancement of natural immune function

The main cellular effectors of natural immunity include epithelial cells, phagocytic cells (monocytes, macrophages, neutrophils), and natural killer cells (NK cells).

Phagocytic cells are effective in eliminating microbial pathogens, whereas NK cells are crucial for defence against viral infections and tumor cells.

The immuno-modulatory properties of *L. rhamnosus* HN001 have been demonstrated in several well-designed human trials on groups of healthy middle-aged or elderly subjects. This confirms the findings of previous animal and *in vitro* studies.

A study was conducted to determine the impact of dietary supplementation with *L. rhamnosus* HN001 on the activity of peripheral blood natural killer (NK) cells in healthy elderly subjects.

The activity of NK cells increased significantly in both male and female subjects after 3 weeks' consumption of *L. rhamnosus* HN001. When the probiotic supplement was no longer taken, responses declined to levels not significantly different from the baseline values (figure 5). This indicates that consumption of *L. rhamnosus* HN001 can enhance an important cellular immune function. Moreover, there was a significant correlation between age and the relative increase in NK cell function due to consumption of *L. rhamnosus* HN001. The greatest increase in NK cell function was observed for the sub-group of people above 70 years of age. This is especially relevant since most people in this age group have a suppressed NK cell function [7,37].

The effect of dietary supplementation with *L. rhamnosus* HN001 on immune cell function was investigated in a study of healthy elderly subjects. Immune function was assessed by measureing blood leukocyte phagocytosis, differentiating between polymorphonuclear (PMN) and mononuclear phagocytes. The activity levels of both types of phagocytes were significantly increased by the probiotic, although levels returned towards baseline during the washout period (figure 6). These results suggested that *L. rhamnosus* HN001 is able to enhance aspects of natural immunity in humans and offers an effective means of improving immunity in a controlled fashion [38].

In a similar study which investigated the effect of *L. rhamnosus* HN001 on immune cell function, half of the participants received the probiotic strain dissolved in the same low-fat milk powder beverage as they consumed during the run-in and washout periods. The other half received a lactose-hydrolysed, low-fat milk powder beverage. Immune function was assessed by measuring blood leukocyte phagocytic activity, both for polymorphonuclear cells and monocytes.

The activity levels of both types of phagocytes were significantly elevated by the probiotic. Again levels returned towards baseline during the washout phaser. The low-fat milk powder matrix – regular vs. lactose-hydrolysed – did not affect probiotic efficacy [39].

Previous findings have been confirmed by a small, pre- and post-intervention trial focussing on short term outcome measures of natural cellular immunity in elderly subjects. Total intake of *L. rhamnosus* HN001 was 5x10E9 cfu/day for three weeks. The phagocytic capacity of monocytes and PMN cells and NK-cell activity was significantly elevated following consumption of *L. rhamnosus* HN001. Levels returned to near baseline levels during the following washout. The study shows that three weeks consumption of 5x10E9 cfu/day *L. rhamnosus* HN001 was sufficient to enhance immunity in elderly subjects [40].

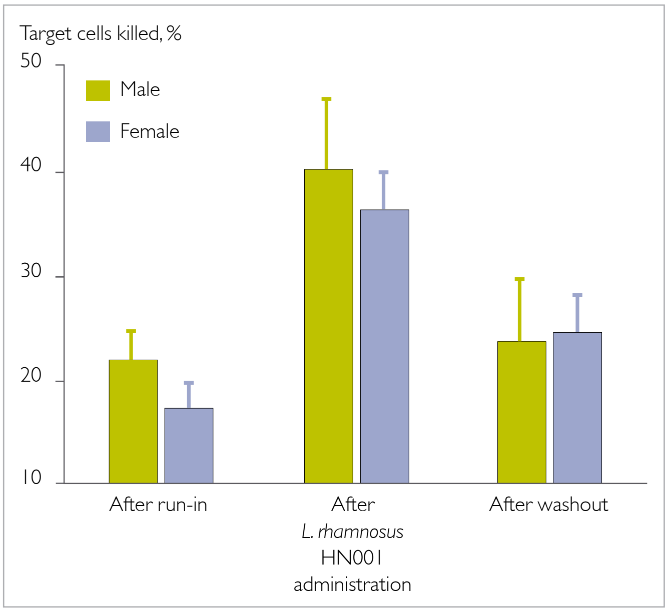

Figure 5. Effect of dietary supplementation with *L. rhamnosus* HN001 on *in vitro* NK activity [37].

