
Lactobacillus plantarum 299v

CONTENTS

- Consumption of live lactic acid bacteria (probiotics) – p. 2
- Lactic acid bacteria, *Lactobacillus*, *Lactobacillus plantarum* and strain 299v – p. 2
- The concept, lactic acid bacteria – p. 2
 - The species, *Lactobacillus plantarum* – p. 3
 - The bacterial strain, *Lactobacillus plantarum* 299v – p. 4
- ProViva and lactic acid fermented oatmeal beverage – p. 5
- Beneficial health effects – p. 6
- Intestinal microflora – p. 6
 - Probiotics and the bacterial balance – p. 6
 - Intestinal mucosal status and reduced translocation – p. 9
 - Risk factors for coronary artery disease – p. 10
 - Irritable bowel syndrome (IBS) – p. 11
 - Inflammatory bowel disease (IBD) – p. 12
 - Immune modulation – p. 13
 - Expression of cytokines in cells *in vitro* – p. 13
 - Experimental models in rat – p. 13
 - Immune response in HIV-positive children – p. 14
 - Attenuation of the systemic inflammatory response in critically ill patients – p. 14
 - Clostridium difficile* associated diarrhoea – p. 14
 - Antioxidative capacity in serum – p. 15
- Safety aspects – p. 15
- References – p. 16

CONSUMPTION OF LIVE LACTIC ACID BACTERIA (PROBIOTICS).

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. In fact, there are archaeological signs that mankind has used this technique from the beginning of time and it was presumably invented 1.5 millions years ago by the early humanoids (Leakey 1993; Leakey 1995). See Figure 1. Thus, humans have in this way consumed large numbers of live LAB, and presumably those associated with plant material were consumed before those associated with milk based foods. Lactic acid fermentation is the simplest and often the safest way of preserving food, and before the Industrial Revolution, lactic acid fermentation was applied just as much in Europe as it still is in Africa. Thus, it could very well be that the human gastro-intestinal (GI) tract evolved to adapt to a more or less daily supply of live LAB. This supply ceased in industrialized countries during the twentieth century, which might have led to GI problems, and even to immunologically dependant ones.

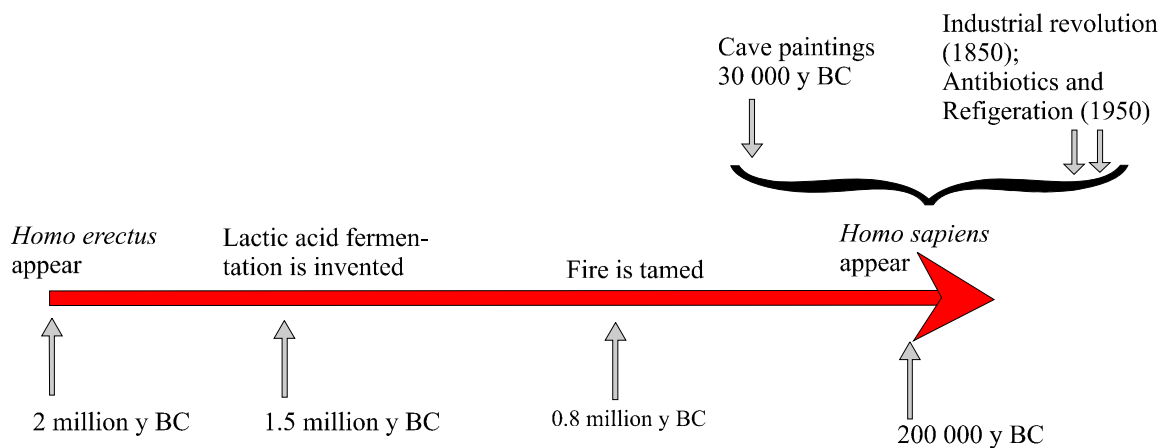


Figure 1. A suggested time scale for the human development, showing how early the technique of lactic acid fermentation probably came into use.

