Technical Memorandum

TM 58-1e

Bifidobacterium lactis HN019 – a probiotic with proven efficacy

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. *Bifidobacterium lactis* HN019 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, 8].

CHARACTERISTICS OF THE GENUS

Bifidobacterium sp. comprises Gram-positive, non-spore forming, anaerobic, pleomorphic bacilli that are dominant microbial residents of the colonic microbiota [9,10].

Bifidobacteria were discovered in 1899 in the faeces of breast-fed infants. This was of particular interest to scientists as these bacteria are typically the most abundant species present in the intestine of breast-fed infants and regarded as a primary reason for the infants' greater resistance to disease. In general, a high proportion of bifidobacteria in the intestinal tract is considered beneficial to health.

Today, bifidobacteria are broadly recognised for their key role in the human intestinal microbiota throughout life and they are widely used in probiotic foods and supplements.

SELECTION AND TAXONOMY

Bifidobacterium lactis was originally described by Meile *et al.* [11] and was recently re-classified as *B. animalis* subsp. *lactis* [12]. In the interests of simplicity, Danisco refers to strains of this species as *B. lactis*.

B. lactis HN019 was originally isolated from a yogurt produced in New Zealand and has been consumed as part of dairy products for a long time.

B. lactis HN019 is the fruit of a five-year research project conducted by the New Zealand Milk and Health Research Centre, the New Zealand Dairy Research Institute and other internationally renowned health researchers.

The New Zealand Dairy Research Institute screened its extensive collection (more than 2000 strains) of *lactobacilli* and *bifidobacteria* for potential probiotic candidates. After an initial screening, 200 strains were investigated more thoroughly. Out of these, *B. lactis* HN019 was considered to have the best probiotic potential based on its ability to resist bile and low pH *in vitro*.

Modern molecular biology methods, such as DNA/DNA homology and species-specific PCR primers, were used to identify the strain [13].

The strain has been deposited with the Australian Government Analytical Laboratories (AGAL) as deposit number NM97/09513.

SAFE FOR CONSUMPTION

Bifidobacterium sp. has long been considered safe and suitable for human consumption with several published studies addressing its safety [14,15]. Furthermore, *B. lactis* has been present in human food for decades and is listed in the Inventory of *Microorganisms With Documented History of Use in Human Food* [16].The European Food Safety Authority (EFSA) has also added the species to the Qualified Presumption of Safety list [17].

No harmful metabolic or toxigenic activities are associated with *B. lactis*.

General safety

In order to assess the safety of *B. lactis* HN019 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 with different doses of *B. lactis* HN019 for 7 days (5x10E7, 10E9 or 5x10E10 cfu/mouse/day). No abnormal clinical signs were observed in any of the groups during the period of the experiment. There were no significant differences in feed intake, water intake, or liveweight gain among mice fed the probiotic, compared to anon-probiotic control group. No bacteria were detected in the spleen of any animals. Histological and haematological parameters also indicated that *B. lactis* HN019 did not adversely affect mice health [18].

The general safety of *B. lactis* HN019 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 *B. lactis* HN019 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 [19].

Another study was performed to evaluate the acute oral toxicity of *B. lactis* HN019 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 [20].

The results suggest that *B. lactis* HN019 is non-pathogenic and non-toxic and has no adverse effect on the health of mice.

No risk for auto-immune disease

B. lactis HN019 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 *B. lactis* HN019 does not induce or enhance an auto-immune response. The strain can therefore be considered safe also for subjects with or at risk of auto-immune disease [21].

No degradation of mucin

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. In a study the ability of *B. lactis* HN019 to degrade mucin *in vitro* was evaluated in order to assess its potential pathogenicity and local toxicity. The results demonstrate that *B. lactis* HN019 is unable to degrade mucin [22]. The strain 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 [19,20].

No induction of platelet aggregation

Further *in vitro* investigations have shown that *B.lactis* HN019 does 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 [23].

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.

