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December 20, 2016

**BY Hand Delivery**

United States Food and Drug Administration  
Center for Food Safety and Applied Nutrition  
Office of Food Additive Safety  
HFS-200  
5001 Campus Drive  
College Park, MD 20740

**Re: GRAS Notification for Lactobacillus plantarum Strain 299v**

Dear Dr. Anderson:

Enclosed is a copy of a GRAS notification submitted on behalf of Probi AB, of Lund, Sweden ("Probi") through its Agent Mark Yacura of the law firm Quarles & Brady LLP in accordance with the requirements of 21 C.F.R. Part 170, Subpart E.

If you have any questions or concerns regarding these minutes, please contact me at (202) 372-9529 or at [mark.yacura@quarles.com](mailto:mark.yacura@quarles.com).

Sincerely,

(b) (6)

Mark Yacura  
Counsel to Probi AB



**Generally Recognized as Safe (GRAS)  
Determination for the Use of  
*Lactobacillus plantarum*  
Strain 299v in Conventional Foods**

**Submitted by  
Probi AB  
Lund, Sweden**

**Submitted to  
United States Food and Drug Administration  
Center for Food Safety and Applied Nutrition  
Office of Food Additive Safety  
HFS-200  
5001 Campus Drive  
College Park, MD 20740**

**Prepared by  
Probi AB  
and  
JHeimbach LLC  
Port Royal, Virginia**

**December 2016**

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# 685

# Generally Recognized as Safe (GRAS) Determination for the Use of *Lactobacillus plantarum* Strain 299v in Conventional Foods

## Part 1 - Signed Statements and Certification

### GRAS Notice Submission

Probi AB, of Lund, Sweden ("Probi") submits this GRAS notification through its Agent Mark Yacura, Partner in the law firm Quarles & Brady LLP in accordance with the requirements of 21 C.F.R. Part 170, Subpart E.

### Name and Address

Probi AB  
Sölvegatan 41 A  
SE-223 70 Lund, Sweden

### Name of Notified Substance

The probiotic bacterium *Lactobacillus plantarum* designated 299v. The strain is also known commercially as Plantarum 299v and Lp299v.

### Intended Conditions of Use

*L. plantarum* 299v is intended to be added as a probiotic microorganism to conventional foods at concentrations consistent with cGMP needed to provide beneficial health effects. Intended food applications include but are not limited to the following:

- Wet chilled and ambient products such as fruit drinks, yogurts, milk and plant based products;
- Dry chilled products;
- Dry and shelf-stable products such as cereals, candy, bars, cookies, gums, and confectionery;
- Delivery systems designed for bacterial stability in room temperature. E.g., the bacterial powder may be enclosed in a cap mounted on a drink bottle (fruit drink, plain or flavored water, etc.) to be mixed prior to consumption.

The intended level of *L. plantarum* in food is up to  $10^{10}$  cfu/serving throughout the shelf-life of the food. In order to allow for loss of viability over time, the intended addition level is up to  $10^{11}$  cfu/serving, which provides for up to 90% loss of viability.

**Statutory Basis for GRAS Status**

*Lactobacillus plantarum* designated 299v has been determined to be GRAS through scientific procedures in accordance with 21 C.F.R. § 170.30(a) and (b).

**Premarket Exempt Status**

*Lactobacillus plantarum* designated 299v is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on a conclusion that the notified substance is GRAS under the conditions of intended use.

**Data Availability**

The data and information that serve as the basis for the conclusion that *Lactobacillus plantarum* designated 299v is GRAS for its intended use, will be made available to FDA upon request. At FDA's option, a complete copy of the information will be sent to FDA in either paper or electronic format, or the information will be available for review at Quarles & Brady LLP's Washington, DC office, located at 1701 Pennsylvania Ave, NW Washington, DC during normal business hours.

**Freedom of Information Act Statement**

None of the information in the GFAS notice is exempt from disclosure under the Freedom of Information Act, 5 U.S.C. 552.

**Certification**

To the best of my knowledge, this GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to me and pertinent to the evaluation of the safety and GRAS status of *Lactobacillus plantarum* designated 299v.

**FSIS Statement**

Not applicable

**Name, Position and Signature of Notifier**

(b) (6)



Mark Yacura  
Partner  
Quarles & Brady LLP  
Counsel to Probi AB



## Part 2 - Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

### 2.1. Name of the GRAS Organism

The subject of this Generally Recognized as Safe (GRAS) determination is a strain of the probiotic bacterium *Lactobacillus plantarum* designated 299v. The strain is also known commercially as Plantarum 299v and Lp299v.

### 2.2. Source of the GRAS Organism

*L. plantarum* 299v was isolated from healthy intestinal mucosa (Molin et al. 1993). It was isolated from a biopsy taken from a patient with polyps, but the biopsy was taken from healthy mucosa. The strain was deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) and referenced as DSM 9843.

### 2.3. Description of the GRAS Organism

*L. plantarum* is a Gram-positive, catalase-negative, bacterium that is a member of the broad classification of lactic acid bacteria (LAB). LAB comprise a group of microbes related by common metabolic functionality—the production of lactic acid as the major metabolic end product of carbohydrate metabolism—and common physiological traits. LAB are Gram-positive, non-spore-forming, and catalase-negative and are devoid of cytochromes (Holzapfel et al. 2001). They are preferential nonaerobes but are aerotolerant, acid-tolerant, and strictly fermentative. Although they are not a strictly defined taxonomic grouping, LAB generally are considered to include the following phylogenetically related genera, which have several biochemical and ecological features in common (Axelsson 1998): *Aerococcus*, *Alloicoccus*, *Carnobacterium*, *Dolosigranulum*, *Enterococcus*, *Globicatella*, *Lactobacillus*, *Lactococcus*, *Lactosphaera*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenoccus*, *Vagococcus*, and *Weissella*. Due to similarities in its biochemistry, physiology, and ecology, the genus *Bifidobacterium* is often considered to be a LAB as well, even though it is phylogenetically unrelated (Axelsson 1998). With the exception of some *Streptococcus* species and possibly some *Enterococcus* strains, most LAB strains are considered to have little or no pathogenic potential (Donohue and Salminen 1996; Adams 1999). LAB have a long history of use in fermented and non-fermented foods and have been noted for their ability to inhibit other microorganisms capable of causing foodborne illness or food spoilage (Adams, 1999; Donohue and Salminen 1996). Furthermore, some LAB are ubiquitous as minor components in the intestinal epithelium and the gastrointestinal tract of humans of all ages. All of these factors lead to the reasonable conclusion that most LAB strains are safe for use in conventional foods that may be consumed by all members of the general population.

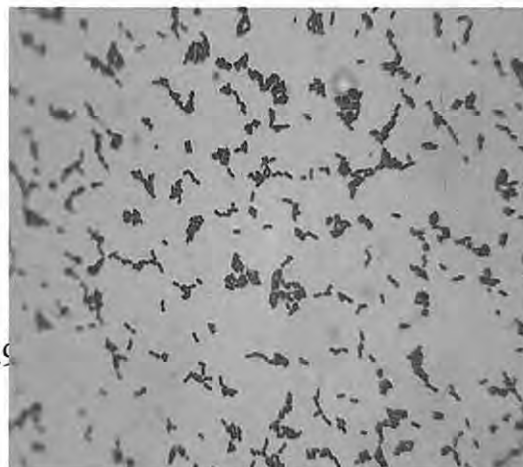
*Lactobacillus* is a non-pathogenic genus, comprising the rod-shaped LAB, that consists of over a hundred species. A report by the European Food Safety Authority in November 2007 (EFSA 2007b) identified 112 species, while Bernardeau et al. (2008), writing the following year, suggested that the genus contains some 135 species and 27 subspecies. *Lactobacillus* is a heterogeneous genus with a large variety of phenotypic, biochemical, and physiological properties; it has been suggested that the extreme diversity of the *Lactobacillus* genomes would

justify recognition of new subgeneric divisions (Bernardeau et al. 2008). Lactobacilli are rod-shaped, non-motile, and non-sporulating. They are used in commercial applications for the fermentation of dairy products, fruits, vegetables, and meats (Aguirre and Collins 1993; Gasser 1994). Lactobacilli grow under reduced oxygen conditions in habitats where ample nutrients exist. Some *Lactobacillus* strains are found in the gastrointestinal tract of healthy humans of all ages (Saxelin et al. 1996; Goldin et al. 1992). Members of the genus may be either homo- or heterofermentative. The former convert carbohydrates to lactic acid through the glycolytic pathway, while the latter convert carbohydrates using phosphoketolase to produce lactic acid, acetic acid, ethyl alcohol, and carbon dioxide. While homofermenters are obligate homofermentative, heterofermentative strains may be either obligate or facultative. *L. plantarum* is a facultative hexose heterofermenter (Cogan 1996).

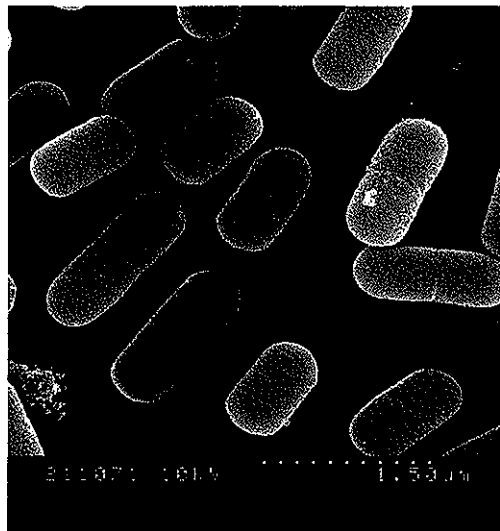
The name of the species originated from its common occurrence in spontaneously fermented plants, which were major food sources long before meat and milk became dominant. The type strain of *L. plantarum* is ATCC 14917 (Kandler and Weiss 1986). *L. plantarum* differs from many other *Lactobacillus* species in that *L. plantarum* has a relatively large genome (Kleerebezem et al. 2003), possesses a striking ability to ferment many different carbohydrates, has a high growth requirement for manganese and can accumulate high intercellular levels of manganese, which can scavenge  $O_2^-$  and thereby confer a high degree of aerotolerance (Archibald and Fridovich 1981), and has a high tolerance to low pH, frequently predominating in spontaneously lactic-acid-fermented foods where the pH is below 4.0. Images of *L. plantarum* 299v at 3 degrees of magnification are shown in Figures 1-3.



Figure 1. *L. plantarum* 299v Colonies on MRS Agar (Magnification 1x).



**Figure 2. Gram-Stained *L. plantarum* 299v (Magnification 1000x).**



**Figure 3. Scanning Electron Micrograph of *L. plantarum* 299v (Magnification 20,000x).**

### **1.3.1. Phenotypic Strain Identification**

According to Bergey's Manual of Systematic Bacteriology (Sneath et al. 1986), *Lactobacillus* identification is performed by standard testing and by API 50 CH profile (Biomérieux, France), based on the ability to grow on different sugars. The results shown in Table 1 are based upon seven days' growth, and identify strain 299v as *L. plantarum*.

Table 1. Growth of *L. plantarum* Strain 299v on Different Sugars.

Sugar	Growth	Sugar	Growth	Sugar	Growth
Control	-	Inositol	-	Melezitose	+
Glycerol	(+)	Mannitol	+	D-raffinose	(+)
Erythritol	-	Sorbitol	+	Starch	-
D-arabinose	-	$\alpha$ -methyl-D-mannoside	+	Glycogen	-
L-arabinose	+	$\alpha$ -methyl-D-glucoside	-	Xylitol	-
Ribose	+	N-acetylglucosamine	+	$\beta$ -gentiobiose	+
D-xylose	-	Amygdalin	+	D-turanose	+
L-xylose	-	Arbutin	+	D-lyxose	-
Adonitol	-	Esculin	+	D-tagatose	-
$\beta$ -methylxylyldose	-	Salicin	+	D-fucose	-
Galactose	+	Cellobiose	+	L-fucose	-
D-glucose	+	Maltose	+	D-arabitol	(+)
D-fructose	+	Lactose	+	L-arabitol	-
D-mannose	+	Melibiose	+	Gluconate	+
L-sorbose	-	Sucrose	+	2-ketogluconate	-
Rhamnose	(+)	Trehalose	+	5-ketogluconate	-
Dulcitol	-	Inulin	-		

The ability of *L. plantarum* to grow on a wide variety of sugars was confirmed in a study by Hedberg et al. (2008) in which the sugar utilization of 6 *Lactobacillus* strains (*L. plantarum* 299v and 931, *L. rhamnosus* GG and LB21, *L. paracasei* ssp. *paracasei* F19, and *L. reuteri* PTA 5289) were compared. The authors reported that the 2 *L. plantarum* strains fermented all tested sugars except raffinose, xylitol, and melibiose (this last result conflicting with the test results reported above), while the 2 *L. rhamnosus* strains were less active and the *L. paracasei* and *reuteri* strains had only weak growth patterns.

### 2.3.2. Genotypic Strain Identification

Randomly Amplified Polymorphic DNA (RAPD) is a PCR-based method that uses short primers to amplify random sections of DNA in the genome of an organism using a low primer annealing temperature. The primer anneals to any site with a similar or identical sequence and amplifies until the next annealing site (on the other DNA strand). A profile based on the location of those annealing sites that are close enough to generate a PCR product in the genome is therefore generated. RAPD is useful to characterize a specific strain and for quick comparison of strains.

A study performed by Johansson et al. (1995c) showed that RAPD is a rapid and useful method for the typing of *L. plantarum*. The method described, using base sequence 5'-CCG CAG CCA A-3', produced a pattern consisting of 5 highly characteristic bands that confirmed strain 299v as a member of the species *L. plantarum*.

Amplified Fragment Length Polymorphism PCR (AFLP) is a PCR-based technique for whole-genome DNA fingerprinting by selective amplification of restriction fragments (Janssen et al. 1996; Johansson et al. 1995d). DNA was prepared using the method of Gevers et al. (2001) with the following restriction enzymes and adaptors:

Hexacutter: Eco RI  
Adaptor: 5'-CTC GTA GAC TGC GTA CC-3'  
3'-CTG ACG CAT GGT TAA -5'

Tetracutter: TaqI  
Adaptor: 5'-GAC GAT GAG TCC TGA C-3'  
3'-TAC TCA GGA CTG GC-5'

*L. plantarum* 299v was identified according to EU-PROSAFE recommendations (Vankerckhoven et al. 2008) as correctly placed at the genus and the species level using the AFPL method.

*L. plantarum* 299v is included in a genetic subgroup within the species *Lactobacillus plantarum* (Johansson et al. 1995a) comprising bacteria that mostly originate from human intestinal mucosa, but also can be found in traditional lactic-acid-fermented foods (Molin et al. 1993; Ahrne et al. 1998). Strains of this subgroup have been shown to have a pronounced ability to attach to human mucosa cells *in vitro* by means of a mannose-binding adherence mechanism (Adlerberth et al. 1996; Ahrne et al. 1998). Gene-specific deletion studies of 14 *L. plantarum* strains, including strain 299v, identified a single gene (lp\_1229) hypothesized to encode the mannose-specific adhesin of *L. plantarum* (Pretzer et al. 2005). Deletion of this gene resulted in a complete loss of mannose adhesion ability, while overexpression enhanced this phenotype.

Moreover, *L. plantarum* strains of this genomic subtype survive passage through the acid conditions of the human stomach (Johansson et al. 1993), frequently dominate the total *Lactobacillus* flora of healthy individuals on both rectal and oral mucosa (Molin et al. 1993; Ahrne et al. 1998), may possess tannase activity (Osawa et al. 2000; Vaquero et al. 2004), and are able to metabolize phenolic acids (Barthelmebs et al. 2000).

### 2.3.3. Plasmids

Plasmid profiling of *L. plantarum* 299v, 7 other *Lactobacillus* strains, and one *Bifidobacterium* strain was performed by Bioneer A/S, Denmark, in September 2004. Analysis of the plasmid profiles of strains A, B, C and E showed 5 plasmids with profiles indistinguishable from the profile observed for *L. plantarum* 299v (Figure 4). The sizes of the 5 plasmids, ranging from 5.5 to 37 kilobases (kb) are the same in the 5 matching strains (Table 2).

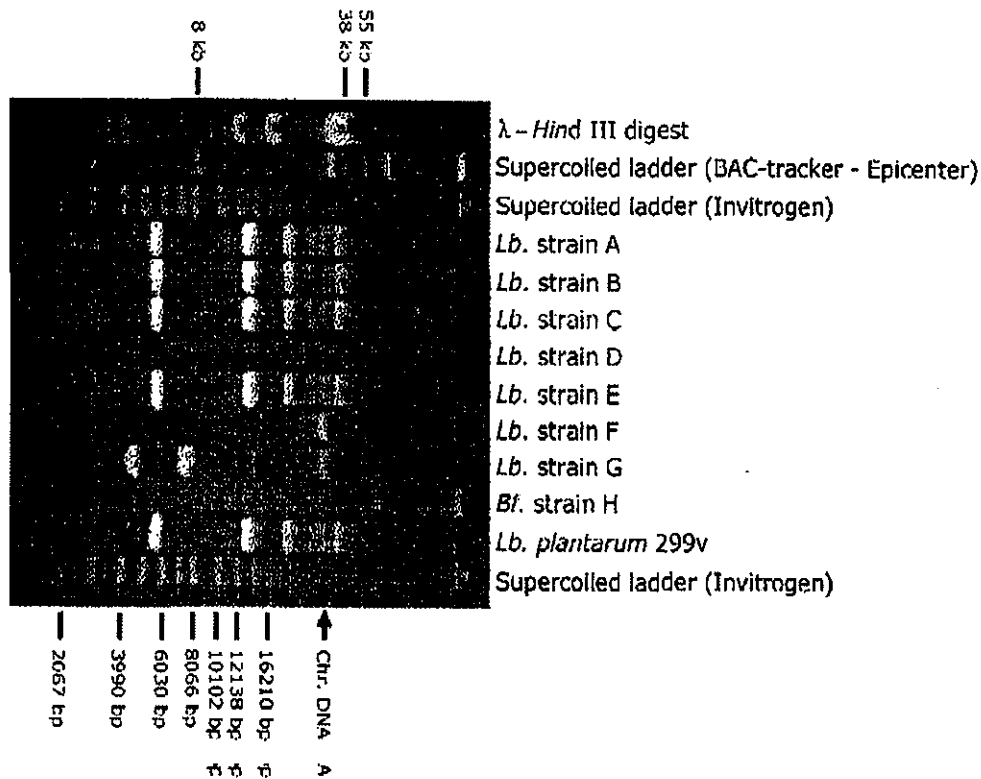


Figure 4. Plasmid Profiles of *L. plantarum* 299v and Other Strains.

Table 2. Sizes of Identified Plasmids.

Strain	Plasmids (kb)
Lb. strain A ( <i>L. plantarum</i> )	37 25 19 13 5.5
Lb. strain B ( <i>L. plantarum</i> )	37 25 19 13 5.5
Lb. strain C ( <i>L. plantarum</i> )	37 25 19 13 5.5
Lb. strain D ( <i>L. plantarum</i> )	24
Lb. strain E ( <i>L. plantarum</i> )	37 25 19 13 5.5
Lb. strain F ( <i>L. paracasei</i> )	50
Lb. strain G ( <i>L. rhamnosus</i> )	50 14 7.5 4.5
Bf. strain H ( <i>Bifidobacterium</i> )	None detected
<i>L. plantarum</i> 299v	37 25 19 13 5.5

#### 2.3.4. Surface-Associated Proteins

Using a proteomic approach, Beck et al. (2009) identified 29 surface-associated proteins from *L. plantarum* 299v. The most abundant protein present on the outer surface was the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase—an enzyme more often located intracellularly and regarded as a housekeeping enzyme essential for glycolysis, but which may aid in allowing *L. plantarum* 299v to adhere to human intestinal mucosa. Other glycolytic proteins identified included phosphoglycerate kinase, triose-phosphate isomerase, enolase, and

glucose-6-phosphate isomerase; these were also suggested to play a role in adherence and in the competitive exclusion of pathogens, as may the ribosomal proteins elongation factor Tu and L12/L7. Finally, stress-related proteins GrpE and DnaK were hypothesized to play roles in the immunomodulatory properties of *L. plantarum* 299v.

Hamon et al. (2011) conducted proteomic analyses of 3 *L. plantarum* strains exhibiting different degrees of bile tolerance—high tolerance (strain 299v), intermediate tolerance (strain LC 804), and low tolerance (strain LC 56). Six proteins were identified that appeared to be associated with bile tolerance: 2 glutathione reductases (GshR1 and GshR4) involved in protection against oxidative injury caused by bile salts; a cyclopropane-fattyacyl-phospholipid synthase (Cfa2) which may aid in maintenance of cell envelope integrity; and a bile salt hydrolase, an ABC transporter, and a F0F1-ATP synthase, all of which participate in the active removal of bile-related stress factors. Of these, the authors identified the ABC transporter, OpuA, as a key agent in the high bile resistance of strain 299v.

## 2.4 Production Method

Production of *L. plantarum* 299v is carried out by Probi AB or by independent suppliers under contract with Probi AB following the process shown in the flowchart (Figure 5). All components of the fermentation media as well as the cryoprotectant are food grade and permitted for these applications. No milk protein or peptides derived from milk protein are used; if the growth medium includes peptides derived from soy protein the possible presence of soy-derived peptides is disclosed in the labeling of the consumer product.

Production starts with prime ampoules; these are used to prepare production ampoules which in turn are used as inoculation material.

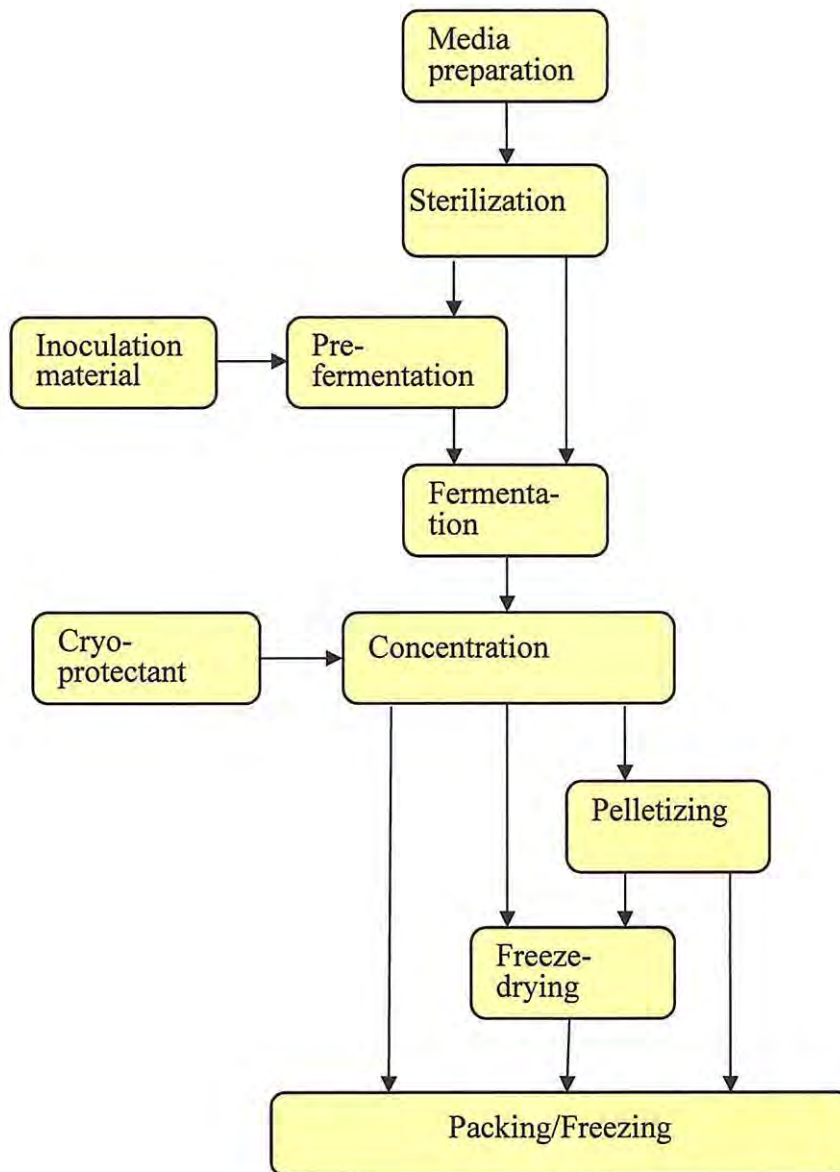


Figure 5. Production Schematic for *L. plantarum* 299v.



Prime ampoule production begins with media preparation and sterilization. The medium used to prepare the bacterium is “non-animal MRS.” Sterilization is achieved by heating the MRS medium to a temperature of 121°C under pressure and maintaining this temperature for 20 minutes. The identity, number of and purity of the organisms in each lot are checked individually by Probi AB using genotypic and phenotypic tests as described in Table 3.

**Table 3. Microbiological Analyses for Prime Ampoules.**

Parameter	Criterion of Acceptance	Method
Viable count of <i>L.plantarum</i> 299v (cfu/ml)	$\geq 1.0 \times 10^{10}$	QM-013, NMKL140/2007 (modified)
Total count of non-lactic acid bacteria (cfu/ml)	< 5	Aerobic bacteria at 30°C Blood Agar, method developed at Probi AB QM-002
Enterococci (cfu/ml)	< 1	NMKL 68/2004 4th ed. QM-004
Enterobacteriaceae (cfu/ml)	< 1	NMKL 144/2005 3rd ed. (modified) QM-005a
Total count of Gram-negative bacteria (cfu/ml)	< 1	Lindberg, A-M. 1997. “Characterisation of <i>Aeromonas</i> , <i>Enterobacteriaceae</i> , <i>Pseudomonas</i> , <i>Bacillus</i> and <i>Lactobacillus</i> spontaneously growing to high numbers in milk, minced meat, fish or cheese.” (modified) QM-005b
Presumptive staphylococci (cfu/ml)	< 5	Mannitol Salt Agar, Egen method QM-006b
Yeasts and molds (cfu/ml)	< 5	Marsall, T. 1992; Standard Methods for Examination of Dairy Products 16th ed. Compendium of Methods for the Microbiological Examination of Foods. QM-007
Presumptive <i>Bacillus cereus</i> (cfu/ml)	< 5	Aerobic bacteria at 30°C Blood Agar, method developed at Probi AB QM-002
API 50CH-profile	Identical to reference profile	Molin, G. et al. 1993; J. of Appl. Bact 74: 314-323
RAPD-profile	Identical to reference profile	Johansson, M.L et al. 1995; Lett. Appl. Microbiol., 21:155-159 (modified)
A mannose-inhiberad agglutination med <i>S cerevisiae</i>	Identical to reference strain	Adlerberth, I. et al. 1996; Appl. Environ. Microbiol. 62:2244-2251 (modified)
1. cfu = colony-forming units		

Production ampoules are prepared from prime ampoules by inoculation of sterile “non-animal MRS” as described for the prime ampoules. Growth of the bacterium is stopped at the

optimum point for harvesting, typically 10-18 hours, and the ampoules are evaluated according to the same criteria as established for the prime ampoules.

The process for making consumer products begins with media preparation and sterilization. The medium used is based on food-grade peptones, salts, and sugars. The inoculation material is the production ampoules, frozen liquid cultures in vials as described above, produced by Probi AB or by a contracted supplier and kept at -80°C.

The fermentation process is performed in multiple steps. An outline example of the process is:

1. Sterile medium in a small laboratory-scale flask is inoculated and undergoes temperature-controlled incubation until the culture reaches stationary phase, typically 10-18 hours.
2. The broth culture from step 1 is used to inoculate a small-size fermenter (pre-fermentation stage). This in turn is allowed to grow and is transferred at its optimum point for re-inoculation, again typically 10-18 hours.
3. Finally, the broth culture from the pre-fermenter is transferred to a main fermenter (fermentation stage) and is allowed to grow under controlled conditions for, again typically 10-18 hours.
4. The cells are harvested.

Since *L. plantarum* produces lactic acid, food-grade NaOH or NH<sub>3</sub> is added continuously and automatically to keep the pH in the broth at a constant desired level in accordance with production parameters. Bacterial growth in fermenters is monitored by measuring acid production. After approximately 10 -18 hours the growth is stopped at the optimum point for harvesting. The broth culture is chilled and concentrated and commonly-used food-grade cryoprotectant agents are added.

