DANISCO

LACTOBACILLUS PARACASEI LPC-37®

Technical Memorandum

TM 56-3E US 10.18

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 the ingestion of probiotics, friendly bacteria, is beneficial for supporting the body's delicate microbial balance. This balance is known to particularly enhance intestinal health and the immune system, as well as other physiological functions, making it a critical factor for general human well-being (Vandenplas et al., 2015; de Moreno de LeBlanc & LeBlanc, 2014; Kechagia et al., 2013).

Definition

Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Hill et al, 2014).

Most probiotics are either lactobacilli or bifidobacteria, although some strains of other microbial genera are also reported to possess probiotic properties.

The beneficial effects of probiotics either involve reducing the 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 the main areas of probiotic research for DuPont:

- 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 strain elicits all the health benefits that have been proposed for probiotics. Furthermore, when one probiotic strain has a certain health benefit, it cannot be assumed that another strain, not even 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 must fulfill certain requirements. These will improve its functionality in the intestine after consumption and enhance its survival in the product.

- The strain must be safe and thus free from potential risk-genes. This requires identification by appropriate molecular techniques
- The strain should be resistant to acid and bile
- The strain must have scientifically proven health benefits
- The strain should have good technological properties, such as the ability to survive in the final consumer product in sufficient counts until end of shelf-life, whether food or dietary supplements.

The only certain way to establish the true benefit of a probiotic strain is by systematic *in vitro* and *in vivo* studies and, in particular, human clinical trials. *Lactobacillus paracasei* Lpc-37 has been subject to several studies.

CHARACTERISTICS OF THE SPECIES

Lactobacillus paracasei is a Gram-positive, non-spore forming, homofermentative rod that is a common inhabitant of the human intestinal tract (Mitsuoka, 1996; Kandler & Weiss, 1986). *L. paracasei* strains are also found naturally in fermented vegetables, milk, and meat. Selected strains of this species are also used in probiotic foods and dietary supplements.

SELECTION AND TAXONOMY

Bacterial taxonomy is in dynamic development as new technologies continue to differentiate closely-related taxonomic groups. This is particularly true for the *L. casei/paracasei* group. Here research in DNA homology and typing has led to several proposals to reject the species *L. paracasei* and to include it in the restored species *L. casei* with a neotype strain (Dicks et al., 1996, Dellaglio et al., 1991). This proposal has, however, not been confirmed by the Judicial Commission of the International Committee on Systematic Bacteriology. Consequently, *Lactobacillus casei* today is restricted to strains ATCC 393 and NCFB 173, while almost all other '*Lactobacillus casei*' strains, are properly named *Lactobacillus paracasei* subsp. *paracasei*.

The *L. paracasei* Lpc-37 genome has recently been sequenced and confirmed to be *Lactobacillus paracasei* (NCBI accession # NOKL00000000).

L. paracasei Lpc-37 strain is isolated from a dairy source and has been deposited in the American Type Culture Collection as SD5275. It is also known as DGCC4981 and Lbc81.

GENOMICS

The whole genome sequences of published *L. paracasei* strains show good overall genomic synteny and core similarity, with unique regions that can be utilized for strain differentiation, including identification of *L. paracasei* Lpc-37 (Broadbent et al., 2012). *L. paracasei* strains isolated from diverse origins show strain-specific features that are related to carbohydrate utilization. The genome has been re-sequenced and publicly deposited (NCBI accession# NOKL00000000). Furthermore, a thorough assessment for safety showed no evidence of pathogenic genetic elements (Morovic et al., 2017).

Consistent strain identity

For a strain with documented probiotic activity, it is very important that it is not subjected to any genetic or physiological change during processing. To maintain the quality, purity, and consistency of each production batch of the strain, DuPont makes rigorous use of frozen bacterial seed inventories to reduce the risk of genetic drift over time and maintain strain integrity. DuPont also performs bacterial identification based on 16s rRNA gene sequence similarity for every produced batch of probiotics.

SAFE FOR CONSUMPTION

General safety

Lactobacillus species have historically been considered safe and suitable for human consumption with several published studies addressing its safety. Very few instances of infection have been associated with these bacteria, and those commonly with immunocompromised subjects with underlying health issues (Aguirre & Collins, 1993; Gasser 1994, Salminen et al., 1998; Borriello et al., 2003; Gueimonde et al., 2004).