B. lactis HN019 has been found not to contain transferable antibiotic resistance and will therefore not contribute to the spread of antibiotic resistance among potential pathogenic microbes [24].

Safety aspects in human infants aged 0–2 years

As part of a double-blind placebo-controlled clinical trial of the effects of

B. lactis HN019 on infant eczema [42], 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 from birth for two years (daily dose 6x 10E9 cfu) had no effect on the general growth, health and tolerance of this sensitive group. The study concluded that *B. lactis* HN019 was safe and well tolerated and did not affect normal growth or gut and immune development when given to infants from birth [25].

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

B. lactis HN019 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 gastrointestinal (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 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 [26,27].

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 both to the highly acidic conditions present in the stomach and to concentrations of bile salts found in the small intestine.

B. lactis HN019 demonstrates high tolerance to low pH and high resistance to bile salts *in vitro* (figure 1) [13].

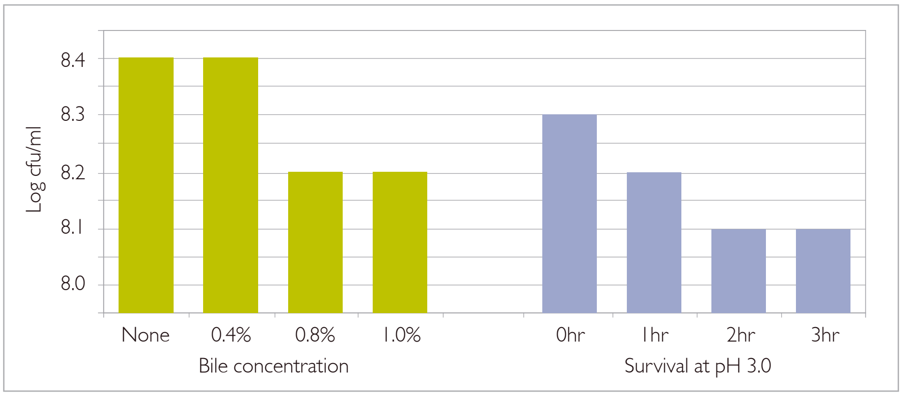

Figure 1. Effect of low pH (pH 3.0) and high bile concentration on the survivability of *B. lactis* HN019 [13].

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 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. 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 (figure 2).

B. lactis HN019 showed strong adhesion to different types of human epithelial cell lines applied in *in vitro* studies (table 1) compared with two competitor probiotic Lactobacillus strains and a negative control (non-probiotic *L. bulgaricus*) [28].

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 *B. lactis* HN019 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 *B. lactis* HN019 reduced the culturable *E. coli* numbers along with the the invasive ability and cell association characteristics of the pathogenic strain [28].

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 *B. lactis* HN019 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 [13].

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.

Modulation of intestinal microbiota
There are two dietary intervention studies that show that dietary consumption of *B. lactis* HN019 indeed modulates the composition of gut microbiota favourably.

The first study investigated the effect of dietary consumption of *B. lactis* HN019 on the microbiota of the GI tract of healthy adults. Subjects in one group consumed a reconstituted milk containing 3x10E10 cfu of *B. lactis* HN019 a day. Subjects in the control group consumed control milk without any supplementation. None of the subjects harboured *B. lactis* prior to the study. Dietary intervention continued for four weeks followed by a washout period of two weeks. An increase in faecal counts of *Bifidobacterium* and *Lactobacillus* was observed after 28 days feeding compared to the control group. Live transit of *B. lactis* HN019 through the GI tract was established by colony hybridisation and *B. lactis* specific probe. The relative proportion of *B. lactis* among *bifidobacteria* varied from 0.1% to 68% with an average of 28%. This study documents that *B. lactis* HN019 survives intestinal transit, can become a significant component of the normal faecal *Bifidobacterium* composition, and can increase total *Lactobacillus* and *Bifidobacterium* counts in the faeces (figure 3) [29].

