DANISCO

LACTOBACILLUS PLANTARUM LP-115®

Technical Memorandum

TM 50-2E US 6.18

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, or friendly bacteria, is beneficial for maintaining the body's delicate microbial balance. This balance is known to 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; LeBlanc & LeBlanc 2014, Sanders 2015, Markowiak & Slizewska 2017).

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 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 the main areas of DuPont's probiotic research:

- 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. 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 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 – this requires identification by appropriate molecular techniques
- the strain has to be able to resist acid and bile
- the strain must have clinically-proven health benefits
- the strain should have good technological properties, such as the ability to survive in the final consumer product, whether food or dietary supplements, and when included in a food either be neutral or contribute favorably to the flavor of that 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. plantarum* Lp-115 has been subject to several studies.

CHARACTERISTICS OF THE SPECIES

L. plantarum is a Gram-positive, non-spore forming, facultative hetero-fermentative rod. *L. plantarum* is one of the most versatile species and found in many different ecological niches (Siezen 2011, Arena et al, 2016).

The species frequently occurs spontaneously in high numbers in most lactic acid fermented, plant-based foods, including brined olives, sauerkraut, traditional African ogi and cassava, and Asian kimchi. It is also often found in the human gastrointestinal and vaginal tract (Seddik et al, 2017).

Its versatility and its metabolic capacity facilitates its use both as probiotic and as industrial starter cultures in several food products, including sourdough bread, fermented meat products, fermented vegetables and wine (Salvetti 2017). It is also the most commonly used species in silage production.

SELECTION AND TAXONOMY

L. plantarum Lp-115 has been genetically characterized and properly classified as *L. plantarum* using modern, genotypic methods including 16S rRNA gene sequence analysis. *L. plantarum* Lp-115 is a strain isolated from plant material and has been deposited in the American Type Culture Collection as SD5209.

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. In order 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 culture.

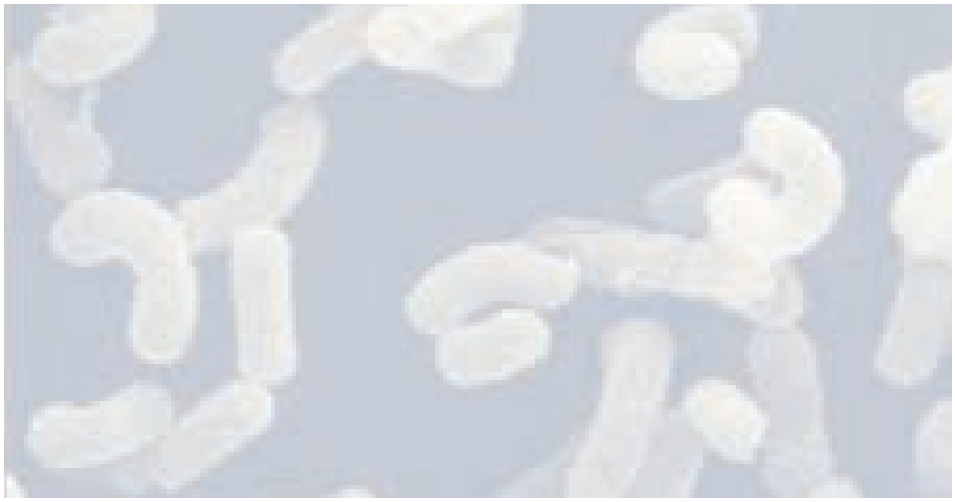


Figure 1. Scanning electron micrograph of Lactobacillus

SAFE FOR CONSUMPTION

General Safety

Lactic acid bacteria have long been considered safe and suitable for human consumption and many *Lactobacillus* species, including *L. plantarum*, are listed in Food fermentations: Microorganisms with technological beneficial use (Bourdichon et al, 2012). The European Food Safety Authority (EFSA) has also included the species to the Qualified Presumption of Safety list (EFSA BIOHAZ 2017).

The fact that many traditional lactic acid fermented vegetable-based foods spontaneously contain high numbers of *L. plantarum* and that these products, all over the world, have a reputation of being safe and wholesome, strongly indicates that *L. plantarum* can safely be consumed. This becomes especially obvious if the long historical tradition of lactic acid fermented foods is taken into account (Bernardeau et al, 2008).

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 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. Antibiotic resistance

L. plantarum Lp-115 has been safely used as a single strain and in combination with other probiotics and/or prebiotics in human clinical trials (Table 1). None of these trials have reported any safety concern related to *L. plantarum* Lp-115.