When beneficial effects of certain types of live bacteria have been discussed, these types of bacteria gradually have been called “probiotics”. The original concept of probiotics implies that the balance between beneficial and harmful bacteria in the microflora of the GI-tract can be positively affected by eating the right type of living microorganisms (Parker 1974; Fuller 1989). However, the concept probiotics is to day used more generally for describing live bacteria that exercise health beneficial effects after ingestion.

LACTIC ACID BACTERIA, *LACTOBACILLUS*, *LACTOBACILLUS PLANTARUM* AND STRAIN 299V.

The concept, lactic acid bacteria

The organisms performing the conversion of carbohydrates to carboxylic acids, mainly

lactic acid, are by tradition called LAB. Food microbiologists used the term early, and in 1919 the Danish bacteriologist Orla Jensen tried to define key features of LAB as “The true lactic acid bacteria form a large natural group of non-motile, non-spore-formers, Gram-positive cocci and rods that at fermentation of sugar mainly produce lactic acid.” Based on definitions like this, different systematically defined taxa have been included in the group LAB. However, LAB is not a systematically defined group based on evolutionary relationships. It is a functional group used by food microbiologists applied to bacteria, harmless to both food quality and human health that occurs spontaneously in traditional lactic acid fermented foods. It has been showed by meta-analyses of published clinical trials that different kind of lactic acid bacteria can be used to prevent antibiotic associated diarrhoea (D’Souza *et al.* 2002) and shorten the duration of acute diarrhoeal illness in children (Huang *et al.* 2002).

From the taxonomic point of view, LAB means a relatively wide variety of taxa. How many genera and species that should be included in LAB depend much on how many different types of lactic acid fermented foods that are included and how strict the quality definitions are set for those food products. For example, the higher the eating quality of a lactic acid fermented food product is, the fewer types of bacteria can generally be involved in the final fermentation. In a product of poorer quality, all types of unwanted organisms can be present in high numbers in the final product. The only absolute condition for the organisms involved in lactic acid fermentation must be that they produce lactic acid and that they are harmless to consume in high numbers, even for consumers with underlying sicknesses that weaken their immunological defence. The taxa frequently occurring in high numbers in traditional and spontaneously fermenting lactic acid fermented foods are *Lactobacillus*, *Pediococcus*, *Weissella*, *Leuconostoc*, *Oenococcus*, *Lactococcus*, and *Streptococcus thermophilus* (and presumably some closely related species). The genera *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Weissella* and *Oenococcus* have a relatively close phylogenetic relationship and might all be included in the trivial expression ”lactobacilli”. However, *Lactococcus* and *S. thermophilus* have from phylogenetic point of view nothing in common with the lactobacilli other than being members of the same general branch of evolution, i.e. the phylum (or division) of *Firmicutes* (Gram-positives having a low ratio of guanine and cytosine in their genome).

The species, *Lactobacillus plantarum*

L. plantarum is a bacterial species in the huge and relatively diverse genus of *Lactobacillus*, which comprises about 90 validly named species. By tradition, the *Lactobacillus* spp. have been divided into three functional groups depending on their fermentation abilities; the obligately homofermentatives (Group I), the facultatively heterofermentatives (Group II) and the obligately heterofermentatives (Group III) (Kandler and Weiss 1986). Group I ferment hexoses exclusively to lactic acid, and can not ferment gluconate or pentoses, while Group II can ferment pentoses and/or gluconate. Group III ferment hexoses to lactic acid, acetic acid and/or ethanol and carbon dioxide. *L. plantarum* is facultatively heterofermentative. The type strain of *L. plantarum* is ATCC 14917 (Kandler and Weiss 1986).