In another study the impact of the consumption of three different daily doses of *B. lactis* HN019 on the microbial ecology of the gastrointestinal tract of elderly human subjects was investigated in another study.

The control group consumed non-supplemented milk while other three other experimental groups consumed milk supplemented with *B. lactis* HN019

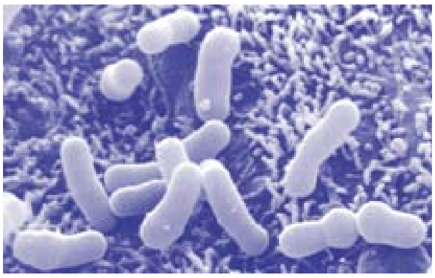

Figure 2. *B. lactis* HN019 adhering to a monolayer of Caco-2 cells [28].

Bacterial strain	HT-29	Caco-2	HT-29 MTX
Bifidobacterium lactis HN019	188	195	310
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* [28].

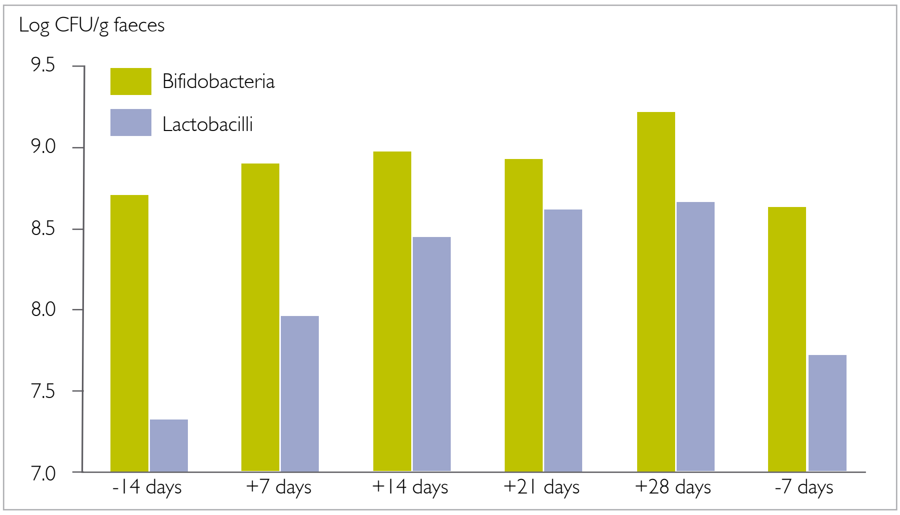

Figure 3. Faecal counts of *bifidobacteria* and *lactobacilli* in healthy subjects, before, during and after the consumption of *B. lactis* HN019 [29].

at a level of 5x10E9 cfu/day (high dose), 1x10E9 cfu/day (medium dose) and 6.5 x10E7 cfu/day (low dose) .

After dietary intervention, statistically significant increases in *bifidobacteria*, *lactobacilli* and enterococci were observed. There were no significant differences between the responses of the different dose groups, indicating that even the lowest dose (6.5 x 10E7 cfu/day) tested is able to confer desired changes in the intestinal microbiota (figure 4) [30].

These two studies provide evidence that dietary consumption of *B. lactis* HN019 helps increase the total *Lactobacillus* and *Bifidobacterium* count in the intestinal microbiota, and, thus, to maintain the gut health of the consumer.

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.

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 represent one of the key elements in the body's immune defence system [31].

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.

There is a significant amount of evidence to suggest that specific strains of probiotics 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.

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 [2,32].