Daily dose of Lp-115	Subjects receiving Lp-115 (n)	Age of subjects (yrs)	Duration of supplementation (days)	Reference
2x 10^{11}	40	17- ›50	30	Costa et al 2014
1x 10^{10}	9	38 (mean)	21	Paineau et al 2008
5x 10^{9}	34	57 (mean)	7	Zhang et al 2013
80 ml with 1.25x 10^{7} cfu/g	12	62 (mean)	90	Barreto et al 2014

Table 1. Use of *L. plantarum* Lp-115, including probiotic blends, in selected human clinical trials.

Table 2. Antibiotic Susceptibility Profile

Antibiogram of *L. plantarum* Lp-115 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 MICs are displayed in the table below. All MIC values are below or equal to the Microbial Break Points (MBPs) defined for *L. plantarum* [EFSA FEEDAP Panel 2012].

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 plantarum Lp-115	8	64	64	32	0.5	2	8	0.5	>128	2
MBP for *Lactobacillus plantarum / pentosus***	16	64	NR***	32	1	2	8	2	NR***	4

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

due to a specific resistance gene is of most concern. In case such resistance is found, it has to be investigated whether it may also be passed on to other (potentially pathogenic) bacteria.

No acquired antibiotic resistance was detected in *L. plantarum* Lp-115 during a screening project (Klare et al, 2007).

The antibiotic susceptibility patterns for *L. plantarum* Lp-115 are summarized in Table 2.

The safety of the strain was further evaluated in a colitis mouse model, using trinitrobenzenesulphonic acid (TNBS) to induce colitis. In TNBS-treated mice, high doses (10^{10} cfu) *L. plantarum* Lp-115 did not result in translocation of the organism, nor did it induce any potential adverse effect on mouse activity, weight, and colon inflammation or abnormal translocation of members from the intestinal microbiota (Daniel et al, 2006).

L/D-LACTIC ACID PRODUCTION

Lactic acid is the most important metabolic end product of fermentation processes by lactic acid bacteria and other microorganisms. 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. The ratio of L- and D-lactate for *L. plantarum* Lp-115 is 45% L-lactate and 55% D-lactate.

In humans, animals, plants and microorganisms, L(+)-lactic acid is a normal intermediate or end product of carbohydrate and amino acid metabolism whereas, D(-)-lactic acid was thought to be "non-physiological" and a possible cause for lactate acidosis. However, mammals (including humans) express D-alpha-hydroxy acid dehydrogenase which is able to metabolize D(-)-lactic acid, albeit at a slower rate than L-lactate dehydrogenase (Ewaschuk et al, 2005).

Although probiotic cultures as nutritional ingredients that produce D(-)-lactic acid can be safely administered to infants, the CODEX recommendation not to use D(-)-lactic acid producing cultures in food for infants below the age of 12 months, should be followed (Connolly et al, 2005).

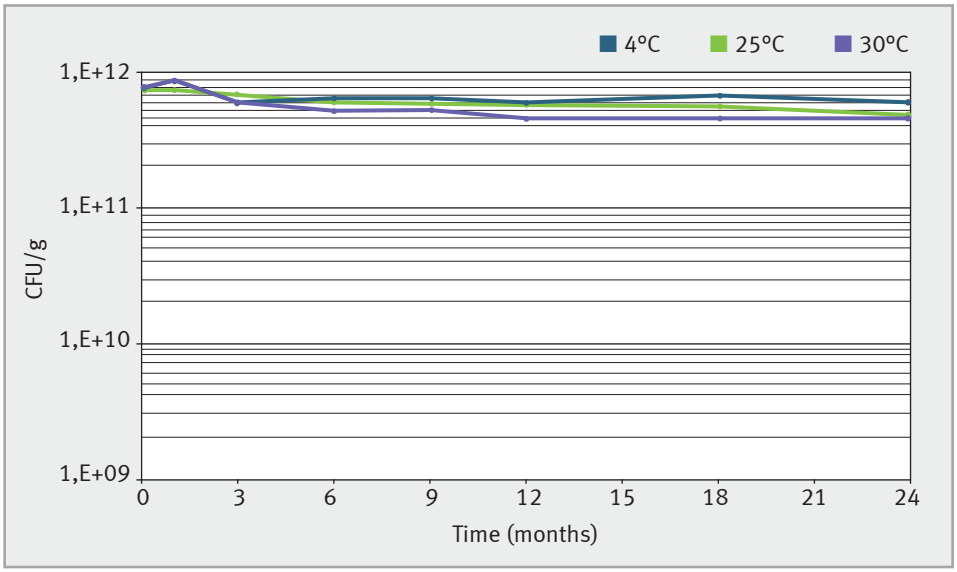
PRODUCTION OF BIOGENIC AMINES

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 histamine 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 the *L. plantarum* Lp-115 genome. Consequently, *L. plantarum* Lp-115 is unlikely to produce histamine or tyramine, theoretically decreasing the risk for adverse reactions in those individuals ingesting *L. plantarum* Lp-115 with sensitivity to either amine.