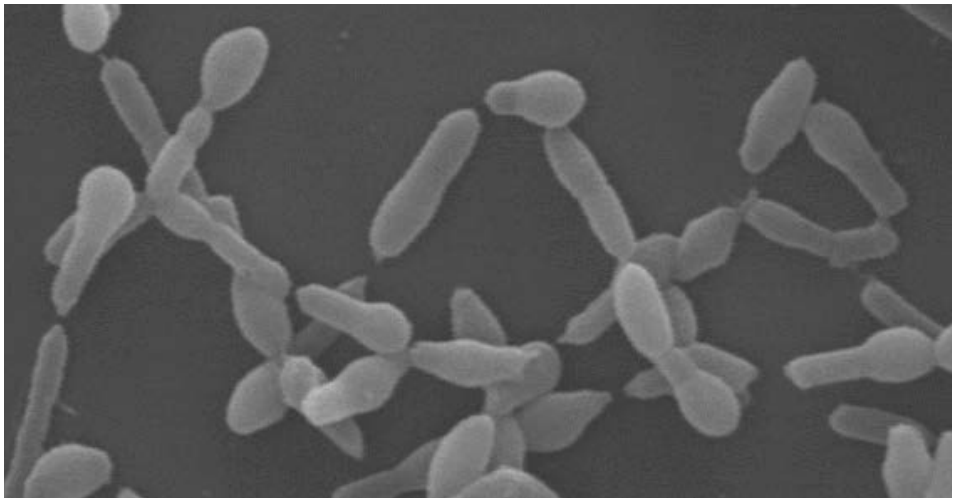


Figure 1. Scanning electron micrograph of *Lactobacillus paracasei* Lpc-37

L. paracasei Lpc-37 is listed in the Inventory of Microorganisms with Documented History of Safe Use in Human Food (Bourdichon et al., 2012) and it is also included on the Qualified Presumption of Safety list approved by European Food Safety Authority (EFSA Panel on Biological Hazards, 2017). Furthermore, *L. paracasei* has been listed in the U.S. FDA Generally Recognized as Safe (GRAS) list, where the FDA has no questions for the addition of *L. paracasei* to a variety of foods for antimicrobial use (U.S. Food and Drug Administration, 2012.)

In any published clinical trials, thus far, no adverse events have been associated with the administration of *L. paracasei* Lpc-37 and no serious adverse events have been reported.

In addition to a long history of safe human consumption of the species, no acquired antibiotic resistance was detected in *L. paracasei* Lpc-37 during screening by the EU-funded PROSAFE project. The strain has been sold commercially for more than 15 years.

Antibiotic susceptibility patterns

Antibiotic susceptibility patterns are an important means of demonstrating the potential of an organism to be readily inactivated by the antibiotics used in human therapy. Antibiotic resistance is a natural property of microorganisms and existed before antibiotics became used by humans. In many cases, resistance is due to the absence of the specific antibiotic target or is a consequence of natural selection. Antibiotic resistance can be defined as the ability of some bacteria to survive or even grow in the presence of certain substances that usually inhibit or kill other bacteria. This resistance may be inherent/intrinsic or acquired.

Inherent or intrinsic resistance

Most, if not all, strains of a certain bacterial species are not normally susceptible to a certain antibiotic. The antibiotic has no effect on these cells, being unable to kill or inhibit the bacterium, for example because the target for the antibiotic may be missing.

Acquired resistance

Most strains of a bacterial species are usually susceptible to a given antibiotic. However, some strains may be resistant, having adapted to survive antibiotic exposure. Possible explanations for this include:

Table 1. *L. paracasei* Lpc-37 Antibiotic Susceptibility Profile

Antibiogram of *L. paracasei* Lpc-37 was established using ISO 10932 IDF223 method and VetMIC Lact-1 and 2 microdilution plates that include all antibiotics that are recommended by the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). Recorded Minimum Inhibitory Concentrations (MICs) are displayed in the table below. All MIC values are below the Microbial Break Points (MBPs) defined for *Lactobacillus casei / paracasei* (EFSA Journal 2012; 10(6):2740). According to the results, ***L. paracasei* Lpc-37 does not bear acquired antibiotic resistance** (Morovic et al., 2017).

APPENDIX: Antibiotic Susceptibility Profile Method used: ISO 10932 IDF 223 with VetMIC Lact 1 and 2 microdilution plates.	Gentamycin	Kanamycin	Streptomycin	Tetracycline	Erythromycin	Clindamycin	Chloramphenicol	Ampicillin	Vancomycin	Virginiamycin*
	Gm	Km	Sm	Tc	Em	Cl	Ch	Amp	Va	Vi*
	Max MIC µg/ml									
Lactobacillus paracasei Lpc-37	4	64	32	1	0.12	0.12	4	1	>128	1
MBP for *Lactobacillus casei / paracasei***	32	64	64	4	1	1	4	4	NR***	4

* Virginiamycin is no more included in the FEEDAP recommended list of antibiotics (June 2012)
** EFSA Journal 2012;10(6):2740
*** NR: not required

- a mutation in the gene coding for the antibiotic's target can make the antibiotic less efficient. This type of antibiotic resistance is usually not transferable
- a resistance gene may have been acquired from another bacterium

Of the acquired resistances, the latter is of most concern, as it may also be passed on to other (potentially pathogenic) bacteria.