In vitro studies

Intestinal permeability and immune markers

The gut acts as an internal barrier preventing pathogenic bacteria and other harmful substances from entering the body. The inner surface of the intestine consists of a layer of cells (epithelium), which are covered by a mucus layer (a viscous-elastic layer consisting mainly of carbohydrates combined with proteins

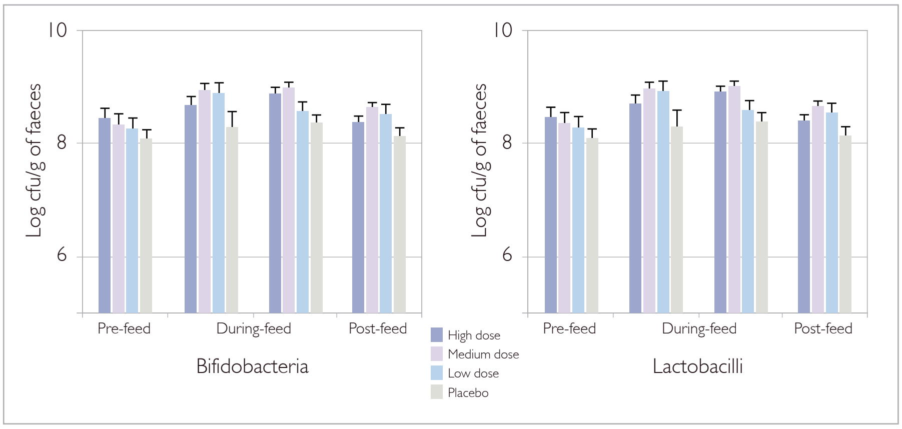

Figure 4. Impact of different doses of *B. lactis* HN019 on faecal counts of *bifidobacteria* and *lactobacilli* in elderly subjects [30].

and lipids) that plays a key role in the barrier effect mechanism.

Tight junctions are protein structures that link the epithelial cells together and control and maintain balanced intestinal permeability. As increased permeability is associated with certain diseases, proper regulation of the function of tight junctions is important in disease prevention.

In an *in vitro* study, it was shown that cell-free supernatant of *B. lactis* HN019 may increase the strength of tight junctions, although not to an extent that is statistically significant, while pathogenic *E. coli* reduces the strength considerably. The results suggest that direct contact between probiotic bacteria and epithelial cells may not always be necessary to obtain a beneficial effect. Cell-free supernatant of *B. lactis* HN019 also showed a slight significant increase in the ratio of inflammatory markers. This indicates that *B. lactis* HN019 has immune-enhancing properties, as also previously observed in clinical studies [33, 41].

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 *B. lactis* HN019 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, which is the primary, immediate, non-specific immune response and the acquired or adaptive immune system, which is a targeted response to a specific antigen and has a memory component.

To explore whether *B. lactis* HN019 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 *B. lactis* HN019 on various indices of the natural and acquired immunity of healthy mice. Supplementation with

B. lactis HN019 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 *B. lactis* HN019 is able to enhance several indices of natural and acquired immunity in mice [34].

Increased resistance to infections

Further studies were conducted to investigate whether this immune enhancement actually results in improved resistance to pathogenic bacteria. Increased resistance to infections and disease as a benefit of immune stimulation can be demonstrated via *in vivo* models and infection-challenge studies. *B. lactis* HN019 has been shown to be effective against common gastrointestinal pathogens in several animal models (see below).

Protection from experimental Salmonella infection.

A group of mice was fed the strain daily for a week, while a control group was not. The mice were subsequently challenged with *Salmonella typhimurium*. Three weeks after the challenge, only 7% of the control mice had survived while 80% of the mice fed *B. lactis* HN019 were still alive (figure 5) [35].

Reduction of E coli infection in mice

In a similar study, where the challenge applied was *E. coli* O157:H7, an important cause of food poisoning, a much lower rate of morbidity was observed among mice fed with *B. lactis* HN019 than for the control group. The probiotic-fed mice also showed significantly higher phagocytic activity [36].

Reduction of diarrhoea in piglets

Escherichia coli and rotavirus are common causes of diarrhoea in infants and young animals. As these two infectious agents commonly cause diarrhoea in piglets during weaning, piglets are an ideal model for studying this type of enteric infection.

This study investigated the protective effectiveness of feeding *B. lactis* HN019 against naturally acquired diarrhoea in weanlings.