Depending on the intended usage of the product, the bacteria concentrate can be packed in liquid form, freeze dried, pelletized in liquid nitrogen, or freeze dried after pelletizing. The identity of the final product is determined according to routines of the producer. Purity tests for the final product are conducted according to the quality control procedures of the producing company.

## Part 3 - Dietary Exposure

*L. plantarum* 299v is intended to be added as a probiotic microorganism to conventional foods at concentrations consistent with cGMP needed to provide beneficial health effects. Intended food applications include but are not limited to the following:

- Wet chilled and ambient products such as fruit drinks, yogurts, milk and plant based products;
- Dry chilled products;
- Dry and shelf-stable products such as cereals, candy, bars, cookies, gums, and confectionery;
- Delivery systems designed for bacterial stability in room temperature. E.g., the bacterial powder may be enclosed in a cap mounted on a drink bottle (fruit drink, plain or flavored water, etc.) to be mixed prior to consumption.

The intended level of *L. plantarum* in food is up to  $10^{10}$  cfu/serving throughout the shelf-life of the food. In order to allow for loss of viability over time, the intended addition level is up to  $10^{11}$  cfu/serving, which provides for up to 90% loss of viability.

*L. plantarum* is thus expected to be present in a limited number of foods at between  $10^9$  and  $10^{11}$  cfu/serving. It will not proliferate in the foods and beverages to which it is added, but instead will decline over the shelf-life of the food. Its likely maximum ingestion is thus less than  $10^{11}$  cfu/day, well within levels that have been shown to be safe.

Labeling of products containing *L. plantarum* 299v will disclose the possible presence of soy proteins from the use of soy-derived peptides in the growth media or soy or milk as a regular ingredient in the product.

## **Part 4 - Self-limiting Use Levels**

*Lactobacillus plantarum* designated 299v does not have any self-limiting use levels under the conditions of use described in this GRAS notification.

## **Part 5 - Experience Based on Common Use in Food**

The statutory basis for the GRAS conclusion for *Lactobacillus plantarum* designated 299v is not based on common use in food.

## Part 6 - Narrative

### 6.1. History of Safe Ingestion

#### 6.1.1. Lactic Acid Bacteria and *Lactobacillus*

Consumption of live lactic acid bacteria (LAB) included in lactic acid fermented foods has been a regular part of the food intake of humans for a long time. There are archaeological signs that *Homo erectus* started fermenting foods with LAB 1.5 million years ago (Leakey 1993; Leakey 1995), to be compared with the start of using fire 800,000 years ago and the appearance of *Homo sapiens* 200,000 years ago. Thus, humans have historically consumed large numbers of live LAB. All through times up until the industrial revolution, lactic acid fermentation was applied as the simplest and often the safest way of preserving food. It is therefore likely that the human gastrointestinal tract has evolved to adapt to a daily supply of live LAB.

Lactobacilli have been consumed on a daily basis since humans started using fermented milks as food, including the probiotic use of certain *Lactobacillus* species for more than 75 years (Salminen et al. 1998), and indeed were almost certainly widely consumed even before that time since they are normal inhabitants of green plant material. Bernardeau et al. (2006) noted that, “lactobacilli are ubiquitous, being found wherever substances rich in carbohydrates are available.” These authors reported that in healthy humans, “lactobacilli are normally present in the oral cavity ( $10^3$ - $10^7$  cfu/g), the ileum ( $10^3$ - $10^7$  cfu/g), and the colon ( $10^4$ - $10^8$  cfu/g) and they are the dominant microorganism in the vagina.”

A Food and Agriculture Organization and World Health Organization expert consultation (FAO/WHO 2001) noted that, “lactobacilli have a long history of use as probiotics without established risk to humans, and this remains the best proof of their safety” (p17) and concluded that, “no pathogenic or virulence properties have been found for lactobacilli” (p17).

Discussing the use of probiotics in primary care pediatrics, Cabana et al. (2006) observed that the optimal dose of probiotics remains an area of active investigation, but noted that, “Although no specific pediatric dose has been established in general, there are no known reports of ‘toxicity’ associated with exceeding a specific dose in either adults or children” (p407).

Vandenplas et al. (2007) observed that lactobacilli and other probiotics “do not colonize the gastro-intestinal tract as they become undetectable a few days after stopping the administration. This results in the absence of any risk for long-term side effects.” As is discussed in more detail later, many studies have demonstrated that lactobacilli are not recovered from feces by 1-2 weeks after administration ceases. One study (Schultz et al. 2004), however, found that infants born to mothers who had received daily oral doses of  $2 \times 10^9$  cfu *L. rhamnosus* strain GG (LGG) during the 30-36 weeks of their pregnancies had detectible LGG strains in their feces for extended periods, with strain identification confirmed by molecular methods. All of the 4 infants delivered vaginally and 1 of 2 infants delivered by Caesarian section were shedding LGG at 1 and 6 months of age. Three children still had detectible fecal LGG at 12 months and 2 at 24 months; none had detectible LGG in their feces at 36 months of age. None of the mothers, on the other hand, exhibited evidence of LGG colonization by 1 month after delivery.

In an article addressing the safety of lactobacilli and bifidobacteria, Borriello et al. (2003) suggested that “classical” approaches to evaluating safety are not appropriate for these commensal bacteria:

“Lactobacilli and bifidobacteria are ubiquitous in the diet and in the healthy large intestine soon after birth. A classical risk assessment approach, similar to that used for pathogens, is not possible or warranted. Some studies of lactobacilli have attempted to define virulence factors. Such classical approaches, although useful for known pathogens, are inherently flawed when applied to normal commensals, lactobacilli, or bifidobacteria. In the case of the risk assessment approach for pathogens, pathogenicity is demonstrated and is normally a consequence of several properties, including colonization factors and virulence factors, acting in concert. Frequently, such factors as adhesion are considered to be virulence factors when pathogens are studied. However, mucosal adhesion and other colonization factors are essential features of most commensals. For example, there is a distinct mucosal-associated flora in the gastrointestinal tract. There is little value in screening organisms of low clinical significance and with no proven virulence determinants for such characteristics as potential virulence factors, particularly in the absence of gastrointestinal commensals as comparative controls” (p777).

Borriello et al. (2003) argued that the risk of bacteremia from probiotic lactobacilli and bifidobacteria is well under 1 in a million and concluded that, based on the overall risk from this or other adverse endpoints, “consumption of such products presents a negligible risk to consumers, including immunocompromised hosts.”

In a similar vein, Bernardeau et al. (2008) suggested that, “The bibliographical data support the hypothesis that the ingestion of *Lactobacillus* is not at all hazardous since lactobacillemia induced by food, particularly fermented dairy products, is extremely rare and only occurs in predisposed patients.”

### **6.1.2. *Lactobacillus plantarum***

*L. plantarum* is one of the facultative heterofermentative lactobacilli which form a mixture of the D and L isomers of lactic acid in the breakdown of various carbohydrates (Kandler and Weiss 1986). This species is not as nutritionally demanding as many other *Lactobacillus* species and is thus used as the starter culture in the industrial production of a large number of fermented foods products (Stiles 1996). *L. plantarum* occurs spontaneously in high numbers in most lactic-acid fermented foods, especially when the food is based on plant material, e.g., brined olives (Fernandez Gonzalez et al. 1993), capers (Pulido et al. 2005), sauerkraut (Dedicatoria et al. 1981), salted gherkins (McDonald et al. 1993), sourdough bread and rolls (Lonner and Ahrne 1995), Nigerian ogi (made from maize or sorghum; Johansson 1995), Ethiopian kocho (made from starch from *Ensete ventricosum*; Gashe 1987), Ethiopian sourdough made from tef (*Eragrostis tef*; Gashe 1987; Nigatu 2000) and cassava (Oyewole and Odunfa 1990). Thus, it is evident that individuals consuming lactic-acid fermented products of plant origin, as well as grape juice and wine (Vaquero et al. 2004) also consume large numbers of live *L. plantarum*.

*L. plantarum* is frequently isolated from human gastrointestinal mucosa from the mouth to the rectum (Molin et al. 1993; Ahrne et al. 1998). Ahrne et al. (1998) took bacterial samples from the back of the tongue and the rectal mucosa of 42 apparently healthy volunteers, 20 males and 22 females aged 23-48 years. *Lactobacillus* species were phenotypically identified by GRAS Determination for  
*Lactobacillus plantarum* 299v

comparing their carbohydrate fermentation patterns with type strains using API 50CH strips and genotypically confirmed by DNA-DNA hybridization to type strains. A total of 123 lactobacilli isolates were analyzed; by far the largest taxum was *L. plantarum*, isolated from 22 of the 42 volunteers (52%), followed by *L. rhamnosus* (26%) and *L. paracasei* (17%). It was also found that *L. plantarum* isolates were most often able to adhere to human colonic cells (line HT-29) by a mannose-sensitive mechanism, a capability rare among other taxa. The authors concluded that “*L. plantarum* is a major colonizer of the human gastrointestinal mucosa, and its capacity to adhere to mannose-containing receptors may be of some ecological importance.”

The fact that many traditional lactic acid fermented foods spontaneously contain high numbers of *L. plantarum* (Dedicatoria et al. 1981; Gashe 1985; Gashe 1987; Oyewole and Odunfa 1990; Fernandez Gonzalez et al. 1993; McDonald et al. 1993; Lonner and Ahrne 1995; Johansson et al. 1995b), and that these products have established a deserved reputation all over the world of being safe and wholesome, strongly demonstrates that live *L. plantarum* can be safely consumed.

### 6.1.3. *L. plantarum* 299v

*L. plantarum* 299v is included in a Swedish functional food product with the brand name ProViva<sup>®</sup> launched on the market in 1994 (Molin 1995; Molin 2001; Molin 2003). The ProViva<sup>®</sup> brand includes a range of fruit beverages sold in Sweden, Finland, Denmark, Belgium, the United Kingdom, and the U.S. by Next Foods under the brand name Good Belly<sup>®</sup>. In Germany the product has been marketed under the brand name PrimaVita<sup>®</sup> and in Belgium, ProVie<sup>®</sup>. Kraft has marketed *L. plantarum* 299v in dry form as an ingredient in the LiveActive<sup>™</sup> range of products in the U.S. and Canada. In addition, *L. plantarum* 299v is today sold as a dietary supplement in about 25 countries in Europe, North America, South America, Oceania, and Asia.

Since 1994, products containing *L. plantarum* 299v have been consumed in millions of daily doses by millions of people worldwide without any reported adverse events. In Sweden alone, with 9 million inhabitants, the consumption of ProViva<sup>®</sup> to date is about 30 million liters per year, corresponding to 150 million daily doses of *L. plantarum* 299v.

## 6.2. Safety-Related Issues

### 6.2.1. Antibiotic Resistance

In a detailed evaluation of the safety of the *Lactobacillus* genus, Bernardeau et al. (2008) addressed all issues pertaining to safety and concluded that, “transferable antibiotic resistance is the only relevant cause for caution . . . Safety assessment requirements for *Lactobacillus* strains of technological interest should be limited to an antibiotic profile and a study to determine whether any antibiotic resistance(s) of medical interest detected is (or are) transferable.”

Salminen et al. (1998) reviewed the safety of lactic acid bacteria, noting that these bacteria have a long history of safe use in foods. Lactic acid bacteria are intrinsically resistant to many antibiotics. In many cases resistances are not, however, transmissible, and the species are also sensitive to many clinically used antibiotics even in the case of a lactic acid bacteria-associated opportunistic infection. Therefore no particular safety concern is associated with intrinsic type of resistance. The primary concern with the presence of phenotypic resistance to antibiotics in probiotic bacteria is the potential for transfer of this resistance to pathogenic or potentially pathogenic organisms *in vivo* (Teuber et al. 1999).



Antibiotic susceptibility of *L. plantarum* 299v was assessed both phenotypically by determination of the minimal inhibitory concentrations (MIC) for a variety of clinically important antibiotics, and genotypically by a search for genes encoding antimicrobial resistances that are known to be transferrable.

### **6.2.1.1. Minimal Inhibitory Concentrations**

#### **6.2.1.1.1. 2005 MIC Testing**

A sample of *L. plantarum* 299v was tested for antimicrobial susceptibility by PROSAFE in 2005 using methods recommended at that time. The inocula of the strain were prepared by suspending several freshly cultivated single colonies in a tube with 5 ml saline up to an optical density of McFarland standard No. 0.5. The corresponding colonies were picked up from MRS agar plates on which the strains were grown for 48 hours at 37°C and at 5% CO<sub>2</sub> atmosphere. Subsequently, this suspension was diluted 1:10 by transferring 4 ml suspension into a suitable inoculum container with 36 ml saline and subsequent mixing. The MIC microtiter test plates (95 wells with different concentrations of the test antibiotics and one well for the growth control without any antibiotic) were prepared before. The nutrient medium was LSM (lactobacilli susceptibility test medium) broth consisting of 90% Iso-sensitest broth + 10% MRS broth (pH = 6.7). The inoculations of the pre-made MIC test plates were performed by a multipoint inoculator producing a final inoculum of the strain in the microtiter plate of about 10<sup>5</sup> bacteria/ml. The plates were subsequently incubated in ambient air or in a 5% CO<sub>2</sub> atmosphere at 37°C for 24-48 hours. The MIC were read as the lowest concentration inhibiting the growth of the test organism, with the results shown in Table 4.

These MIC were compared with the 2 markers used to differentiate intrinsic and acquired resistance, shown in the third and fourth columns of Table 4. The third column shows the microbiological breakpoints defined by the European Food Safety Authority's (EFSA) Panel on Additives and Products or Substances Used in Animal Feed (EFSA 2008a), while the fourth column shows the tentative epidemiological cut-off (ECOFF) values established based on strains deposited in the PROSAFE collection (Klare et al. 2007).

Table 4. Results of 2005 MIC Testing of *L. plantarum* 299v.

Antimicrobial Agent	MIC	EFSA Break Point <sup>1</sup>	ECOFF <sup>2</sup>
Ampicillin	0.25	2	2
Ampicillin/sulbactam	0.25		2
Chloramphenicol	4	8	8
Clindamycin	0.25	1	0.5
Erythromycin	0.125	1	0.5
Fusidic acid	16		32
Gentamicin	≤ 1	16	8
Linezolid	1	n.r. <sup>3</sup>	4
Oxytetracycline	8	32	32
Penicillin	1		2
Quinupristin/dalfopristin	0.25		1
Streptomycin	16	n.r.	64
Sulfamethoxazole/trimethoprim	0.5		IE <sup>4</sup>
Teicoplanin	256		IE
Trimethoprim	≤ 0.25	n.r.	IE
Vancomycin	> 256	n.r.	IE

1. EFSA 2008a  
2. Epidemiological Cut-Off (Klare et al. 2007)  
3. n.r. = not required  
4. IE = insufficient evidence

None of the MIC for *L. plantarum* 299v exceeded either the EFSA break point or the ECOFF, indicating that all resistances are intrinsic rather than acquired. It is important to note that many strains of *Lactobacilli* are naturally (intrinsically) resistant to vancomycin and teicoplanin and *L. plantarum* is considered inherently resistant to these antimicrobials. It is accepted that antibiotic resistance is not, in itself, a hazard. The resistance genes of *Lactobacillus* species appear to be chromosomally located and are not easily transferable to other genera (Borriello et al. 2003), in opposition to acquired resistances mediated by plasmids and transposons.

#### 6.2.1.1.2. 2016 MIC Testing

MIC testing of *L. plantarum* 299v was repeated in 2016 using updated methods in conformance with current EFSA recommendations (EFSA 2012a). MIC values were read as the lowest concentration of an antimicrobial agent at which visible growth was inhibited. The test was performed with two replicates. The accuracy of susceptibility testing was monitored by parallel use of the quality control strain *L. plantarum* ATCC 14917T. The results of antimicrobial susceptibility testing of *L. plantarum* 299V and quality control strain are shown in Figure 6. The MIC values for *Lactobacillus plantarum* 299V for the tested antibiotics, with the exception of kanamycin, were equal or lower than the established EFSA cut-off. Exceptions were recorded for kanamycin. Both repetitions of the test for kanamycin showed MIC values 1-fold higher than the EFSA breakpoint. According to ISO 10932:2010 for the interpretation of the

results “at least 95% of the values should be included in the proposed range and will include mode  $\pm$  1 log.” *L. plantarum* 299V was therefore classified as sensitive to kanamycin.

Antibiotic	MIC for <i>L. plantarum</i> ATCC 14917T (control strain) ( $\mu$ g/ml)		*EFSA cut-off for <i>L. plantarum</i> ( $\mu$ g/ml)	MIC for <i>Lactobacillus plantarum</i> 299V ( $\mu$ g/ml)		ISO 10932:2010 range of results for the control strain	Sensitive / Resistant (R or S)
	1 <sup>st</sup> repetition	2 <sup>nd</sup> repetition		1 <sup>st</sup> repetition	2 <sup>nd</sup> repetition		
Gentamycin	2	2	16	4	4	1-16	S
Kanamycin	32	32	64	128	128	32-512	S (●)
Streptomycin	8	8	n.r.	64	64	16-256	n.r.
Tetracycline	0.5	0.5	32	32	32	8-16	S
Erythromycin	0.12	0.12	1	0.5	0.5	0.25-2	S
Clindamycin	0.5	0.5	2	1	1	0.5-4	S
Chloramphenicol	8	8	8	8	8	2-8	S
Ampicillin	2	2	2	1	0.5	0.25-2	S
Vancomycin	>128	>128	n.r.	>128	>128	128	n.r.
Quinopristin/Dalfopristin	2	2	\	2	2	1-4	\
Linezolid	4	4	\	4	2	1-32	\
Trimethoprim	32	32	\	>64	>64	2-128	\
Ciprofloxacin	4	4	\	32	32	32-64	\
Rifampicin	0.5	0.5	\	2	4	1-8	\
Neomycin	4	4	\	4	8	1-64	\
Penicillin	0.5	1	\	2	2	0.5-4	\

a= EFSA cut-off for *L. plantarum* as listed in EFSA Scientific Opinion “Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance” FSA Journal 2012;10(6):2740.

n. r.= not required by EFSA

(●) = MIC has been found 1-fold higher than EFSA breakpoint (considering ISO 10932:2010 criteria for the interpretation of the results, the mode  $\pm$  1 log is expected, and the strain is therefore considered Sensitive S for that antibiotic).

Figure 6. Results of 2016 MIC Testing of *L. plantarum* 299v.

### 6.2.1.2. Genetic Analysis

The possible presence of known genes specifying resistance in Gram-positive bacteria was examined using PCR as specified in Table 5 by the Danish Institute for Food and Veterinary Research. It was not possible to detect any amplicons in the PCR reaction, indicating that none of these genes is present in *L. plantarum* 299v. This further supports the conclusion that transferable antimicrobial resistance is not present in the strain.

**Table 5. Resistance Genes Determined Not To Be Present in *L. plantarum* 299v.**

Resistance	Resistance gene	Primer-pair
<i>Gentamicin</i>	<i>aac(6)-aph(2)</i>	5' -CCAAGAGCAATAAGGGGCATA-3' 5' -CACTATCATAAACCCTACCG-3'
<i>Kanamycin</i>	<i>AphA3</i>	5' -GCCGATGTGGATTGCGAAAA-3' 5' -GCTTGATCCCCAGTAAGTCA-3'
<i>Kanamycin</i>	<i>aadD</i>	5' -TGCGTTTTGACACATCCAC-3' 5' -GGTGTATTATGGCTCTCTTGG-3'
<i>Streptomycin</i>	<i>aadE</i>	5' ATGGAATTATTCCCACCTGA-3' 5' -TCAAACCCCTATTAAAGCC-3'
<i>Trimethoprim</i>	<i>dfrA</i>	5' -AAAAGGGGCAGAGCATG-3' 5' -AGAAAATGGCGTAATCGGTA-3'
<i>Tetracycline</i>	<i>tet(K)-1</i> <i>tet(K)-2</i>	5' -TTAGGTGAAGGGTTAGGTCC-3' 5' -GCAAACCTCATTCCAGAAGCA-3'
<i>Tetracycline</i>	<i>tet(L)-1</i> <i>tet(L)-2</i>	5' -GTTGCGCGCTATATTCCAAA-3' 5' -TTAAGCAAACCTCATTCCAGC-3'
<i>Tetracycline</i>	<i>tet(M)-1</i> <i>tet(M)-2</i>	5' -GTTAAATAGTGTCTTGGAG-3' 5' -CTAAGATATGGCTCTAACAA-3'
<i>Tetracycline</i>	<i>tet(O)-1</i> <i>tet(O)-2</i>	5' -GATGGCATAACAGGCACAGAC-3' 5' -CAATATCACCAGAGCAGGCT-3'
<i>Tetracycline</i>	<i>tet(S)-1</i> <i>tet(S)-2</i>	5' -TGGAACGCCAGAGAGGTATT-3' 5' -ACATAGACAAGCCGTTGACC-3'

### 6.2.2. Production of Bacteriocins

Bacteriocins are proteinaceous compounds produced by bacteria that exhibit a bactericidal or bacteriostatic mode of action against sensitive bacterial species. They are defined as protein antibiotics of relatively high molecular weight mainly working against the same or closely related species by adsorption to receptors on the target cells. They are divided into 4 classes:

Class I- Lantibiotics,

Class II- Small hydrophobic, heat stable peptides (<13kDa),

Class III- Large heat-labile proteins (>30 kDa), and

Class IV- Complex bacteriocins; proteins with lipid and/or carbohydrate (Fooks and Gibson, 2002).

Most of the bacteriocins of *Lactobacillus* species belong to Class II, which contains a wide variety of bacteriocins and has therefore been subdivided into three subclasses. A number of Class II bacteriocins have been shown to be membrane-active peptides which destroy the integrity of the membrane by the formation of pores. Other mechanisms exist within this class, but they can all be linked to a common pattern: dissipation of proton motive force. Some strains of *L. plantarum* have been reported to be bacteriocin producers; e.g., *L. plantarum* strains ALC01 and WHE produce pediocin (Ennahar et al. 1998). Additionally, different kinds of plantaricin (a pediocin-like bacteriocin) are produced by *L. plantarum* strains; e.g., plantaricin C19 by *L. plantarum* C19 (Atrih et al. 2001), plantaricin UG1 by *L. plantarum* UG1 (Enan 1996), plantaricin TF711 by *L. plantarum* TF711 (Hernandez et al. 2005), plantaricin NC8 by *L.*

*plantarum* NC8 (Maldonado et al. 2004), and plantaricin 423 by *L. plantarum* 423 (Van Reenen et al. 2003).

*L. plantarum* 299v is considered a potential bacteriocin producer, but such production has not been demonstrated. The possible production of bacteriocins by *L. plantarum* 299v would in case represent a positive health benefit rather than a risk.

### 6.2.3. Production of Carboxylic Acids

#### 6.2.3.1. Total Lactic Acid and Acetic Acid

*L. plantarum* 299v produces only lactic acid and acetic acid. Other short-chain organic acids such as propionic acid or butyric acid may be produced in the gastrointestinal tract, but this is due to normal metabolism from other intestinal bacteria (Berggren 1996).

According to a study by Johansson et al. (1998), ingestion of *L. plantarum* 299v does not increase the total lactic acid concentration in the colon. Acetic acid has been consumed by man for centuries at about 1 g/day, as it is present in vinegar and other items of food and drink (WHO 1974). The increased production of acetic acid resulting from consumption of *L. plantarum* 299v has no negative health effect since the acceptable daily intake for man is not limited as a food additive (WHO 1974) and since the increased mean acetic acid concentration in the active group is still within the physiologically achievable range after 3 weeks of application (Johansson et al. 1998).

#### 6.2.3.1. D-Lactic Acid

All LAB, by definition, produce lactate from carbohydrate fermentation. Lactate exists in two enantiomeric forms, a dextrorotary enantiomer (D-lactate) and a levorotary enantiomer (L-lactate). *L. plantarum* 299v produces a mixture of L- and D-lactate, with the latter accounting for about 61.9% of total lactate production. All LAB produce some amount of D-lactate, ranging from 1 to 97% of all lactate produced, depending on the strain, with 40% being a typical amount. Other indigenous intestinal bacteria also produce D-lactate, including *E. coli*, *Klebsiella* spp., and *Bacteroides* spp. (Duzgun et al. 2007). Only nanomolar concentrations of D-lactate are produced endogenously in mammals due to the absence of the isomer-specific enzyme D-lactate dehydrogenase (D-LDH) needed for its production (Ewaschuk et al. 2005; Petersen 2005), but D-lactate may be present in serum due to exogenous sources such as fermented foods and microbial fermentation in the colon.

Intestinal bacteria express either or both a D- or L-lactate specific dehydrogenase (Hove and Mortonsen 1995; Kochar et al. 1992). Carbohydrates such as hexoses are fermented by bacterial glycolytic pathways to pyruvate and either L- or D-lactate. Additionally, some *Lactobacillus* strains have DL-lactate racemase which catalyzes the conversion between D- and L-lactate (Hove and Mortonsen 1995). Thus, colonic D-lactate may be formed from pyruvate by bacterial D-lactate dehydrogenase or from L-lactate by racemization (Hove 1998). L- and D-lactate are intermediary products that other colonic bacteria can metabolize to short-chain fatty acids (Hove and Mortonsen 1995).

Under normal circumstances, lactate generated by bacterial fermentation in the intestine does not result in clinically significant elevation of lactate in the blood or stool of humans. The normal serum concentration of lactate, nearly entirely L-lactate, is about 500-6000  $\mu\text{mol/L}$  (Anderson et al. 1997; Ewaschuk et al. 2005). The normal serum concentration of D-lactate is

variously estimated as 0-250  $\mu\text{mol/L}$  (McLellan et al. 1992; De Vrese and Barth 1991; Hove and Mortensen 1995; Vella and Farrugia 1998; Connolly et al. 2005) or as about 1.5% of the L-lactate concentration (Anderson et al. 1997).

In the past, it was believed that D-lactate in humans is metabolized only slowly by the enzyme D- $\alpha$ -hydroxy-acid dehydrogenase and is mainly excreted in the urine, but newer studies have identified putative human mitochondrial D-lactate dehydrogenases, most importantly D-2-hydroxy acid dehydrogenase (D-2-HDH), which is found in high concentration in the kidney cortex and the liver and provides a large capacity to metabolize D-lactate to pyruvate (Petersen 2005, Ewaschuk et al. 2005). As a result, most of the studies of the factors resulting in D-lactic acidosis and other effects of severely elevated serum levels of D-lactate have concluded that D-lactate is metabolized and hence does not accumulate (Hove and Mortensen 1995; Uribarri et al. 1998) and is unlikely to occur absent impaired D-lactate metabolism (Uribarri et al. 1998). In humans who do not have impaired D-lactate metabolism, de Vrese et al. (1990) found that with bolus consumption of up to 12.8 mmol/kg bw of racemic DL-lactic acid, D-lactate reached a maximum plasma concentration of 0.45 mmol/l and was eliminated from plasma with an average half-life of 40.4 minutes. Daily consumption of 6.4 mmol/kg bw/day of racemic DL-lactic acid for 5 weeks did not result in the accumulation of plasma D-lactate (de Vrese et al. 1990).

The only medical indications arguing against the use of D-lactate producing strains as probiotics are derived from older studies in which infants were fed formula acidified with known amounts of D- and L-lactate (Stolley and Droese 1971). Subsequent studies with acidified formulas have not supported these initial findings. The acidification was a direct result of the addition of chemical lactic acid and not naturally occurring acidification due to the fermentation of food matter.

Uribarri et al. (1998), after reviewing the literature, concluded that “impaired metabolism of D-lactate is almost a prerequisite for the development of the syndrome.” However, the activity of D-2-HDH is inhibited by oxalate and by low pH (Petersen 2005), and the presence of these conditions may lead to accumulation of D-lactate. Additionally, the 2 organs that provide the highest concentrations of D-2-HDH, the kidney and liver, are often compromised in short-bowel patients and it is not uncommon for both renal and hepatic function to fluctuate significantly in these patients (Petersen 2005). Connolly and Lonnerdal (2004), in a review of the metabolism and possible toxicity of D-lactic acid, reached a similar finding, concluded that there is no evidence to show that the normal gastrointestinal tract microbiota can induce D-lactic acidosis in the healthy human adult or infant. D-lactic acid acidosis only occurs in subjects with a disturbed gastrointestinal function following bowel resection. Connolly and Lonnerdal (2004) further noted that bacterial overgrowth and a disturbed gastrointestinal microbiota in the large bowel is a prerequisite for D-lactic acidosis in humans. There is no evidence that exogenous probiotics can induce *Lactobacillus* overgrowth or imbalance in the bacterial flora of healthy newborn infants, children, or adults.