Figure 2. Stability of *L. plantarum* Lp-115 culture concentrate.



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. *L. plantarum* Lp-115 has demonstrated to be a very versatile strain. Food and supplement manufacturers find the strain particularly attractive for several reasons, including:

- available as a high-count freeze-dried material
- excellent stability in a variety of food and supplement applications

BENEFITS TO INTESTINAL HEALTH AND WELL-BEING

Health-related properties

The health benefits of probiotic bacterial strains have been demonstrated over the years, including a range of health improvements and inhibition of infection. *In vitro*, animal and human clinical studies have established the efficacy of *L. plantarum* Lp-115 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 surfaces, antimicrobial activity and efficacy in human clinical trials. The key findings from research on *L. plantarum* Lp-115 are summarized below.

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 the excretion of waste end-products. In recent years, however it has become recognized that the GI tract fulfills many other functions, which are essential to our well-being. The GI tract harbors a vast number of microbial cells (10^{14}). 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 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 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.

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, to reduce the risk for a range of diseases or unfavorable conditions (Guarino et al, (eds) 2013, Scott et al, 2015, Lin et al, 2014).

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 to confer a health benefit. This definition implies that a probiotic must survive GI tract passage and, according to some interpretations, transiently colonize the gut mucosa of the host.

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

In vitro studies have shown that *L. plantarum* Lp-115 is extremely resistant to low pH conditions and survives the presence of bile at concentrations present in the duodenum (Table 3).

The ability of *L. plantarum* Lp-115 to remain viable under a variety of conditions, all recognized as characteristics of probiotic bacteria, allowing survival in the gastrointestinal tract have been demonstrated in a further study (Ding and Shah 2007).

Another study investigated 61 healthy subjects to obtain information regarding the quantity and maintenance of *L. plantarum* Lp-115 in the fecal microbiota after ingestion of fermented milk containing $2x10^{11}$ cfu of *L. plantarum* Lp-115 for different periods of time. The target microorganism was monitored in the fecal microbiota via quantitative PCR (qPCR). *L. plantarum* Lp-115 was detected and quantified in all of the subjects during the ingestion periods. The differences between the *L. plantarum* Lp-115 levels at baseline and during all the different ingestion periods were statistically significant. However, at 15 and 45 days after discontinuing supplementation, the number of *L. plantarum* Lp-115 was reduced to the baseline level. A longer period with *L. plantarum* Lp-115 in the diet did neither result in increased levels of this bacterium in the stool nor in prolonged fecal excretion (Costa et al, 2014).

Table 3. Resistance to acid and bile of *L. plantarum* Lp-115

++++ Excellent +++ Very good ++ Good + Fair	
Acid tolerance	**+++** Very good (80-89% survival in hydrochloric acid and pepsin (1%) at pH 3 for 1 hour at 37°C)
Bile salt tolerance	**++++** Excellent (›90% survival in 0.3% bile salt containing medium)
Pepsin resistance	**+++** Very good (›40% in 0.3% pepsin containing medium at pH 2 for 1 hour)
Pancreatin resistance	**+++** Very good (›60% survival in 0.1% pancreatin containing medium at pH 8 for 2 hour)

Source: DuPont, internally generated data

Adhesion to intestinal mucosa

While adhesion is not a prerequisite 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.

L. plantarum Lp-115 was shown to adhere to human intestinal mucus applied in *in vitro* studies (Table 4); albeit only 'fair.'

Table 4. Adherence to human intestinal mucus *in vitro* of *L. plantarum* Lp-115

Adhesion of *L. plantarum* Lp-115 to Intestinal Mucus		
Measured by fluorescence labelling of bacteria. Adhesion rate [%] is calculated comparing the fluorescence of the adhered bacterial cells with the fluorescence of labelled bacteria applied.	**0.8%**	**++**
+ Poor (‹0.5) **++ Fair** (0.5 - 2%) **+++ Good** (2 - 3.5%) **++++ Very good** (3.5 - 5%) **+++++ Excellent** (›5%)		

Source: DuPont, internally generated data

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 ability to aggregate and coaggregate are desirable properties for probiotics as they are related to the ability to interact closely with pathogens and could avoid or reduce their adhesion to the intestinal mucosa. Coaggregation may also improve antimicrobial activity against (potential) pathogens, due to the close proximity.

An *in vitro* study was conducted to investigate the abilities of individual probiotic strains, including *L. plantarum* Lp-115, to disrupt the adhesion of eight selected pathogens to immobilized human

Table 5. *L. plantarum* Lp-115 displayed *in vitro* inhibition of selected pathogens.

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

Source: DuPont, internally generated data

mucus. *L. plantarum* Lp-115 demonstrated the ability to adhere to intestinal human mucus, to inhibit pathogen adhesion, and to displace pathogens (Collado et al, 2007).