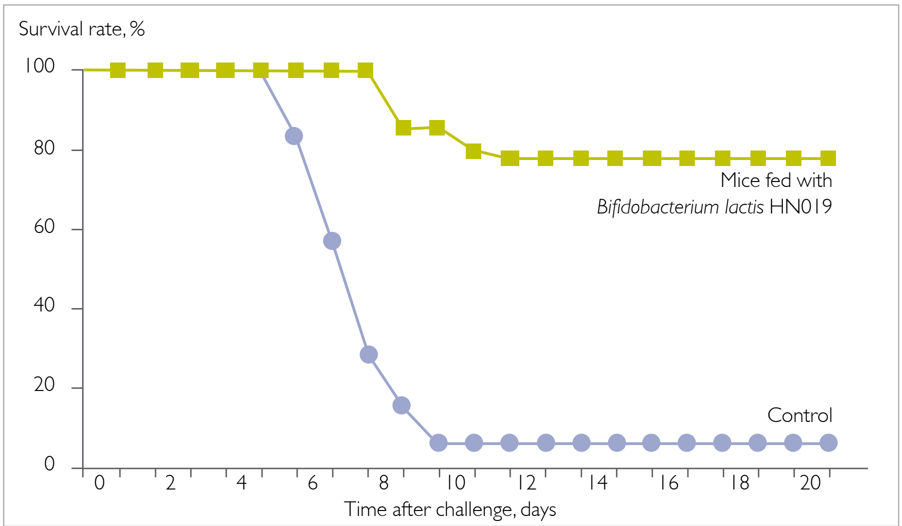

Figure 5. Survival of *Salmonella* infected mice with and without supplementation *B. lactis* HN019 [35].

The administration of *B. lactis* HN019 to weaning piglets resulted in a significantly lower incidence of diarrhoea during the first two days after weaning. The faecal levels of *E. coli* and rotavirus were also lower in the treatment group. This was shown by the significantly higher titre of specific antibodies in the faeces [37].

These results show that dietary treatment using *B. lactis* HN019 can reduce the severity of weanling diarrhoea associated with rotavirus and *E. coli*, possibly via a mechanism of enhanced immune-mediated protection.

Reduction of blood glucose levels in diabetic rats

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 *B. lactis* HN019, in combination with *L rhamnosus* HN001 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 [38].

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 *B. lactis* HN019 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 examine the impact of *B. lactis* HN019 on natural immunity of healthy, elderly subjects. Increases in interferon-alpha produced by peripheral blood mononuclear cells and phagocytic activities of polymorphonuclear cells were observed in subjects consuming milk containing *B. lactis* HN019 (figure 6). The results suggest that *B. lactis* HN019 may enhance natural immunity in elderly subjects, and that a relatively short-term dietary regime (6 weeks) is suffcient to impart measurable improvements in immunity that may offer significant health benefits to consumers. In addition, this study also documented the ability of *B. lactis* HN019 to survive gastrointestinal transit, using RAPD-DNA analysis with a strain-specific probe [39].

The impact of *B. lactis* HN019 on immune function was investigated in a further study. Two groups on probiotic supplemented diets were assessed: one group consumed *B. lactis* HN019 in low-fat milk, the other consumed the strain in lactose-hydrolysed low-fat milk. *B. lactis* HN019 consumption increased phagocytosis in both groups after three weeks. This level of activity was sustained three weeks after dietary supplementation with *B. lactis* HN019 had stopped. Natural killer (NK) cell activity was significantly improved at six and nine weeks in the group consuming the lactose-hydrolysed low-fat milk with *B. lactis* HN019, but only at nine weeks in the non-hydrolysed low-fat milk group. These results confirm the ability of *B. lactis* HN019 to enhance phagocytic activity in an age group including middle-aged subjects. It also expands on the previous study by showing a statistically significant improvement in NK cell activity [40].