L. plantarum differs from many other *Lactobacillus* spp. in the following points:

- 1) *L. plantarum* has a relatively large genome.
- 2) *L. plantarum* possess a striking ability to ferment many different carbohydrates.
- 3) *L. plantarum* has a high growth requirement for manganese and can accumulate high intercellular levels of manganese (Archibald and Fridovich 1981b). Manganese provides a defence for *L. plantarum* against oxygen toxicity by the reduction of oxygen radicals to H₂O₂ (Archibald and Fridovich 1981a.). The produced H₂O₂ can then be converted to O₂ and water by manganese cofactored pseudocatalase (Kono and Fridovich 1983a, 1983b).
- 4) *L. plantarum* have a high tolerance to low pH (Daeschel and Nes 1995). The fact that *L. plantarum* frequently predominate in spontaneously, lactic acid fermented foods where the pH usually is below 4.0 and also survive the passage through the acid conditions of the human stomach (Johansson *et al.* 1993), point to their high resistance to acid conditions.
- 5) *L. plantarum* can possess tannase activity (Osawa *et al.* 2000; Vaquero *et al.* 2004) and are also able to metabolise phenolic acids (Barthelmebs *et al.* 2000; Barthelmebs *et al.* 2001).

L. plantarum frequently occurs spontaneously, in high numbers, in most lactic acid fermented foods, especially when the food is based on plant material, for example, in brined olives (Fernández Gonzalez *et al.* 1993), capers (caper berries; Pulido *et al.* 2005), sauerkraut (Dedicatoria *et al.* 1981), salted gherkins (McDonald *et al.* 1993), sour-dough (Lönner and Ahrné 1995), Nigerian ogi (made from maize or sorghum) (Johansson 1995a), Ethiopian kocho (made from starch from *Ensete ventricosum*) (Gashe 1987; Nigatu 1998), Ethiopian sour-dough made out of tef (*Eragrostis tef*) (Gashe 1987; Nigatu 1998) and cassava (Oyewole and Odunfa 1990; Moorthy and Mathew 1998). Thus, it is obvious that individuals consuming lactic acid fermented products of plant origin also consume large amounts of *L. plantarum*. Furthermore, *L. plantarum* occurs in grape juice and wine (Vaquero *et al.* 2004). *L. plantarum* frequently occurs on the human gastro intestinal mucosa, from the mouth to the rectum (Molin *et al.* 1993; Ahrné *et al.* 1998).

Genotyping of twenty different strains of *L. plantarum* from various sources have been assessed by microarrays containing a subset of small genomic fragments of the strain *L. plantarum* WCFS1 (Molenaar *et al.* 2005). It was shown that genes involved in sugar transport and catabolism were highly variable between strains while those involved in biosynthesis or degradation of structural compounds like proteins, lipids and DNA were conserved (Molenaar *et al.* 2005).

The bacterial strain, *Lactobacillus plantarum* 299v.

The *L. plantarum* strain 299v (=DSM 9843) was isolated from human intestinal mucosa (Molin 1993), and is included in a genetic subgroup within the species *L. plantarum* (Johansson *et al.* 1995b) where the members mostly originate from human intestinal mucosa, but also can be found in traditional lactic acid fermented foods (Molin *et al.* 1993; Ahrné *et al.* 1998). The strains of this subgroup have been shown to have a pronounced ability to attach to human mucosa cells *in vitro* and the adhesion is dependant on a mannose-binding adherence mechanisms (Adlerberth *et al.* 1996; Ahrné *et al.* 1998).

Moreover, *L. plantarum* strains of this particular genomic subtype frequently dominate the total *Lactobacillus* flora of healthy individuals, both on rectal and on oral mucosa (Molin *et al.* 1993; Ahrné *et al.* 1998). The mannose-binding adherence mechanism was shown to be crucial for the immune modulating ability of *L. plantarum* 299v in the human HT-29 colonic epithelial cell line (McCracken *et al.* 2002).

The strain *L. plantarum* 299v, that has been isolated from healthy human intestinal mucosa (Molin *et al.* 1993; Johansson *et al.* 1993; Johansson *et al.* 1995b), have been granted patent in Europe and USA amongst others (possessor of all rights are Probi AB, Lund, Sweden). Closely related strains of *L. plantarum* can be defined and identified by restriction endonuclease analysis (REA) of total chromosomal DNA by the use of relatively frequently cutting restriction enzymes such as *EcoRI* and *ClaI*, and traditional agarose gel electrophoresis (Johansson *et al.* 1995b). This method was successfully used for strain-definition and re-isolation of *L. plantarum* 299v from mucosal biopsies obtained in an administration study in humans (Johansson *et al.* 1993). *L. plantarum* 299v could be re-isolated from mucosal biopsies taken from jejunum and rectum after oral administration (Johansson *et al.* 1993). In some individuals *L. plantarum* 299v could be found even as a dominating part of the mucosal lactobacilli-flora 11 days after the end of administration (Johansson *et al.* 1993).