These same authors conducted a second study to investigate the ability of select strains of probiotics to aggregate and displace pathogens. *L. plantarum* Lp-115 showed high aggregation ability and coaggregation with pathogens (Collado et al, 2008).

L. plantarum Lp-115 decreases the potential of human fecal water to induce DNA damage

DNA damage is an essential component of the genesis of colon cancer. It is likely that the agents involved in the aetiology of colon cancer are associated with the aqueous phase of the fecal stream (fecal water) in the gut, as it has been shown to contain biologically active substances that are cytotoxic to mammalian cells (Gratz et al, 2011). The use of fecal water in conjunction with human colon cell lines thus provides a useful and highly relevant *in vitro* model to investigate dietary components for their potential anti-cancer activity in the colon (Pearson et al, 2009).

A study involving a number of probiotic strains, including *L. plantarum* Lp-115, was conducted to investigate the effect of probiotics on DNA damage. The probiotic strains were each incubated with (human) genotoxic fecal water. Human colon cancer cells were then challenged with these probiotic mixtures, and DNA damage was assessed. DNA damage was significantly decreased by all bacteria, but *L. plantarum* Lp-115 was one of the strains which showed the greatest effect. As a subsequent analysis, prebiotics were also introduced and mixed with the probiotic strains to investigate DNA damage. Results with prebiotics also demonstrated that incubations of *L. plantarum* Lp-115 with fructo-oligosaccharide (FOS)-based prebiotics were the most effective, decreasing the cellular genotoxicity in fecal water (Burns & Rowland 2004).

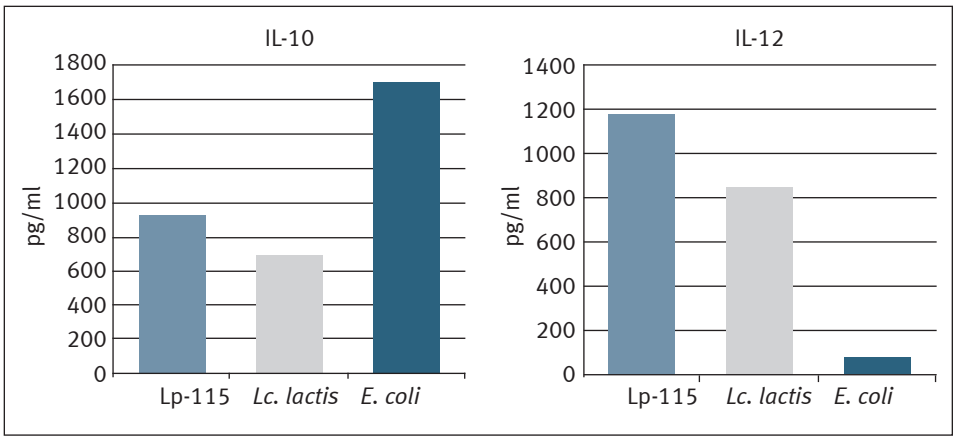
BENEFICIAL MODULATION OF THE IMMUNE SYSTEM

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 (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 (Geuking et al, 2014, 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

Figure 3. *In vitro* cytokine expression induced by *L. plantarum* Lp-115 in human PBMCs.



Source: Foligne et al, 2007

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).

Intestinal permeability and immune markers

The gut acts as an internal barrier, preventing pathogenic bacteria and other harmful substances from entering the body. Intestinal barrier integrity is a prerequisite for homeostasis of mucosal function, which is balanced to maximize absorptive capacity, while maintaining efficient defensive reactions against chemical and microbial challenges.

The inner surface of the intestine consists of a layer of cells (epithelium), which are covered by a mucus layer (a visco-elastic layer consisting mainly of protein-linked carbohydrates) which plays a key role in the barrier effect mechanism. Tight junctions are protein structures that form the continuous intercellular barrier between epithelial cells.

These structures control and maintain balanced intestinal permeability. Increased permeability is associated with certain diseases (such as allergies and inflammatory bowel disease), so a proper regulation of the function of tight junctions is important in disease prevention (Bron et al, 2017).

Using a Caco-2 human adenocarcinoma cell line as a model for intestinal epithelia, Commane and coworkers investigated the effect of probiotics, including *L. plantarum* Lp-115, on tight junction integrity. While the effect was strain specific, the fermentation product of probiotic strains was shown to raise tight juncture integrity, which may be associated with prevention of colorectal tumor formation. *L. plantarum* Lp-115 demonstrated an increase of the integrity of the barrier function *in vitro*, especially when prebiotics were included (Commane et al, 2005).