Much concern has arisen in recent years regarding vancomycin resistance, as vancomycin-resistant enterococci are a leading cause of hospital-acquired infections and are refractory to treatment. The transmissible nature of genetic elements that encode vancomycin resistance in these enterococci is an important mechanism of pathogenicity.

Resistance to vancomycin in certain lactobacilli, including *L. paracasei*, pediococci and leuconostoc, is due to intrinsic factors related to the composition of their cell wall. It is not due to any transmissible elements (Delcour et al., 1999). Through PCR testing, *L. paracasei* Lpc-37 has been found to be free of *Enterococcus* - like vancomycin-resistant genes. As yet, no case of antibiotic resistance transfer has ever been identified and reported for lactic acid bacteria used in foods and feed.

Measurements of antibiotic sensitivity did not demonstrate resistance for *L. paracasei* Lpc-37 at levels exceeding any breakpoints defined by EFSA (Morovic et al., 2017). Genomic mining of *L. paracasei* Lpc-37 showed lack of transfer potential of antibiotic resistance. The antibiotic susceptibility patterns for *L. paracasei* Lpc-37 are summarized in the Table 1.

Production of biogenic amines

Histamine and tyramine are biogenic amines that occur naturally in a wide range of foods including fermented products. They are formed by the enzymes present in the raw material or are generated by microbial decarboxylation of amino acids. The consumption of food containing large amounts of these amines can induce adverse reactions such as nausea, headaches, rashes, and changes in blood pressure (Ladero et al., 2010).

In lactic acid bacteria, production of histamine results from the catabolism of histidine by a histidine decarboxylase, and production of tyramine results from the catabolism of tyrosine by tyrosine decarboxylase. A specific detection method for histidine and tyrosine decarboxylase genes has been developed internally in DuPont based on the scientific literature and on the most updated genomic databases. With this method, no histidine or tyrosine decarboxylase gene was identified in *L. paracasei* Lpc-37 genome (Morovic et al. 2017). Consequently, *L. paracasei* Lpc-37 is unlikely to produce histamine or tyramine.

L/D-lactic acid production

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

Table 2. L/D-Lactic acid production

L/D lactic acid production molar ratio	100/0
	Boehringer Mannheim/ R-Biopharm D-lactic acid/ L-lactic acid UV-method

Source: DuPont, internally generated data

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 intermediate or end product of the carbohydrate and amino acid metabolic processes whereas, D(-)-lactic acid was thought to be "non-physiological" and a possible cause for lactate acidosis.

Although some species of probiotic cultures, as nutritional ingredients that may produce D(-)-lactic acid, have been safely administered to infants (Connolly et al., 2005), Codex specifies that only L(+)-lactic acid producing cultures can be used for infant formula and follow up formula.

L. paracasei Lpc-37 only produces L(+)-lactic acid (Table 2).

PRODUCT STABILITY

Today there is a general consensus that probiotics have to be consumed in sufficient numbers to provide the desired health benefit. It is likely that different strains and different effects require different dosages. Delivering the proper dose of *L. paracasei* Lpc-37 over the shelf-life of a product can be dependent on many factors such as formulation, processing, packaging, and storage temperature and humidity. It is important to consider these factors and run stability trials to develop sound products.

From technical qualifications perspective, *L. paracasei* Lpc-37 is amendable for large scale production in a variety of consumer end-products with sufficient shelf-life thus making it a suitable for manufacturing processes.

HEALTH-RELATED PROPERTIES

The health benefits of probiotic bacterial strains have been demonstrated over the years, including a range of health improvement and inhibition of infection. *In vitro*, animal, and human clinical studies have established the efficacy of strain *L. paracasei* Lpc-37 as a probiotic with demonstrated health benefits. Research has focused on characteristics that indicate beneficial effects such as acid and bile resistance, adhesion to intestinal and oral surfaces, antimicrobial activity, and efficacy in human clinical trials. The key findings from research on *L. paracasei* Lpc-37 are summarized below.