Another study has extended the understanding of immune-enhancing characteristics of *B. lactis* HN019. Here, the dose-dependent expression of immune cell subsets of mononuclear leukocytes and ex vivo phagocytic activ-

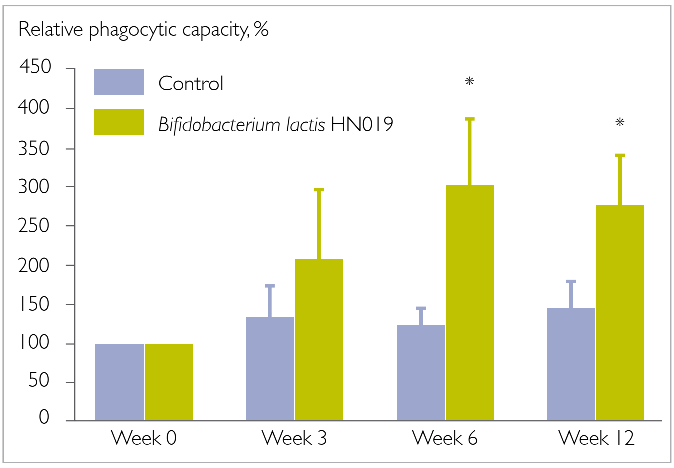

Figure 6. Effect of the consumption of *B. lactis* HN019 on phagocytic activity relative to base-line levels [39].

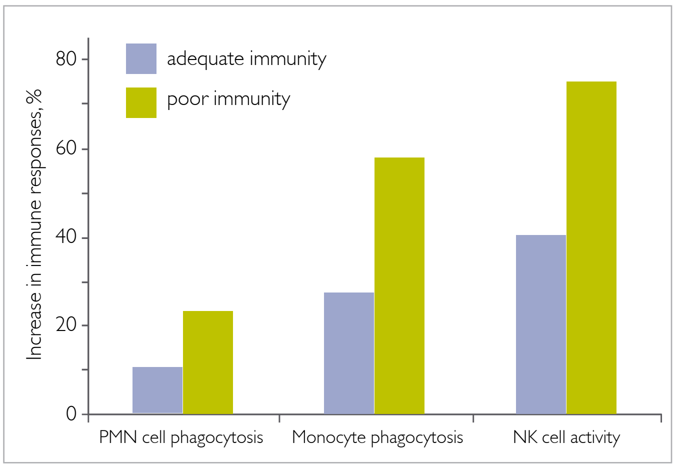

Figure 7. Influence of pre-treatment immune status on increases in immune response after consumption of *B. lactis* HN019 [41].

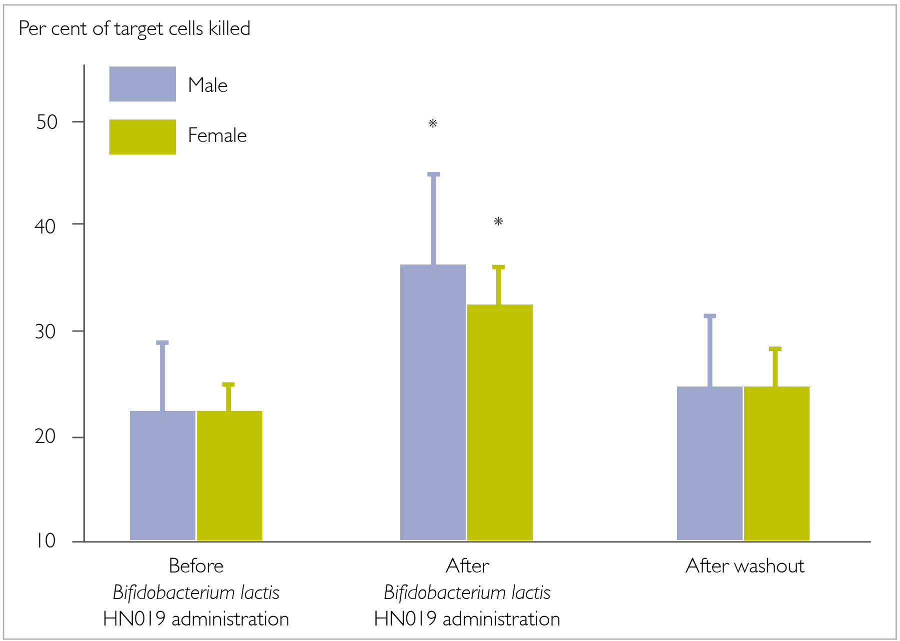

Figure 8. Effect of the consumption of *B. lactis* HN019 on natural killer cell activity [42].