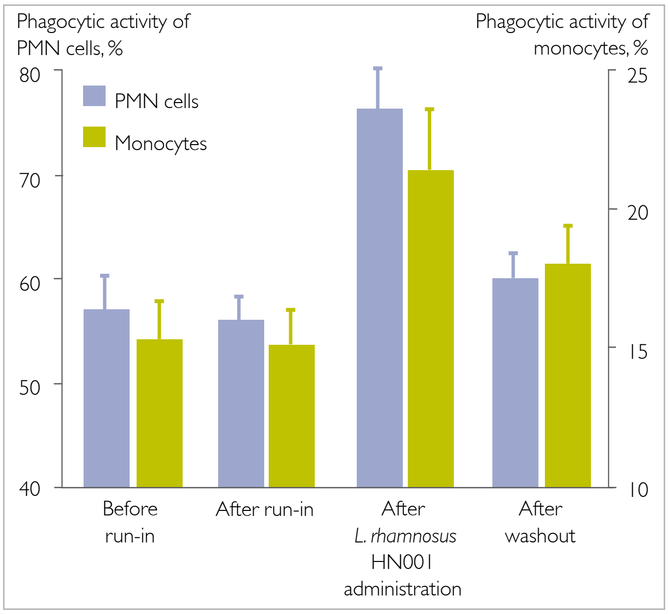

Figure 6. Effect of dietary supplementation with *L. rhamnosus* HN001 on *in vitro* phagocytic activity of PMN cells and monocytes [38].

These studies show *L. rhamnosus* HN001 consumption has a consistent impact on important markers of immune function in middle-aged and elderly adults. Furthermore, results suggest that the greatest benefit is likely to be experienced by consumers with an initially poor immune status.

Influence of L. rhamnosus HN001 on atopic dermatitis

A study was conducted to examine the effect of a combination of two probiotics (*L. rhamnosus* HN001 and *B. lactis* HN019) on established atopic dermatitis (AD) in children.

SCORAD (SCORing Atopic Dermatitis) a measure of the extent and severity of AD, was assessed at baseline, 2 and 12 weeks after starting treatment and 4 weeks after treatment was discontinued. In this study a combination of *L. rhamnosus* HN001 and *B. lactis* HN019 improved AD, but only in a sub-group of food sensitized children [41].

Recently, the efficacy of *L. rhamnosus* HN001 has been demonstrated against early childhood eczema. A double-blind, randomised, placebo-controlled clinical trial studied the ability of *L. rhamnosus* HN001 to prevent the development of eczema and atopy in infants at risk of allergic disease. In this study, dietary supplementation with 6x10E9 cfu/day *L. rhamnosus* HN001 was given to pregnant mothers, who were randomly chosen to take *L. rhamnosus* HN001 or a placebo daily from 35 weeks into the pregnancy and, if breastfeeding, for up to 6 months. Their infants were also randomly selected to receive the same treatment from birth until 2 years of age. The infant's cumulative prevalence of eczema and point prevalence of atopy was assessed using skin prick tests for common allergens at 2 years of age.

The results showed that infants receiving *L. rhamnosus* HN001 had a significantly reduced risk (approx. 50%) of eczema compared with those who received the placebo (figure 7). Children who developed atopic dermatitis also had less severe eczema in the *L. rhamnosus* HN001 group (figure 8). *L. rhamnosus* HN001 had no significant effect on atopy [42]. This study suggests that *L. rhamnosus* HN001 effectively reduces the risk of eczema development in high-risk infants.

The study also explored the effects of maternal probiotic supplementation on immune markers in cord blood and breast milk to gain insight into the possible mechanisms of probiotics in allergy prevention.

In this study, *L. rhamnosus* HN001 induced particular immune parameters that are considered to contribute to reduced allergy risk, tumour necrosis factor (TNF)-α in cord blood, immunoglobuline (Ig) A and transforming growth factor (TGF)-β in breast milk.

The results demonstrate that probiotic supplementation from pregnancy may have effects through different pathways. While the most obvious mechanism of action is through maternal-infant colonisation, the findings here suggest that maternal supplementation could also have antenatal effects on foetal immune function and potential postnatal influences through immunomodulators in breast milk [43].

L/D- LACTIC ACID PRODUCTION

Lactic acid is the most important metabolic end product of fermentation processes by lactic acid bacteria and other microorganisms. For thousands of years, lactic acid fermentation has been used in the production of fermented foods.