BENEFITS TO INTESTINAL HEALTH AND WELL-BEING

The importance of the intestinal microbiota for health

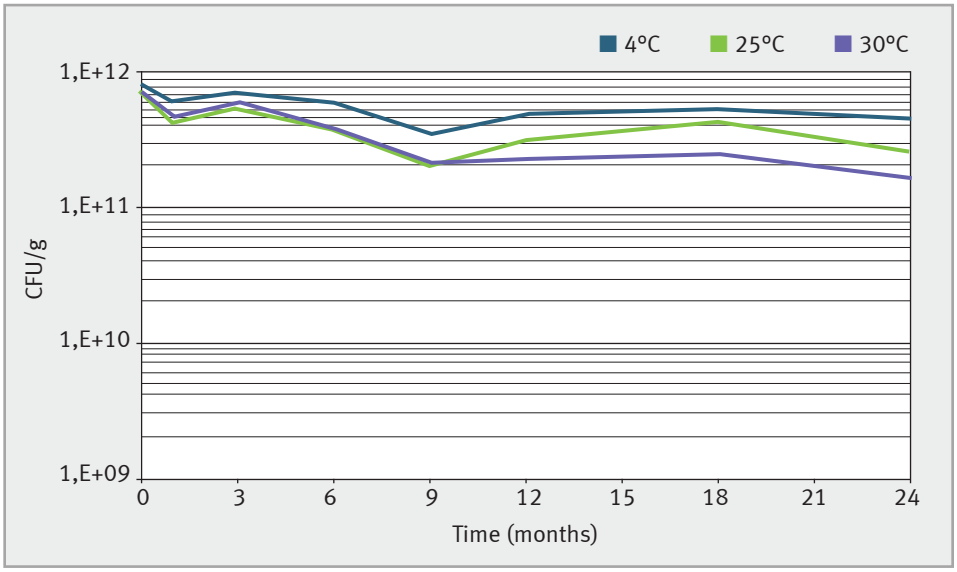
The human gastrointestinal (GI) tract is an extremely complex ecosystem and represents the host's greatest area of contact with the environment. This ecosystem is comprised of:

- the gastrointestinal epithelium
- immune cells
- resident microbiota

The primary function of the human GI tract has long been considered the digestion and absorption of nutrients and the excretion of waste end-products. However, in recent years it has become recognized that the GI tract fulfills many other functions that are essential to our well-being. The GI tract harbors a vast number of microbial cells (10^{14}), which may surpass the number of cells that make up the human body (Sender et al., 2016).

The intestinal microbiota is estimated to consist of at least 1000 species, although 95-99% of all bacteria belong to just 10 genera. Many members of the intestinal microbiota are beneficial, while others are potentially detrimental or their function not known. A higher concentration of

Figure 2. Stability of *L. paracasei* Lpc-37 culture concentrate



Source: DuPont, internally generated data

certain genera, including *Lactobacillus* and *Bifidobacterium*, is generally thought to be associated with a healthier GI tract. The resident microbes are involved in many metabolic processes, such as the fermentation of undigested carbohydrates into short-chain fatty acids and 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 and potentially pathogenic microbes. 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 optimize the composition and activity of the intestinal microbiota and, thus, reduce the risk of a range of diseases or unfavorable conditions (Ouwehand et al., 2006; Guarino et al., 2013; Lin et al., 2014; Scott et al., 2015).

Table 3. Selected characteristics of *L. paracasei* Lpc-37

++++ Excellent +++ Very good ++ Good + Fair	
Acid tolerance	**++++** (›90% survival in hydrochloric acid and pepsin (1%) at pH 3.5 for 1h at 37°C)
Bile salt tolerance	**+** (‹69% survival in 0.3% bile salt containing medium)
Pepsin resistance	**+** (‹60% in 0.3% pepsin containing medium at pH 2 for 1h)
Pancreatin resistance	**++++** (›60% survival in 0.1% pancreatin containing medium at pH 8 for 2h)

Source: DuPont, internally generated data

Resistance to acid and bile and survival in the intestinal passage

According to the generally accepted definition of a probiotic, the probiotic microorganism should be viable at the time of ingestion in order to confer a health benefit. This implies that a probiotic should survive passage through the GI tract and, according to some interpretations, transiently colonize the host epithelium.

A variety of traits are believed relevant for surviving GI tract passage, the most important of which is tolerance to the highly acidic conditions present in the stomach and the concentrations of bile salts found in the small intestine. *In vitro* studies have shown that *L. paracasei* Lpc-37 is very resistant to low pH conditions and shows moderate resistance to bile at the concentrations present in the duodenum (Ding & Shah, 2007; Table 3). In addition, *L. paracasei* Lpc-37 has been recovered in high numbers in feces after supplementation, which indicates that *L. paracasei* Lpc-37 is able to colonize the intestine transiently (Hemalatha et al., 2014; Roessler et al., 2008).

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 colonize the intestine.