ity were investigated. The results were similar for both doses of probiotic used. Both phagocytic activity and NK cell activity increased significantly after *B. lactis* HN019 consumption. The data was also analysed by looking at subjects divided into sub-groups with poor or adequate pre-treatment immune response. This analysis showed that increases in immune response after consumption of *B. lactis* HN019 was greater in subjects with poor pre-intervention immune status (figure 7) [41].

In another study conducted with elderly subjects, the ability of *B. lactis* HN019 to improve cellular immune function was tested. Statistically significant increases in NK cells activity were observed after probiotic feeding among both male and female subjects. While absolute responses tended to be higher among males, there were no significant differences between the genders (figure 8). The subgroup of subjects over the age of 70 years showed greater improvement in NK cells activity than those below 70 years [42].

These studies consistently show that *B. lactis* HN019 consumption has an impact on important markers of immune function in middle-aged and elderly subjects. Furthermore, the results suggest that the greatest benefit is likely to be experienced by consumers with poor immune status.

Prevention of infections and improvement of growth in young children

An extensive, long-term intervention trial on healthy children was conducted in India. Both *B. lactis* HN019 and galacto-oligosaccharides (GOS) were used to supplement a dried milk formulation. The ability of the strain to grow on components of the GOS preparation was confirmed in *in vitro* trials [49]. Consumption of this enriched milk resulted in reduced bloody diarrhoea, fewer days with fever and lower prevalence of ear infections. Furthermore, improved iron status and growth were observed for the group consuming the enriched milk. This study compliments previous human studies conducted with *B. lactis* HN019 as it expands tested populations to include healthy, young children, documents effects with lower doses (min. 9.6x10E6 cfu/day), evaluates long-term consumption and extends documentation in humans beyond biomarkers to include disease and growth endpoints. However, it is not possible to differentiate the relative contributions of *B. lactis* HN019 and GOS to the effects observed [43,44].

Influence of B. lactis HN019 in a probiotic combination 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-sensitised children [45].

B. lactis HN019 was also included in a double-blind, randomised placebo-controlled clinical trial, where the ability of probiotic supplementation to preventthe development of eczema and atopy in infants at risk of allergic disease was studied. Supplementation of the expecting mother and subsequently the infant with *B. lactis* HN019 had no effect on the incidence or severity of eczema compared to the placebo group [46]. However, higher levels of interferon (IFN) -γ in cord blood and transforming growth factor (TGF)-β and immunoglobulin (Ig) A in breast milk were observed [47]. The results indicate that probiotic supplementation begun in pregnancy may influence the antenatal and postnatal immune development.