Nevertheless, some concern was expressed by Mack (2004) regarding the use of probiotic bacteria that produce D-lactic acid. (It is to be noted, however, that no case of D-lactic acidosis due to an intake of food containing D-lactic acid producing bacteria has been reported in the literature [Haschke-Becher et al. 2000]. This statement remains true as of 2011, based on a Medline search.) The author noted that there are no reports of healthy infants or children developing D-lactic acidosis, but urged that controlled clinical studies involving primary analysis

of this issue be undertaken to set aside this concern. In response, Connolly et al. (2005) compared the blood D-lactic acid levels of 14 infants who had received  $10^8$  cfu/day *L. reuteri* ATCC 55730 (a D-lactic acid producer) from birth with those of 10 infants who had received placebo, at the age of 6 months and at 12 months. In both groups, blood D-lactate levels were within the normal range; they were insignificantly higher in the *L. reuteri* treated group at 6 months, but insignificantly lower at 12 months. The authors concluded that the findings provide evidence that there is no elevation of D-lactic acid in the blood of healthy infants given *L. reuteri* at a dose of  $10^8$  cfu/day from birth to 12 months.

In another study, urinary D-lactate excretion of infants ingesting *L. johnsonii* strain Lal added to infant formula was evaluated by Haschke-Becher et al. (2008). Like *L. plantarum* and *L. reuteri*, *L. johnsonii* produces both the D- and L-isomers of lactic acid. A total of 71 healthy infants with gestational ages of 36-44 weeks and birth weights >2500 g was enrolled; the average age of the infants was about 106 days. Twenty-six infants were breast fed, and the remaining infants were randomly assigned to receive formula containing 0 (n = 26) or  $10^8$  cfu probiotic/g powder (n = 19). Parents were instructed to provide 3-4 200-ml formula feedings/day to achieve daily intakes of  $0.8-1.1 \times 10^{10}$  cfu *L. johnsonii*. Morning urine samples were taken at baseline and after 4 weeks and analyzed for D- and L-lactate as well as creatinine; lactate excretion was expressed per mol creatinine.

Thirteen infants were withdrawn from the study, none for reasons attributed to the feeding. There were no differences in formula intake between the 2 formula groups nor among the 3 groups in growth. There were no differences in urinary D-lactate concentrations among the 3 groups at baseline, but after 4 weeks D-lactate excretion increased significantly in both formula groups as compared to the breastfed group, but the 2 formula groups did not differ in D-lactate excretion. There were no differences among the groups in L-lactate excretion at any time. The authors concluded that “current evidence does not point at any risk of lactate acidosis in healthy infants fed a formula supplemented with the probiotic strain Lal.”

It may be concluded that there is no valid reason to exclude the supplementation of indigenous human *Lactobacillus* species to the newborn human infant, nor to children or adults, on the basis of the stereoisomers of lactic acid these bacteria produce.

#### **6.2.4. Production of Biogenic Amines**

Microbial biogenic amine formation occurs via the decarboxylation of amino acids. While this is a common function of microorganisms, high concentrations of biogenic amines can cause undesirable physiological effects. The primary precursor amino acids are histidine, tyrosine, hydroxytryptophane, tryptophane, lysine, ornithine, and arginine, which may be catalyzed by specific decarboxylases into histamine, tyramine, serotonin, tryptamine, cadaverine, putrescine, and spermine/spermidine, respectively.

Some strains of *L. plantarum* have been reported to produce tyramine, putrescine, and agmatine (Arena et al. 2001; Buncic et al. 1993; Masson et al. 1996). According to the Hazardous Substances Data Bank (2002) for histamine and tyramine, the risk of allergic-like response after ingestion of biogenic amines is very low. Biogenic amines can reach high concentrations in fish, especially scombrides (e.g., tuna, mackerel), but poisoning following consumption of scombrides occurs rarely, even if high amounts of histamine are ingested.

While it is not certain whether *L. plantarum* 299v has the capability of producing biogenic amines, this cannot be regarded as a safety hazard.

#### 6.2.5. Allergic Potential

According to FAO/WHO (2001), no population of healthy individuals is known to be sensitized (i.e., IgE-mediated) to bacterial proteins, and there have been no reports of allergenicity following consumption of *L. plantarum* 299v.

#### 6.2.6. Infectivity

Cases of infection by lactic acid bacteria are extremely rare. Reid and Hammond (2005) asserted that, “The safety record of probiotics is remarkable considering that more than 20 billion doses are estimated to be used each year.” Over the past 30 years there have been about 180 published cases of bacteremia and 69 cases of endocarditis putatively caused by lactobacilli (Aguirre and Collins, 1993; Gasser, 1994; Donohue and Salminen, 1996). The majority of these cases have occurred in patients with compromised immune status and/or mucosal barrier function due to underlying conditions such as heart disease or diabetes or therapeutic treatment (e.g., dental surgery). Boyle et al. (2006) stated firmly, “All cases of probiotic bacteremia or fungemia have occurred in patients with underlying immune compromise, chronic disease, or debilitation, and no reports have described sepsis related to probiotic use in otherwise healthy persons.”

Eleven case reports have been published on clinical infections in patients consuming probiotics, most commonly *L. rhamnosus* or *L. casei* strains. However, in only some of these cases was the strain isolated from the infection confirmed to be identical to the strain that was consumed. The species *L. plantarum* has been involved in a few cases of infections. It has been associated with cases of single and mixed (more than one species of bacteria) bacteremia and was the cause of some endocarditis, all in patients with underlying conditions (Adiego and Wessels 2002).

It is clear that all reported cases of clinical infections with suspected *Lactobacillus* involvement occurred in subjects with one or more severe underlying diseases or health conditions. While these reports indicate that *Lactobacillus* has the potential to be an opportunistic pathogen in severely compromised subjects, it is equally clear that the genus is safe in healthy subjects and those with less severe medical conditions, where adverse events have never been reported.

This conclusion is strongly supported by surveillance studies that have failed to discover any evidence of increased rates of clinical infection correlated with increased consumption of *Lactobacillus* species. One of the most comprehensive such studies (Saxelin et al. 1996; Salminen et al. 2002) showed that over a nine year period in which consumption of *L. rhamnosus* increased 10-fold in Finland (a country with an excellent reporting system for health-related events), the number of infections involving *Lactobacillus* species reported to Helsinki health authorities was unchanged.

Positive blood cultures for lactobacilli have also been regarded as indicators of serious or fatal underlying disease (Husni et al. 1997). With regard to cases of endocarditis, strains of lactobacilli are only rarely involved (0.05 – 0.4% of total) compared to bacteria shown to be most highly associated with endocarditis (e.g., >79% by the *Streptococcus-Staphylococcus*



group). Cases of lactobacilli endocarditis are typically associated with serious underlying health conditions, such as structural heart disease, that predisposed the patient to opportunistic infections (Donohue and Salminen, 1996). These observations suggest that lactobacilli are much less capable of adhering to intact cardiac valves than other bacteria and only become involved in infections when a predisposing circumstance exists. Although lactobacilli play a minor etiologic role in the context of all cases of endocarditis, in cases where etiologic strains were identified at the species level (a procedure that is not always done), the majority of cases were caused by vancomycin-resistant strains of *L. rhamnosus*, *L. plantarum*, and *L. casei* (Gasser, 1994; Donohue and Salminen, 1996). Saxelin et al. (1996) studied the prevalence of bacteremia due to *Lactobacillus* species during the period 1989-1992. Among 3,317 blood culture isolates, lactobacilli were identified in 8 patients, 5 of whom had severe diseases predisposing to bacteremic complications.

No case has been described of a *Lactobacillus* infection derived from food or feed fermented with *Lactobacillus* cultures (Adams and Marteau 1995). The participants in the 2007 EU-PROSAFE project (Vankerckhoven et al. 2007) observed, “It was argued that clinical cases of LAB endocarditis were so rare that they were more medical exceptions, or even curiosities, than a genuine public health issue, especially with regard to the huge worldwide daily consumption of LAB in regular food intake.”

### **6.3. Research Studies of *L. plantarum* 299v**

#### **6.3.1. *In Vitro* Studies**

A number of *ex vivo* and *in vitro* studies of *L. plantarum* 299v have elucidated the strain’s probiotic properties as well as its capacity to up- or down-regulate immune responses

Jensen et al. (2012) tested *in vitro* 5 commercial probiotic strains and 13 potential probiotic strains for gastric and intestinal tolerance, adhesion capacity to human intestinal cell lines, and effect on epithelial barrier function. *L. plantarum* 299v exhibited 93% survival after 90 minutes in simulated gastric juice and 62% survival after 180 minutes; in simulated small intestine fluid survival was 95% after 240 minutes. Its adhesion to Caco2 cells was only about 2%, the lowest value of any strain tested, but its adhesion to HT-29 cells was about 3% and to LS 174T was about 2%, both about average compared to other strains tested. In summary, the authors concluded that both *L. plantarum* 299v and *L. rhamnosus* GG “performed relatively poor compared to other strains in our assays.”

In an *ex vivo* study using macroscopically normal colonic tissue from the distal region of resected intestine from patients undergoing adenocarcinoma surgery, Bauerl et al. (2013), studied the immunological response to infusion of *L. paracasei* BL23 and *L. plantarum* 299v. The 2 strains were cultured in MRS broth in the laboratory, harvested at early stationary phase, and added to tissue culture wells at a concentration of  $10^6$  cfu/ml of incubation medium for 4 hours, after which RNA samples were harvested for genetic analysis.

Treatment with either probiotic resulted in down-regulation of genes encoding several proinflammatory effector molecules, including IL-2, IL-17A, IFN- $\gamma$ , members of the CXC chemokine family, and 2 members of the tumor necrosis factor receptor superfamily, TNFRSF4 and TNFRSF9. The authors concluded that the changes in gene expression “could be explained

by primary downregulation of IFN- $\gamma$  . . . by the probiotic lactobacilli. Reduction in gene expression in IL-2 and IL-2RA suggests that these lactobacilli are also counteracting the molecular events leading to T cell activation and proliferation.”

The mucosal adhesion properties of *L. plantarum* 299v were studied in an *in vitro* investigation of the adhesive properties of the enzymes glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and enolase isolated from its cell surface (Glenting et al. 2013). The probiotic bacteria were grown in MRS medium for 40-48 hours and harvested by centrifugation; proteins extracted from the cell surface were concentrated on spin columns and genes encoding GAPDH, phospho-glycerate kinase (PGK), and enolase were PCR amplified for study of their activity.

The role of GAPDH and enolase in adhesion to Caco-2 cells was found to be pH-dependent, with binding occurring at pH 5 but not at pH 7. Both GAPDH and enolase showed specific binding to plasminogen and fibronectin, while GAPDH also showed weak binding to mucin. The authors concluded that, “The results showed that these glycolytic enzymes could play a role in the adhesion of the probiotic bacterium *L. plantarum* 299v to the gastrointestinal tract of the host.”

In a follow-up to the 2012 study (Jensen et al. 2012), again based on *in vitro* testing, Jensen et al. (2014) to assess immune stimulating abilities of the same 18 *Lactobacillus* strains based on cytokine secretion from the monocytic cell line THP-1 (IL-8, IL-10, and TNF $\alpha$ ) and NF- $\kappa$ B activation in the monocytic cell line U937-3xkB-LUC. *L. plantarum* 299v produced very little secretion of IL-8—less than all but one of the tested strains. Secretion of TNF $\alpha$  was also very low, with only two strains showed lower levels. The NF- $\kappa$ B activation capacity of *L. plantarum* 299v was also among the lowest of any strain tested. The authors concluded that, “Well-known probiotic strains such as *L. plantarum* 299v and *L. rhamnosus* GG had little effect on cytokine secretion from THP-1 cells and activation of NF- $\kappa$ B in the U937-3xkB-LUC cell line.”

Diana et al. (2015) tested the probiotic properties of *Leuconostoc mesenteroides* in *in vitro* experiments, using *L. plantarum* 299v as a “reference probiotic strain.” Tests included survival in simulations of the stomach and small intestine, adherence to intestinal mucosa, hydrophobicity, adhesion to mucin, hemolytic activity, antibiotic resistance, and antimicrobial activity (against pathogenic strains of *Salmonella enterica* ssp. *enterica*, *Listeria monocytogenes*, *Escherichia coli*, and *Staphylococcus aureus*).

The data on *L. plantarum* 299v showed about 77% and 99% survival in the stomach and intestinal simulations, respectively (significantly higher than *L. mesenteroides*). On the other hand, 299v showed less adherence to intestinal mucosa (about 75%) and about the same (~93%) adhesion to mucin. *L. plantarum* was significantly lower in hydrophobicity than the experimental strains and exhibited no hemolytic activity. The 299v tested showed susceptibility to erythromycin, tetracycline, and ampicillin and resistance to 9 other antibiotics tested (dicloxacillin, gentamicin, pefloxacin, trimethoprim, penicillin, cephalothin, cefotaxime, ceftazidime, and cefuroxime). Based on the sizes of the inhibition zones on agar, the authors determined that *L. plantarum* 299v and the test strains all showed “medium levels of inhibition against the five pathogens tested.”

In summary, *ex vivo* and *in vitro* experiments with *L. plantarum* 299v have confirmed its probiotic characteristics such as gastric and intestinal survival along with a relatively low level of adherence to intestinal mucosa and an absence of hemolytic activity. The studies have also shown that the strain has little tendency to upregulate proinflammatory cytokines; rather, it more often downregulates them.

### 6.3.2. Animal Studies

The studies discussed in this section are summarized in Table 6 on page 35.

Studies on acute toxicity have been carried out on a number of *Lactobacillus* strains (Salminen and von Wright 1998). In all cases, the LD<sub>50</sub> value was > 6000 mg/kg bw, equivalent to the intake of > 400 g pure bacterial culture for a person with a body weight of about 70 kg.

Kasravi et al. (1996) used a rat model of acute liver injury to study the effect of oral supplementation with lactobacilli on bacterial translocation. Forty Sprague-Dawley rats, divided into 5 groups of n = 8 rats/group, received 5 ml saline/day, 5 ml 20% lactose solution/day, 20 mg neomycin sulfate/day, 2.5-5.0x10<sup>9</sup> cfu *L. reuteri* R2LC/day, or 2.5-5.0x10<sup>9</sup> cfu *L. plantarum* 299v/day for 7 days. On day 7, acute liver injury was induced by intraperitoneal injection of D-galactosamine. A control group of n = 8 rats received only saline. After 24 hours, the animals underwent laparotomy; aortal blood samples were taken for bacteriological study and measurement of serum endotoxin, bilirubin, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase while portal-vein samples were taken for bacteriological analysis. Samples were taken from the liver's left and caudate lobes, as well as from mesenteric lymph nodes, and mucosa of the distal small intestine and cecum.

Liver injury significantly increased levels of bilirubin, aspartate aminotransferase, and alanine aminotransferase, but these were significantly reduced by administration of *L. plantarum*, neomycin, or lactulose, as was injury-related bacterial translocation. The authors concluded that ingestion of *L. plantarum* 299v "improved the overall proliferative state of the mucosa in the small intestine and cecum . . . and the subsequent bacterial translocation."

Mao et al. (1996a) studied the effects of 2 *Lactobacillus* strains, along with oat fiber, on methotrexate-induced enterocolitis in Sprague-Dawley rats. A total of 126 male rats weighing 200-250 g were housed individually in wire-bottom cages and given free access to a fiber-free diet. After 108 of the rats received gastrostomies, they were randomized to 6 groups (n = 18 rats/group) in a 2x3 factorial design: daily infusions with or without oat fiber, and daily infusions with no probiotic, with 4x10<sup>9</sup> cfu *L. plantarum* 299v, or with 4x10<sup>9</sup> cfu *L. reuteri* R2LC. The remaining rats, without gastrostomies, constituted a control group. Methotrexate was injected intraperitoneally on day 3 to induce enterocolitis. Three days later 6 rats from each group were weighed, sacrificed, and subjected to histopathologic examination and analysis for mucosal protein, DNA, RNA, and nucleotides; 6 rats from each group were anesthetized for permeability measurement; and 6 rats from each group were sacrificed for analysis of myeloperoxidase, microbiota, bacterial translocation, and endotoxins.

Rats receiving *L. plantarum* and oat fiber showed significantly less lethargy and diarrhea than did the other rats with induced enterocolitis, lost significantly less weight, had less mucosal inflammation and ulceration, significantly higher levels of mucosal protein, DNA, RNA, and nucleotide, less permeability in the proximal and distal small intestine and colon, significantly

less bacterial translocation to the mesenteric lymph nodes, liver, spleen, and aortic blood, and significantly reduced plasma endotoxin levels. No adverse effects were reported due to ingestion of either probiotic.

In a similar study (Mao et al. 1996b), 42 male Sprague-Dawley rats weighing 200-250 g were housed individually in wire-bottom cages and given free access to a fiber-free diet. Six rats were assigned to a control group and the other 36 were gastrostomized and assigned to 6 groups of n = 6 animals/group. The 6 groups included a gastrostomy control group that received no further intervention and 5 groups that received intraperitoneal injections of methotrexate on day 3 after receiving 1% pectin, 4 ml oat fiber/day,  $4 \times 10^9$  cfu *L. plantarum* 299v/day, or  $4 \times 10^9$  cfu *L. reuteri* R2LC/day on days 1-2 and on days 4-5. All rats were sacrificed on day 6; the small intestine was excised and divided into jejunum and ileum and mucosa from the ileum and colon were tested for secretory IgA, T-helper lymphocytes (CD4) and T-suppressor lymphocytes (CD8). Colon and ileum samples were subjected to histopathological assessment.

All 4 treatments—pectin, oat fiber, and both probiotics—produced significantly less weight loss and histopathological damage due to enterocolitis. Injection of methotrexate produced significantly lowered levels of sIgA CD4, and CD8, which were mostly restored by ingestion of either of the two strains of *Lactobacillus* but not by either fiber. No adverse effects from ingestion of either probiotic were reported, and both treatments improved mucosal immunity.

Adawi et al. (1997) studied the effects of 5 different *Lactobacillus* strains on liver damage and bacterial translocation in a rat model of acute liver injury. Male Sprague-Dawley rats weighing 200-300 g were assigned to 13 groups (n = 6 rats/group). One group was left as a normal control while the other 12 groups were given intraperitoneal injections of D-galactosamine to induce acute liver injury. Six of the groups received arginine supplementation. One arginine-supplemented group and one unsupplemented group were assigned to each of the following treatments: no prebiotic, *L. reuteri* R2LC, *L. rhamnosus* DSM 6594, *L. fermentum* 8704:3, *L. reuteri* 108, or *L. plantarum* DSM 9843 (= strain 299v). Probiotic doses were  $3 \times 10^9$  cfu/day administered rectally; treatment began 8 days before induction of the acute liver injury. Samples were collected 24 hours post-injection; aortic and portal blood were taken and the caudate lobe of the liver, mesenteric lymph nodes, and cecal and colonic contents were taken. All samples were subjected to bacteriological analysis, liver was studied histopathologically, and blood was analyzed for bilirubin, alkaline phosphatase, aspartate transaminase, and alanine transaminase.

Four additional groups of n = 6 rats were assigned in a 2x2 factorial design to undergo the same protocol with or without arginine supplementation and with or without *L. plantarum* 299v. The only difference from the main experiment is that samples were collected 48 hours rather than 24 hours after induction of acute liver injury.

No mortality occurred in either phase of the study. Significant lowering of the level of aspartate transaminase was observed 24 hours after liver injury in the group receiving *L. plantarum* + arginine; at 48 hours, both aspartate and alanine transaminase were significantly lower in the groups receiving arginine and/or *L. plantarum*. Bacterial translocation to portal and arterial blood, liver, and mesenteric lymph nodes was significantly increased by liver injury, but significantly reduced at both 24 and 48 hours in the groups receiving *L. plantarum*, with or without arginine, as well as at 24 hours in several of the other probiotic groups. Numbers of

*Enterobacteriaceae* were significantly reduced in the cecum and colon and liver histopathology was significantly lessened by administration of *L. plantarum* 299v. The authors concluded that:

“Rectal supplementation of some *Lactobacillus* strains with and without arginine significantly reduced the liver enzyme release observed in the ALI after administration of d-galactosamine. This decrease in the liver enzyme release indicates improvement in the liver injury and failure. The most pronounced effects were obtained in the groups supplemented with *Lb. plantarum* DSM 9843 [strain 299v] + arginine and with *Lb. plantarum* DSM 9843 [strain 299v] alone.”

Herias et al. (1999) colonized gnotobiotic rats with *E. coli* alone or along with *L. plantarum* 299v to investigate the effect of the probiotic on immune function. Fourteen male and female AGUS rats were maintained in isolators and colonized by gavage with  $6.8 \times 10^8$  cfu *E. coli* O6:K13:H1; half of the rats were also gavaged with  $7.8 \times 10^8$  cfu *L. plantarum* 299v. The rats were sacrificed at either 1 week (n = 6) or at 5 weeks (n = 8); bacterial populations of the small intestine, cecum, colon, mesenteric lymph node were determined; serum antibody titers against *E. coli* were measured; total IgA, IgG, and IgM concentrations were assessed; and proliferation of CD8<sup>+</sup>, CD4<sup>+</sup>, CD25<sup>+</sup>, and mitogen-induced spleen cells was determined.

In the rats sacrificed at 1 week, *L. plantarum* 299v significantly reduced *E. coli* counts in the small intestine and cecum, but this effect was not seen at 5 weeks. At both 1 and 5 weeks total serum IgA and CD25<sup>+</sup> cells were significantly increased in the rats receiving the probiotic. The authors concluded that “*L. plantarum* colonization competes with *E. coli* for intestinal colonization and can influence intestinal and systemic immunity,” and suggested that, “It is possible that the presence of the mannose-dependent adherence mechanism in *L. plantarum* was partly responsible for the competition with *E. coli* early after colonization.”

The ability of *L. plantarum* 299v to inhibit microbial translocation in acute pancreatitis was studied in an animal model by Mangiante et al. (2001). Acute pancreatitis was induced in adult pathogen-free Lewis rats weighing 250-350 g by isolation and ligation of the biliopancreatic duct; 4 days before and 4 days after the induction the rats received daily gavage of 5 ml/day of either saline (n = 28) or  $2.5-5.0 \times 10^9$  cfu of *L. plantarum* (n = 27). Animals had free access to water and chow. The animals were sacrificed after 96 hours and subjected to histological studies and microbiological analyses. Administration of the probiotic significantly reduced the number of animals showing bacterial translocation to the pancreatic tissue and mesenteric lymph nodes. No *L. plantarum* were identified in blood, pancreatic tissue, or lymph nodes.

Adawi et al. (2001) investigated the effect of different strains of lactobacilli and bifidobacteria on the intestinal ecology, bacterial translocation, and extent of liver injury in an animal model of acute liver injury. Male Sprague-Dawley rats weighing 200 to 300 g were divided into 5 groups (n = 6 rats/group): a control group and test groups that received daily rectal administrations of  $3 \times 10^9$  cfu of *B. animalis* NM2, *L. acidophilus* NM1, *L. rhamnosus* ATCC 53103, or *L. rhamnosus* DSM 6594 + *L. plantarum* DSM 9843 (strain 299v) for 8 days, during which they had free access to feed and water. On the 8<sup>th</sup> day, liver injury was induced by intraperitoneal injection of D-galactosamine; the animals were sacrificed 24 hours later and portal and arterial blood was taken for analysis of liver enzymes alkaline phosphatase and alanine transaminase, bilirubin, and bacterial translocation; liver and mesenteric lymph nodes

were analyzed for bacterial translocation; and intestinal samples were analyzed for effects on microbial ecology.

There was no mortality and no effect on bilirubin or alkaline phosphatase, but alanine transaminase was significantly lower in the rats that had received *Lactobacillus* than the control rats or those receiving bifidobacteria. Enterobacteriaceae were significantly decreased in the colon by all treatments as compared to controls, but were significantly decreased in the cecum only by treatment with *B. animalis* or the combination of *L. rhamnosus* + *L. plantarum*. Administration of bacteria, as compared with the control condition, significantly reduced bacterial translocation to portal and arterial blood, liver, and mesenteric lymph nodes.

The ability of *L. plantarum* 299v to speed anastomotic healing and reduce mucosal atrophy associated with radiation therapy of patients with rectal carcinoma was studied in an animal model (Liu et al. 2001). Seventy-two adult male Sprague-Dawley rats weighing about 300 g were assigned to 4 groups in a 2x2 design (n = 18 rats/group) in which they did or did not receive radiation and were given by gavage saline providing 0 or  $4 \times 10^9$  cfu *L. plantarum* 299v. Those animals scheduled for radiation received it on days 3 and 7 and all animals underwent distal colon resection and anastomosis on day 11. A third of the animals in each group were sacrificed on days 15, 18, and 22. Bodyweight and white blood cell counts were measured on days 1, 7, 11, 15, 18, and 22. Tissues were collected and analyzed for myeloperoxidase, collagen, and histopathology; and samples of blood, liver, spleen, and mesenteric lymph nodes were taken for bacterial translocation evaluation.

Rats receiving radiation had significantly lower bodyweights and white blood cell counts on days 11 and 15, which had resolved by day 18; probiotic treatment had no effect. Radiation significantly reduced myeloperoxidase activity, which was further lowered by administration of *L. plantarum* 299v. Probiotic treatment significantly increased collagen content of the anastomosis area, but there were no significant differences in healing of the anastomosis or in bacterial translocation. The authors concluded that administration of lactobacilli “seems to reduce the inflammatory reaction and increase the deposition of collagen at the anastomosis-healing wound.”

Wang et al. (2001) studied bacterial translocation in an acute liver injury rat model and the ability of pretreatment with *L. plantarum* 299v to reduce it. Twelve adult male Sprague-Dawley rats weighing 200-300 g received rectal administrations of 3 ml saline containing either 0 or  $1.5 \times 10^9$  cfu *L. plantarum* 299v (n = 6 rats/group) for 8 days, at which time liver injury was induced by intraperitoneal injection of D-galactosamine. After 24 hours the rats were anesthetized and samples were taken of arterial and portal blood, mesenteric lymph nodes, the caudate lobe of the liver, cecum, and colon; all samples were subjected to microbiological analysis including RAPD analysis and 16S rDNA sequencing.

Lactobacilli dominated the intestinal biota and were the most frequently isolated genus in the liver and the lymph nodes. Administration of *L. plantarum* 299v significantly decreased counts of *Enterobacteriaceae* in the cecum and colon and significantly reduced translocation of all bacterial types to the liver, mesenteric lymph nodes, and both arterial and portal blood.

Mangell et al. (2002) studied the effect on intestinal permeability of *L. plantarum* 299v administered to Sprague-Dawley rats alone or with *E. coli*. Adult male Sprague-Dawley rats weighing 385-456 g and housed 2 rats/cage were assigned to receive regular feeding (n = 8),

regular feeding with gavage administration of 2 ml oatmeal drink twice a day (n = 9), or regular feeding with oatmeal drink mixed in the drinking water (n = 8). The oatmeal drink provided  $10^9$  cfu *L. plantarum* 299v/ml. After a week the rats were sacrificed and their small intestines harvested and treated with *L. plantarum* 299v, *E. coli* F131, both, or neither, along with the permeability marker  $^{14}\text{C}$ -mannitol. Passage of radiation was determined at 20, 40, 60, and 120 minutes.

Exposure to *E. coli* significantly increased intestinal permeability, which was not reduced by simultaneous exposure to *L. plantarum* 299v but was significantly reduced by 1 week's administration of the probiotic. The authors reported, "It is noteworthy that neither pretreatment nor acute administration of *L. plantarum* 299v *per se* had any effect on mannitol passage."