Currently, adherence is measured using *in vitro* human intestinal epithelial cell lines, Caco-2 and HT-29 and human colonic mucus. While these are not a thorough test of the ability of probiotics to adhere to intestinal mucosa in the body, attachment to these cell lines and mucus *in vitro* is considered a good indicator of their potential to attach. *L. paracasei* Lpc-37 has demonstrated excellent adhesion to human epithelial cell lines (Caco-2 and HT-29) and fair adhesion to human colonic mucus (Table 4) applied in *in vitro* studies.

Table 4. Adherence of *L. paracasei* Lpc-37 to human intestinal cells and human colonic mucus *in vitro*

++++ Excellent +++ Very good ++ Good + Fair	
Adherence of *L. paracasei* Lpc-37 to human intestinal cells *in vitro*	HT-29: **+++** Caco-2: **++++**
+++++ Excellent ++++ Very good +++ Good ++ Fair	
Adherence of *L. paracasei* Lpc-37 to human colonic mucus *in vitro*	**++** (0.5 – 2% of added bacteria)

Source: DuPont, internally generated data

Table 5. Pathogen inhibition *in vitro*

++++ Excellent +++ Very good ++ Good + Fair	
Pathogen inhibition *in vitro*	**++++ *Salmonella typhimurium*** **++++ *Staphylococcus aureus*** **++++ *Escherichia coli*** **++ *Listeria monocytogenes***

Source: DuPont, internally generated data

Inhibition of pathogens

The protective role that probiotic bacteria provide 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 co-aggregate with pathogens, and the stimulation of the immune system (Gibson et al., 2005).

In vitro inhibition is usually investigated using an agar inhibition assay, where soft agar containing the pathogen is laid over colonies of probiotic cultures, causing the development of inhibition zones around the colonies. This effect may be due to the production of acids, hydrogen peroxide, bacteriocins and other substances that act as antibiotic agents as well as competition for nutrients. It should be pointed out, however, that extending such results to the *in vivo* situation is not straightforward. The assessment in the Table 5 is based on such an *in vitro* assay.

L. paracasei Lpc-37 displayed *in vitro* inhibition of selected pathogens using an agar inhibition assay. These pathogens included *Salmonella typhimurium, Staphylococcus aureus, Escherichia coli,* and *Listeria monocytogenes* (Table 5).

Forssten et al. (2015) studied the effects of polydextrose, *L. paracasei* Lpc-37, and *Lactobacillus acidophilus* NCFM on the growth of *Clostridium difficile* in an *in vitro* model of infected human large intestine. According to the results from the qPCR assays, *L. paracasei* Lpc-37 and *L. acidophilus* NCFM did not have any significant effects on the growth of *C. difficile* in the colonic model. This was assumed to result from strain-specific features or possibly too low inoculation levels (Forssten et al., 2015).

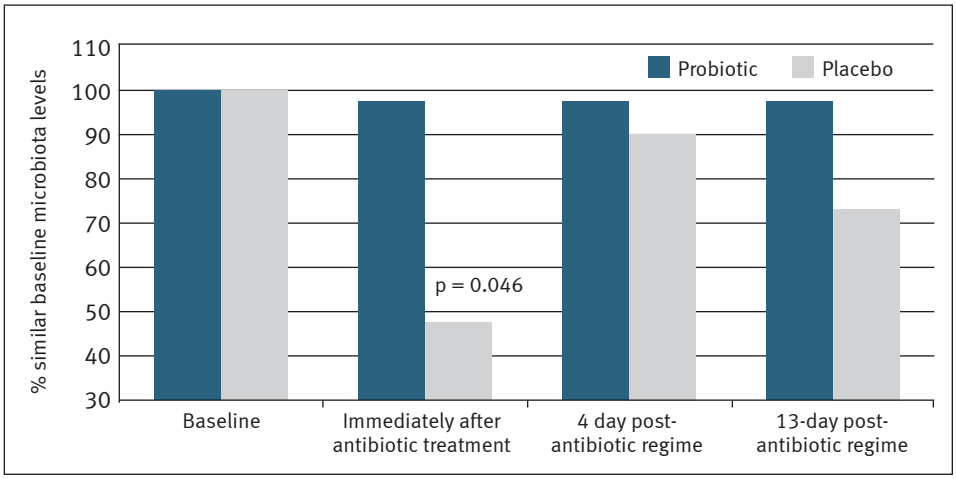
Although *L. paracasei* Lpc-37 may have antimicrobial activity, it does not appear to influence the survival of other probiotic bacteria when studied as a single strain or multi-strain combination using a simulated colonic environment (Forssten & Ouwehand, 2017).