L. plantarum 299v contains four plasmids of the size 4, 9, 15 and 21 Mda (Johansson *et al.* 1995d). The strain has the same genomic ribopattern (Restriction fragment length polymorphism of the 16S rRNA gene) as the type strain of *L. plantarum* (ATCC 14917^T) with four bands (operons) showed after cleavage with the endonuclease *EcoRI* and five bands after cleavage with *HindIII* (Johansson *et al.* 1995d).

When the genome of *L. plantarum* 299v was compared with 19 other *L. plantarum* strains by microarrays containing a subset of small genomic fragments of the strain *L. plantarum* WCFS1 (Molenaar *et al.* 2005). *L. plantarum* 299v was shown to be genomically different from all the tested strains, and was closest related to the strain, *L. plantarum* 299 (=DSM6595) (Molenaar *et al.* 2005).

PROVIVA, A FRUIT BASED, LACTIC ACID FERMENTED OATMEAL BEVERAGE

L. plantarum 299v is included in a functional food product with the brand name ProViva[®] (Molin 1995; Molin and Ahrné 1999; Molin 2001; Molin 2003). ProViva[®] is primarily a fruit-beverage that today is marketed in Sweden, Finland and Denmark. In Germany it has been marketed under the brand name PrimaVita[®] and in Belgium, ProVie[®]. ProViva[®] is produced and marketed in Scandinavia by the company, Skånemejerier (Malmö, Sweden) while the holder of the rights to the strain, *L. plantarum* 299v, is the company, Probi AB (Lund, Sweden). *L. plantarum* 299v has been included in yoghurt (Skånemejerier) and a low-fat ice cream, God Hälsa[®] that was marketed by the company, SIA Ice Cream AB, (Slöinge, Sweden).

The lactic acid fermented component in the drink ProViva is an oatmeal beverage that has been fermented with *L. plantarum* 299v. The lactic acid fermentation produces about 1 x

10^9 colony forming units [CFU] of *L. plantarum* 299v per ml of oatmeal beverage. This fermented oatmeal formula was originally developed as a new concept for enteral feeding (nasogastric feeding) (Molin *et al.* 1991a). The lactic acid fermented oatmeal formula is an integral part of ProViva, where 5% fermented oatmeal beverage has been mixed with different types of fruit drinks, including rose hip, strawberry, blueberry, blackcurrant, raspberry and tropical fruits. In the final product (ProViva) there is about 5×10^7 CFU of *L. plantarum* 299v per ml of fruit drink.

The process to produce the lactic acid fermented oatmeal beverage is patented (Molin *et al.* 1991a). The viscosity of the products is lowered by a supplement of malt flour (malted barely) in combination with a heat treatment followed by the decreased pH in the lactic acid fermentation. The fermented oatmeal beverage was originally intended as a base for a nutritional formula for enteral feeding, a low viscosity, and high energy liquid were prerequisites (Molin *et al.* 1991a). Without added malt flour, oat meal beverage of the stated concentration of flour will form a thick porridge impossible to administer through a thin tube (Molin *et al.* 1991a; Marklinder and Lönner 1994; Marklinder 1996). The decrease in viscosity is presumably in large part due to degradation of starch. Malt is rich in amylases. There is also an increased solubility of β -glucans, and if higher amounts of malt are used, or extra malt flour is added after the heat treatment, there is also a substantial reduction in total amount of β -glucans (Marklinder and Lönner 1994; Marklinder 1996). However, the β -glucans are considered valuable as they are believed to delay intestinal absorption and beneficially affect cholesterol and glucose metabolism. The process does cause a relatively small, if any, reduction of the total content of β -glucans even if the viscosity is significantly affected.