The risk of bacterial endocarditis from *L. plantarum* strain 299v was tested in an experimental rat model (Adawi et al. 2002). Thirty male Sprague-Dawley rats weighing 250-300 g received catheters reaching into the lumen of the left ventricle, given 48 hours to recover from surgery, and divided into 3 groups—an untreated control group of n = 6 rats that received an inoculum of saline, an endocarditis control group that received an inoculum containing  $10^8$  cfu of *Staphylococcus lugdunensis* CCUG 25349T (n = 12), and a test group that received an inoculum containing  $10^8$  cfu *L. plantarum* 299v (n = 12). The rats were given free access to rat chow and water until sacrifice 96 hours post-inoculum. Blood, heart tissue, and the catheter were sampled for bacteria.

There was no mortality in any of the groups. In the endocarditis control group, the total aerobic and anaerobic bacterial count in the blood, in the heart, and on the catheter increased significantly more than in the normal control or *L. plantarum* groups, which did not differ from each other. No *L. plantarum* 299v was found in any of the sample sites. The authors concluded that, "All these results indicate the safety of *L. plantarum* 299v as it is shown that the bacteria have no role in this endocarditis animal model."

Osman et al. (2004) studied the ability of probiotic bacteria to prevent colitis induced by dextran sulfate sodium in a rat model. Three *Lactobacillus* strains (*L. plantarum* 299v, *L. paracasei* DSM 13434, and *L. gasseri* 5B3) and 2 *Bifidobacterium* strains (*B. infantis* DSM 15158 and strain 3B1 of an unidentified *Bifidobacterium* species) were evaluated in 200-g Sprague-Dawley rats (age and sex not specified). Ten rats were assigned to receive daily gavage of  $6 \times 10^9$  cfu of each probiotic for 7 days while 10 additional rats served as controls, then dextran sulfate sodium was administered to induce colitis and treatment continued for another 7 days. Colitis severity was assessed daily; after sacrifice samples were taken of ileum and colon, arterial and portal blood, mesenteric lymph nodes, and liver for bacterial evaluation.

There was no mortality. The severity of the colitis as well as the incidence of total aerobic, anaerobic, and lactobacilli bacterial translocation to the mesenteric lymph nodes and the liver were significantly lower in all probiotic groups as compared to controls. The microbiome of the ileum, proximal colon, and distal colon did not differ among the groups. No adverse effects from the probiotic administration were reported.

In a follow-up to the previous study (Osman et al. 2004), Osman et al. (2005) studied the effects of the same 5 probiotics on translocation and intestinal load of *Enterobacteriaceae* in a rat model of acute liver injury. Thirty-six male Sprague-Dawley rats weighing about 200 g were assigned to receive  $1.2 \times 10^9$  cfu/day of one of the probiotics or to serve as control animals (n = 6

rats/group). After 8 days, D-galactosamine was given by intraperitoneal injection to induce liver injury; animals were sacrificed 24 hours later and samples were collected of ileum, cecum, and colon; aortic and portal blood; and the mesenteric lymph nodes and caudate lobe of the liver.

No differences between groups were seen in the microbiota of the ileum, but the administration of probiotics significantly reduced the counts of *Enterobacteriaceae* in the cecum and colon. Translocation of total aerobic and anaerobic bacteria to the liver and mesenteric lymph nodes were significantly reduced by ingestion of *L. plantarum* 299v, as well as *L. gasseri* and the 2 bifidobacteria. No probiotic bacteria translocated to either the liver or lymph nodes.

Mangell et al. (2006) studied the effect of *L. plantarum* 299v on bacterial translocation in endotoxemic rats and the influence of the strain's adhesive capability. Forty-four adult male Sprague-Dawley rats weighing 321-423 g were assigned to one of 5 groups: negative (n = 10) and positive (n = 8) control groups that received regular drinking water, the oatmeal group (n = 8) that received water mixed with 18.5% oatmeal drink, the probiotic group (n = 8) that received water mixed with oatmeal drink with  $10^9$  cfu *L. plantarum* 299v/ml, and the nonadhesion group (n = 10) that received water and oatmeal drink with  $10^9$  cfu/ml of a modified *L. plantarum* 299v strain that had lost its adhesion ability. After 1 week all rats received an intraperitoneal injection of either saline or lipopolysaccharide from *E. coli* O111:B4. One day later, the rats were sacrificed and a mesenteric lymph node, a liver tissue sample, and 2 cm of distal ileum were harvested; all tissue samples were subjected to bacterial enumeration.

All cultures from both liver and lymph nodes were negative in the negative control group, while rats in the positive control group (which had received lipopolysaccharide) had positive bacterial translocation to 25% of the lymph nodes and 88% of the livers. Pretreatment with oatmeal drink had no benefit but actually increased translocation nonsignificantly. Pretreatment with *L. plantarum* 299v significantly reduced translocation to the liver tissue to 12.5% and to the lymph nodes to zero, but this effect disappeared when the nonadhesive strain replaced the normal strain. The authors concluded that "our data demonstrate the capability of a probiotic bacterium, *L. plantarum* 299v, to prevent translocation of intestinal flora in a septic state and that this effect correlates with the ability of *L. plantarum* 299v to interact with the intestinal mucosa."

In an investigation into the potential use of phytohemagglutinin (PHA) in animal feed and infant nutrition, which has been reported to induce intestinal bacterial overgrowth, Gross et al. (2008) investigated whether oral administration of mannose-adhering *L. plantarum* 299v could reduce *E. coli* stimulation by mannose-specific competitive exclusion. Nine-week-old specific-pathogen-free male Wistar rats were housed individually in metabolic cages and given *ad libitum* access to chow and water. Test animals (n = 8) received broth containing  $10^{10}$  cfu of *L. plantarum*, while control rats (n = 8) received bacteria-free broth. Forty-eight and 72 hours later, the rats were given oral gavages of 33 mg PHA and were sacrificed 24 hours after the second gavage. Fecal samples from before and during PHA administration and excised samples from jejunum and ileum were analyzed to enumerate lactobacillus and *E. coli*.

*L. plantarum* was abundant in fecal samples from test-group rats, but no inhibition of *E. coli* was observed. Further, oral administration of  $10^{10}$  cfu of *L. plantarum* 299v did not increase lactobacillus counts in either the jejunum or ileum. The value of this study for evaluating the safety of addition of *L. plantarum* 299v to foods is the authors' observation that "*L. plantarum* did not colonize the intestinal mucosa" and therefore could not prevent *E. coli* overgrowth based on competitive exclusion.



In a study of the effect of *L. plantarum* 299v on gastrointestinal function in suckling rats, Fak et al. (2008a) fed 59 Sprague-Dawley pups representing 7 litters 0.05-0.15 ml/day of a preparation of *L. plantarum* 299v (dose =  $3 \times 10^6$  cfu/kg bw) or saline on post-natal days 3-10, 7-14, or 14-21. At the end of dosing the pups were gavaged with a marker solution containing bovine serum albumin and bovine immunoglobulin; after 3 hours they were sacrificed, blood was taken, and the small intestine, cecum, pancreas, spleen, liver, thymus, and adrenals excised and examined.

Administration of *L. plantarum* significantly increased counts of lactobacilli in the cecum. The authors reported that there was no effect of treatment on bodyweight and that, "The bacteria did not appear to cause any adverse effects, such as diarrhoea or behavioural changes, and all rats survived the experimental treatment periods." No effect was seen in stomach pH or in relative organ weights for the liver, stomach, small intestine, cecum, spleen, and thymus. The relative weights of the pancreas and adrenals were significantly decreased in the rats fed *L. plantarum* between days 7 and 14. Treatment with the probiotic significantly reduced absorption of bovine immunoglobulin in the youngest pups, but there was no significant effect on absorption of bovine albumin. The authors observed that the data showed "treatment during the first week of life leading to an improved gut barrier function."

Fak et al. (2008b) further investigated the effect of *L. plantarum* 299v on gastrointestinal function in suckling rats by beginning administration of the probiotic to 2 pregnant Sprague-Dawley rats (weighing about 200 g at mating) one week prior to parturition and continuing it through the suckling period. *L. plantarum* was administered in drinking water at a concentration of  $7 \times 10^6$  cfu/ml while 2 control dams received only tap water. Water consumption throughout the study was about 40 ml/day; thus, the achieved dose was about  $2.8 \times 10^8$  cfu/day. After birth, each litter was culled to 6-8 pups and kept with its dam for 2 weeks. Fecal samples from the dam were taken 1 week before parturition, on the day of parturition, and 14 days after parturition. On day 14, the pups were separated from their dam and given a marker solution containing bovine serum albumin and bovine immunoglobulin. After 3 hours they were sacrificed; blood was taken by heart puncture, the pancreas, stomach, small intestine, cecum, liver, spleen, thymus, and adrenals were removed and weighed. The small intestine was divided into proximal and distal halves.

Administration of *L. plantarum* 299v had no observed effect on the microbial ecology of the dams, nor on that of the pups except that the administered strain was recovered in the ceca of all but one pup in the test group and no pups in the control group. There was no mortality, and no effects on the bodyweight of the pups, nor the relative weight of the stomach, adrenals, or thymus. The small intestine, liver, pancreas, and spleen were significantly heavier in pups whose dams had received the probiotic, and absorption of bovine immunoglobulin was significantly reduced but with no effect on bovine albumin. The authors concluded that administration of *L. plantarum* 299v to the dams during pregnancy and lactation significantly accelerated gastrointestinal development of their pups, including increased gut-barrier function.

Waugh et al. (2009) used a murine model to study the effect of *L. plantarum* 299v on the symptoms of irritable bowel syndrome (IBS). Male wild-type mice aged 8-10 weeks from a 129/SvEv background were rectally administered 1% isothiocyanate in 30% ethanol to induce IBS. Mice in the experimental and control pretreatment groups received daily gavage administration of  $10^9$  cfu of *L. plantarum* 299v or pure saline solution beginning 7 days before

the isothiocyanate instillation and continuing to day 20 post-instillation; mice in the experimental and control posttreatment groups received daily gavage beginning 8 days after instillation and continuing to day 20. The exact number of mice in each group was not reported, but was indicated to be between 5 and 10. Colonic inflammation was measured on days 4 and 20 after isothiocyanate instillation and intestinal motility was assessed on day 20.

Isothiocyanate instillation produced an eightfold increase in IFN $\gamma$  levels, confirming an intense inflammatory response, as well as decreased intestinal motility. These responses were almost entirely prevented by pretreatment with *L. plantarum* 299v and they were significantly reduced to control levels when *L. plantarum* 299v was given 8 days after instillation. The authors concluded that “*L. plantarum* 299v treatment decreases both the inflammatory response and the delayed intestinal transit seen in the oil of mustard murine IBS model.” The authors did not report if any adverse effects were observed related to the probiotic treatment.

Dykstra et al. (2011) compared the effects of *L. plantarum* 299v, *L. rhamnosus* R0011, and *Bifidobacterium bifidum* LrR0011 and BbR0071 on gene modulation in pathogen-free male Sprague-Dawley rats weighing ~250 g (number of rats not reported). Rats received doses of either 10<sup>7</sup> or 10<sup>9</sup> cfu/day of their assigned probiotic in their drinking water for from 2 to 7 days. Another group of rats received 3x10<sup>9</sup> cfu of *L. plantarum* 299v by rectal gavage for up to 7 days. Animals were killed by CO<sub>2</sub> suffocation and sections of the proximal jejunum, distal ileum, proximal colon, and distal colon were excised and mucosal scrapings were taken for RNA extraction. RNA was analyzed for *Muc1*, *Muc2*, *Muc3*, human inhibitor of apoptosis protein 1 and 2, neuronal apoptosis inhibitor protein, and X-linked inhibitor of apoptosis protein.

All tested probiotics produced a significant up-regulation of *Muc3* transcript in jejunum and ileum after 2 days of ingestion. *Muc1* expression was significantly increased in jejunum, but not in ileum or colon; *Muc2* expression was not affected. Rectal delivery of *L. plantarum* 299v resulted in significantly increased *Muc2* expression in the distal colon, but not in other gastrointestinal segments, and no change in *Muc1* or *Muc3*. No dose-effects were reported; i.e., the effects were similar after oral-gavage administration of either 10<sup>7</sup> or 10<sup>9</sup> cfu/day. *L. plantarum* 299v administration also up-regulated expression of human inhibitor of apoptosis protein 1 and 2.

Dykstra et al. (2011) also examined the ability of daily oral administration of *L. plantarum* 299v to sustain up-regulation of *Muc3* by continuing administration of 10<sup>7</sup> cfu of the probiotic for 20 days. Although the up-regulation was seen after 2 days of administration, it was no longer significantly different from zero by day 10, and continued to be ineffective to day 20. However, “pulse administration,” in which the probiotic was given for 2 days, withheld for 8 days, and given for 2 days again, appeared to sustain the up-regulation of *Muc3*. The authors concluded that “live viable probiotics [including *L. plantarum* 299v] can induce innate protective mechanisms of the intestinal epithelial cell in a reproducible but time-limited manner.”

Recognizing that alteration of the intestinal microbiota, dysbiosis, is associated with multiple disease states, Lam et al. (2012) studied the ability of a combination of *L. plantarum* 299v and *B. lactis* Bi-07 to reduce the size of myocardial infarcts resulting from ischemia/reperfusion in rats. Male Dahl S rats weighing 200-220 g (number and age not reported) were given vancomycin in their drinking water for 7 days, during which the test group of rats also received *L. plantarum* 299v and *B. lactis* Bi-07 (dose not reported) in their drinking water. Under anesthesia, a thoracotomy was performed and a silk ligature was placed distal to

the left atrial appendage; the area was occluded for 30 minutes and then reperfused for 2 hours before being again occluded. The heart was then excised and examined to determine infarct sizes.

Probiotic treatment reduced infarct size by 29% (statistically significant). It is not clear to what extent the effect was due to *L. plantarum* 299v or *B. lactis* Bi-07. The authors did not report any adverse effects of the probiotic treatment.

In a study of the effect of *L. plantarum* on biliary obstruction, Badger et al. (2013) randomized 24 male Wistar rats (age and bodyweight not reported) into 3 groups of 8 rats to receive oatmeal containing viable *L. plantarum* 299v at a concentration of  $2 \times 10^9$  cfu/ml, oatmeal with heat inactivated 299v, or water via daily 2-ml gavage for 7 days. At the end of this period, all rats underwent laparotomy and bile duct ligation, which was followed by an additional 7 days of the assigned gavage regimen. Rats were gavaged with  $^{14}\text{C}$  PEG 4000 at baseline, after bile duct ligation, and on the last day of feeding to measure intestinal permeability. At the end of the gavage administrations, rats were killed and subjected to isolated in-situ hepatic perfusion with *E. coli* endotoxin for 2 hours, after which livers were tested for bilirubin, alkaline phosphatase, and aspartate aminotransferase and sections were excised and processed for histological and transmission electron microscopic assessment. TNF $\alpha$ , IL-6, and IL-10 were measured in perfusate and plasma.

Two rats in the 299v and water groups died from non-intervention-related causes. Rats in all groups gained weight up to the bile duct ligation surgery, then lost weight thereafter; there were no significant differences between groups. No differences were reported in levels of bilirubin, alkaline phosphatase, aspartate aminotransferase, intestinal permeability, urine volume, liver morphology, TNF $\alpha$ , or IL-6, but IL-10 levels were lower in rats receiving *L. plantarum* 299v. The authors suggested that the probiotic may have “slightly altered inflammatory response to portal endotoxaemia . . . with a reduction in the anti-inflammatory cytokine IL-10,” but conceded that “these results are not completely conclusive of effect.”

Gotteland et al. (2014) isolated 421 strains of *Lactobacillus* from stools of healthy infants and adults, human milk, goat cheese, fruits, and vegetables and tested them for tolerance to gastric pH and bile salts, adherence to Caco-2 and gastric epithelial cells, and antioxidant, anti-inflammatory, and anti-*Helicobacter pylori* activity. Five strains were selected for testing in male BALB/c mice aged 6-8 weeks and weighing 17-21 g; for comparison purposes, two known probiotic reference strains, *L. plantarum* 299v and *L. rhamnosus* GG, were also tested.

The mice were acclimatized for 2 weeks, then randomized into 8 groups of 6-11 animals/group, group caged, and given free access to feed and water. Bacterial strains were suspended in 10% skim milk at a concentration of  $10^{11}$  cfu/ml and mice received 50  $\mu\text{l}$  of the suspension twice daily ( $10^{10}$  cfu) by gavage for 7 days, followed by a 7-day washout period prior to sacrifice. Animals were weighed and clinically observed at the start and end of the feeding period and after the washout. Blood samples were taken under anesthesia by cardiac puncture before being killed by cervical dislocation; mesenteric lymph nodes, liver, and spleen samples were taken.

The authors reported that “The condition of the animals was not affected by the treatments.” No differences were reported in bodyweight; thickness of ileal, cecal, or colonic mucosa; or integrity of the epithelium. There was no indication of infiltration of neutrophils or monocytes in the lamina propria, edema, or venous congestion. *L. plantarum* 299v and *L.*

*rhamnosus* GG had no effect on erythrocyte or leukocyte concentration, mean corpuscular volume, hematocrit, hemoglobin concentration, or zinc protoporphyrin. Signs of bacterial translocation did not differ between probiotic groups and the control group with the exception of a novel strain of *L. plantarum*, N221. The authors concluded that, “Four strains of *L. rhamnosus* [in addition to *L. plantarum* 299v and *L. rhamnosus* GG] were found to be safe and could be used in human studies.”

Hulst et al. (2015) used the pig as a model for humans in order to study interactions between *L. plantarum* 299v and the host in the jejunum, ileum, and proximal colon. Weaned male 4-week-old Duroc x Topigs 20 pigs were fed *ad libitum* during a 14-day adaptation period, after which fecal samples were collected and the pigs were given either 0 or  $10^{12}$  cfu of *L. plantarum* 299v in phosphate-buffered saline by syringe injection into the oral cavity. They received no further dosing for 10 days, and then the treatment was repeated for 3 days. The animals were euthanized 12 hours after the last administration and mucosal scrapings were collected for RNA extraction.

Fecal samples at the end of the 10-day interval contained little *L. plantarum*, and the authors suggested that “no significant colonization took place in the GI tract of pigs and that the survival rate of *L. plantarum* 299v in the GI tract is low.” This conclusion was supported by the absence of bacterial counts in the mucosal scrapings after 3 consecutive days’ administration of the probiotic. RNA analysis of ileal scrapings identified 303 up-regulated and 104 down-regulated genes; in the jejunum and colon, only 179 genes were differentially expressed—54 up-regulated and 125 down-regulated. There was little response to *L. plantarum* 299v by inflammatory mediators, while up-regulation of cell-specific marker genes such as BCOR resulted in an increase in progenitor B cells that proliferate to IgA-plasma cell progenitors. The authors concluded that, “these metabolites may play a role in the crosstalk between intestinal immune cells and sub-mucosal adipocytes [which] may contribute to tempering of inflammatory reactions.”

In summary, *L. plantarum* 299v has been administered to rats (pups, pregnant, lactating, adult), as well as mice and pigs, with doses as high as  $10^{10}$  cfu/day for up to 4 weeks without any sign of adverse effects. In experimental models where acute liver injury or colitis had been initiated, the bacteria significantly reduced translocation and inflammation. Furthermore, no adverse effects were observed even after injection of *L. plantarum* 299v directly into the blood stream in an animal endocarditis model. These findings support the safety of *L. plantarum* 299v.

**Table 6. Studies of *L. plantarum* 299v in Animals.**

Reference	Study Objective	Animal Model	Daily Dose	Duration	Results
Adawi et al. (1997)	Study the effects of 5 <i>Lactobacillus</i> strains on liver damage and bacterial translocation in a rat model of acute liver injury induced by injections of D-galactosamine	Rat model of acute liver injury; 78 male Sprague-Dawley rats weighing 200-300 g	3x10 <sup>9</sup> cfu <i>L. plantarum</i> 299v administered rectally	9 days	No mortality occurred. Significant lowering of the level of aspartate transaminase was observed 24 hours after liver injury in the group receiving <i>L. plantarum</i> + arginine.. Bacterial translocation to portal and arterial blood, liver, and mesenteric lymph nodes was significantly increased by liver injury, but significantly reduced at 24 hours in the groups receiving <i>L. plantarum</i> with or without arginine. Numbers of <i>Enterobacteriaceae</i> were significantly reduced in the cecum and colon and liver histopathology was significantly lessened by administration of <i>L. plantarum</i> 299v.
Adawi et al. (1997)	Study the effects of 5 <i>Lactobacillus</i> strains on liver damage and bacterial translocation in a rat model of acute liver injury induced by injections of D-galactosamine	Rat model of acute liver injury; 24 male Sprague-Dawley rats weighing 200-300 g	3x10 <sup>9</sup> cfu <i>L. plantarum</i> 299v administered rectally	10 days	No mortality occurred. Significant lowering of the levels of aspartate transaminase and alanine transaminase was observed 48 hours after liver injury in the group receiving <i>L. plantarum</i> or arginine. Bacterial translocation to portal and arterial blood, liver, and mesenteric lymph nodes was significantly increased by liver injury, but significantly reduced at 48 hours in the groups receiving <i>L. plantarum</i> with or without arginine. Numbers of <i>Enterobacteriaceae</i> were significantly reduced in the cecum and colon and liver histopathology was significantly lessened by administration of <i>L. plantarum</i> 299v.
Adawi et al. (2001)	Study the effect of lactobacilli and bifidobacteria on the intestinal ecology, bacterial translocation and extent of liver injury in a rat model of acute liver injury induced by injections of D-galactosamine	Rat model of acute liver injury; 30 male Sprague-Dawley rats weighing 200 to 300 g	3x10 <sup>9</sup> cfu <i>L. plantarum</i> 299v administered rectally	9 days	There was no mortality and no effect on bilirubin or alkaline phosphatase, but alanine transaminase was significantly lower in the rats that had received <i>Lactobacillus</i> than the control rats or those receiving bifidobacteria. Enterobacteriaceae were significantly decreased in the colon by all treatments as compared to controls, but were significantly decreased in the cecum only by treatment with <i>B. animalis</i> or the combination of <i>L. rhamnosus</i> + <i>L. plantarum</i> . Administration of bacteria, as compared with the control condition, significantly reduced bacterial translocation to portal and arterial blood, liver, and mesenteric lymph nodes.

**Table 6. Studies of *L. plantarum* 299v in Animals.**

Reference	Study Objective	Animal Model	Daily Dose	Duration	Results
Adawi et al. (2002)	Study the risk of bacterial endocarditis from <i>L. plantarum</i> 299v	30 male Sprague-Dawley rats weighing 250-300 g	10 <sup>8</sup> cfu <i>L. plantarum</i> 299v by inoculum into the lumen of the left ventricle	single dose	There was no mortality in any of the groups. In the endocarditis control group, the total aerobic and anaerobic bacterial count in the blood, in the heart, and on the catheter increased significantly more than in the normal control or <i>L. plantarum</i> groups, which did not differ from each other. No <i>L. plantarum</i> 299v was found in any of the sample sites. The authors concluded that, "All these results indicate the safety of <i>L. plantarum</i> 299v as it is shown that the bacteria have no role in this endocarditis animal model."
Badger et al. (2013)	Study the effect of <i>L. plantarum</i> on biliary obstruction in rats	24 male Wistar rats (age and bodyweight not reported) with bile duct ligation	2x10 <sup>9</sup> cfu <i>L. plantarum</i> 299v /ml	14 days	2 rats in the 299v and water groups died from non-intervention-related causes. Rats in all groups gained weight up to the bile duct ligation surgery, then lost weight thereafter; there were no significant differences between groups. No differences were reported in levels of bilirubin, alkaline phosphatase, aspartate aminotransferase, intestinal permeability, urine volume, liver morphology, TNF $\alpha$ , or IL-6, but IL-10 levels were lower in rats receiving <i>L. plantarum</i> 299v. The authors suggested that the probiotic may have "slightly altered inflammatory response to portal endotoxaemia . . . with a reduction in the anti-inflammatory cytokine IL-10," but conceded that "these results are not completely conclusive of effect."
Dykstra et al. (2011)	Compared the effects of <i>L. plantarum</i> 299v, <i>L. rhamnosus</i> R0011, and <i>Bifidobacterium bifidum</i> LrR0011 and BbR0071 on gene modulation	Pathogen-free male Sprague-Dawley rats weighing ~250 g (number of rats not reported)	either 10 <sup>7</sup> or 10 <sup>9</sup> cfu of the assigned probiotic orally or 3x10 <sup>9</sup> cfu <i>L. plantarum</i> 299v by rectal gavage	7 days	All tested probiotics produced a significant up-regulation of <i>Muc3</i> transcript in jejunum and ileum. <i>Muc1</i> expression was significantly increased in jejunum; <i>Muc2</i> expression was not affected. Rectal delivery of <i>L. plantarum</i> 299v resulted in significantly increased <i>Muc2</i> expression in the distal colon.. <i>L. plantarum</i> 299v up-regulated expression of human inhibitor of apoptosis protein 1 and 2. The authors concluded that "live viable probiotics [including <i>L. plantarum</i> 299v] can induce innate protective mechanisms of the intestinal epithelial cell in a reproducible but time-limited manner."

**Table 6. Studies of *L. plantarum* 299v in Animals.**

Reference	Study Objective	Animal Model	Daily Dose	Duration	Results
Fak et al. (2008a)	Study the effect of <i>L. plantarum</i> 299v on gastrointestinal function in suckling rats	59 Sprague-Dawley pups representing 7 litters	3x10 <sup>6</sup> cfu <i>L. plantarum</i> 299v/kg bw	7 days on post-natal days 3-10, 7-14, or 14-21	There was no mortality. Administration of <i>L. plantarum</i> significantly increased counts of lactobacilli in the cecum. There was no effect on body-weight and the bacteria did not cause any adverse effects, such as diarrhea or behavioral changes. No effect was seen in stomach pH or in relative organ weights for the liver, stomach, small intestine, cecum, spleen, and thymus. The relative weights of the pancreas and adrenals were significantly decreased in the rats fed <i>L. plantarum</i> between days 7 and 14. Treatment with the probiotic significantly reduced absorption of bovine immunoglobulin in the youngest pups, but there was no significant effect on absorption of bovine albumin. The data showed improved gut barrier function.
Fak et al. (2008b)	Study the effect of <i>L. plantarum</i> 299v on gastrointestinal function in suckling rats	4 pregnant Sprague-Dawley rats (weighing about 200 g at mating) one week prior to parturition; each litter was culled to 6-8 pups	2.8x10 <sup>8</sup> cfu <i>L. plantarum</i> 299v	4 weeks	Administration of <i>L. plantarum</i> 299v had no effect on the microbial ecology of the dams or on that of the pups except that the administered strain was recovered in the ceca of all but one pup in the test group. There was no mortality, and no effects on the bodyweight of the pups, nor the relative weight of the stomach, adrenals, or thymus. The small intestine, liver, pancreas, and spleen were significantly heavier in pups whose dams had received the probiotic, and absorption of bovine immunoglobulin was significantly reduced but with no effect on bovine albumin. The authors concluded that administration of <i>L. plantarum</i> 299v to the dams during pregnancy and lactation significantly accelerated gastrointestinal development of their pups, including increased gut-barrier function.