Modulation of the human intestinal microbiota

L. paracasei Lpc-37 was included in a five-strain probiotic formulation and investigated for its ability to stabilize the intestinal microbiota during and after antibiotic therapy (Engelbrektson et al., 2006; Engelbrektson et al., 2009). Participants were randomly assigned to either the placebo or the probiotic test product, which consisted of a capsule containing a dried bacterial preparation consisting of *Bifidobacterium bifidum* Bb-02, *Bifidobacterium lactis* Bl-04, *Bifidobacterium lactis* Bi-07, *L. acidophilus* NCFM, and *L. paracasei* Lpc-37.

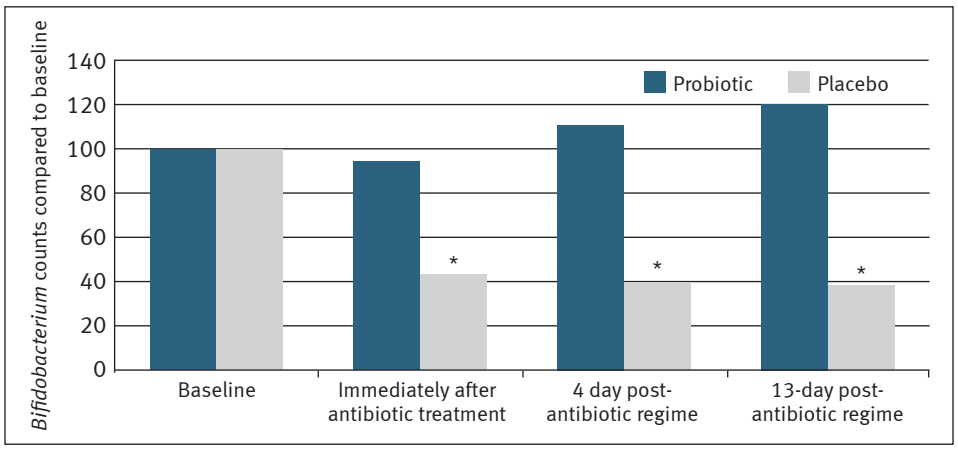
In this human trial, the probiotic mixture was found to reduce the antibiotic-induced disturbance of the total microbiota population (Figure 3). In addition, the probiotic product still maintained bifidobacteria at significantly higher levels than that found in the placebo group two weeks after the cessation of antibiotic therapy (Figure 4). This study indicated that the mixture of probiotics, including *L. paracasei* Lpc-37, promoted a more rapid return to pre-antibiotic baseline fecal microbiota composition (Engelbrektson et al., 2009).

Figure 3. The probiotic mixture containing *L. paracasei* Lpc-37 protects the fecal microbiota from disruption by antibiotics, as indicated by the greater dissimilarity of the microbiota of the placebo group compared to the baseline microbiota composition.



Source: Engelbrektson et al, 2009

Figure 4. The probiotic mixture containing *L. paracasei* Lpc-37 promotes the maintenance of bifidobacterial levels in the feces of antibiotic-consuming subjects during post-treatment (*p=0.030) (Adapted from Engelbrektson et al., 2009).



Source: Engelbrektson et al, 2009

L. paracasei Lpc-37 was the main probiotic component in a placebo-controlled cross-over study with healthy adults and patients with atopic dermatitis (AD) (Roessler et al., 2012). A combination of probiotics *L. paracasei* Lpc-37, *Lactobacillus acidophilus* 74-2, and *Bifidobacterium animalis* subsp. *lactis* DGCC 420 (*B. lactis* 420) resulted in a significant increase in fecal lactobacilli. The probiotic combination significantly reduced the genotoxic potential of fecal water in AD patients and may therefore contribute to a less carcinogenic environment.

Reduction of incidence of diarrhea

Ouwehand et al. (2014) have reported that a four-strain supplement HOWARU® Restore including *L. paracasei* Lpc-37 appears to lower the risk of antibiotic-associated diarrhea, *C. difficile*-associated diarrhea, and diarrhea-associated symptoms in a dose-dependent manner in adult in-patients requiring antibiotic therapy (Ouwehand et al., 2014). Another study also demonstrated a significant improvement of *C. difficile* infection outcomes with a probiotic combination treatment including *L. paracasei* Lpc-37, *L. acidophilus* NCFM, *B. lactis* Bi-07, and *B. lactis* Bl-04 (Barker et al., 2017). Duration and rate of diarrhea were significantly worse for participants treated with placebo compared with participants treated with probiotics.