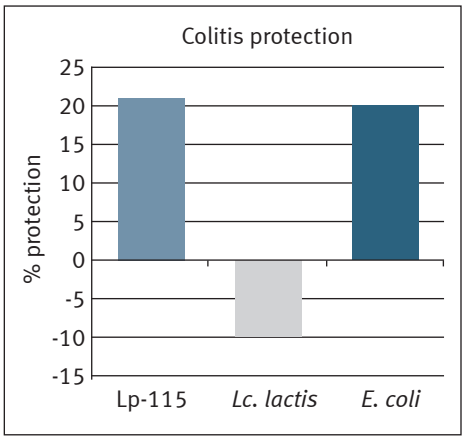
Expression of cytokines and other immune markers

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.

By measuring the impact of probiotic bacteria during interaction with cytokine-expressing peripheral blood mononucleocytes (PBMCs), information is generated that is useful in determining the ability of each strain to contribute to the balance of immune health.

L. plantarum Lp-115 was investigated *in vitro* for its ability to induce the PBMC secretion of selected cytokines: interleukin (IL)-10 and IL-12. The IL-10/IL-12 ratio is commonly used to distinguish between strains exhibiting pro- or anti-inflammatory profile. The results were compared with a strain of *Lactococcus lactis* and a non-pathogenic *E. coli* strain. *L. plantarum* Lp-115 and *Lc. lactis* induced moderate levels of IL-10 and higher levels of IL-12 secretion in PBMCs compared with *E. coli* (Figure 3). This type of cytokine expression may shift the immune system towards a so-called Th1 type of response; which plays a key role in, for example warding off tumors and viruses and the anti-allergy response (Foligne et al, 2007).

Figure 4. Percentage of protection in an acute murine model of inflammation (TNSBS)



Source: Foligne et al, 2007

The same strains were also assessed for their protective effect using the murine TNBS model of acute colitis. *L. plantarum* Lp-115 resulted in high levels of IL-12 production with the PBMCs, moderate levels of IL-10, and was demonstrated to be moderately effective in prevention of the TNBS-induced colitis (Figure 4) (Foligne et al, 2007).

Improved effect of vaccinations

The ability of *L.plantarum* Lp-115 to stimulate antibody responses has been evaluated in a human study measuring primary immune reaction following vaccination (Paineau, 2008).

Human volunteers were orally vaccinated (cholera vaccine was used as the vaccination model) and then received either a placebo or *L. plantarum* Lp-115. Supplementation with *L. plantarum* Lp-115 or the placebo started on day 0 and continued for 21 days. The subjects consumed two capsules a day with 10^{10} cfu *L. plantarum* Lp-115 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. plantarum* Lp-115 resulted in faster IgG induction than in the control group. This indicates stimulation of specific immunity by *L. plantarum* Lp-115.

L. plantarum Lp-115 increases the anti-inflammatory effect of sulfasalazine

A study was done to evaluate n-3 fatty acids from fish oil and/or the probiotic, *L. plantarum* Lp-115, combined with the anti-inflammatory drug sulfasalazine, at inflammatory markers in TNBS-induced colitis in rats. The rats were divided into five groups: (A), control group; (B), sulfasalazine; (C), sulfasalazine with *L. plantarum* Lp-115 fermented milk; (D), sulfasalazine with n-3 fatty acids and (E) sulfasalazine, n-3 fatty acids and *L. plantarum* Lp-115. Tissue samples were collected for Myeloperoxidase (MPO, a widely used inflammatory marker) determination. A significant reduction in MPO levels was observed in the adjunct treatments compared to the treatment with sulfasalazine only. Thus, the use of sulfasalazine in combination with *L. plantarum* Lp-115 improved the anti-inflammatory response, however, sulfasalazine in combination with n-3 fatty acids resulted in an even more favorable response (Paroschi et al, 2015).

Metabolic syndrome

An animal model was used to study metabolic parameters and oxidative and nitrosative stress in metabolic syndrome (MetS) treated with prebiotic, probiotic or synbiotic. MetS was induced in rats by supplementing the diet with 66% fructose. The animals were divided into five groups: G1 received a standard diet without inducing MetS. Animals from G2, G3, G4 and G5 were fed with 66% fructose supplement. G2 had no therapeutic interventions; G3 received treatment with *L. plantarum* Lp-115 (10^9 cfu); G4 received prebiotic yacon (high inulin contents) powder and G5 (synbiotic group) was treated with a beverage containing *L. plantarum* Lp-115 and yacon. All diets were administered for eight weeks. In relation to G1, rats fed a high-fructose diet (G2) showed laboratorial features compatible with MetS; G2 showed reduced nitric oxide metabolites (NOx) levels and increased levels of sulfhydryl (SH) and total radical-trapping antioxidant parameter/uric acid (TRAP/UA). In relation to G2, *L. plantarum* Lp-115 decreased insulin and HOMA-IR (a method used to quantify insulin resistance). Synbiotic resulted in increased HOMA-IR, reduced hydroperoxides and increased levels of NOx and SH (compared to G3. In conclusion, *L. plantarum* Lp-115 reduces insulin resistance and yacon or synbiotic reduces lipid oxidation and increases antioxidant defenses in rats with high-fructose diet-induced MetS (Mari et al, 2015).