The lactic acid fermented oatmeal beverage provides about 76% of the energy and 70% and 99% of the protein and carbohydrate content, respectively, compared to the average nutrient content in commercial nutritive solutions intended for enteral feeding (Marklinder and Lönner 1994). The beverage is also relatively rich in β -glucans, thiamine, phosphorus, iron, copper and manganese (Marklinder and Lönner 1994).

BENEFICIAL HEALTH EFFECTS

The intestinal bacterial flora

Probiotics and the bacterial balance

It is a well established fact that high numbers of lactobacilli counteract many pathogenic and potential pathogenic bacteria, regardless of whether the system is a lactic acid fermented food or the human intestine (De Vuyst and Vandamme 1994a, 1994b). The original concept of probiotics implies that the balance between beneficial and harmful bacteria in the microflora of the GI-tract can be positively affected by eating the right type of living micro-organism (Parker 1974; Fuller 1989). *L. plantarum* 299v is after oral administration to humans found in high numbers on the rectal mucosa (Nobeck *et al.* 2000) and in faeces (Johansson *et al.* 1998; Nobeck *et al.* 2000; Önning *et al.* 2003; Goossens *et al.* 2003; Berggren *et al.* 2003; Goossens *et al.* 2005). *L. plantarum* 299v already adhere to the tonsillar mucosa directly after oral intake (Stjernquist-Desatnik *et al.* 2000). *L. plantarum* 299v increases the total viable count of lactobacilli in faeces (Goossens *et al.* 2003; Berggren *et al.* 2003; Goossens *et al.* 2005).

Moreover, the presence of live and metabolic active *L. plantarum* 299v on human intestinal mucosa after ingestion of the bacteria in a drink has been verified by hybridization to a DNA microarray comprising clones covering the *L. plantarum* genome (de Vries *et al.*, 2006). It was shown that about 10% of the genes were expressed and genes were detected for all functional classes. The expression differed between individuals and to a lower degree between the small and large intestine (de Vries *et al.*, 2006).

Even if the definition of probiotics today is used in a broader sense; probiotics are the same as live micro-organisms with beneficial health effects when administered to animals and humans; the original concept of counteracting deleterious bacteria in the GI-tract still remains interesting. In any case, the key-question is: what components of the intestinal flora should be suppressed? That the probiotics should inhibit pathogens is self-evident, but the normal intestinal flora is much more than pathogens. Unfortunately the human intestinal flora is poorly defined and many components have not been systematically described, not even on the hierarchical level of genus (Langendijk *et al.* 1995). Examples of frequently occurring components of the human intestinal flora that presumably can have negative health implications and therefore should be counteracted are *Bacteroides fragilis* (and maybe other *Bacteroides* spp.) and species of the family *Enterobacteriaceae* (for example, *Escherichia coli* and *Klebsiella pneumoniae*). These groups that are found in the normal flora are also frequently involved in abdominal infections and sepsis.

Lactobacillus spp. are frequently present in varying numbers in the human GI tract, but are usually present in lower numbers than many other components of the normal flora such as, for example, *Bacteroides*, clostridia/eubacteria/ruminococci (Moore and Holdeman 1974; Finegold *et al.* 1983; Wang *et al.* 2005). However, ingested probiotics will not only work in the colon, but will come in contact with the mucosa of the mouth and then the gut mucosa and its microbial inhabitants all along the small intestine. This means the probiotics have exposure to a huge interface that is harbouring a lower population of resident bacteria than that found in the colon. The effects and actions in the small intestine will probably also have an influence in the colonic environment.

L. plantarum 299v (DSM 9843) have been shown *in vitro* to possess anti-microbial activity against potentially pathogenic species such as *Listeria monocytogenes*, *Bacillus cereus*, *Escherichia coli*, *Yersinia enterocolitica*, *Citrobacter freundii*, *Enterobacter cloacae* and *Enterococcus faecalis* (Jacobsen *et al.* 1999). Furthermore, when healthy volunteers consumed a mixture of lactobacilli strains, including *L. plantarum* 299v, the level of lactobacilli in the intestine increased, and there was also a decrease in the level of Gram-negative anaerobes, *Enterobacteriaceae* and sulphite-reducing clostridia (Johansson *et al.* 1993).