Due to its molecular structure, lactic acid has two optical isomers .One is known as L(+)-lactic acid and the other, its mirror image, is D(-)-lactic acid.

In humans, animals, plants, and microorganisms, L(+) lactic acid is a normal

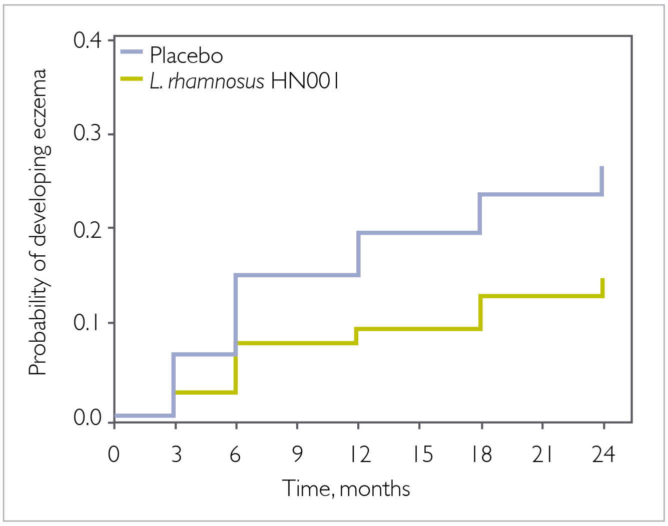

Figure 7. 2-year cumulative prevalence of eczema in infants taking placebo or *L. rhamnosus* HN001 [42].

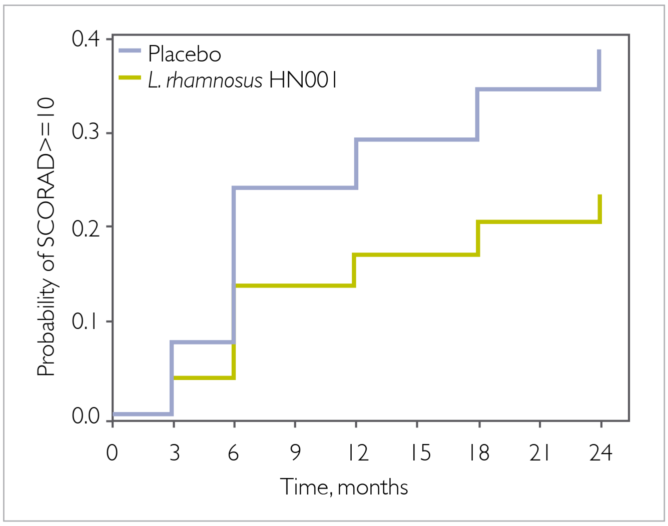

Figure 8. 2-year cumulative prevalence of SCORAD >10 in infants taking placebo or *L. rhamnosus* HN001 [42].