Postmenopausal women (n=24) were recruited to investigate the influence of milk fermented with *L. plantarum* Lp-115 on the classical parameters related to MetS, as well as in other parameters related to cardiovascular risk. The main findings of the present study were that fermented milk with *L. plantarum* Lp-115 decreased two important cardiovascular risk factors, such as glucose and homocysteine in postmenopausal patients with MetS (Barreto et al, 2014).

Influence of *L. plantarum* Lp-115 in a probiotic combination on postoperative bacterial infection

In liver transplantation, postoperative bacterial infection is still a frequent complication, which contributes to an increased risk of fatality. Earlier studies on preoperative use of probiotics in liver transplant patients suggests a reduction in postoperative sepsis and wound infection, but relevant clinical experience with pre- and probiotics is still limited.

To assess the effect of fiber and probiotic on the risk for bacterial sepsis and wound complications, 67 patients undergoing liver transplantation were recruited. The synbiotic composition of fiber and probiotics, which consisted of six different strains, including *L. plantarum* Lp-115 ($5x10^9$ cfu), was administered twice daily to one group (n=34) while another group (n=33) received only the fiber instead of the synbiotic.

As a result, the group receiving the combined fiber and probiotics had a lower incidence of bacterial infections and shorter duration of antibiotic therapy following liver transplantation in comparison to the fiber-only group. Patients in the synbiotic group were also discharged earlier from hospital, but this was statistically significant compared to the fiber-only group (Zhang et al, 2013). The probiotics were also well tolerated by this sensitive population; confirming the safety.

Oxalate-degrading activity

Oxalate (Ox) is a very common component of the human diet, capable to accumulate in the renal tissue and bind calcium to form calcium oxalate (CaOx) crystals. In humans, an accumulation of oxalic acid can result in a number of pathological conditions, including hyperoxaluria kidney stones, renal failure, cardiomyopathy and cardiac conductance disorders.

A study was undertaken to evaluate the oxalate-degrading activity of 60 *Lactobacillus* strains, including *L. plantarum* Lp-115. The oxalate-degrading activity of *L. plantarum* Lp-115 was found to be 40%, compared to the positive control *Oxalobacter formigenes* DSM 4420, which was set as 100%. This suggests that the use of probiotic strains with oxalate-degrading activity may be of benefit to individuals suffering from or at risk for oxalate-associated disorders (Turroni et al, 2007).

BENEFITS SUMMARY

Based on the data generated supporting *L. plantarum* Lp-115 strain qualities, the following health-related attributes can be summarized:

- Well suited for intestinal survival
- High tolerance to acid and bile as present in the intestine
- Immune modulation
- *L. plantarum* Lp-115 may improve specific immune response, as demonstrated in a human clinical study
- *L. plantarum* Lp-115 may have an influence on immune regulation, as demonstrated through balancing of IL-10/IL-12 *in vitro*
- *L. plantarum* Lp-115 may decrease important cardiovascular risk factors, such as glucose and homocysteine
- *L. plantarum* Lp-115 may reduce risk for postoperative infections

REFERENCES

Barreto, F.M., Colado Simão, A.N., Morimoto, H.K., Batisti Lozovoy, M.A., Dichi, I., Helena da Silva Miglioranza, L. (2014). Beneficial effects of *Lactobacillus plantarum* on glycemia and homocysteine levels in postmenopausal women with metabolic syndrome. Nutrition: 30(7-8):939-42.

Arena, M.P., Silvain, A., Normanno, G., Grieco, F., Drider, D., Spano, G. and Fiocco, D. (2016). Use of *Lactobacillus plantarum* Strains as a Bio-Control Strategy against Food-Borne Pathogenic Microorganisms. Front. Microbiol.7:464. doi: 10.3389/fmicb.2016.00464.

Bron, P.A., Kleerebezem, M., Brummer, R-J., Cani, P.D., Mercenier, A., MacDonald, T.T., Garcia-Ródenas, C.L. and J.M. Wells (2017). Can probiotics modulate human disease by impacting intestinal barrier function? British Journal of Nutrition 117, 93–107.

Bernardeau, M., Vernoux, J.P., Henri-Dubernet, S. and Guéguen, M. (2008). Safety assessment of dairy microorganisms: The *Lactobacillus* genus. International Journal of Food Microbiology 126: 278–285.