**Table 6. Studies of *L. plantarum* 299v in Animals.**

Reference	Study Objective	Animal Model	Daily Dose	Duration	Results
Gotteland et al. (2014)	Tested 421 strains of <i>Lactobacillus</i> for tolerance to gastric pH and bile salts, adherence to Caco-2 and gastric epithelial cells, and antioxidant, anti-inflammatory, and anti- <i>Helicobacter pylori</i> activity <i>in vitro</i> ; test 5 strains <i>in vivo</i>	Male BALB/c mice aged 6-8 weeks and weighing 17-21 g, 6-11 mice/group	10 <sup>10</sup> cfu of each tested strain	7 days	The condition of the animals was not affected by the treatments. No differences were seen in bodyweight; thickness of ileal, cecal, or colonic mucosa; or integrity of the epithelium. There was no infiltration of neutrophils or monocytes in the lamina propria, edema, or venous congestion. <i>L. plantarum</i> 299v and <i>L. rhamnosus</i> GG had no effect on erythrocyte or leukocyte concentration, mean corpuscular volume, hematocrit, hemoglobin concentration, or zinc protoporphyrin. Signs of bacterial translocation did not differ between probiotic groups and the control group with the exception of a novel strain of <i>L. plantarum</i> , N221. The authors concluded that, "Four strains of <i>L. rhamnosus</i> [in addition to <i>L. plantarum</i> 299v and <i>L. rhamnosus</i> GG] were found to be safe and could be used in human studies."
Gross et al. (2008)	Study whether <i>L. plantarum</i> 299v could prevent <i>E. coli</i> overgrowth associated with the use of phytohemagglutinin (PHA) in animal feed and infant nutrition	16 9-week-old specific-pathogen-free male Wistar rats	10 <sup>10</sup> cfu <i>L. plantarum</i> 299v	3-4 days	<i>L. plantarum</i> was abundant in fecal samples from test-group rats, but no inhibition of <i>E. coli</i> was observed. Further, oral administration of 10 <sup>10</sup> cfu of <i>L. plantarum</i> 299v did not increase lactobacillus counts in either the jejunum or ileum. The authors' observed that " <i>L. plantarum</i> did not colonize the intestinal mucosa" and therefore could not prevent <i>E. coli</i> overgrowth based on competitive exclusion.
Herias et al. (1999)	Study the effect of <i>L. plantarum</i> 299v on <i>E. coli</i> colonization and immune function in germ-free rats	14 male and female AGUS gnotobiotic rats	7.8x10 <sup>8</sup> cfu <i>L. plantarum</i> 299v	Single dose	<i>L. plantarum</i> 299v reduced <i>E. coli</i> counts in the small intestine and cecum at 1 week but not at 5 weeks. At both 1 and 5 weeks total serum IgA and CD25 <sup>+</sup> cells were increased in the rats receiving the probiotic. The authors concluded that " <i>L. plantarum</i> colonization competes with <i>E. coli</i> for intestinal colonization and can influence intestinal and systemic immunity," and suggested that, "It is possible that the presence of the mannose-dependent adherence mechanism in <i>L. plantarum</i> was partly responsible for the competition with <i>E. coli</i> early after colonization."



**Table 6. Studies of *L. plantarum* 299v in Animals.**

Reference	Study Objective	Animal Model	Daily Dose	Duration	Results
Hulst et al. (2015)	Study interactions between <i>L. plantarum</i> 299v and the host in inaccessible locations including the jejunum, ileum, and proximal colon in a pig model	Weaned male 4-week-old Duroc x Topigs 20 pigs	10 <sup>12</sup> cfu <i>L. plantarum</i> 299v by syringe injection into the oral cavity	4 days total (1 day + 3 days after 1 10-day interval)	No significant colonization took place in the GI tract of pigs and the survival of <i>L. plantarum</i> 299v was low. No bacteria were found in the mucosal scrapings after 3 consecutive days' administration of the probiotic. RNA analysis of ileal scrapings identified 303 up-regulated and 104 down-regulated genes; in the jejunum and colon, only 179 genes were differentially expressed—54 up-regulated and 125 down-regulated. There was little response to <i>L. plantarum</i> 299v by inflammatory mediators, while up-regulation of cell-specific marker genes such as BCOR resulted in an increase in progenitor B cells that proliferate to IgA-plasma cell progenitors. The authors concluded that, "these metabolites may play a role in the crosstalk between intestinal immune cells and sub-mucosal adipocytes [which] may contribute to tempering of inflammatory reactions."
Kasravi et al. (1996)	Study the effect of oral supplementation with lactobacilli on bacterial translocation in a rat model of acute liver injury induced by D-galactosamine	40 Sprague-Dawley rats with acute liver injury induced by D-galactosamine	2.5-5.0x10 <sup>9</sup> cfu <i>L. plantarum</i> 299v	8 days	Liver injury significantly increased levels of bilirubin, aspartate aminotransferase, and alanine aminotransferase, but these were significantly reduced by administration of <i>L. plantarum</i> , neomycin, or lactulose, as was injury-related bacterial translocation. The authors concluded that ingestion of <i>L. plantarum</i> 299v "improved the overall proliferative state of the mucosa in the small intestine and cecum . . . and the subsequent bacterial translocation."
Lam et al. (2012)	Study the ability of a combination of <i>L. plantarum</i> 299v and <i>B. lactis</i> Bi-07 to reduce the size of myocardial infarcts resulting from ischemia/reperfusion in rats	Male Dahl S rats weighing 200-220 g (number and age not reported) with ligature distal to left atrial appendage	Dose of <i>L. plantarum</i> 299v and <i>B. lactis</i> Bi-07 not reported	7 days	Probiotic treatment reduced infarct size by 29% (statistically significant). It is not clear to what extent the effect was due to <i>L. plantarum</i> 299v or <i>B. lactis</i> Bi-07. The authors did not report any adverse effects of the probiotic treatment.

**Table 6. Studies of *L. plantarum* 299v in Animals.**

Reference	Study Objective	Animal Model	Daily Dose	Duration	Results
Liu et al. (2001)	Study the ability of <i>L. plantarum</i> 299v to speed anastomotic healing and reduce mucosal atrophy associated with radiation therapy of patients with rectal carcinoma in an animal model	72 adult male Sprague-Dawley rats weighing about 300 g with colon resection and anastomosis	4x10 <sup>9</sup> cfu <i>L. plantarum</i> 299v	15, 18, or 22 days	Rats receiving radiation had lower bodyweights and white blood cell counts on days 11 and 15; probiotic treatment had no effect. Radiation significantly reduced myeloperoxidase activity, which was further lowered by administration of <i>L. plantarum</i> 299v. Probiotic treatment significantly increased collagen content of the anastomosis area, but there were no significant differences in healing of the anastomosis or in bacterial translocation. The authors concluded that administration of lactobacilli "seems to reduce the inflammatory reaction and increase the deposition of collagen at the anastomosis-healing wound."
Mangell et al. (2002)	Study the effect of <i>L. plantarum</i> 299v on intestinal permeability	25 adult male Sprague-Dawley rats weighing 385-456 g exposed to <i>E. coli</i>	4x10 <sup>9</sup> cfu <i>L. plantarum</i> 299v	7 days	Exposure to <i>E. coli</i> significantly increased intestinal permeability, which was not reduced by simultaneous exposure to <i>L. plantarum</i> 299v but was significantly reduced by 1 week's administration of the probiotic. The authors reported, "It is noteworthy that neither pretreatment nor acute administration of <i>L. plantarum</i> 299v <i>per se</i> had any effect on mannitol passage."
Mangell et al. (2006)	Study the effect of <i>L. plantarum</i> 299v on bacterial translocation in endotoxemic rats and the influence of the strain's adhesive capability	44 adult male Sprague-Dawley rats weighing 321-423 g with endotoxemia induced by <i>E. coli</i>	10 <sup>9</sup> cfu <i>L. plantarum</i> 299v /ml water	8 days	Rats in the positive control group had positive bacterial translocation to 25% of the lymph nodes and 88% of the livers. Pretreatment with oatmeal drink had no benefit. Pretreatment with <i>L. plantarum</i> 299v reduced translocation to the liver tissue to 12.5% and to the lymph nodes to zero, but this effect disappeared when the nonadhesive strain replaced the normal strain. The authors concluded that "our data demonstrate the capability of a probiotic bacterium, <i>L. plantarum</i> 299v, to prevent translocation of intestinal flora in a septic state and that this effect correlates with the ability of <i>L. plantarum</i> 299v to interact with the intestinal mucosa."

**Table 6. Studies of *L. plantarum* 299v in Animals.**

Reference	Study Objective	Animal Model	Daily Dose	Duration	Results
Mangiante et al. (2001)	Study the ability of <i>L. plantarum</i> 299v to inhibit microbial translocation in acute pancreatitis induced by isolation and ligation of the bilio-pancreatic duct in a rat model	55 adult pathogen-free Lewis rats weighing 250-350 g with acute pancreatitis induced by isolation and ligation of the bilio-pancreatic duct	2.5-5.0x10 <sup>9</sup> cfu <i>L. plantarum</i> 299v	8 days	Administration of the probiotic significantly reduced the number of animals showing bacterial translocation to the pancreatic tissue and mesenteric lymph nodes. No <i>L. plantarum</i> were identified in blood, pancreatic tissue, or lymph nodes.
Mao et al. (1996a)	Study the effects of <i>Lactobacillus</i> , along with oat fiber, on methotrexate-induced enterocolitis in a rat model	126 male Sprague-Dawley rats weighing 200-250 g with enterocolitis induced by methotrexate	4x10 <sup>9</sup> cfu <i>L. plantarum</i> 299v	6 days	Rats receiving <i>L. plantarum</i> 299v and oat fiber showed less lethargy and diarrhea, lost less weight, had less mucosal inflammation and ulceration, had higher levels of mucosal protein, DNA, RNA, and nucleotide, had less permeability in the proximal and distal small intestine and colon, had less bacterial translocation to the mesenteric lymph nodes, liver, spleen, and aortic blood, and had reduced plasma endotoxin levels. No adverse effects were reported due to ingestion of probiotic bacteria.
Mao et al. (1996b)	Study the effects of <i>Lactobacillus</i> , along with oat fiber, on methotrexate-induced enterocolitis in a rat model	42 male Sprague-Dawley rats weighing 200-250 g with enterocolitis induced by methotrexate	4x10 <sup>9</sup> cfu <i>L. plantarum</i> 299v	6 days	Injection of methotrexate produced significantly lowered levels of sIgA CD4, and CD8, which were mostly restored by ingestion of <i>L. plantarum</i> 299v. Administration of <i>L. plantarum</i> 299v resulted in less weight loss and histopathological damage due to enterocolitis. No adverse effects from ingestion of the probiotic were reported, and the treatment improved mucosal immunity.
Osman et al. (2004)	Study the ability of probiotic bacteria to prevent colitis induced by dextran sulfate sodium in a rat model	60 Sprague-Dawley rats weighing about 200 g with colitis induced by dextran sulfate sodium	6x10 <sup>9</sup> cfu <i>L. plantarum</i> 299v	14 days	There was no mortality. The severity of the colitis as well as the incidence of total aerobic, anaerobic, and lactobacilli bacterial translocation to the mesenteric lymph nodes and the liver were significantly lower in all probiotic groups as compared to controls. The microbiome of the ileum, proximal colon, and distal colon did not differ among the groups. No adverse effects from the probiotic administration were reported.

**Table 6. Studies of *L. plantarum* 299v in Animals.**

Reference	Study Objective	Animal Model	Daily Dose	Duration	Results
Osman et al. (2005)	Study the effects of probiotics on translocation and intestinal load of <i>Enterobacteriaceae</i> in a rat model of acute liver injury induced by D-galactosamine	36 male Sprague-Dawley rats weighing about 200 g with acute liver injury induced by D-galactosamine	1.2x10 <sup>9</sup> cfu <i>L. plantarum</i> 299v	9 days	No differences between groups were seen in the microbiota of the ileum, but the administration of probiotics significantly reduced the counts of <i>Enterobacteriaceae</i> in the cecum and colon. Translocation of total aerobic and anaerobic bacteria to the liver and mesenteric lymph nodes were significantly reduced by ingestion of <i>L. plantarum</i> 299v, as well as <i>L. gasseri</i> and the 2 bifidobacteria. No probiotic bacteria translocated to either the liver or lymph nodes.
Wang et al. (2001)	Study the ability of pretreatment with <i>L. plantarum</i> 299v to reduce bacterial translocation in a D-galactosamine-induced acute liver injury rat model	12 adult male Sprague-Dawley rats weighing 200-300 g with acute liver injury induced by D-galactosamine	1.5x10 <sup>9</sup> cfu <i>L. plantarum</i> 299v administered rectally	9 days	Lactobacilli dominated the intestinal biota and were the most frequently isolated genus in the liver and the lymph nodes. Administration of <i>L. plantarum</i> 299v significantly decreased counts of <i>Enterobacteriaceae</i> in the cecum and colon and significantly reduced translocation of all bacterial types to the liver, mesenteric lymph nodes, and both arterial and portal blood.
Waugh et al. (2009)	Study the effect of <i>L. plantarum</i> 299v on the symptoms of IBS in a murine model	Male wild-type mice aged 8-10 weeks from a 129/SvEv background rectally administered 1% isothiocyanate in 30% ethanol to induce IBS; 5-10 mice per group	10 <sup>9</sup> cfu <i>L. plantarum</i> 299v	27 days	Isothiocyanate instillation produced an eightfold increase in IFN $\gamma$ levels, confirming an intense inflammatory response, as well as decreased intestinal motility. These responses were almost entirely prevented by pretreatment with <i>L. plantarum</i> 299v and they were significantly reduced to control levels when <i>L. plantarum</i> 299v was given 8 days after instillation. The authors concluded that " <i>L. plantarum</i> 299v treatment decreases both the inflammatory response and the delayed intestinal transit seen in the oil of mustard murine IBS model." The authors did not report if any adverse effects were observed related to the probiotic treatment.

### 6.3.3. Human Studies

The studies discussed in the following sections are summarized in Table 7 on page 58.

#### 6.3.3.1. Healthy Adults

Thirteen apparently healthy volunteers (4 males and 9 females) aged 31-56 years consumed 100 ml of fermented oatmeal soup once a day for 10 days (Johansson et al. 1993). The soup contained  $5 \times 10^8$  cfu of each of 19 bacterial strains: *L. plantarum* 299v; 2 additional *plantarum* strains; 2 strains each of *L. salivarius*, *L. reuteri*, *L. jenseni*, and *L. rhamnosus*; one strain each of *L. casei*, *L. acidophilus*, and *L. agilis*; and 5 *Lactobacillus* strains not classified at species level. Biopsies were taken from the upper jejunum using a biopsy capsule and from the rectum by rectoscopy at baseline, one day after the end of administration, and 11 days after the end of administration.

Five strains were re-isolated from mucosa both 1 and 11 days after cessation of ingestion—*L. plantarum* 299v, one other *L. plantarum*, and one strain each of *L. agilis*, *L. reuteri*, and *L. rhamnosus*. Total counts of lactobacilli increased significantly in the jejunum as compared to baseline, but there were no other significant changes in the jejunal microecology. The rectal mucosa failed to show significant increases in lactobacilli but did have significant decreases in total anaerobic bacteria and gram-negative anaerobic bacteria. These changes were detected both one and 11 days after the end of probiotic administration.

In a randomized, prospective, double-blind, placebo-controlled study, Johansson et al. (1998) enrolled 48 apparently healthy adults (11 males and 37 females) with a mean age of 37 years to consume a rose-hip drink either unsupplemented ( $n = 22$ ) or containing oats fermented with *L. plantarum* 299v,  $5 \times 10^9$  cfu of the strain, and 35 mg DL-lactic acid ( $n = 26$ ) for 21 days. Fecal samples were taken at baseline, at 1 and 3 weeks, and 8 days after the end of intake, and analyzed for pH, concentrations of individual short-chain fatty acids, total content of carboxylic acids, and bacterial counts. At the end of administration, participants self-reported their overall bowel function regarding stool frequency, volume, and consistency; difficulty of defecation; and degree of flatulence.

No participants dropped out and there were no differences in adverse events reported by the 2 groups. Five individuals in each group reported transient nausea or abdominal discomfort. Those receiving the fermented oats and live bacteria experienced a significant increase in stool volume and a decrease in flatulence. They also had significant increases in fecal levels of total carboxylic acids, particularly acetic, propionic, and lactic acid, but no significant change in fecal pH. *L. plantarum* 299v was found in large numbers in the feces of the test group at weeks 1 and 3, but in only 5 of the 26 individuals 8 days after cessation of ingestion. No other significant changes were seen in the fecal microbiota in comparison with the control group.

In a prospective, randomized, double-blind, placebo-controlled study, Bukowska et al. (1998) assigned 30 apparently healthy males with a mean age of 42.6 years to consume 200 ml/day of either a rose hip drink containing fermented oatmeal and  $10^{10}$  cfu *L. plantarum* 299v or an unsupplemented rose hip drink for 6 weeks. Members of both groups had moderately elevated levels of fibrinogen, total cholesterol, and LDL-cholesterol.

By the end of 6 weeks, total and LDL-cholesterol levels and fibrinogen decreased significantly in the probiotic group. It was not clear whether the beneficial change could be ascribed to the *L. plantarum* 299v or the fermented oat fiber. No adverse effects were reported from the ingestion of  $10^{10}$  cfu/day of *L. plantarum* 299v.

Stjernquist-Desatnik et al. (2000) conducted 3 experiments with apparently healthy adults to assess the persistence of *L. plantarum* 299v on human tonsillar surfaces. In the first experiment, 6 adults aged 33-42 years (1 man and 5 women, mean age = 38 years) gargled for 2 minutes with 100 ml fermented oatmeal gruel containing about  $2 \times 10^{11}$  cfu *L. plantarum* 299v and then swallowed it; tonsillar scrapings were taken before intake and hourly for 8 hours. In the second study, 2 women aged 41 and 42 years drank 50 ml fermented oatmeal gruel containing about  $10^{11}$  cfu *L. plantarum* 299v mixed with 50 ml fruit juice; tonsillar scrapings were again taken hourly for 8 hours. In the third experiment, the same 2 women consumed only 5 ml fermented oatmeal gruel containing about  $10^{10}$  cfu *L. plantarum* 299v mixed with 95 ml fruit juice and tonsillar scrapings were taken each hour for 8 hours. All samples were analyzed for *L. plantarum* 299v based on morphological assessment of electron microphotographs.

In the first experiment, all 6 volunteers had detectable levels of *L. plantarum* 299v on their tonsillar epithelia after gargling and ingestion and all 6 had the bacteria present at 4 hours; however, only 1 person still had detectable levels at 8 hours after intake. Similarly, both women had *L. plantarum* 299v on their tonsillar epithelia after ingesting fermented gruel mixed with fruit juice and for 4 hours thereafter, but only intake of  $10^{11}$  cfu resulted in detectable levels remaining at 8 hours. The authors concluded that, since the bacteria could be isolated from tonsillar epithelia up to 8 hours after ingestion despite the constant flow of saliva and beverages over the tonsils, "the bacteria under investigation may possess the capacity to adhere to tonsillar cells."

Naruszewicz et al. (2002) studied the effect of *L. plantarum* 299v on cardiovascular disease risk factors in heavy smokers in a prospective, randomized, double-blind, placebo-controlled trial. Thirty-six apparently healthy 25-45-year-old smokers (18 of each sex) were randomized to receive either rose-hip drink with  $2 \times 10^{10}$  cfu *L. plantarum* 299v or the same beverage without the bacteria for 6 weeks. Fecal samples were collected at the end of the study for identification of the probiotic, blood pressure was taken, and blood was drawn for measurement of homocysteine, fibrinogen, triacylglycerol, total and HDL cholesterol, IL-6, and plasma F<sub>2</sub>-isoprostane (a marker of lipid peroxidation and oxidant stress) and extraction of mononuclear cells for use in an adhesion assay with human umbilical vein endothelial cells.

The probiotic drink was well accepted and no adverse events were reported; *L. plantarum* 299v was found in fecal samples from 13 of the 18 members of the probiotic group. The probiotic group had significantly lower systolic blood pressure compared with before intake. No differences were apparent in total cholesterol, triacylglycerol, or lipoprotein(a), but HDL levels increased in the probiotic group while leptin and insulin concentrations decreased; only the leptin change was statistically significant. There were large and significant decreases in F<sub>2</sub>-isoprostanes, IL-6, and fibrinogen concentrations among smokers ingesting *L. plantarum* 299v, as well as the adherence capability of monocytes. All of the biochemical changes attributed to the probiotic intervention were regarded as beneficial and no adverse changes were observed.

Onning et al. (2003), in a prospective, randomized, double-blind, placebo-controlled study, studied the effect of consuming a test beverage for 4 weeks on plasma total antioxidant

capacity, selenium status, and fecal bacteria. A total of 114 volunteers between the ages of 18 and 65 were enrolled and 106 completed the study; four participants withdrew due to antibiotic treatment, three for lack of time, and one due to an experience of increased appetite after consumption of the product. Eight of the participants were excluded according to previously determined exclusion parameters, leaving 98 subjects (50 in the test group and 48 controls) in the final analysis. The analyzed participants included 39 men and 59 women aged 21-61 years (mean age = 35 years). The test drink was a complex mixture that included beta-carotene, alpha-tocopherol, ascorbic acid, pyridoxine, magnesium, manganese, zinc, copper, and selenium in addition to providing  $2.2 \times 10^{10}$  cfu *L. plantarum* 299v; as a result, it was not possible to assign causality for effects to any one component. The level of *L. plantarum* 299v in feces increased significantly after 4 weeks consumption, but the only significant findings regarding the probiotic were that there was no difference between the test and control groups in the incidence or nature of adverse effects and there were no adverse events that could reasonably be attributed to ingestion of *L. plantarum* 299v.

The effect of *L. plantarum* 299v on gut ecology and microbiota was studied in a prospective, randomized, double-blind, placebo-controlled study (Goossens et al. 2003) with 22 apparently healthy adults. All participants received 2 servings/day of 100 ml fermented oatmeal drink providing 0 (n = 11) or  $2 \times 10^{11}$  cfu *L. plantarum* 299v/day (n = 11) for 4 weeks with a 2-week run-up and a 4-week follow-up. Two of the members of the placebo groups failed to complete the study; information was provided only on the 20 individuals (9 males and 11 females; mean age = 32.9 years) who did. Fecal samples were collected weekly from week 1 through week 10 and participants completed questionnaires on defecation patterns (frequency and stool consistency) and side effects. Feces were analyzed for bacterial enumeration, including identification of *L. plantarum* 299v, assessment of  $\beta$ -glucosidase,  $\beta$ -glucuronidase, and azoreductase activities, assays of endotoxins and short-chain fatty acids, and pH determination.

Compliance neared 100%, and no side effects were reported attributable to the probiotic. There were no differences in frequency or consistency of stooling. The individuals consuming *L. plantarum* 299v all had the strain in their feces, but the it could be recovered from only one person a week after the end of ingestion. The probiotic group also had a significant increase in total lactobacilli compared with the placebo group, but there were no differences in total aerobes, total anaerobes, enterobacteriaceae, spore-forming clostridia, *Enterococcus* spp., or *Bacteroides* spp.,  $\beta$ -glucosidase or  $\beta$ -glucuronidase activity, endotoxin concentrations, short-chain fatty acid concentrations, or pH. The authors concluded that

“A fermented oatmeal drink containing *L. plantarum* 299v increases the number of lactobacilli in the faeces of healthy volunteers, but has no influence on other bacterial counts or on metabolic activities... The observed effect of *L. plantarum* 299v on the intestinal flora appears within 1 week after the start of consumption of the probiotic drink and disappears completely 1 week after cessation of consumption of the drink” (Goossens et al. 2003).

Goossens et al. (2005) studied the survival of *L. plantarum* 299v in the gastrointestinal tract and its effects on fecal microbiota, with and without a previous 1-week exposure to pantoprazole to neutralize gastric acidity. Twenty-nine apparently healthy volunteers (9 males and 20 females, mean age = 28.5 years) participated in a prospective, randomized, double-blind, placebo-controlled study and started on pantoprazole or placebo. After 1 week, all participants

consumed a fermented oatmeal drink twice daily, providing a daily total of  $3 \times 10^{11}$  cfu *L. plantarum* 299v, for 2 weeks while continuing on the pantoprazole or placebo. Fecal samples were collected prior to study initiation, prior to probiotic administration, at the end of administration, and 4 weeks following cessation of probiotic administration; at the same time, questionnaires were completed on stool frequency and consistency as well as any side effects.

No side effects were reported and there were no differences between groups in defecation frequency or stool consistency, nor in fecal pH or concentrations of short-chain fatty acids. Pantoprazole did not affect either the number or variety of bacteria found in fecal samples, nor the increase in lactobacilli that occurred after the initiation of *L. plantarum* 299v administration. The administered strain was detected in the feces of all participants at the end of administration, but in only one 4 weeks later. The authors concluded that *L. plantarum* 299v survives passage through the gastrointestinal tract irrespective of gastric acidity.

In a second study, Goossens et al. (2006) conducted a prospective, randomized, double-blind, placebo-controlled trial to assess the effect of ingestion of *L. plantarum* 299v on fecal bacterial ecology and mucosal adhesion of bacteria in the rectum and ascending colon. The study enrolled 29 apparently healthy patients (16 males and 13 females with a mean age of 56.9 years) undergoing colonoscopic examination for polyps, who consumed a twice-daily drink with ( $n = 15$ ) or without ( $n = 14$ ) *L. plantarum* 299v for 2 weeks; *L. plantarum* ingestion in the probiotic group totaled  $2 \times 10^{11}$  cfu/day. Fecal samples were collected at enrollment and after the 2-week treatment period, along with questionnaires regarding bowel habits and possible side effects, and biopsies were collected from the ascending colon and the rectum during the colonoscopy.

No side effects were reported, and no differences were seen between groups in reported defecation frequency or stool consistency. Feces from the probiotic group showed significant increases in clostridia, total lactic-acid bacteria, and lactobacilli after ingestion of *L. plantarum* 299v for 2 weeks, but lactobacilli could be cultured in rectal and ascending-colon biopsies from only 3 and 2 patients, respectively, in the probiotic group. There were no significant differences in the total bacterial load or the presence of other bacterial species in the feces or mucosa. The authors concluded that “*L. plantarum* 299v survives passage through the gastrointestinal tract... [and] the probiotic strain did colonize the colonic mucosa to a minor extent.”

Bering et al. (2006) offered fermented oatmeal with *L. plantarum* 299v to healthy women of childbearing age to assess its effect on absorption of non-heme iron from a phytate-rich meal. Twenty-four apparently healthy non-anemic women with a mean age of 25 years were enrolled in a prospective, randomized, double-blind crossover-design study, in which each participant consumed whole-wheat rolls and 100 g of each of 4 gruels for 4 days. The 4 gruels were: (1) oatmeal fermented by *L. plantarum* 299v and providing  $1.1 \times 10^{11}$  cfu of the probiotic, (2) similar fermented oatmeal pasteurized to kill the bacteria, (3) non-fermented oatmeal with lactic-acid-adjusted pH, and (4) non-fermented oatmeal with added lactic and acetic acids. One meal with each type of gruel was labeled with  $^{55}\text{Fe}$  while another was labeled with  $^{59}\text{Fe}$  (2 isotopes used to avoid confounding with residual isotope from a previous meal). Blood samples were drawn from the cubital vein and analyzed for serum ferritin and radioactivity. Iron absorption was found to be significantly higher with ingestion of *L. plantarum* 299v than with any other condition, and the authors concluded, that “the observed increase in Fe absorption in the present study seems to be attributable to a specific effect of the live *L. plantarum* 299v. Whether this effect is caused by



colonisation of *L. plantarum* 299v in the intestine, or by production of organic acids during passage through the intestine remains to be established.”

In a follow-up prospective, randomized, double-blind, placebo-controlled crossover study, Bering et al. (2007) tested the effect of lyophilized viable *L. plantarum* 299v on absorption of non-heme iron. Eighteen apparently healthy women with a mean age of 22 years consumed each of 2 meals for 2 days; the meals included whole-wheat rolls and 100 g fermented oatmeal gruel that had been pasteurized after to kill all bacteria, with or without the addition of  $10^{11}$  cfu viable lyophilized probiotic. One meal with each type of gruel was labeled with  $^{55}\text{Fe}$  or  $^{59}\text{Fe}$ . Blood samples were drawn from the cubital vein and analyzed for serum ferritin and radioactivity. Iron absorption was found to be no higher with ingestion of viable lyophilized *L. plantarum* 299v than without. The authors suggested that the lack of effect of the probiotic could be explained by the bacteria not being in an active state. A side study supported this hypothesis by showing that the metabolic activity of lactic acid bacteria added to gruel in lyophilized form was retarded by about 1 hour, “which potentially could affect the metabolic activity of the bacteria in the duodenum,” where iron absorption occurs.

Hoppe et al. (2015) investigated the ability of *L. plantarum* 299v to improve iron absorption from a fruit drink in 2 prospective, randomized, single-blind, placebo-controlled, cross-over trials. Twenty-two women described only as “healthy Swedish women of reproductive age recruited from students at the University of Gothenburg” were enrolled. In the first trial, 11 women consumed fruit drink fortified with  $^{55}\text{Fe}$  and  $^{59}\text{Fe}$  as ferrous lactate and containing either 0 or  $10^9$  cfu *L. plantarum* 299v for 4 days; the second trial was identical except the probiotic dose was increased to  $10^{10}$  cfu. Retention of the iron isotopes was measured with whole-body counting and in blood.