The inhibitory effect of *L. plantarum* 299v against *Enterobacteriaceae* (Mao *et al.* 1996a; Adawi *et al.* 1997; Wang *et al.* 2001; Osman *et al.* 2005) and Gram-negative anaerobes (Mao *et al.* 1996a) has been demonstrated, in rat models simulating severe clinical conditions. *L. plantarum* 299v have also been shown to inhibit enteropathogenic and enterohemorrhagic *Escherichia coli* adhesion *in vitro* to intestinal epithelial cells in culture by inducing mucin expression, i.e. intestinal epithelial cells produce more mucin that limits access of pathogens to their surface (Mack *et al.* 1999; Mack *et al.* 2003). The ability of *L. plantarum* 299v to reduce secretory response of intestinal epithelial cells to

enteropathogenic *E. coli* (EPEC) was shown *in vitro* (Michail and Abernathy, 2002). The observed effect was due to reduced attachment of EPEC to epithelial cells (Michail and Abernathy, 2002). It has also been shown that the colonization of *L. plantarum* 299v competes with that of *E. coli* in gnotobiotic rats (Herías *et al.* 1999).

Gram-negative anaerobes are noxious from the viewpoint that they often are involved in secondary infections after abdominal surgery (Nichols 1980; Offenbartl and Bengmark 1990; Wittman 1991). Furthermore, Gram-negative bacteria always contain endotoxins and they initiate, even when present in small numbers, violent inflammatory reactions. Gram-negative anaerobes are also suggested to be producers of carcinogenic substances in the intestine (Rowland 1992; Roberfroid and Gibbson 1994). Rats pre-treated with the Gram-negative, *Bacteroides fragilis*, before the onset of an acute liver injury, developed a significantly poorer liver status than control rats with the liver injury but without bacterial pre-treatment (Adawi *et al.* 1999a). Some strains of *B. fragilis* can also secrete a toxin that has shown to activate T-cell factor dependant β -catenin nuclear signalling in intestinal epithelial cells, and it has been suggested that this event may contribute to oncogenic transformation in the colon (Wu *et al.* 2003). The group of sulphite-reducing clostridia can contain subgroups that produce toxins. Sulphite-reducing clostridia also produce hydrogen sulphide that has a general toxicity. Furthermore, clostridia can produce carcinogenic substances in the intestine (Rowland 1992). *Enterobacteriaceae* is a genetically close family including many pathogenic taxa, and even normally non-pathogenic taxa have a pathogenic potential in situations where the immunological defence of the host is failing.

In a study in Tanzania, *L. plantarum* 299v was used as a starter culture for producing the cereal based lactic acid fermented beverage Togwa. *L. plantarum* 299v was used for producing 50% of the test-togwa while the other 50% was made by traditional back sloping (Kingamkono *et al.* 1999). Spontaneously fermented togwa is frequently dominated by *L. plantarum* (Mugula 2001). The product was given to children (<5 years) once a day for 13 consecutive days and the presence of faecal entero-pathogens such as *Campylobacter*, entero-pathogenic *Escherichia coli*, *Salmonella* and *Shigella* was evaluated. The proportion of children with isolated faecal entero-pathogens decreased significantly ($P < 0.001$) during the study period (Kingamkono *et al.* 1999).

The ingestion of probiotics can positively alter the GI microflora as has been seen by the decreased plate counts of *Enterobacteriaceae* and sulphite reducing clostridia after ingestion of lactobacilli (Johansson *et al.* 1993). In a randomized, placebo controlled, double blinded study in healthy volunteers that consumed *L. plantarum* 299v in a fruit drink (2×10^{10} CFU/day for 3 weeks), the total level of carboxylic acids in faeces increased (Johansson *et al.* 1998), and it was the concentration of acetic acid and propionic acid that increased (Johansson *et al.* 1998). The carboxyl acids are produced by the GI microflora, and this change in acid composition points at significant changes in the flora. *L. plantarum* 299v are not known to be able to produce propionic acid. The increased concentration of acetic acid and propionic acid must be regarded as beneficial from a health-perspective. Both types of short-fatty acids are utilized as an energy source by the mucosa cells of the intestine. Short-chain fatty acids are in fact the major energy source of the colonic mucosa cells. An increased level of short-chain fatty acids in the lumen is therefore beneficial for the condition of the mucosa. Moreover, absorbed propionic acid comes via the portal blood to the liver and there it can have positive effects on both the lipid metabolism and inflammatory responses in the liver.