Bourdichon, F., Casaregola, S., Farrokh, C., Frisvad, J.C., Gerds, M.L., Hammes, W.P., Harnett, J., Huys, G., Laulund, S., Ouwehand, A. *et al.* (2012). Food fermentations: microorganisms with technological beneficial use. International Journal of Food Microbiology 154, 87-97.

Burns, A.J., Rowland, I.R. (2004). Antigenotoxicity of probiotics and prebiotics on fecal water-induced DNA damage in human colon adenocarcinoma cells. Mutat Res. 551: 233-243.

Calder, P.C. (2013) Feeding the immune system. Proc Nutr Soc. 72(3):299-309. doi: 10.1017/S0029665113001286.

Collado, M.C., Meriluoto, J., Salminen, S. (2007). Role of commercial probiotic strains against human pathogen adhesion to intestinal mucus. Letters in Appl Microbiol. 45: 454-460.

Collado, M.C., Meriluoto, J., Salminen, S. (2008). Adhesion and aggregation properties of probiotic and pathogen strains Eur Food Res Technol. 226: 1065-1073.

Commane, D.M., Shortt, C.T., Silvi, S., Cresci, A., Hughes, R.M., Rowland, I.R. (2005). Effects of fermentation products of pro- and prebiotics on trans-epithelial electrical resistance in an *in vitro* model of the colon. Nutr Cancer. 51(1):102-9.

Connolly, E., Abrahamsson, T. and Bjorksten, B. (2005). Safety of D(-)-lactic acid producing bacteria in the human infant. J Pediatr Gastroenterol Nutr 41, 489-492.

Costa, G.N., Marcelino-Guimarães, F.C., Vilas-Bôas, G.T., Matsuo, T., Miglioranza, L.H. (2014). Potential fate of ingested *Lactobacillus plantarum* and its occurrence in human feces. Appl Environ Microbiol. 80(3):1013-9.

Daniel, C., Poiret, S., Goudercourt, D., Dennin, V., Leyer, G., Pot, B. (2006). Selecting lactic acid bacteria for their safety and functionality by use of a mouse colitis model. Appl Environ Microbiol. 72: 5799-5805.

Ding, W.K. and Shah, N.P. (2007). Acid, bile, and heat tolerance of free and microencapsulated probiotic bacteria. J Food Sci 72(9): M446-450.

Duerkop, B.A., Vaishnava, S. and Hooper, L.V. (2009) Immune Responses to the Microbiota at the Intestinal Mucosal Surface. Immunity. 31: 368–376.

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Ricci, A., *et al.* (2017). Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA. EFSA Journal;15(3):4664, 177 pp. doi:10.2903/j.efsa.2017.4664.

Ewaschuk, J. B., Naylor, J. M. and Zello, G. A. (2005). D-lactate in human and ruminant metabolism. J.Nutr., 135, 1619-1625.

Foligné, B., Nutten, S., Grangette, C., Dennin, V., Goudercourt, D., Poiret, S., Dewulf, J., Brassart, D., Mercenier, A., Pot, B. (2007). Correlation between *in vitro* and *in vivo* immune modulatory properties of lactic acid bacteria. World J Gastroenterol. 13: 236-243.

Geuking, M.B., Koller, Y., Rupp, S. *et al.* The interplay between the gut microbiota and the immune system. Gut Microbes 2014; 5: 411–418.

Guarino, A., Quigley, E.M.M., Walker, W.A. (eds). World Review of Nutrition and Dietetics, Vol. 107. Probiotic Bacteria and Their Effect on Human Health and Well-Being. Karger, Basel 2013.

Hardy, H., Harris, J., Lyon, E., Beal, J. and Foey, A.D. (2013) Probiotics, Prebiotics and Immunomodulation of Gut Mucosal Defences: Homeostasis and Immunopathology. Nutrients 5, 1869-1912; doi:10.3390/nu5061869.

Hill, C. *et al.* (2014). The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat. Rev. Gastroenterol. Hepatol. 11, 506–514, doi:10.1038/nrgastro.2014.66.

Klare, I., Konstabel, C., Werner, G., Huys, G., Vankerckhoven, V., Kahlmeter, G., Hildebrandt, B., Müller-Bertling, S., Witte, W. and Goossens, H. (2007). Antimicrobial susceptibilities of *Lactobacillus*, *Pediococcus* and *Lactococcus* human isolates and cultures intended for probiotic or nutritional use. Journal of Antimicrobial Chemotherapy 59, 900–912.

Ladero, V., Calles-Enriquez, M., Fernandez, M. and Alvarez, M.A. (2010). Toxicological Effects of Dietary Biogenic Amines. Current Nutrition & Food Science 6, 145-156.

LeBlanc, A., LeBlanc, J.G. (2014). Effect of probiotic administration on the intestinal microbiota, current knowledge and potential applications. World J Gastroenterol. 20(44): 16518-16528.