Iron absorption and retention was significantly higher with either  $10^9$  or  $10^{10}$  cfu of *L. plantarum* 299v than with the control fruit drink ( $28.6\pm 12.5$  and  $29.1\pm 17.0\%$  vs.  $18.5\pm 5.8$  and  $20.1\pm 6.4\%$ , respectively), but there was no significant difference in iron retention with the two probiotic doses. No adverse effects were reported.

### 6.3.3.2. Healthy Children

In a prospective, placebo-controlled trial (Ribeiro and Vanderhoof 1998), 143 children aged 6 months to 3 years attending daycare in a region of Brazil with a high incidence of infectious diarrhea received a beverage containing fermented oatmeal and  $10^{10}$  cfu *L. plantarum* 299v ( $n = 71$ ) or a control beverage containing unfermented oatmeal ( $n = 72$ ) for 3 months. The beverage was given once a day during the 1<sup>st</sup> month, every other day during the 2<sup>nd</sup>, and weekly during the 3<sup>rd</sup>.

Significant reductions in the incidence of diarrhea and respiratory infections were seen in both the test and control groups, with no differences between groups. The authors speculated that colonization of half of the children in the daycare setting with *L. plantarum* may have reduced the dissemination of infectious diseases. No adverse effects were reported from the ingestion of  $10^{10}$  cfu/day of *L. plantarum* 299v by these young children.

Kingamkono et al. (1999) enrolled apparently healthy children aged 6 months to 5 years in a randomized, double-blind, placebo-controlled study of the effects of fermented and

unfermented cereal gruel on the presence of fecal enteric bacteria. Fifty children were assigned to receive cereal fermented by *L. plantarum* 299v, 50 children received cereal fermented by a traditional unidentified starter culture, and 51 children received unfermented cereal. Children received daily servings of cereal for 13 days. Rectal swabs were taken at baseline, on days 7 and 13 during the feeding period, and 14 days after cessation. Since neither the amount of cereal consumed nor the concentration of *L. plantarum* 299v in the cereal were reported, the daily ingestion of *L. plantarum* 299v could not be determined.

Six children receiving the cereal fermented with *L. plantarum* 299v failed to complete the study, as did 7 children receiving the other fermented cereal and 9 receiving unfermented cereal. The results from the 2 fermented cereals did not appear to differ significantly and the 2 groups were combined to increase statistical power. Nevertheless, the proportion of children in the probiotic and control groups harboring enteric bacteria (campylobacter, salmonella, shigella, *E. coli* O157, and enterotoxigenic *E. coli*) did not differ. There were no reported adverse effects due to consumption of fermented cereals.

Berggren et al. (2003), in a prospective, randomized, double-blind, placebo-controlled study, evaluated the effect of consumption of a fermented oat product containing *L. plantarum* 299v on children's intestinal function and microbiota. A total of 84 children aged 6 months to 3 years were enrolled; half of the children were assigned to receive fermented oatmeal providing  $1.4 \times 10^{11}$  cfu *L. plantarum* 299v/day or placebo unfermented oatmeal for 3 weeks. Fecal samples were taken at baseline and at the end of the test period.

Four enrolled children did not start the study, and 11 withdrew because they did not like the product (4 from the probiotic group and 7 controls). Product-related adverse events were reported for 5 children, 4 in the probiotic group and 1 control: 3 children in the probiotic group developed constipation and one child had regurgitations (which, however, had begun before feeding commenced); one placebo-group child had softer than normal stool. No differences were seen between groups in measures of intestinal function—stool frequency and consistency, flatulence, vomiting, and intestinal pain. *L. plantarum* 299v was present in the feces of all but one member of the probiotic group and none of the controls. The level of lactobacilli was significantly higher in the probiotic than the control group. The authors concluded that “the children tolerated the fermented oat product well.”

### **6.3.3.3. Compromised Adults**

Nobaek et al. (2000) enrolled 60 adult patients with irritable bowel syndrome (IBS) in a prospective, randomized, double-blind, placebo-controlled trial of the effect of attempted alteration of the gastrointestinal microecology. Half of the patients received 400 ml/day of rose hip drink providing  $2 \times 10^{10}$  cfu *L. plantarum* 299v and 3.6 g oat flour while the other half received plain rose-hip drink. Administration lasted for 4 weeks; patients began recording their self-assessments of gastrointestinal function (including frequency of defecation, stool consistency, bloating, flatulence, and pain) 2 weeks before administration and continued every day through administration. Fecal samples and rectal biopsies were taken for microbiological analysis at baseline and at termination of administration. Patients completed a follow-up questionnaire one year after the study.

*L. plantarum* 299v was found in the fecal samples from 84% of the test group and in 32% of their rectal biopsies, but there were no changes or differences between test and control groups

in other bacterial counts. Flatulence decreased significantly in the test group while defecation frequency and self-assessed overall gastrointestinal function improved significantly. One year later, patients in the test group still had significantly better function than at study entry while those in the placebo group had no improvement. The authors noted that the products were well tolerated and no treatment-related adverse effects were reported from ingestion of  $2 \times 10^{10}$  cfu/day of *L. plantarum* 299v for 4 weeks.

In another prospective, randomized, double-blind, placebo-controlled trial of the effect of *L. plantarum* 299v on patients with IBS (Niedzielin et al. 2001), 40 patients (8 males and 32 females aged 27-63 years, mean = 45 years) received either  $2 \times 10^{10}$  cfu probiotic or placebo in 2 daily doses for 4 weeks. The bacteria or placebo were delivered in a fruit beverage containing 5% oatmeal soup. All patients were clinically examined at baseline and at the end of the study, and provided weekly self-assessments of IBS symptoms.

The patients receiving *L. plantarum* 299v showed significantly greater improvement in their IBS symptoms than did the placebo group, and the authors noted that “No treatment related side-effects were observed.”

In a prospective, randomized, unblinded study with no placebo, McNaught et al. (2002) evaluated the ability of *L. plantarum* 299v administered both before and after major abdominal surgery to reduce the incidence of bacterial translocation and septic complications. A total of 129 patients (75 males and 54 females with median age = 68 years) entered the study; 64 were randomized to the probiotic group and received 500 ml/day of an oatmeal-based drink providing  $2.5 \times 10^{10}$  cfu *L. plantarum* 299v from study entry to the day of surgery, and again after surgery, while 65 patients were assigned to the control group, which received no non-traditional intervention but did receive standard antibiotic prophylaxis. Nasogastric aspirates were analyzed to assess gastric colonization and bacterial translocation. After the peritoneum was opened during surgery, a lymph node was excised from the ileocolic mesentery and a serosal scraping was taken from the antimesenteric border of the terminal ileum. C-reactive protein levels were measured preoperatively and on postoperative days 1 and 7 as measures of systemic inflammatory response.

No differences were seen between the probiotic and control groups in bacterial translocation to the lymph nodes or ileal serosa, gastric colonization, C-reactive protein levels, septic complications, or mortality. The authors concluded that “preoperative administration of the probiotic *Lactobacillus plantarum* 299v for two weeks has no effect [either beneficial or adverse] on the human gut mucosal barrier ... and the systemic inflammatory response.”

Sen et al. (2002) studied the effect of *L. plantarum* 299v on colonic fermentation of IBS patients in a prospective, double-blind, placebo-controlled crossover trial. Twelve patients (1 male and 11 female aged 23-61 years, mean age = 40.6 years) gastroenterologic outpatients diagnosed with IBS but otherwise apparently healthy received a placebo drink for 4 weeks and then a rose-hip drink providing  $6.3 \times 10^9$  cfu *L. plantarum* 299v for 4 weeks. Patients reported their symptoms daily; at the end of each phase they were given a calorimetry test and a lactulose challenge.

No difference was seen between the groups on any measure: exhalation of hydrogen and methane during calorimetry, breath hydrogen after lactulose ingestion, or daily symptom scores.

The authors concluded that “*Lactobacillus plantarum* 299v in this study did not appear to alter colonic fermentation.”

The ability of *L. plantarum* 299v to reduce the likelihood of further recurrent episodes of *Clostridium difficile*-associated diarrhea was studied in a prospective, randomized, double-blind, placebo-controlled study (Wullt et al. 2003). Twenty-nine patients testing positive for *C. difficile* toxin and having a history of previous *C. difficile*-associated diarrhea were enrolled; 8 patients failed to complete the study (none due to possible side-effects of the treatment) and information was provided only on the 21 patients with complete records; these include 20 females and only 1 male with a mean age of 63.8 years. Patients were given metronidazole for 10 days and were assigned to receive either a fruit drink containing fermented oats and *L. plantarum* 299v twice a day, providing a total of  $5 \times 10^{10}$  cfu ( $n = 12$ ) or a placebo ( $n = 9$ ), for 38 days. Patients were assayed for *C. difficile* toxin on about day 12, about day 39, and upon any report of symptoms.

There was a small but statistically insignificant reduction in the risk of recurrence among the patients receiving *L. plantarum* 299v, and the authors noted that “Treatment with the lactobacilli had no apparent side-effects.”

Fecal samples collected in this study were analyzed for short-chain fatty acids, lactate, and succinate and for the presence of *L. plantarum* 299v (Wullt et al. 2007). The strain was isolated in the feces of 11 of the 12 individuals consuming the probiotic but none of the controls. The authors reported “a trend toward higher butyrate, total short-chain fatty acids, and total organic acids in the group of patients receiving lactobacilli,” but these concentrations were not significantly higher than those in the placebo group.

Woodcock et al. (2004) assessed the effect of *L. plantarum* 299v on gut immune function in a subset of the patients participating in a prospective, randomized, unblinded study with no placebo, discussed previously (McNaught et al. 2002), in which *L. plantarum* 299v was administered both before and after major abdominal surgery. Twenty-two patients (10 males and 12 females with median age = 69 years) undergoing small-bowel resection were studied, 11 each from the probiotic and control groups. The probiotic group received 500 ml/day of an oatmeal-based drink providing  $2.5 \times 10^{10}$  cfu *L. plantarum* 299v from study entry to the day of surgery, and again after surgery, while the control group received no non-traditional intervention but did receive standard antibiotic prophylaxis. A 5-cm section of macroscopically normal small intestine was excised and the mucosa was exposed and examined for plasma cell concentration, IgA-positive cell concentration, IgM-positive cell concentration, and IgA and IgM levels at the mucosal surface.

There were no differences between the probiotic and control groups in numbers of plasma cells or either IgA- or IgM-positive cells, or in mucosal-surface IgA levels, but the concentration of IgM was significantly reduced in the group receiving *L. plantarum* 299v. The authors concluded that there is no evidence from this study that administration of the probiotic has any effect on gut-associated lymphoid tissue, an important component of the gut barrier.

The ability of *L. plantarum* 299v to adhere to the gut mucosa of critically ill patients was studied in a small prospective, randomized, unblinded study (Klarin et al. 2005) with 17 critically ill patients admitted to the ICU, 8 males and 9 females aged 33 to 84 years (mean = 64.6 years). Nine patients received enteral formula with added fermented oatmeal and *L. plantarum* 299v in 6 daily administrations providing a total of  $2 \times 10^{11}$  cfu of the probiotic, while

8 control patients received enteral formula alone. Treatment continued through the time the patient remained in the ICU. Rectal biopsies were taken at enrollment and twice weekly; the laboratory analysis of the biopsies was blinded.

All patients tolerated total or partial enteral feeding, and there were no differences in diarrhea, bloating, illness severity, hospital mortality, length of stay in the ICU (4-37 days; median = 11 days), or 6-month mortality, nor in levels of C-reactive protein or leukocyte count. Three of 8 patients receiving *L. plantarum* 299v tested positive for the strain in the samples of rectal mucosa taken during the treatment. The authors concluded that “*L. plantarum* 299v administered to critically ill, antibiotic-treated patients can survive and colonise the gut mucosa, and repeated administration of the bacteria is necessary to obtain this effect.”

Another study in which *L. plantarum* 299v was administered to critically ill patients was conducted by McNaught et al. (2005), who studied the effect of the probiotic on gut barrier function and systemic inflammatory response. This prospective, randomized, but unblinded study enrolled 103 patients (58 males and 45 females aged 28-90 years; median age = 71 years) within 24 hours of admission to the ICU; 52 were assigned to the probiotic group and 51 to the control group. The probiotic group received 122-315 ml/day of Proviva fruit drink with fermented oatmeal and *L. plantarum* 299v orally or by nasogastric tube; the median intake of 213 ml provided about  $10^{10}$  cfu of the probiotic. The control group received conventional therapy including adjuvant enteral or parenteral nutritional support as deemed clinically appropriate. Treatment continued until discharge from the hospital—a range of 3-17 days with median = 9 days. Nasogastric aspirates were obtained at baseline (day 1) and on days 4 and 8 and cultured; intestinal permeability was assessed by means of a lactulose challenge on days 1 and 8; and blood was drawn on day 1 and weekly thereafter for measurement of IgM, C-reactive protein, and IL-6.

*L. plantarum* 299v was cultured from gastric aspirates of 26% of probiotic-group patients at day 3 and 33% at day 8; aside from this strain there were no differences between the 2 groups in the numbers or species of the detected microorganisms. The lactulose test revealed no difference between the 2 groups in intestinal permeability, nor were differences seen in levels of IgM or C-reactive protein, but IL-6 levels were significantly lower in the probiotic group than in the controls at day 15. The mortality rate was 35% in both groups, and a total of 68 septic complications occurred, most commonly chest infections, but there were no differences in incidence, causes, or severity between patients receiving *L. plantarum* 299v and controls. The authors concluded that “the results of this prospective randomised trial suggest that *Lactobacillus plantarum* 299v may attenuate the systemic inflammatory response in critically ill patients. This was not accompanied, however, by any significant changes in gastrointestinal microflora, endotoxin exposure, intestinal permeability, septic morbidity or mortality.”

Klarin et al. (2008) enrolled 44 ICU patients (26 males and 18 females aged 18-89 years; mean age = 64.7 years) receiving antibiotic therapy in a prospective, randomized, double-blind, placebo-controlled study of the capacity of *L. plantarum* 299v to reduce *Clostridium difficile*-associated disease. The patients received 200 ml fermented oatmeal gruel/day for 3 days and 100 ml/day thereafter; half of the patients received gruel providing  $1.6 \times 10^{11}$  cfu (later  $8 \times 10^{10}$  cfu) *L. plantarum* 299v while the other half received gruel without the probiotic. Treatment continued for the duration of the stay in the ICU. Blood samples were drawn daily for analysis of clinical biochemistries and cytokines, fecal samples were collected at baseline and twice weekly for

culturing of *C. difficile*, *L. plantarum* 299v, total lactobacilli, *Enterobacteriaceae*, sulfite-reducing clostridia, enterococci, and total anaerobes. Fifteen patients took lactulose tests to assess gut permeability.

Two patients from each group died in the ICU; 1 patient from the probiotic group died in the hospital, and 4 patients from the control group died within 6 months. There were no differences between the groups in sequential organ failure, length of ICU stay (the range in the probiotic group was 2.5 to 22 days with mean = 5.5 days), or days on ventilators. In 71 fecal samples from the probiotic group, none tested positive for *C. difficile*, while 4 emergent cases were found in the 80 samples from control group patients, a statistically significant difference. Control group patients also harbored a number of potential pathogens not found in the probiotic group, including *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Bacillus cereus*. There were no differences in C-reactive protein, TNF- $\alpha$ , IL-1 $\beta$ , or IL-6; IL-10 and white blood cell counts were significantly higher in the control group than in patients receiving *L. plantarum* 299v. Gut permeability was significantly higher in the control group than in the probiotic group. The study product was well tolerated and the authors stated that “We found no adverse impact of the given probiotic preparation.” They also noted that *L. plantarum* 299v has “been given to hospitalised patients for many years, but there have been no reports of associated bacteraemia or sepsis or any other adverse events.”

Sawant et al. (2010), in a prospective, randomized, double-blind, placebo-controlled, multi-center study, enrolled 200 IBS patients (141 males and 59 females; mean age = 37.8 years) to evaluate the capacity of *L. plantarum* 299v to reduce symptoms of the disease. For 4 weeks the patients ingested 1 capsule containing either  $10^{10}$  cfu *L. plantarum* 299v (n = 101) or placebo (n = 99). The frequency and intensity of abdominal pain, bloating, feeling of incomplete bowel movement, and stooling frequency were assessed at baseline and conclusion of treatment.

Ten patients failed to complete the study, 3 from the probiotic group and 7 from the control group. The patients ingesting the probiotic showed significant improvement in all assessed symptoms as well as in overall assessment as compared with the control group. No changes were observed in pulse or respiratory rates, blood pressure, or body temperature, and no side effects were reported.

In a prospective, randomized, double-blind, placebo-controlled study (Lonnermark et al. 2010), 239 patients (93 males and 146 females; median age = 45 years) receiving antibiotic therapy for infectious disease were given a fruit drink containing oats gruel and providing 0 (n = 121) or  $10^{10}$  cfu *L. plantarum* 299v/day (n = 118). Treatment began within 48 hours of the initiation of antibiotic therapy and continued until 7 days after termination of antibiotics. Patients maintained a diary of defecation frequency, stool consistency, blood in stools, nausea, vomiting, abdominal pain, and flatulence; fecal samples were collected at baseline and at 7-10 days after termination of antibiotics as well as in any case of reported diarrheal symptoms.

Seventy-six patients withdrew or were excluded from the study, 38 each from the probiotic and placebo groups; reasons for withdrawal did not differ between the groups. Diary data were available for a median of 22 days. Diarrhea was infrequent (only 5 and 6 patients in the placebo and probiotic groups, respectively). However, the incidence of loose or water stools but not meeting the criteria for diarrhea and the incidence of nausea were significantly lower in the group receiving *L. plantarum* 299v than in the control group. The authors reported that “No side effects of the treatment were recorded.”

A total of 254 ICU patients (157 males and 97 females aged 17 to 90 years; mean age = 62.7 years) participated in a prospective randomized open-label trial comparing the effects of *L. plantarum* 299/299v (n = 130) and routine selective decontamination of the digestive tract (n = 124) in reducing the infection rate (Oudhuis et al 2011). Patients in the probiotic group received 2 doses/day of the bacteria via nasogastric tube, totaling  $10^{10}$  cfu/day; controls received 4 treatments daily or an oral paste composed of polymyxin B, gentamicin, amphotericin B, an enteral solution containing the same antibiotics, and cefotaxime administered intravenously. Treatment continued for the duration of the stay in the ICU, a mean of 11 days. Cultures of sputum and urine were taken twice weekly and rectal swabs were taken weekly.

There were no differences between the 2 groups in length of ICU or hospital stay, need for mechanical ventilation, or mortality. Although there was a “tendency” toward more infections in patients receiving probiotic therapy than in those receiving antibiotics, this difference was not statistically significant. Although no adverse effects were reported from the probiotic treatment, the authors interpreted the results as suggesting that probiotic treatment might be inferior to selective decontamination.

In a prospective, randomized, double-blind, placebo-controlled, multi-center, parallel study (Ducrotte et al. 2012), 214 patients (151 men and 63 women; mean age  $37.5 \pm 12.6$  years) with irritable bowel syndrome (IBS) were enrolled to receive daily one capsule containing  $10^{10}$  cfu of *L. plantarum* 299v (n = 108) or potato starch placebo (n = 106) for 4 weeks. The primary endpoint was reduction in the frequency of abdominal pain episodes, but measures also included pain severity, abdominal bloating, feelings of incomplete rectal emptying, daily number of stools, and blood tests for red and white blood cell counts, glycemia, BUN, and liver function (specific assays not reported). Patients were evaluated weekly during the intervention and for 3 weeks thereafter.

Three patients in the probiotic group and 7 in the placebo group dropped out, none for treatment-related reasons. The frequency of pain episodes was reduced to a significantly greater degree in the 299v group (by 51.9%) than in the placebo group (by 13.6%). Reductions in severity of abdominal pain, stool frequency, bloating, and feeling of incomplete emptying were also significantly greater in the probiotic group. The authors reported that, “No significant side-effect was reported in any group during the 4 wk of treatment. The only adverse event reported was a transient vertigo onset by one of the patients who received *L. plantarum* 299v (DSM 9843). No change in blood parameters was detected throughout the study.”

Mangell et al. (2012), in a prospective, randomized, double-blind, placebo-controlled trial, investigated the effect of prophylactic *L. plantarum* 299v on the intestinal load of pathogenic bacteria, bacterial translocation, and cell proliferation in colon surgery. The 64 enrolled patients included 36 males and 28 females aged 64 to 80 years (median age = 72 years) who were referred for colonic resection. Starting 8 days prior to surgery, each patient received 100 ml/day of the assigned preparation, which provided either 0 or  $10^{11}$  cfu of *L. plantarum* 299v (32 patients per group), and continued until 5 days post-surgery. Rectal swabs and mucosal biopsies were taken 9 days and 1 day before surgery and 6 days, 6 weeks, and 6 months after surgery for bacterial enumeration. Bacterial translocation was assessed by DNA extraction from a mesenteric lymph node harvested at the ileocecal junction during surgery.

No benefit was reported from administration of *L. plantarum* 299v to colon surgery patients; there were no differences between groups in the incidence of enteric pathogenic

bacteria, bacterial translocation, or postoperative complications. The authors noted that, “No adverse effects were recorded after the administration of high doses of *L. plantarum* 299v.”

In a prospective open-label study of treatment of ulcerative colitis with *L. plantarum* 299v (Krag et al. 2012), 39 patients (15 males and 24 females aged 19-50 years [median age – 35 years]) were given increasing doses of Profermin®, providing  $10^8$  cfu/ml. Patients received 2 doses per day, totaling  $2.5 \times 10^{10}$  cfu for 2 days, then  $5 \times 10^{10}$  cfu thereafter. The median dose of Profermin® was 445 ml/day, providing  $4.45 \times 10^{10}$  cfu of *L. plantarum* 299v. Treatment was continued for up to 176 days.

The treatment significantly reduced the severity of the disease by 56.5%. The authors reported:

“No major AEs were reported and there were no dropouts due to AEs. An increased number of bowel movements were reported by 11 patients (28%), bloating by four (10%) and an increased number of bowel movements and bloating by three (8%). All AEs were self-limiting or managed by dose adjustments. For example, if a patient experienced a presumable AE during the introduction of Profermin®, the period with the low Profermin® dose was prolonged for up to 2 wk. None of the eight dropout or four excluded patients left the trial due to deterioration in UC symptoms” (Krag et al. 2012).

Krag et al. (2013) carried out a similar study as a prospective randomized single-blind two-arm study comparing Profermin® and Fresubin®, a high-energy and protein drink that is an accepted treatment. (In a departure from the usual single blinding, the staff and statisticians were blinded but the patients were not, because the two products are different in appearance and texture.) In this study, 73 ulcerative-colitis patients (33 males and 40 females aged 20-78 years; mean age 41 years) were enrolled, 32 assigned to received Profermin® and 41 to receive Fresubin®. The median dose of Profermin® was 489 ml/day, providing  $4.89 \times 10^{10}$  cfu of *L. plantarum* 299v.

Treatment with Profermin® was significantly more effective in reducing the severity of ulcerative colitis than was Fresubin®. The authors reported that, “No major adverse events (AEs) were reported, but 3 patients experienced AEs. In the Fresubin group, one experienced an ‘obvious weight gain’ and one felt it induced vomiting. In the Profermin group, one suffered from rumbling and bloating.” They concluded that, “Supplementation with Profermin is safe, well tolerated, palatable.”

Seventeen patients (12 men, 5 women aged 48 to 75 years [median = 52 years]) with obstructive jaundice undergoing biliary drainage completed a prospective, randomized, double-blind, placebo-controlled trial as a pilot study (Jones et al. 2013). Patients were divided into 3 groups to receive live *L. plantarum* 299v (n = 5), inactivated 299v (n = 5), or water (n = 7) from day 1 to 7 after biliary drainage. The dose of *L. plantarum* 299v was not reported. On hospital admission, on the day before drainage, and on days 1 and 7 after drainage, intestinal permeability was tested, peripheral blood was drawn for hematological and biochemical analysis, and urine was collected for assay of TNF p55 receptor levels.

The findings of the hematological and biochemical analyses were not reported, suggesting that the findings were not remarkable. While trends toward reduced intestinal permeability and reduced TNF p55 receptors with administration of *L. plantarum* 299v were



reported, neither effect reached statistical significance. There were no reports of adverse effects from the probiotic treatment.

Treatment of irritable bowel syndrome (IBS) with *L. plantarum* 299v was assessed in a prospective, randomized, double-blind, placebo-controlled trial (Stevenson et al. 2014). A total of 81 IBS patients (2 males and 79 females aged 47.9±12.1 years) were randomized to receive *L. plantarum* 299v or placebo in a 2:1 ratio (n = 54 probiotic, n = 27 placebo); the probiotic dose was 10<sup>10</sup> cfu/day while the placebo was microcrystalline cellulose, both in 2 daily capsules. Patients consumed their assigned capsules for 8 weeks with clinical evaluations at baseline and at week 2, 4, 6, and 10 (after a 2-week washout phase).

Patients in both the probiotic and placebo groups showed significant improvement, reflected in reduced reported pain, but there was no difference between groups and the authors concluded that “No significant beneficial effects by the probiotic were seen on the severity of symptoms (abdominal pain) and quality of life.” At the same time, there was no difference between groups in compliance and “the rate of AEs was very low. The tolerability of the test product was good.”

In a retrospective open-label study, Kujawa-Szewieczek et al. (2015) compared incidence of *Clostridium difficile* infection in hospital patients receiving therapy during the year before and the year after introduction of the use of *L. plantarum* 299v as a prophylactic at a level of 10<sup>9</sup> cfu/day. The observation population was the high-risk group of patients after organ transplantation or receiving immunosuppressive drugs for any other reason, 174 patients in the year prior to introduction of *L. plantarum* 299v and 182 in the year after. Of these patients, 21 in the first year and 2 in the second year were diagnosed with *C. difficile* infection, infection rates of 12.1 and 1.1%, respectively. Patients suffering from *C. difficile* infection included 13 males and 10 females with a mean age of 56.7±15.1 years. No adverse effects were reported due to probiotic treatment and the authors concluded that, “Routine use of [*L. plantarum* 299v] during treatment with antibiotics may prevent *C. difficile* infection in the nephrology and transplantation ward.”

A total of 149 patients with *Salmonella* infections were enrolled in a prospective, randomized, double-blind, placebo-controlled, multi-center study of the ability of *L. plantarum* 299v to treat the infection (Lonnermark et al. 2015). The patients included 40 males and 109 females aged 5 to 68 years (median age = 36 years) who ingested sachets of skim milk powder containing 0 (n = 72) or 5x10<sup>10</sup> cfu of *L. plantarum* 299v (n = 77) until 4 consecutive fecal samples tested negative for *Salmonella*. The median time to clearance (and so the median time patients ingested *L. plantarum* 299v) was 26 days in the probiotic group and 25 days in the placebo group. The authors reported a non-statistically significant tendency for a greater number of gastrointestinal symptoms to be reported by patients consuming *L. plantarum* 299v. The conclusion of the authors was that, “Our results give little support for positive effects of *L. plantarum* 299v treatment in nontyphoid salmonellosis.”

Bengtsson et al. (2016) reported a prospective, randomized, double-blind, placebo-controlled trial of the effects of *L. plantarum* 299v and *Bifidobacterium infantis* Cure 21 on patients with poor ileal pouch function. Thirty-two patients with impaired pouch function, 24 men and 8 women, aged 27-70 years (median age = 50 years) were enrolled; half were randomly assigned to receive 10<sup>10</sup> cfu each of *L. plantarum* 299v and *B. infantis* Cure 21 per day for 21 days while the control group received a maltodextrin placebo. Fecal samples were taken at

baseline and at study termination for assays of calprotectin, lactoferrin, myeloperoxidase, and eosinophilic cationic protein and pouch endoscopy was performed to assess mucosa morphology and to take biopsies for histopathology.

There were no significant differences on any measures between the probiotic and placebo groups and the authors concluded that, “The current study failed to confirm the hypothesis that probiotics improve function in patients with poor pouch function.” There was no discussion of any adverse effects of the treatment.