The effect of probiotics *L. paracasei* Lpc-37 or *Bifidobacterium lactis* HN019 on diarrhea and fever was studied in preschool children in a community setting in a developing country (Hemalatha et al., 2014). During the rainy season, when incidence of fever and diarrhea was highest, administration of both *L. paracasei* Lpc-37 and *B. lactis* HN019 reduced the incidence of diarrhea and fever. Outside the rainy season, risk of diarrhea was low and no benefit was observed for probiotic supplementation during the whole 9-month study period. The probiotic intervention with *L. paracasei* Lpc-37 influenced the levels of fecal short chain fatty acids (SCFA) and branch chain fatty acids (BCFA) in a different manner (Hemalatha et al., 2017). There was also a differential response in SCFA and BCFA levels in those who developed diarrhea and those who did not. After the intervention, *L. paracasei* Lpc-37 supplementation correlated positively with total *Bifidobacterium* counts and isovalerate levels in fecal samples.

Modulation of abdominal pain

Abdominal pain is common in the general population and among patients with diarrhea or irritable bowel syndrome. In a human clinical trial, a four-strain supplement HOWARU® Restore including *L. paracasei* Lpc-37 reduced abdominal pain in adult in-patients with diarrhea in a dose-dependent manner (Ouwehand et al. 2014).

L. paracasei Lpc-37 was studied among other probiotic strains for the ability to induce the expression of analgesic receptors in human HT-29 intestinal epithelial cells (Rousseaux et al., 2007). Some *Lactobacillus* strains were observed to induce the expression of μ-opioid and cannabinoid receptors in bacteria-stimulated HT-29 cells, however *L. paracasei* Lpc-37 was found ineffective.

The effect of synbiotic product on constipation

The clinical response of chronically constipated women to a commercially available synbiotic, combining fructo-oligosaccharide (FOS) with *Lactobacillus* and *Bifidobacterium* strains were assessed (Waitzberg et al., 2013). The synbiotic product containing FOS and 10^8-10^9 bacteria of the strains *L. paracasei* Lpc-37, *L. rhamnosus* HN001, *L. acidophilus* NCFM, and *B. lactis* HN019 was consumed for 30 days. The synbiotic product improved evacuation parameters and constipation intensity of chronically constipated women relative to placebo after the 30-day supplementation. There were no significant differences between groups in the frequency of abdominal symptoms before or after the intervention.

BENEFICIAL MODULATION OF THE IMMUNE SYSTEM

The probiotic concept and 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 GI 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 defense system (Calder et al., 2013).

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 and normal resistance to disease. Reduced microbial exposure in Western societies has also been associated with an increased incidence of atopic and autoimmune disorders (Calder et al., 2013; Versini et al., 2015).

There is a significant amount of evidence to suggest that specific probiotic 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 (Lei et al., 2015).

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 (Duerkop et al., 2009; Hardy et al., 2013).

Modulation of the immune system is an area of intense study in relation to the DuPont™ Danisco® range of probiotics. The goal is to understand how each strain contributes to the maintenance and balance of optimal immune function. The immune system is controlled by compounds known as cytokines. Cytokines are hormone-like proteins made by cells that affect the behavior of other cells and, thereby, play an important role in the regulation of immune system functions. Cytokine expression can be modulated by specific probiotic bacteria. However, interpreting the health relevance of changes in cytokine levels, both from *in vivo* and human studies, remains a challenge.

Response to vaccination

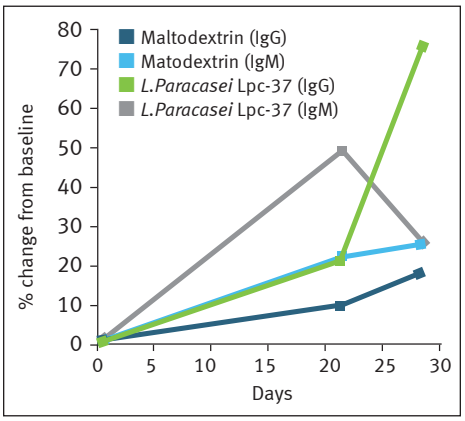
The ability of *L. paracasei* Lpc-37 to stimulate specific immunity has been evaluated in a randomized, double-blind, controlled human study measuring primary immune reaction following vaccination (Paineau et al., 2008). Human volunteers were orally vaccinated using cholera vaccine as the vaccination model. Supplementation with *L. paracasei* Lpc-37 or placebo started on day 0 and continued for 21 days. The subjects consumed two capsules a day with 10^{10} CFU *L. paracasei* Lpc-37 or two capsules a day with maltodextrin (control). On day 7 and 14, the subjects received the oral vaccine. Blood samples were collected on day 0, 21, and 28 and antigen-specific antibodies (immunoglobulins, IgA, IgG, IgM) were determined.