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

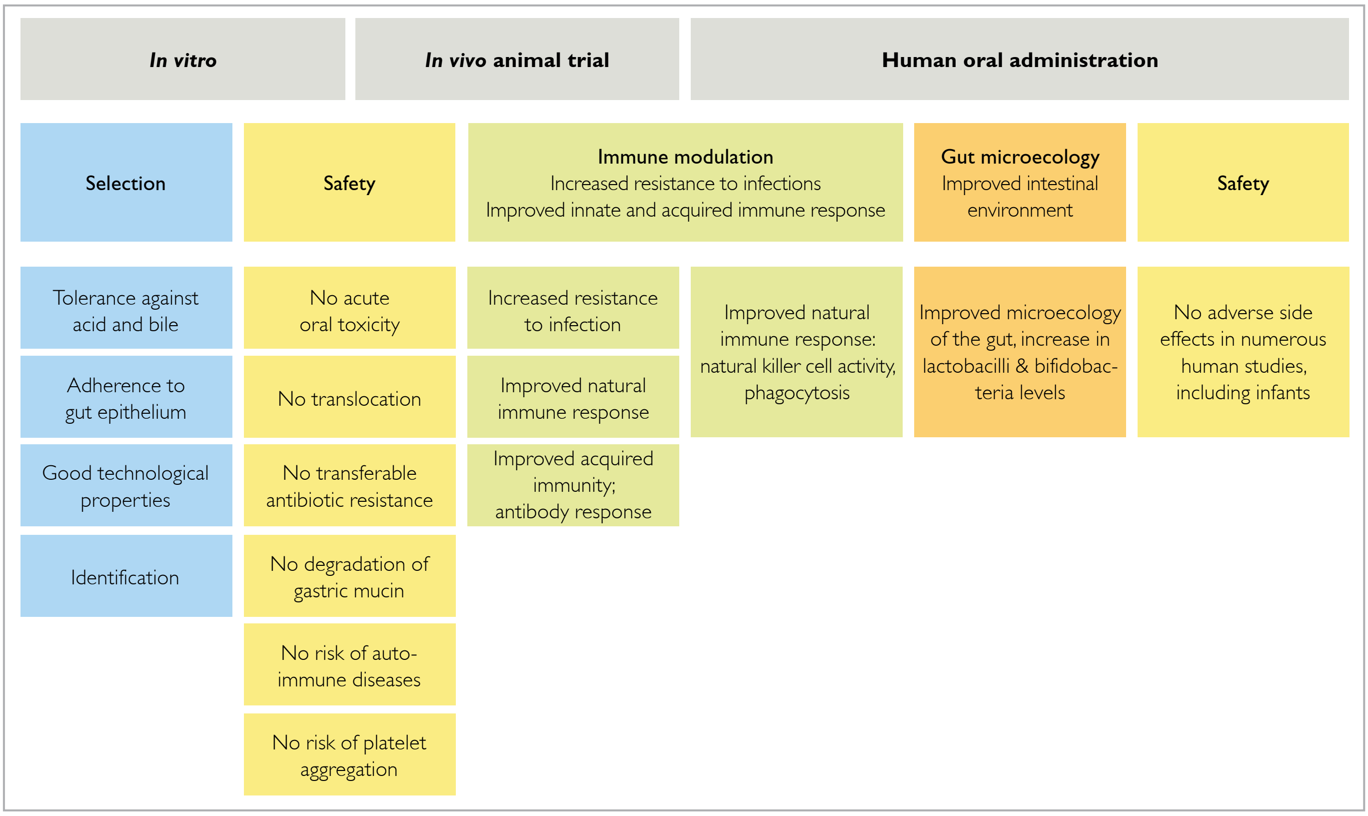

Figure 9. Summary of study findings.

known as L(+)-lactic acid and the other, its mirror image, is D(-)-lactic acid.

In humans, animals, plants, and micro-organisms, L(+) lactic acid is a normal 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.

B. lactis HN019 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 [48].

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 *B. lactis* HN019 can utilise galacto-oligosaccharides from dairy products (commercial milk powder) to support its growth *in vitro* [49]. This means galacto-oligosaccharides may be a potential prebiotic for *B. lactis* HN019.

APPLICATIONS & STABILITY

B. lactis HN019 shows very good stability in a variety of food applications, including yogurt, cheese and others [50,51,52,53], as well as in non-liquid products, such as powder supplements, capsules and tablets.

BENEFIT SUMMARY

B. lactis HN019 is a well-characterised strain with documented probiotic effects. Several published studies describe the strains 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 the natural defences in the elderly
- contributes to enhance the body's resistance
- helps to promote a healthy immune system
- helps to improve the composition of the intestinal microbiota

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Note:
B. lactis HN019 appears in the literature also as DR10 or HOWARU® Bifido.

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