#### **6.3.3.4. Compromised Children**

Two case studies in which  $10^{10}$  cfu/day of *L. plantarum* 299v was given to children with small-bowel bacterial overgrowth as a complication of short-bowel syndrome were reported by Vanderhoof et al. (1998). The 1<sup>st</sup> case was a 7-year-old boy who had been receiving successful antibiotic therapy with trimethoprim-sulfamethoxazole and metronidazole, but was switched to the probiotic. Within 2-3 weeks stool consistency improved, primarily in reduction of water content. No adverse effects were noted due to the probiotic therapy, which continued for 2 months. The 2<sup>nd</sup> case was an 16-year-old boy suffering from bacterial-overgrowth-linked arthritis of the interphalangeal joints, who had received some relief from sulfasalazine treatment. Replacement of the antibiotic with *L. plantarum* 299v produced good therapeutic response with no reported adverse effects. It is noteworthy that no indication of D-lactic acidosis was reported in either of these short-bowel patients receiving *L. plantarum*.

*L. plantarum* 299v was given to 15 immunocompromised children age 11.5 months to 14 years (5 males and 10 females) with HIV (Cunningham-Rundles et al. 2000). The first child (an 11-year-old boy) received the probiotic in fruit juice in an unblinded administration, but the other 14 received packets containing about  $2 \times 10^{10}$  cfu *L. plantarum* 299v or placebo. All but one patient consumed the packet contents mixed into a beverage and ingested orally; the remaining patient received the supplement via percutaneous enteral feeding tube. Treatment continued for about 1 month and colonization was tested by culture of rectal swabs.

The first treated patient showed significantly improved growth, improved appetite, and resolution of mouth ulcers, candidiasis, and diarrhea. This success led to the randomized, double-blind, placebo-controlled study with the remaining patients. No patient experienced bloating or other symptoms of intolerance, and none had to be withdrawn. Although residence of *L. plantarum* 299v was established, colonization was not permanent and no bacteria were detected in rectal swabs by the end of the first month after cessation of administration. Evaluation of mononuclear cells isolated from peripheral blood showed a natural immune response to *L. plantarum* 299v in 60% of the children, but not in the other 40%. The authors concluded that, “The data suggest that *L. plantarum* 299v may be given safely to the immunocompromised host and may indeed have a positive effect on immune response.”

Ladas et al. (2015) evaluated the safety and efficacy of prophylactic use of *L. plantarum* 299v in children and adolescents undergoing hematopoietic cell transplantation in a prospective open-label multi-center pilot study. A convenience sample of 30 children and adolescents (16 males and 14 females aged  $7.7 \pm 4.7$  years; the age range was 2.2 to 17.3 years). Twelve patients were being treated for leukemia and 9 for sickle cell disease, 4 suffered from severe aplastic anemia, and the rest from a variety of severe conditions. Study participants received, orally or by

enteral feeding tube,  $10^8$  cfu of *L. plantarum* 299v/kg bw/day from 7 days prior to transplantation to 14 days after the operation.

The incidence of graft-versus-host disease was 30%, lower than is usually encountered. No episodes of *L. plantarum* bacteremia were observed, but 6 patients had non-*Lactobacillus* bacteremia. The authors reported that, “We did not observe any serious adverse events or unexpected severe adverse events attributed to [*L. plantarum*] in any patient enrolled to the study.” Although there were 3 deaths, the authors reported that “none of these deaths were attributed to [*L. plantarum*].” The authors concluded that “Our study provides preliminary evidence that administration of [*L. plantarum*] is safe and feasible in children and adolescents undergoing [hematopoietic cell transplantation].”

#### **6.3.3.5. Conclusions from Human Studies**

*L. plantarum* 299v has been administered to humans in 37 prospective studies—nearly all of them double-blinded and placebo-controlled; 1502 individuals aged 6 months to 90 years have received the probiotic at daily dose levels up to  $2 \times 10^{11}$  cfu for as long as 90 days. In studies of healthy adults, 250 participants received *L. plantarum* 299v at daily levels from  $5 \times 10^8$  to  $2 \times 10^{11}$  cfu for between 1 and 42 days; 154 healthy children received the probiotic at levels of  $10^{10}$  to  $1.4 \times 10^{11}$  cfu/day for 13 to 90 days. The bacterium appears to have the capability to establish short-term residence in the intestinal mucosa, but it is rarely isolated in feces a week after administration has ended. Administration of the probiotic may lead to minor alterations in the intestinal microbiota and a slight increase in the production of short-chain fatty acids, which in turn may reduce the luminal pH and improve absorption of non-heme iron. No adverse effects were reported in any studies of healthy adults or children.

In the 20 studies reported in the literature in which *L. plantarum* 299v was administered to compromised adults, 1068 individuals with IBS, *C. difficile* infection, or severe illness requiring ICU placement received the probiotic at levels up to  $2 \times 10^{11}$  cfu/day for durations ranging from a few days to 56 days. Administration of the probiotic was generally regarded as beneficial, and no trial resulted in any adverse effect on these severely compromised patients. The human studies also corroborated findings from *in vitro* and animal studies that *L. plantarum* 299v has the capacity to down-regulate proinflammatory cytokines.

The totality of the evidence from human intervention studies demonstrates the safety of ingestion of *L. plantarum* 299v at levels as high as  $2 \times 10^{11}$  cfu/day.

**Table 7. Studies of *L. plantarum* 299v in Humans.**

Reference	Study Design & Objective	Subjects	Daily Dose	Duration	Results
Bengtsson et al. 2016	Prospective, randomized, double-blind, placebo-controlled trial of the effects of <i>L. plantarum</i> 299v and <i>Bifidobacterium infantis</i> Cure 21 on patients with poor ileal pouch function	32 patients with impaired pouch function, 24 men and 8 women, aged 27-70 years (median age = 50 years) Lp299v: 16	10 <sup>10</sup> cfu each of <i>L. plantarum</i> 299v and <i>B. infantis</i> Cure 21	21 days	There were no significant differences on any measures between the probiotic and placebo groups and the authors concluded that, "The current study failed to confirm the hypothesis that probiotics improve function in patients with poor pouch function." There was no discussion of any adverse effects of the treatment.
Berggren et al. (2003)	Prospective, randomized, double-blind, placebo-controlled study to evaluate the effect of a fermented oat product containing <i>L. plantarum</i> 299v on children's intestinal function and microbiota	69 apparently healthy children aged 6 months to 3 years Lp299v: 33	1.4x10 <sup>11</sup> cfu <i>L. plantarum</i> 299v	3 weeks	Product-related adverse events were reported for 5 children, 4 in the probiotic group and 1 control: 3 children in the probiotic group developed constipation and one child had regurgitations (which had begun before feeding commenced); one placebo-group child had softer than normal stools. No differences were seen between groups in stool frequency and consistency, flatulence, vomiting, or intestinal pain. <i>L. plantarum</i> 299v was present in the feces of all but one member of the probiotic group and in none of the controls. The level of lactobacilli was significantly higher in the probiotic than in the control group. The authors concluded that "the children tolerated the fermented oat product well."
Bering et al. (2006)	Prospective, randomized, double-blind crossover-design study to assess the effect of <i>L. plantarum</i> 299v on absorption of non-heme iron from a phytate-rich meal	24 apparently healthy women with a mean age of 25 years. All received Lp299v.	1.1x10 <sup>11</sup> cfu <i>L. plantarum</i> 299v	2 days	Iron absorption was found to be significantly higher with ingestion of <i>L. plantarum</i> 299v than with any other condition, and the authors concluded, that "the observed increase in Fe absorption in the present study seems to be attributable to a specific effect of the live <i>L. plantarum</i> 299v. Whether this effect is caused by colonisation of <i>L. plantarum</i> 299v in the intestine, or by production of organic acids during passage through the intestine remains to be established."

**Table 7. Studies of *L. plantarum* 299v in Humans.**

Reference	Study Design & Objective	Subjects	Daily Dose	Duration	Results
Bering et al. (2007)	Prospective, randomized, double-blind, placebo-controlled crossover study to test the effect of lyophilized <i>L. plantarum</i> 299v on absorption of non-heme iron	18 apparently healthy women with a mean age of 22 years. All received Lp299v.	10 <sup>11</sup> cfu viable lyophilized <i>L. plantarum</i> 299v	2 days	Iron absorption was found to be no higher with ingestion of viable lyophilized <i>L. plantarum</i> 299v than without. The authors suggested that the lack of effect of the probiotic could be explained by the bacteria not being in an active state. A side study supported this hypothesis by showing that the metabolic activity of lactic acid bacteria added to gruel in lyophilized form was retarded by about 1 hour, "which potentially could affect the metabolic activity of the bacteria in the duodenum."
Bukowska et al. (1998)	Prospective, randomized, double-blind, placebo-controlled study to evaluate the effect of <i>L. plantarum</i> 299v on markers of CVD	30 apparently healthy males with a mean age of 42.6 years Lp299v: 15	1x10 <sup>10</sup> cfu <i>L. plantarum</i> 299v	6 weeks	Fibrinogen, total and LDL-cholesterol level decreased significantly in the probiotic group. It was not clear whether the beneficial change could be ascribed to the <i>L. plantarum</i> or the fermented oat fiber. No adverse effects were reported from the ingestion of 1x10 <sup>10</sup> cfu/day of <i>L. plantarum</i> 299v.
Cunningham-Rundles et al. (2000)	Prospective, randomized, double-blind, placebo-controlled study of the effect of <i>L. plantarum</i> 299v on the symptoms of AIDS	15 immunocompromised children (5 males and 10 females) with HIV, aged 11.5 months to 14 years. All received Lp299v.	2x10 <sup>10</sup> cfu <i>L. plantarum</i> 299v	About 1 month	No patient experienced bloating or other symptoms of intolerance, and none had to be withdrawn. Although residence of <i>L. plantarum</i> 299v was established, colonization was not permanent and no bacteria were detected in rectal swabs by the end of the first month after cessation of administration. Mononuclear cells isolated from peripheral blood showed a natural immune response to <i>L. plantarum</i> 299v in 60% of the children. The authors concluded, "The data suggest that <i>L. plantarum</i> 299v may be given safely to the immunocompromised host and may indeed have a positive effect on immune response."

**Table 7. Studies of *L. plantarum* 299v in Humans.**

Reference	Study Design & Objective	Subjects	Daily Dose	Duration	Results
Ducrotte et al. 2012	Prospective, randomized, double-blind, placebo-controlled, multi-center, parallel study of <i>L. plantarum</i> 299v in reduction in the frequency of abdominal pain episodes in IBS	214 patients (151 men and 63 women; mean age 37.5±12.6 years) with IBS Lp299v: 108	10 <sup>10</sup> cfu of <i>L. plantarum</i> 299v	4 weeks	3 patients in the probiotic group and 7 in the placebo group dropped out for non-treatment-related reasons. Frequency of pain episodes was reduced to a significantly greater degree in the 299v group (by 51.9%) than in the placebo group (by 13.6%). Reductions in severity of abdominal pain, stool frequency, bloating, and feeling of incomplete emptying were also significantly greater in the probiotic group. The authors reported that, "No significant side-effect was reported in any group during the 4 wk of treatment. The only adverse event reported was a transient vertigo onset by one of the patients who received <i>L. plantarum</i> 299v (DSM 9843). No change in blood parameters was detected throughout the study."
Goossens et al. 2003	Prospective, randomized, double-blind, placebo-controlled study of the effect of <i>L. plantarum</i> 299v on gut ecology and microbiota	20 apparently healthy adults (9 males and 11 females); mean age = 32.9 years). Lp299v: 10	2x10 <sup>11</sup> cfu <i>L. plantarum</i> 299v	4 weeks	No side effects were reported attributable to the probiotic. Individuals consuming <i>L. plantarum</i> 299v all had the strain in their feces, but the it could be recovered from only one person a week after the end of ingestion. The probiotic group also had increased total lactobacilli compared with the placebo group, but there were no differences in total aerobes, total anaerobes, enterobacteriaceae, spore-forming clostridia, <i>Enterococcus</i> spp., or <i>Bacteroides</i> spp., β-glucosidase or β-glucuronidase activity, endotoxin concentrations, SCFA concentrations, or pH. The authors concluded that "A fermented oatmeal drink containing <i>L. plantarum</i> 299v increases the number of lactobacilli in the faeces of healthy volunteers, but has no influence on other bacterial counts or on metabolic activities... The observed effect of <i>L. plantarum</i> 299v on the intestinal flora appears within 1 week after the start of consumption of the probiotic drink and disappears completely 1 week after cessation of consumption of the drink."

**Table 7. Studies of *L. plantarum* 299v in Humans.**

Reference	Study Design & Objective	Subjects	Daily Dose	Duration	Results
Goossens et al. (2005)	Prospective, randomized, double-blind, placebo-controlled study of the survival of <i>L. plantarum</i> 299v in the GI tract and its effects on fecal microbiota, with and without gastric acid inhibition.	29 apparently healthy volunteers (9 males and 20 females, mean age = 28.5 years). All received Lp299v.	2x10 <sup>11</sup> cfu <i>L. plantarum</i> 299v	2 weeks	No side effects were reported and there were no differences between groups in defecation frequency or stool consistency, nor in fecal pH or concentrations of short-chain fatty acids. Pantoprazole did not affect either the number or variety of bacteria found in fecal samples, nor the increase in lactobacilli that occurred after the initiation of <i>L. plantarum</i> 299v administration. The administered strain was detected in the feces of all participants at the end of administration, but only in one 4 weeks later. The authors concluded that <i>L. plantarum</i> 299v survives passage through the gastrointestinal tract irrespective of gastric acidity.
Goossens et al. (2006)	Prospective, randomized, double-blind, placebo-controlled study to assess the effect of ingestion of <i>L. plantarum</i> 299v on fecal bacterial ecology and mucosal adhesion of bacteria	29 apparently healthy patients (16 males and 13 females with a mean age of 56.9 years) undergoing colonoscopic examination for polyps. Lp299v: 15	2x10 <sup>11</sup> cfu <i>L. plantarum</i> 299v	2 weeks	No side effects were reported, and no differences were seen between groups in reported defecation frequency or stool consistency. Feces from the probiotic group showed increases in clostridia, total lactic-acid bacteria, and lactobacilli, but lactobacilli could be cultured in rectal and ascending-colon biopsies from only 3 and 2 patients, respectively, in the probiotic group. There were no differences in the total bacterial load or the presence of other bacterial species in the feces or mucosa. The authors concluded that " <i>L. plantarum</i> 299v survives passage through the gastrointestinal tract... [and] the probiotic strain did colonize the colonic mucosa to a minor extent."
Hoppe et al. 2015	2 prospective, randomized, single-blind, placebo-controlled, cross-over trials to assess the ability of <i>L. plantarum</i> 299v to improve iron absorption	22 apparently healthy Swedish women of reproductive age, 11 in each trial Lp299v: 11	10 <sup>9</sup> or 10 <sup>10</sup> cfu <i>L. plantarum</i> 299v in trials 1 and 2	4 days	Iron absorption and retention was significantly higher with either 10 <sup>9</sup> or 10 <sup>10</sup> cfu of <i>L. plantarum</i> 299v than with the control fruit drink (28.6±12.5 and 29.1±17.0% vs. 18.5±5.8 and 20.1±6.4%, respectively), but there was no significant difference in iron retention with the two probiotic doses. No adverse effects were reported.

**Table 7. Studies of *L. plantarum* 299v in Humans.**

Reference	Study Design & Objective	Subjects	Daily Dose	Duration	Results
Johansson et al. (1993)	Open-label study of mucosal colonization of various strains of <i>Lactobacillus</i>	13 apparently healthy volunteers (4 males and 9 females) aged 31-56 years. All received Lp299v.	5x10 <sup>9</sup> cfu of each of 19 bacterial strains: <i>L. plantarum</i> 299v; 2 additional <i>plantarum</i> strains; 2 strains each of <i>L. salivarius</i> , <i>L. reuteri</i> , <i>L. jenseni</i> , and <i>L. rhamnosus</i> ; one strain each of <i>L. casei</i> , <i>L. acidophilus</i> , and <i>L. agilis</i> ; and 5 <i>Lactobacillus</i> strains not classified as to species	10 days	5 strains were re-isolated from mucosa both 1 and 11 days after cessation of ingestion— <i>L. plantarum</i> 299v, one other <i>L. plantarum</i> , and one strain each of <i>L. agilis</i> , <i>L. reuteri</i> , and <i>L. rhamnosus</i> . Total counts of lactobacilli increased significantly in the jejunum as compared to baseline, but there were no other significant changes in the jejunal microecology. The rectal mucosa failed to show significant increases in lactobacilli but did have significant decreases in total anaerobic bacteria and gram-negative anaerobic bacteria. These changes were detected both one and 11 days after the end of probiotic administration.
Johansson et al. (1998)	Prospective, randomized, double-blind, placebo-controlled study of the effects of <i>L. plantarum</i> 299v on metabolic endpoints and fecal bacteria	48 apparently healthy adults (11 males and 37 females) with a mean age of 37 years. Lp299v: 26	2x10 <sup>10</sup> cfu <i>L. plantarum</i> 299v	21 days	No participants dropped out and there were no differences in adverse events reported by the 2 groups. Five individuals in each group reported transient nausea or abdominal discomfort. Those receiving the fermented oats and live bacteria experienced a significant increase in stool volume and a decrease in flatulence. They also had significant increases in fecal levels of total carboxylic acids, particularly acetic, propionic, and lactic acid, but no significant change in fecal pH. <i>L. plantarum</i> 299v was found in large numbers in the feces of the test group at weeks 1 and 3, but in only 5 of the 26 individuals 8 days after cessation of ingestion. No other significant changes were seen in the fecal microbiota in comparison with the control group.



**Table 7. Studies of *L. plantarum* 299v in Humans.**

Reference	Study Design & Objective	Subjects	Daily Dose	Duration	Results
Jones et al. 2013	Prospective, randomized, double-blind, placebo-controlled pilot study of effects of <i>L. plantarum</i> 299v on obstructive jaundice during biliary drainage	17 biliary-drainage patients (12 men, 5 women aged 48 to 75 years [median = 52 years]) Lp299v: 5	The daily dose of <i>L. plantarum</i> 299v was not reported	7 days	The findings of the hematological and biochemical analyses were not reported, suggesting that the findings were not remarkable. While trends toward reduced intestinal permeability and reduced TNF p55 receptors with administration of <i>L. plantarum</i> 299v were reported, neither effect reached statistical significance. There were no reports of adverse effects from the probiotic treatment.
Kingamkono et al. (1999)	Prospective, randomized, double-blind, placebo-controlled study of the effects of fermented and unfermented cereal gruel with <i>L. plantarum</i> 299v on the presence of fecal enteric bacteria	151 apparently healthy children aged 6 months to 5 years. Lp299v: 50	The daily dose of <i>L. plantarum</i> 299v was not reported	13 days	The results from the 2 fermented cereals did not appear to differ significantly and the 2 groups were combined to increase statistical power. Nevertheless, the proportion of children in the probiotic and control groups harboring enteric bacteria (campylobacter, salmonella, shigella, <i>E. coli</i> O157, and enterotoxigenic <i>E. coli</i> ) did not differ. There were no reported adverse effects due to consumption of fermented cereals.
Klarin et al. (2005)	prospective, randomized, unblinded study of the ability of <i>L. plantarum</i> 299v to adhere to the gut mucosa of critically ill patients	15 critically ill patients admitted to the ICU, 8 males and 9 females aged 33 to 84 years (mean = 64.6 years). Lp299v: 8	$2 \times 10^{11}$ cfu <i>L. plantarum</i> 299v/day	Duration of stay in the ICU—4-37 days; median = 11 days	All patients tolerated total or partial enteral feeding, and there were no differences in diarrhea, bloating, illness severity, hospital mortality, length of stay in the ICU, or 6-month mortality, nor in levels of C-reactive protein or leukocyte count. Three of eight patients receiving <i>L. plantarum</i> 299v tested positive for the strain in the samples of rectal mucosa taken during the treatment. The authors concluded that " <i>L. plantarum</i> 299v administered to critically ill, antibiotic-treated patients can survive and colonise the gut mucosa, and repeated administration of the bacteria is necessary to obtain this effect."

**Table 7. Studies of *L. plantarum* 299v in Humans.**

Reference	Study Design & Objective	Subjects	Daily Dose	Duration	Results
Klarin et al. (2008)	Prospective, randomized, double-blind, placebo-controlled study of the capacity of <i>L. plantarum</i> 299v to reduce <i>Clostridium difficile</i> -associated disease in critically ill patients	44 ICU patients (26 males and 18 females aged 18-89 years; mean age = 64.7 years) receiving antibiotic therapy. Lp299v: 22	1.6x10 <sup>11</sup> cfu (later 8x10 <sup>10</sup> cfu) <i>L. plantarum</i> 299v/day	Duration of stay in the ICU—2.5-22 days; mean = 5.5 days	Two patients from each group died in the ICU; 1 patient from the probiotic group died in the hospital, and 4 patients from the control group died within 6 months. There were no differences between the groups in sequential organ failure, length of ICU stay, or days on ventilators. In 71 fecal samples from the probiotic group, none tested positive for <i>C. difficile</i> , while 4 emergent cases were found in the 80 samples from control group patients. Control group patients also harbored a number of potential pathogens not found in the probiotic group, including <i>Pseudomonas aeruginosa</i> , <i>Klebsiella pneumoniae</i> , and <i>Bacillus cereus</i> . There were no differences in C-reactive protein, TNF- $\alpha$ , IL-1 $\beta$ , or IL-6; IL-10 and white blood cell counts were higher in the control group than in patients receiving <i>L. plantarum</i> 299v. Gut permeability was higher in the control group than in the probiotic group. The study product was well tolerated and the authors stated that "We found no adverse impact of the given probiotic preparation."
Krag et al. 2012	Prospective open-label study of treatment of ulcerative colitis with <i>L. plantarum</i> 299v	39 ulcerative colitis patients (15 males and 24 females aged 19-50 years [median age – 35 years]) Lp299v: 39	2.5x10 <sup>10</sup> cfu for 2 days, then 5x10 <sup>10</sup> cfu thereafter	Up to 176 days	The treatment significantly reduced the severity of the disease by 56.5%. The authors reported: "No major AEs were reported and there were no dropouts due to AEs. An increased number of bowel movements were reported by 11 patients (28%), bloating by four (10%) and an increased number of bowel movements and bloating by three (8%). All AEs were self-limiting or managed by dose adjustments. For example, if a patient experienced a presumable AE during the introduction of Profermin®, the period with the low Profermin® dose was prolonged for up to 2 wk. None of the eight dropout or four excluded patients left the trial due to deterioration in UC symptoms."

**Table 7. Studies of *L. plantarum* 299v in Humans.**

Reference	Study Design & Objective	Subjects	Daily Dose	Duration	Results
Krag et al. 2013	Prospective randomized single-blind two-arm study comparing Profermin® and Fresubin as treatments for ulcerative colitis	73 ulcerative-colitis patients (33 males and 40 females aged 20-78 years; mean age 41 years) Lp299v: 32	Median = $4.89 \times 10^{10}$ cfu of <i>L. plantarum</i> 299v		carried out a similar study as a, an accepted treatment. (In a departure from the usual single blinding, the staff and statisticians were blinded but the patients were not, because the two products are different in appearance and texture.) In this study, were enrolled, 32 assigned to received Profermin® and 41 to receive Fresubin. The median dose of .Profermin® was 489 ml/day, providing. Treatment with Profermin® was significantly more effective in reducing the severity of ulcerative colitis than was Fresubin. The authors reported that, "No major adverse events (AEs) were reported, but 3 patients experienced AEs. In the Fresubin group, one experienced an 'obvious weight gain' and one felt it induced vomiting. In the Profermin group, one suffered from rumbling and bloating." They concluded that, "Supplementation with Profermin is safe, well tolerated, palatable."
Kujawa-Szewieczek et al. 2015	Retrospective open-label study of the use of <i>L. plantarum</i> 299v to reduce the incidence of <i>Clostridium difficile</i> infection	356 organ transplant patients, 174 before <i>L. plantarum</i> 299v and 182 after Lp299v: 182	$10^9$ cfu <i>L. plantarum</i> 299v	Not reported	Of these patients, 21 in the first year and 2 in the second year were diagnosed with <i>C. difficile</i> infection, infection rates of 12.1 and 1.1%, respectively. No adverse effects were reported due to probiotic treatment.
Ladas et al. 2015	Prospective open-label multi-center pilot study of safety and efficacy of prophylactic use of <i>L. plantarum</i> 299v in children and adolescents undergoing hematopoietic cell transplantation	30 children and adolescents (16 males and 14 females aged $7.7 \pm 4.7$ years; the age range was 2.2 to 17.3 years) Lp299v: 30	$10^8$ cfu <i>L. plantarum</i> 299v/kg bw/day	21 days	The incidence of graft-versus-host disease was 30%, lower than is usually encountered. No episodes of <i>L. plantarum</i> bacteremia were observed. The authors reported that, "We did not observe any serious adverse events or unexpected severe adverse events attributed to [ <i>L. plantarum</i> ] in any patient enrolled to the study." The authors concluded that "Our study provides preliminary evidence that administration of [ <i>L. plantarum</i> ] is safe and feasible in children and adolescents undergoing [hematopoietic cell transplantation]."

**Table 7. Studies of *L. plantarum* 299v in Humans.**

Reference	Study Design & Objective	Subjects	Daily Dose	Duration	Results
Lonnermark et al. (2010)	Prospective, randomized, double-blind, placebo-controlled study of the effect of <i>L. plantarum</i> 299v on GI symptoms during antibiotic therapy	239 patients (93 males and 146 females; median age = 45 years) receiving antibiotic therapy for infectious disease. Lp299v: 80	1x10 <sup>10</sup> cfu <i>L. plantarum</i> 299v/day	Until 7 days after the end of antibiotics	76 patients withdrew or were excluded from the study, 38 each from the probiotic and placebo groups; reasons for withdrawal did not differ between the groups. Diarrhea was infrequent (only 5 and 6 patients in the placebo and probiotic groups, respectively). The incidence of loose or water stools but not meeting the criteria for diarrhea and the incidence of nausea were lower in the group receiving <i>L. plantarum</i> 299v than in the control group. The authors reported that "No side effects of the treatment were recorded."
Lonnermark et al. 2015	Prospective, randomized, double-blind, placebo-controlled, multi-center study of the ability of <i>L. plantarum</i> 299v to treat <i>Salmonella</i> infection	149 patients with <i>Salmonella</i> infections (40 males and 109 females aged 5 to 68 years; median age = 36 years) Lp299v: 77	5x10 <sup>10</sup> cfu <i>L. plantarum</i> 299v	Median of 26 days	The authors reported a non-statistically significant tendency for a greater number of gastrointestinal symptoms to be reported by patients consuming <i>L. plantarum</i> 299v. The conclusion of the authors was that, "Our results give little support for positive effects of <i>L. plantarum</i> 299v treatment in nontyphoid salmonellosis."
Mangell et al. 2012	Prospective, randomized, double-blind, placebo-controlled trial of the effect of prophylactic <i>L. plantarum</i> 299v on pathogenic bacteria, translocation, and cell proliferation in colon surgery	64 patients (36 males and 28 females aged 64 to 80 years; median age = 72 years) referred for colonic resection Lp299v: 32	10 <sup>11</sup> cfu <i>L. plantarum</i> 299v	14 days	No benefit was obtained from administration of <i>L. plantarum</i> 299v to colon surgery patients; there were no differences between groups in the incidence of enteric pathogenic bacteria, bacterial translocation, or postoperative complications. The authors noted that, "No adverse effects were recorded after the administration of high doses of <i>L. plantarum</i> 299v."

**Table 7. Studies of *L. plantarum* 299v in Humans.**

Reference	Study Design & Objective	Subjects	Daily Dose	Duration	Results
McNaught et al. (2002)	Prospective, randomized, unblinded study to test if <i>L. plantarum</i> 299v administered before and after abdominal surgery reduces the incidence of sepsis	129 patients (75 males and 54 females with median age = 68 years). Lp299v: 64	2.5x10 <sup>10</sup> cfu <i>L. plantarum</i> 299v/day	median = 2 weeks	No differences were seen between the probiotic and control groups in bacterial translocation to the lymph nodes or ileal serosa, gastric colonization, C-reactive protein levels, septic complications, or mortality. The authors concluded that "preoperative administration of the probiotic <i>Lactobacillus plantarum</i> 299v for two weeks has no effect [either beneficial or adverse] on the human gut mucosal barrier ... and the systemic inflammatory response."
McNaught et al. (2005)	Prospective, randomized, unblinded study of the effect of <i>L. plantarum</i> 299v on gut barrier function and systemic inflammatory response in critically ill patients	103 patients (58 males and 45 females aged 28-90 years; median age = 71 years) within 24 hours of admission to the ICU. Lp299v: 52	10 <sup>10</sup> cfu <i>L. plantarum</i> 299v/day	Until discharge from the hospital—3-17 days; median = 9 days	<i>L. plantarum</i> 299v was cultured from gastric aspirates of 26% of probiotic-group patients at day 34 and 33% at day 8; there were no other differences between the 2 groups in the numbers or species of detected microorganisms, in intestinal permeability, or in levels of IgM or C-reactive protein, but IL-6 levels were lower in the probiotic group than in the controls. The mortality rate was 35% in both groups. 68 septic complications occurred; there were no differences in incidence, causes, or severity between test patients and controls. The authors concluded that "the results of this prospective randomised trial suggest that <i>Lactobacillus plantarum</i> 299v may attenuate the systemic inflammatory response in critically ill patients. This was not accompanied, however, by any significant changes in gastrointestinal microflora, endotoxin exposure, intestinal permeability, septic morbidity or mortality."