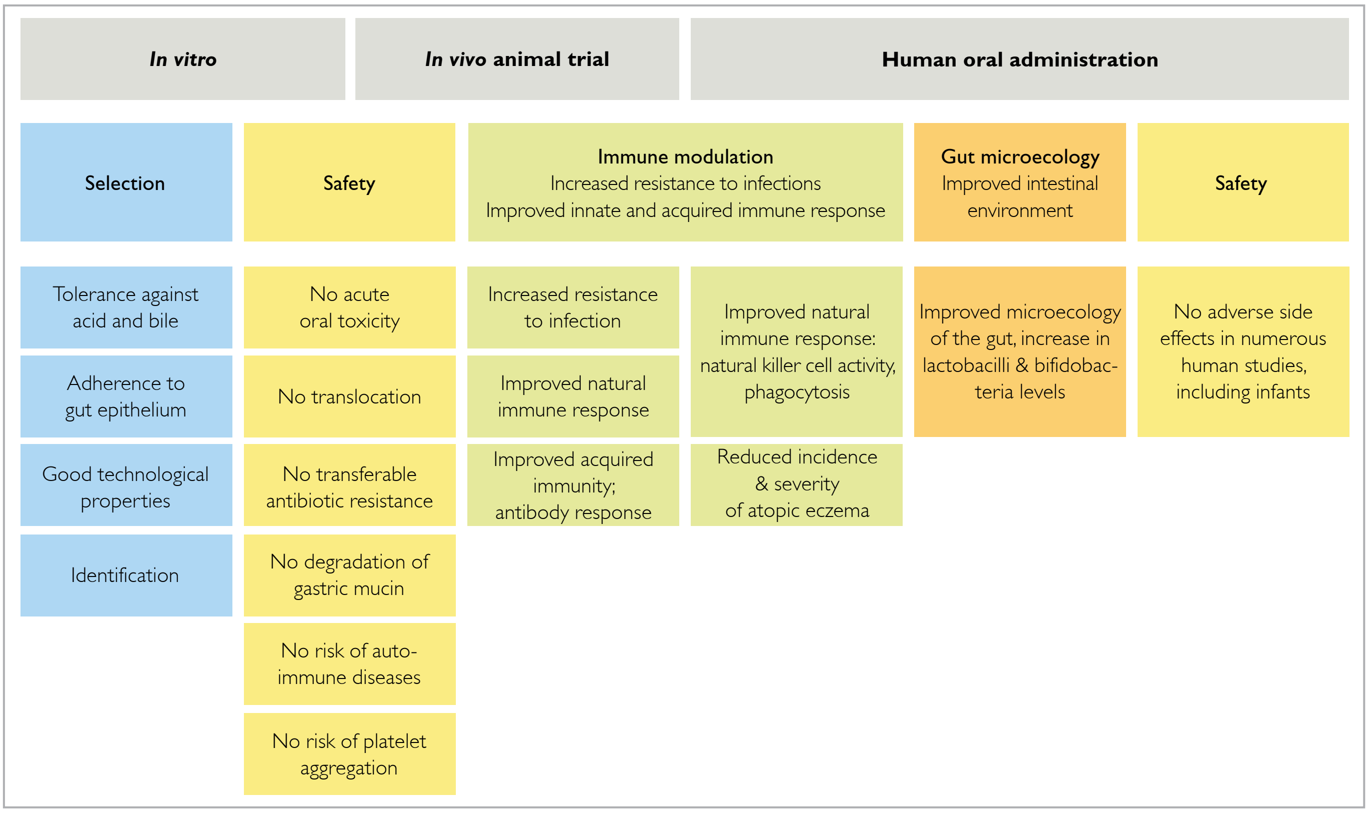

Figure 9. Summary of study findings.

intermediate or end product of the carbohydrate and amino acid metabolisms. It is important for the generation of energy under anaerobic conditions.

In the organs of humans and animals, the endogenous synthesis of D(-)-lactic acid is very low in quantity. The isomer is normally present in the blood of mammals at nanomolar concentrations and may be formed from methylglyoxal, derived from lipid or amino acid metabolism.

L. rhamnosus HN001 only produce L(+)-lactic acid.

UTILISATION OF PREBIOTICS

The ability of gastrointestinal bacteria to utilise diverse carbohydrates successfully in the intestinal tract may provide a competitive advantage. Prebiotics are non-digestible food ingredients that selectively stimulate the growth and/or activity of beneficial microbial strains residing in the host intestine [44].

The presence of galacto-oligosaccharides in human milk is believed to support the establishment of bifidobacteria in the gut of breast-fed infants. Apart from most bifidobacteria, only a few strains from other genera, including lactobacilli, possess the ability to make use of galacto-oligosaccharides.

It was demonstrated that *L. rhamnosus* HN001 can utilise galacto-oligosaccharides from dairy products (commercial milk powder) to support its growth *in vitro* [45]. This means galacto-oligosaccharides may be a potential prebiotic for *L. rhamnosus* HN001.

APPLICATIONS & STABILITY

L. rhamnosus HN001 shows very good stability in a variety of food applications, including yogurt and cheese [46,47,48], as well as in non-liquid products, such as powder supplements, capsules and tablets.

BENEFIT SUMMARY

L. rhamnosus HN001 is a well-characterised strain with documented probiotic effects. Several published studies describe the strain's properties, especially in the area of immune system modulation.

Figure 9 is a summary of attributes, based on these studies.

The health-related benefits can be summarised as follows:

- helps to strengthen the body's natural defences
- helps to strengthen natural defences in the elderly
- contributes to enhance the body's resistance
- helps to positively influence a healthy immune system
- reduces the incidence and severity of atopic eczema
- helps to improve the composition of the intestinal microbiota

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Note:
L. rhamnosus HN001 appears in the literature also as DR 20 or HOWARU® Rhamnosus.

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