Lei, Y.M., Nair L., Alegre, M.L. (2015) The interplay between the intestinal microbiota and the immune system. Clin Res Hepatol Gastroenterol; 39:9–19.

Lin, Chuan-Sheng *et al.* (2014) Impact of the Gut Microbiota, Prebiotics, and Probiotics on Human Health and Disease Biomed J ;37:259-268.

Mari, N.L., Bregano, J.W., Simão, A.N.C., Lozovoy, M.A.B., Bonifacio, K.L., Alfieri, D.F., Dichi, I. and Miglioranza, L.H.S. (2015). *Lactobacillus plantarum* reduces insulin resistance and yacon or symbiotic reduces oxidative stress in rats with metabolic syndrome. Nutrition & Energy Balance 1:12-17.

Markowiak, P. and Slizewska, K. (2017). Effects of Probiotics, Prebiotics, and Synbiotics on Human Health. Nutrients 9, 1021; doi:10.3390/nu9091021.

Paineau, D., Carcano, D., Leyer, G., Darquy, S., Alyanakian, M.A., Simoneau, G., Bergmann, J.F., Brassart, D., Bornet, F., Ouwehand, A.C. (2008). Effects of seven potential probiotic strains on specific immune responses in healthy adults: a double-blind, randomized, controlled trial. FEMS Immunol Med Microbiol. 53: 107-13.

Paroschi, T.P., Breganó, J.W., Simão, A.N.C., Dichi, I. and Miglioranza, L.H.S. (2015). Effects of Sulfasalazine, *Lactobacillus Plantarum* (Lp-115) and Fish Oil in Experimental Colitis. SM J Food Nutri Disord. 1(1): 1005.

Pearson, J.R., Gill, C.I.R. and Rowland, I.R. (2009). Diet, fecal water, and colon cancer – development of a biomarker. Nutrition Reviews 67: 9, 509–526.

Salvetti, E. and O'Toole, P.W. The Genomic Basis of *Lactobacilli* as Health-Promoting Organisms. Microbiol Spectr. 2017 Jun;5(3). doi: 10.1128/microbiolspec.BAD-0011-2016.

Sanders, M.E. (2015). Probiotics in 2015: Their Scope and Use. Journal of Clinical Gastroenterology 49: Supp. 1, S2-S6.

Scott, K.P., Antoine, J.M., Midtvedt, T. and van Hemert, S. (2015). Manipulating the gut microbiota to maintain health and treat disease. Microbial Ecology in Health & Disease. 26: 25877 - http://dx.doi.org/10.3402/mehd.v26.25877.

Seddik, H.A., Bendali, F., Gancel, F. *et al.* (2017). *Lactobacillus plantarum* and Its Probiotic and Food Potentialities. Probiotics & Antimicro. Prot. 9: 111. https://doi.org/10.1007/s12602-017-9264-z.

Siezen, R.J. and van Hylckama Vlieg, J.E.T. (2011). Genomic diversity and versatility of *Lactobacillus plantarum*, a natural metabolic engineer. Microbial Cell Factories, 10 (Suppl 1):S3

Turroni, S., Vitali, B., Bendazzoli, C., Candela, M., Gotti, R., Federici, F., Pirovano, F., Brigidi, P. (2007). Oxalate consumption by *lactobacilli*: evaluation of oxalyl-CoA decarboxylase and formyl-CoA transferase activity in *Lactobacillus acidophilus*. J Appl Microbiol. 103: 1600-1609.

Vandenplas, Y., Huys, G., Daubec, G. (2015). Probiotics: an update. J Pediatr (Rio J). 91(1):6-21.

Versini, M., Jeandel, P.Y., Bashi, T., Bizzaro, G., Blank, M., Shoenfeld, Y. (2015). Unraveling the Hygiene Hypothesis of helminthes and autoimmunity: origins, pathophysiology, and clinical applications. BMC Med.13:81. doi: 10.1186/s12916-015-0306-7.

Zhang, Y., Chen J., Wu J., Chalson H., Merigan L., Mitchell A. (2013). Probiotic use in preventing postoperative infection in liver transplant patients. Hepatobiliary Surg Nutr. 2(3):142-7.

About DuPont™ Danisco®

DuPont™ Danisco® is the brand for a range of products that help provide enhanced bioprotection, an improved nutritional profile, and better taste and texture with greater cost efficiency and lower environmental impact, meeting the needs of manufacturers of food and beverages, dietary supplements and pet food. Through the work of the global network of food scientists and technologists in DuPont™, the Danisco® range is supported by a uniquely broad spectrum of know-how across applications and processing.

TM 50-2E US 6.18

DuPont Nutrition & Health
health&protection@danisco.com • www.danisco.com/probiotics