**Table 7. Studies of *L. plantarum* 299v in Humans.**

Reference	Study Design & Objective	Subjects	Daily Dose	Duration	Results
Naruszewicz et al. (2002)	Prospective, randomized, double-blind, placebo-controlled trial of the ability of <i>L. plantarum</i> 299v to reduce symptoms of CVD risk factors in smokers	36 apparently healthy 25-45-year-old smokers (18 of each sex). Lp299v: 18	2x10 <sup>10</sup> cfu <i>L. plantarum</i> 299v/day	6 weeks	No adverse events were reported; <i>L. plantarum</i> 299v was found in fecal samples from 13 of the 18 members of the probiotic group. The probiotic group had lower systolic blood pressure compared with before intake. No differences were apparent in total cholesterol, triacylglycerol, or lipoprotein(a), but HDL levels increased in the probiotic group while leptin and insulin concentrations decreased; only the leptin change was statistically significant. There were decreases in F2-isoprostanes, IL-6, and fibrinogen concentrations among smokers ingesting <i>L. plantarum</i> 299v, as well as the adherence capability of monocytes. All of the biochemical changes attributed to the probiotic intervention were regarded as beneficial; no adverse changes were observed.
Niedzielin et al. (2001)	Prospective, randomized, double-blind, placebo-controlled trial of the effect of <i>L. plantarum</i> 299v on IBS patients	40 IBS patients (8 males and 32 females aged 27-63 years, mean = 45 years). Lp299v: 20	2x10 <sup>10</sup> cfu <i>L. plantarum</i> 299v/day	4 weeks	The patients receiving <i>L. plantarum</i> 299v showed significantly greater improvement in their IBS symptoms than did the placebo group, and the authors noted that "No treatment related side-effects were observed."
Nobaek et al. (2000)	Prospective, randomized, double-blind, placebo-controlled trial of the effect of attempted alteration of the gastrointestinal microecology of IBS patients with <i>L. plantarum</i> 299v	52 adult patients with IBS. Lp299v: 25	2x10 <sup>10</sup> cfu <i>L. plantarum</i> 299v/day	4 weeks	<i>L. plantarum</i> 299v was found in the fecal samples from 84% of the test group and in 32% of their rectal biopsies, but there were no changes or differences between test and control groups in other bacterial counts. Flatulence decreased in the test group while defecation frequency and self-assessed overall gastrointestinal function improved. One year later, patients in the test group still had significantly better function than at study entry while those in the placebo group had no improvement. The authors noted that the products were well tolerated and no treatment-related adverse effects were reported from ingestion of 2x10 <sup>10</sup> cfu/day of <i>L. plantarum</i> 299v for 4 weeks.

**Table 7. Studies of *L. plantarum* 299v in Humans.**

Reference	Study Design & Objective	Subjects	Daily Dose	Duration	Results
Onning et al. (2003)	Prospective, randomized, double-blind, placebo-controlled study of the effect of a test beverage with <i>L. plantarum</i> 299v on plasma antioxidant capacity and fecal bacteria	98 volunteers with a high working pace (39 men and 59 women aged 21-61 years [mean age = 35 years] Lp299v: 50	2.2x10 <sup>10</sup> cfu <i>L. plantarum</i> 299v/day	4 weeks	There was no difference between the test and control groups in the incidence or nature of adverse effects and there were no adverse events that could reasonably be attributed to ingestion of <i>L. plantarum</i> 299v.
Oudhuis et al (2011)	Prospective randomized open-label trial comparing the effects of <i>L. plantarum</i> 299/299v and decontamination of the digestive tract in reducing infection rates in ICU patients	254 ICU patients (157 males and 97 females aged 17 to 90 years; mean age = 62.7 years). Lp299v: 130	5x10 <sup>9</sup> cfu <i>L. plantarum</i> 299v/day	Duration of the stay in the ICU; mean = 11 days	There were no differences between the 2 groups in length of ICU or hospital stay, need for mechanical ventilation, or mortality. Although there was a "tendency" toward more infections in patients receiving probiotic therapy that in those receiving antibiotics, this difference was not statistically significant. No adverse effects were reported from the probiotic treatment.
Ribeiro and Vanderhoof (1998)	Prospective, randomized, single-blind placebo-controlled trial of the ability of <i>L. plantarum</i> 299v to reduce the incidence of infective diarrhea among children	143 children aged 6 months to 3 years attending daycare in a region of Brazil with a high incidence of infectious diarrhea. Lp299v: 71	10 <sup>10</sup> cfu <i>L. plantarum</i> 299v/day	3 months	Reductions in the incidence of diarrhea and respiratory infections were seen in both the test and control groups, with no differences between groups. The authors speculated that colonization of half of the children in the daycare setting with <i>L. plantarum</i> may have reduced the dissemination of infectious diseases. No adverse effects were reported from the ingestion of 10 <sup>10</sup> cfu/day of <i>L. plantarum</i> 299v by these young children.

**Table 7. Studies of *L. plantarum* 299v in Humans.**

Reference	Study Design & Objective	Subjects	Daily Dose	Duration	Results
Sawant et al. (2010)	Prospective, randomized, double-blind, placebo-controlled, multi-center study of the capacity of <i>L. plantarum</i> 299v to reduce symptoms of IBS	200 IBS patients (141 males and 59 females; mean age = 37.8 years). Lp299v: 98	10 <sup>10</sup> cfu <i>L. plantarum</i> 299v/day	4 weeks	Patients ingesting the probiotic showed improvement in all assessed symptoms as well as in overall assessment as compared with the control group. No changes were observed in pulse or respiratory rates, blood pressure, or body temperature, and no side effects were reported.
Sen et al. (2002)	Prospective, double-blind, placebo-controlled crossover trial of the effect of <i>L. plantarum</i> 299v on colonic fermentation of IBS patients	12 (1 male and 11 female aged 23-61 years, mean age = 40.6 years) gastroenterologic IBS outpatients. All received Lp299v.	6.3x10 <sup>9</sup> cfu <i>L. plantarum</i> 299v/day	4 weeks	All subjects started with the placebo product. No difference was seen between the groups on any measure: exhalation of hydrogen and methane during calorimetry, breath hydrogen after lactulose ingestion, or daily symptom scores. The authors concluded that " <i>Lactobacillus plantarum</i> 299v in this study did not appear to alter colonic fermentation."
Stevenson et al. 2014	Prospective, randomized, double-blind, placebo-controlled trial of the effect of <i>L. plantarum</i> 299v on IBS patients	81 IBS patients (2 males and 79 females aged 47.9±12.1 years) Lp299v: 54	10 <sup>10</sup> cfu <i>L. plantarum</i> 299v/day	8 weeks	Patients in both groups showed significantly reduced reported pain, but there was no difference between groups and the authors concluded that "No significant beneficial effects by the probiotic were seen on the severity of symptoms (abdominal pain) and quality of life." At the same time, there was no difference between groups in compliance and "the rate of AEs was very low. The tolerability of the test product was good."



**Table 7. Studies of *L. plantarum* 299v in Humans.**

Reference	Study Design & Objective	Subjects	Daily Dose	Duration	Results
Stjernquist-Desatnik et al. (2000)	3 open label experiments on the ability of <i>L. plantarum</i> 299v to colonize tonsillar epithelia	1 <sup>st</sup> experiment: 6 adults aged 33-42 years (1 man and 5 women, mean age = 38 years);  2 <sup>nd</sup> experiment: 2 women aged 41 and 42 years;  3 <sup>rd</sup> experiment: same 2 women All received Lp299v.	1 <sup>st</sup> experiment: 1 dose of $2 \times 10^{11}$ cfu <i>L. plantarum</i> 299v;  2 <sup>nd</sup> experiment: 1 dose of $10^{11}$ cfu <i>L. plantarum</i> 299v;  3 <sup>rd</sup> experiment: 1 dose of $10^{10}$ cfu <i>L. plantarum</i> 299v	Single doses	In the 1 <sup>st</sup> experiment, all 6 volunteers had detectable levels of <i>L. plantarum</i> 299v on their tonsillar epithelia after gargling and ingestion and all 6 had the bacteria present at 4 hours; however, only 1 person still had detectable levels at 8 hours after intake. Similarly, both women had <i>L. plantarum</i> 299v on their tonsillar epithelia after ingesting fermented gruel mixed with fruit juice and for 4 hours thereafter, but only intake of $10^{11}$ cfu resulted in detectable levels remaining at 80 hours. The authors concluded that, since the bacteria could be isolated from tonsillar epithelia up to 8 hours after ingestion despite the constant flow of saliva and beverages over the tonsils, "the bacteria under investigation may possess the capacity to adhere to tonsillar cells."
Vanderhoof et al. (1998)	Case study of short-bowel patient with small-bowel bacterial overgrowth	7-year-old boy. Received Lp299v	$10^{10}$ cfu <i>L. plantarum</i> 299v/day	2 months	Within 2-3 weeks stool consistency improved, primarily in reduction of water content. No adverse effects were noted due to the probiotic therapy. It is noteworthy that no indication of D-lactic acidosis was reported in this short-bowel patient receiving <i>L. plantarum</i> .
Vanderhoof et al. (1998)	Case study of short-bowel patient with small-bowel bacterial overgrowth	16-year-old boy Received Lp299v	$10^{10}$ cfu <i>L. plantarum</i> 299v/day	Not reported	Replacement of the antibiotic with <i>L. plantarum</i> 299v produced good therapeutic response with no reported adverse effects. No indication of D-lactic acidosis was reported in this short-bowel patient receiving <i>L. plantarum</i> .
Woodcock et al. (2004) [further analysis of participants in McNaught et al. (2002)]	Prospective, randomized, unblinded study of the effect of <i>L. plantarum</i> 299v on gut immune function of patients receiving abdominal surgery	22 patients (10 males and 12 females with median age = 69 years) undergoing small-bowel resection Lp299v: 11	$2.5 \times 10^{10}$ cfu <i>L. plantarum</i> 299v/day	median = 2 weeks	There were no differences between the probiotic and control groups in numbers of plasma cells or either IgA- or IgM-positive cells, or in mucosal-surface IgA levels, but the concentration of IgM was reduced in the group receiving <i>L. plantarum</i> 299v. The authors concluded that there is no evidence from this study that administration of the probiotic has any effect on gut-associated lymphoid tissue, an important component of the gut barrier.

**Table 7. Studies of *L. plantarum* 299v in Humans.**

Reference	Study Design & Objective	Subjects	Daily Dose	Duration	Results
Wullt et al. (2003 ) Wullt et al. (2007)	prospective, randomized, double-blind, placebo-controlled study of the ability of <i>L. plantarum</i> 299v to reduce the likelihood of further recurrent episodes of <i>Clostridium difficile</i> -associated diarrhea	21 patients (1 male and 20 females; mean age = 63.8 years) testing positive for <i>C. difficile</i> toxin and having a history of previous <i>C. difficile</i> -associated diarrhea. Lp299v: 12	5x10 <sup>10</sup> cfu of <i>L. plantarum</i> 299v/day	38 days	There was a statistically insignificant reduction in the risk of recurrence among the patients receiving <i>L. plantarum</i> 299v, and the authors noted that "Treatment with the lactobacilli had no apparent side-effects."

#### 6.4. Evaluations by Authoritative Bodies

Noting that a wide variety of microbial species are used in food, some with a long history of apparent safe use, and facing the need to set priorities for risk assessment, the European Food Safety Authority (EFSA) proposed a system referred to as “Qualified Presumption of Safety” (QPS; EFSA 2007a, 2007b). This system proposed basing the safety assessment of a defined taxonomic group (e.g., a genus or a species) on 4 pillars: established identity, body of knowledge, possible pathogenicity, and end use. If the taxonomic group did not raise safety concerns or, if safety concerns existed, but could be defined and excluded, the grouping could be granted QPS status. Thereafter, “any strain of microorganism the identity of which could be unambiguously established and assigned to a QPS group would be freed from the need for further safety assessment other than satisfying any qualifications specified” (EFSA 2007a, p1).

EFSA’s Scientific Committee was asked to recommend organisms regarded as suitable for QPS status. The list of such organisms proposed by the Committee included *L. plantarum*. In listing *L. plantarum* and other species of *Lactobacillus* as suitable for QPS status, the Committee stated, “Where QPS status is proposed, the Scientific Committee is satisfied that the body of knowledge available is sufficient to provide adequate assurance that any potential to produce adverse effects in humans, livestock or the wider environment is understood and capable of exclusion” (EFSA 2007a, p8) and that the recommendations are “based on a thorough review of the available scientific literature and the knowledge and experience of the scientists involved” (EFSA 2007a, p8).

It is also to be noted that EFSA has issued annual updates of the QPS status of bacterial strains from 2008 through 2016; no need for either review or change of the QPS status of *L. plantarum* was reported (EFSA 2008b, 2009, 2010, 2011, 2012b, 2013, 2014, 2015a, 2015b, 2016).

## 6.5. Safety Assessment and GRAS Determination

### 6.5.1. Introduction

This section presents an assessment that demonstrates that the addition of *Lactobacillus plantarum* strain 299v to conventional foods and dietary supplements as probiotic bacteria under the conditions of use described is safe and is GRAS based on scientific procedures.

This safety assessment and GRAS determination entail two steps. In the first step, the safety of the intended use of *L. plantarum* 299v is demonstrated. Safety is established by demonstrating a reasonable certainty that the exposure of adults, children, and infants to *L. plantarum* 299v under its intended conditions of use is not harmful. In the second step, the intended use of *L. plantarum* 299v is determined to be GRAS by demonstrating that the safety of this product under its intended conditions of use is generally recognized among qualified scientific experts and is based on publicly available and accepted information.

The regulatory framework for establishing whether the intended use of a substance (or organism) is GRAS, in accordance with Section 201(s) of the Federal Food Drug and Cosmetic Act, is set forth under 21 CFR §170.30. This regulation states that general recognition of safety may be based on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. A GRAS determination may be made either: 1) through scientific procedures under §170.30(b); or 2) through experience based on common use in food, in the case of a substance used in food prior to January 1, 1958, under §170.30(c). This GRAS determination employs scientific procedures established under §170.30(b).

A scientific procedures GRAS determination requires the same quantity and quality of scientific evidence as is needed to obtain approval of the substance as a food additive. In addition to requiring scientific evidence of safety, a GRAS determination also requires that this scientific evidence of safety be generally known and accepted among qualified scientific experts. This “common knowledge” element of a GRAS determination consists of two components:

1. data and information relied upon to establish the scientific element of safety must be generally available; and
2. there must be a basis to conclude that there is a consensus among qualified experts about the safety of the substance for its intended use.

The criteria outlined above for a scientific-procedures GRAS determination are applied below in an analysis of whether the addition of *L. plantarum* 299v to foods and dietary supplements is safe and is GRAS.

### 6.5.2. Safety Evaluation

The body of evidence supporting the safety of oral administration of *Lactobacillus* strains in general, and *L. plantarum* 299v in particular, is large and convincing. Numerous commentators—in addition to authoritative bodies such as EFSA—have noted the safe history of human ingestion of *L. plantarum* over many hundreds of years. *L. plantarum* produces no deleterious metabolites and is not destructive of mucin. It has the capacity to downregulate proinflammatory cytokines. Any effects that this probiotic microorganism has on intestinal permeability appear to be beneficial in strengthening barrier function. While it is theoretically possible for biogenic amines to be produced as a result of fermentation of dairy products by lactobacilli, this phenomenon has not been observed and, when *Lactobacillus* strains are ingested as probiotics, they produce lactic acid, lowering the intestinal pH and reducing the opportunity for production of harmful biogenic amines by putrefactive bacteria.

Lactobacilli are not regarded as pathogens, although some strains are capable of opportunistic infection in extremely favorable circumstances invariably involving severe underlying disease states and most often also involving a facilitated pathway such as surgical intervention or the presence of central lines. Documented cases of *Lactobacillus* bacteremia are so rare, in comparison to the widespread use of *Lactobacillus* strains in the environment, in food production, and in probiotic applications, that the participants in the 2007 EU-PROSAFE project (Vankerckhoven et al. 2008) suggested that “they are more medical exceptions, or even curiosities, than a genuine public health issue.”

Consumption of live lactic acid bacteria included in lactic-acid-fermented foods has been a regular part of the food intake of humans for a long time and it is evident that individuals consuming lactic-acid-fermented products of plant origin also consume large amounts of *L. plantarum*. Furthermore, *L. plantarum* frequently occurs on the human gastrointestinal mucosa from the mouth to the rectum (Molin et al. 1993; Ahrne et al. 1998).

The strain *L. plantarum* 299v originates from human intestinal mucosa (Molin et al. 1993; Ahrne et al. 1998) and is sold as a dietary supplement in about 20 countries in Europe, North America, and Asia. Since 1994, products containing *L. plantarum* 299v have been consumed in millions of daily doses by people worldwide without any adverse events.

The safe history of human exposure to *L. plantarum* strains is strongly supported by a large body of published research. In addition to *in vitro* work, the published literature includes 20 experimental studies in several strains of rats, mice, and pigs, most often as models of acute liver injury, endocarditis, acute pancreatitis, enterocolitis, or other conditions, as well as 40 studies in human adults and children; 1502 individuals aged 6 months to 90 years have received the probiotic at daily dose levels up to  $2 \times 10^{11}$  cfu for as long as 90 days. In studies of healthy adults, 250 participants received *L. plantarum* 299v at daily levels from  $5 \times 10^8$  to  $2 \times 10^{11}$  cfu for between 1 and 42 days; 154 healthy children received the probiotic at levels of  $10^{10}$  to  $1.4 \times 10^{11}$  cfu/day for 13 to 90 days. The bacterium appears to have the capability to establish short-term residence in the intestinal mucosa, but it is rarely isolated in feces a week after administration has ended. No adverse effects were reported in any studies of healthy adults or children.

In the 20 studies reported in the literature in which *L. plantarum* 299v was administered to compromised adults, 1068 individuals with IBS, *C. difficile* infection, or severe illness requiring ICU placement received the probiotic at levels up to  $2 \times 10^{11}$  cfu/day for durations

ranging from a few days to 56 days. Administration of the probiotic was generally regarded as beneficial, and no trial resulted in any adverse effect on these severely compromised patients.

In one study (Cunningham Rundles et al. 2000), *L. plantarum* 299v was given for about a month to children infected with HIV. No adverse effects were reported in these children, who have very weak immune defense; they exhibited a conserved immunological response to *L. plantarum* 299v, which is evidence of the harmlessness of the strain, even in individuals with poor immune competence.

All of the available evidence demonstrates clearly that there is no reason to suspect harm to individuals consuming conventional foods or dietary supplements containing *Lactobacillus plantarum* 299v.

### 6.5.3. General Recognition of the Safety of *L. plantarum* 299v

The intended use of *L. plantarum* 299v, to be added as a probiotic to a variety of conventional foods and dietary supplements, has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b). This safety was shown by establishing the identity and probiotic characteristics of the strain, demonstrating its freedom from pathogenic or other risk factors, and concluding that the expected exposure to *L. plantarum* 299v by adults and children is without significant risk of harm. Finally, because this safety assessment satisfies the common knowledge requirement of a GRAS determination, this intended use can be considered GRAS.

Determination of the safety and GRAS status of the addition of *L. plantarum* 299v to food and dietary supplements has been made through the deliberations of an Expert Panel consisting of Joseph F. Borzelleca, Ph.D., James T. Heimbach, Ph.D., and Michael W. Pariza, Ph.D., who reviewed a monograph prepared by Probi AB and Dr. Heimbach as well as other information available to them. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients, including probiotic bacteria. They independently critically reviewed and evaluated the publicly available information and the potential human exposure to *L. plantarum* 299v anticipated to result from its intended uses, and individually and collectively determined that no evidence exists in the available information on *L. plantarum* 299v that demonstrates, or suggests reasonable grounds to suspect, a hazard to either adults or children under the intended conditions of use of *L. plantarum* 299v.

The Expert Panel applied a recently developed decision tree (Pariza et al. 2015) to the intended use of *L. plantarum* 299v as follows:

1. Has the strain been characterized for the purpose of assigning an unambiguous genus and species name using currently accepted methodology? YES
2. Has the strain genome been sequenced? YES
3. Is the strain genome free of genetic elements encoding virulence factors and/or toxins associated with pathogenicity? YES
4. Is the strain genome free of functional and transferable antibiotic resistance gene DNA? YES
5. Does the strain produce antimicrobial substances? NO
6. Has the strain been genetically modified using rDNA techniques? NO
7. Was the strain isolated from a food that has a history of safe consumption for which the species, to which the strain belongs, is a substantial and characterizing component (not simply an 'incidental isolate')? NO (THE STRAIN WAS ISOLATED FROM HEALTHY HUMAN INTESTINAL MUCOSA)

8. Does the strain induce undesirable physiological effects in appropriately designed safety evaluation studies? NO

The outcome of this decision-tree analysis is a confirmation that “the strain is deemed to be safe for use in the manufacture of food, probiotics, and dietary supplements for human consumption” (Pariza et al. 2015).

It is the Expert Panel’s opinion that other qualified scientists reviewing the same publicly available data would reach the same conclusion regarding the safety of the strain under its intended conditions of use. Therefore, the intended use of *L. plantarum* 299v is GRAS by scientific procedures. A signed copy Expert Panel's conclusion is attached at Appendix A.

## Part 7 - List of Supporting Data and Information

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**GENERALLY RECOGNIZED AS SAFE (GRAS)  
DETERMINATION FOR THE USE OF  
*LACTOBACILLUS PLANTARUM* STRAIN 299V  
IN CONVENTIONAL FOODS:  
CONCLUSION OF THE EXPERT PANEL**

**Prepared for:  
Probi AB  
Lund, Sweden**

**05 December 2016**

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We, the members of the Expert Panel, have individually and collectively critically evaluated the publicly available information on *Lactobacillus plantarum* strain 299v summarized in a monograph prepared by Probi AB and JHEIMBACH LLC, as well as other material deemed appropriate or necessary. Our critical evaluation included review of the identity and characteristic properties of *L. plantarum*, including *L. plantarum* strain 299v, the potential exposure resulting from the intended use of *L. plantarum* strain 299v, and published research bearing on the safety of *L. plantarum* and strain 299v. A teleconference involving the members of the Expert Panel (Professors Joseph F. Borzelleca and Michael W. Pariza), the technical advisor to the Expert Panel (Dr. James Heimbach) and Mark M. Yacura (counsel to Probi AB) was held on 05 December 2016. The Expert Panel presented a summary of its findings (which appears below) and the basis for its unanimous opinion (which also appears below) on the GRAS determination of the proposed uses of *Lactobacillus plantarum* strain 299v.

**Summary**

- The probiotic bacterium that is the subject of this generally recognized as safe (GRAS) determination is *L. plantarum* strain 299v, a Gram-positive and non-spore-forming heterofermentative lactic acid bacterium also known commercially as Plantarum 299v and Lp299v; it has been sold as a component of the functional food product ProViva® since 1994.
- *L. plantarum* strain 299v is intended to be added as a probiotic to foods that can sustain viability of the organism, including but not limited to wet chilled products such as fruit drinks and yogurts; dry chilled products; dry and shelf-stable products such as cereals, candy, bars, cookies, gums, and confectionery; and delivery systems designed for bacterial stability at room temperature at concentrations consistent with cGMP needed to provide up to  $10^{10}$  cfu/serving throughout the shelf life of the product. The estimated daily intake of the strain is less than  $10^{11}$  cfu/day.
- *L. plantarum* strain 299v was isolated from healthy intestinal mucosa and was deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) and referenced as DSM 9843..
- *Lactobacillus* is a non-pathogenic genus consisting of over a hundred species with a large variety of phenotypic, biochemical, and physiological properties. *L. plantarum* commonly occurs in spontaneously fermented plants such as sauerkraut, pickles, and kimchi, and so is

ubiquitous in the GI tracts of both humans and infra-human mammalian species and has long been ingested during normal food-consumption activities with no apparent adverse effects.

- No genes encoding for antibiotic resistance, nor any sequences showing significant homology with known antibiotic resistance genes, were identified in *L. plantarum* strain 299v. This finding is consistent with phenotypic testing that showed that the strain has no resistance not found in most other members of the species and thus regarded as intrinsic rather than acquired. As a result, no antimicrobial resistance is likely to be transferable from *L. plantarum* strain 299v.
- *L. plantarum* strain 299v produces only lactic acid and acetic acid, neither at levels presenting a potential hazard. Lactic acid produced by *L. plantarum* strain 299v consists of approximately 62% D-lactate and 38% L-lactate. While humans do not metabolize D-lactate as readily as L-lactate, it has been shown in several studies that ingestion of D-lactate-producing bacteria does not result in increased risk of D-lactic acidosis.
- Production of *L. plantarum* strain 299v is based on standard fermentation techniques, and all components of the fermentation medium are approved food-grade materials. Probi AB has provided appropriate food-grade specifications for *L. plantarum* strain 299v.
- Published studies in which *L. plantarum* strain 299v was administered to rats, mice, and pigs, most often as models of acute liver injury, endocarditis, acute pancreatitis, enterocolitis, irritable bowel syndrome, or other conditions, at doses up to  $10^{10}$  cfu/day, produced no adverse effects. The animal studies confirmed *in vitro* findings that *L. plantarum* strain 299v tends to downregulate proinflammatory cytokines.
- The safety of *L. plantarum* strain 299v at daily doses up to  $2 \times 10^{11}$  cfu for as long as 90 days was also reported in 37 studies with human adults and children, both healthy and compromised with HIV, irritable bowel syndrome, *C. difficile* infection, ulcerative colitis, small-bowel resection, or other serious conditions. A total of 1,501 individuals ranging in age from 6 months to 90 years ingested the probiotic in these studies. The bacterium appeared to establish short-term residence in the intestinal mucosa, but was rarely isolated in feces a week after administration ended. No adverse effects were reported in any of these studies.
- The European Food Safety Authority (EFSA) classified *L. plantarum* as an organism having a Qualified Presumption of Safety (QPS) and thus being “freed from the need for further safety assessment.” This conclusion has been maintained through all annual reappraisals to date.
- An analysis of the strain using a recently developed decision tree, addressing issues regarding the strain’s genome, potential virulence or pathogenicity, transferable antibiotic resistance, and induction of undesirable physiological effects in safety studies, supports the safety of the intended use of *L. plantarum* strain 299v.

## Conclusion

We, the undersigned members of the Expert Panel, are qualified by scientific education and experience to evaluate the safety of the addition of probiotic bacteria to conventional foods. We have individually and collectively critically evaluated the materials summarized above and discussed our conclusion among ourselves.

We recognize that *Lactobacillus* species have a long history of safe use and are appropriately regarded as non-pathogenic and non-toxicogenic. We conclude that *Lactobacillus plantarum* strain 299v has been adequately identified and characterized and that both phenotypic and genotypic research confirm that no concerns exist regarding the safety of ingestion of this probiotic bacterium at levels up to  $10^{11}$  cfu/day. We have applied a decision-tree analysis that confirms the safety of the strain. Therefore, we conclude that addition of *L. plantarum* strain 299v to conventional foods, at concentrations consistent with cGMP needed to provide up to  $10^{10}$  cfu/serving throughout the shelf life of the product, is safe. We further conclude that the intended use of *L. plantarum* strain 299v is GRAS based on scientific procedures

It is also our opinion that other qualified and competent scientists reviewing the same publicly available information would reach the same conclusion.

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Signature: \_\_\_\_\_

Date: 05 December 2016

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Date: December 5, 2016



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