Supplementation with *L. paracasei* Lpc-37 resulted in a relatively higher but non-significant induction of specific serum IgG compared to the control group (Figure 5). The stronger increase in specific serum IgM from day 0 to day 21 was not significant. A late decrease in serum (IgM after day 21) was observed in the group consuming *L. paracasei* Lpc-37 compared with the control group, but the difference was not significant. Changes in the levels of IgA were not different from those of the control group (Paineau et al., 2008; Figure 5).

Allergy

L. paracasei Lpc-37 was the main probiotic component in a double-blind, placebo controlled, randomized cross-over study with 15 healthy adults and 15 patients with atopic dermatitis (AD) (Roessler et al., 2008). The purpose of the study was to elucidate the effect of a probiotic drink containing a combination of the probiotics *L. paracasei* Lpc-37, *L. acidophilus* 74-2, and *B. lactis* 420 on clinical and immunological parameters and microbiology in feces. The SCORAD (Scoring Atopic Dermatitis) scale was used for assessing the severity (i.e., extent, intensity) of AD. In patients, the SCORAD tended to decrease after supplementation. Differences were not observed in the expression of lymphocyte subsets resulting from probiotic intervention. In healthy subjects, *L. paracasei* Lpc-37 increased the frequency of CD57 positive cells (marker of NK-cells) in the blood. The phagocytic activity of monocytes and granulocytes was significantly increased in healthy subjects but not in patients with AD upon probiotic intervention. This study suggests that the mix of probiotics may modulate peripheral immune parameters in healthy subjects.

Figure 5. Relative change in specific IgG and IgM titre in orally vaccinated humans after supplementation with *L. paracasei* Lpc-37



Source: Paineau et al., 2008

Cellular immunity

A milk drink containing *L. paracasei* Lpc-37 was studied for its effects on immune function and intestinal microbiota in healthy elderly subjects (Forssten et al., 2011). Both the placebo and probiotic milk drinks increased significantly the NK cell activity at the end of the intervention when compared to the baseline. However, there was no significant difference in NK cell activity between groups. At the end of the intervention, despite the slightly higher values of total fecal IgA, PGE2 and calprotectin observed in the probiotic group compared to the placebo group, the differences did not reach statistical

significance at any time point. Cytokine levels showed a large inter-individual variability and, as well as C-reactive protein (CRP), did not show any statistically significant differences between groups or time points.

OTHER HEALTH-RELATED PROPERTIES

Oral health

Dental plaque is a biofilm structure which gradually forms on the tooth surface. This structure is a complex environment in which streptococci and other caries-related microorganisms produce acid, which is the direct cause of tooth decay. Probiotic bacteria have been proposed to affect the oral ecology by specifically preventing the adherence of *Streptococcus mutans*.

Five probiotic lactobacilli strains, including *L. paracasei* Lpc-37, inhibited the growth and biofilm formation of *S. mutans in vitro* (Lin et al., 2015). This inhibitory effect of *L. paracasei* Lpc-37 on *S. mutans* growth and multispecies biofilm formation was also demonstrated *in vitro* for clinically isolated *S. mutans* strains from children with active caries (Lin et al., 2017). It is possible that the antibacterial substances in probiotics may be primarily bacteriocins or bacteriocin-like proteins, however the mechanism of probiotic lactobacilli against *S. mutans* is not fully understood.

Cholesterol lowering

The influence of *L. paracasei* Lpc-37 (10^{10} CFU/d) alone or in combination with a calcium supplement on fecal lactobacilli colonization and beneficial health effects were studied in a double-blind, placebo-controlled, cross-over study with 32 men and women (Trautvetter et al., 2012). The fecal concentration of *L. paracasei* and all lactobacilli increased significantly after *L. paracasei* Lpc-37 and *L. paracasei* Lpc-37 + pentacalcium hydroxytriphosphate (CaP) interventions compared to placebo. The combination of *L. paracasei* Lpc-37 and CaP decreased total cholesterol and low-density lipoprotein (LDL)-cholesterol concentration in plasma compared to *L. paracasei* Lpc-37 and placebo. These results indicate that CaP modulates the colonization of *L. paracasei* Lpc-37 in the human gut under combinatory supplementation of CaP and *L. paracasei* Lpc-37. However, lowering blood cholesterol could be also due to the CaP supplementation.

BENEFITS SUMMARY

Based on the data generated supporting *L. paracasei* Lpc-37 strain qualities, the following health-related attributes can be summarized:

- Long history of safe use
- Well suited for intestinal survival:
  - High tolerance to acid and bile as present in the intestine
  - Transient colonization after consumption
  - Strong adhesion to intestinal cell lines
- May inhibit *S. mutans* growth and biofilm formation as demonstrated *in vitro*
- Promotes gut health and healthy microflora restoration
- Beneficial modulation of immune functions:
  - May have an influence on immune function regulation

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