The healthy subjects receiving *L. plantarum* 299v also experienced a decrease in flatulence during the treatment period (Johansson *et al.* 1998), which might indicate that the concentration of gas-producing microorganisms in the GI tract decreased.

Intestinal mucosal status and reduced translocation

The effect of *L. plantarum* 299v on the mucosal status and barrier function has been extensively studied in rat models. When the status of the intestinal mucosa was evaluated using the content of protein, or content of rRNA and DNA as an indicator, an improvement in status was shown in rats with acute liver injury that had been pre-treated with *L. plantarum* 299v (Kasravi *et al.* 1997; Adawi *et al.* 1999b). An improved mucosal status was also seen in rats with enterocolitis that had been treated with *L. plantarum* 299v (Mao *et al.* 1996a). In this study the permeability of EDTA through the mucosa was measured and found to decrease in animals receiving *L. plantarum* 299v (Mao *et al.* 1996a).

Translocation (the passage of viable bacteria through the epithelial mucosa into the *lamina propria* and then to the mesenteric lymph nodes and possibly other tissues [Berg and Garlington 1979]), can be reduced due to the improved status of the intestinal mucosa. Translocation can be studied in rats with an acute liver injury induced by an injection with D-galactose-amine which causes a severe liver inflammation (Kasravi *et al.* 1996a; Kasravi *et al.* 1996b). Twenty-four hours after the onset of the liver injury, translocating bacteria can be found in organs such as the liver and spleen, and in the portal and arterial blood. The liver injury does not directly affect the intestinal mucosa but the immunological defence of the animal is severely weakened, which allows the translocating bacteria to travel beyond the mesenteric lymph-nodes and the liver. However, by pre-treating of the animals with *L. plantarum* 299v, the translocation can be significantly decreased (Adawi *et al.* 1997; Adawi *et al.* 1999a; Kasravi *et al.* 1997; Wang *et al.* 2001; Osman *et al.* 2005). Some other strains of other *Lactobacillus* spp. have been shown to have an effect in the liver failure model (Adawi *et al.* 1997). However, *L. plantarum* 299v seems to be an especially effective strain in this respect.

It is interesting to identify what type of bacteria is translocating in the rats with liver failure (Wang *et al.* 2001). In rats that had not received any lactobacilli treatment, the majority of the bacteria found in the liver originated from the dominating population of the intestinal mucosa-flora, i.e. *L. animalis*, *L. reuteri* and *L. acidophilus* (*Lactobacillus* are much more dominant in rats than in humans) but also *Proteus vulgaris*, *Bacteroides distasonis*, *Enterococcus faecalis* and *Staphylococcus aureus* were found in the liver. *P. vulgaris* and *S. aureus* were also found in the arterial blood (Wang *et al.* 2001). However, pre-treatment for 8 days with *L. plantarum* 299v before the liver injury not only decreased the rate of translocation to the liver, but no bacteria translocated to the blood and only *L. animalis*, *L. reuteri* and *L. acidophilus* were found in the liver (Wang *et al.* 2001). Thus, the *L. plantarum* treatment not only decreased the rate of translocation, it obviously had a controlling impact on the intestinal microflora and enhanced the domination of *Lactobacillus*. It can also be noted that *L. plantarum* 299v never was found in extra-intestinal sites in spite of the large pre-treatment dose (Wang *et al.* 2001).

Pre-treatment of rats with *L. plantarum* 299v in their drinking water for a week, inhibited *E. coli*-induced permeability of the intestine (Mangell *et al.* 2002). This was shown in

intestinal segments mounted in Ussing chambers where the permeability of mannitol was measured. Exposure to *E. coli* in the Ussing chamber normally increases the permeability, but the pre-treatment of the living rats with *L. plantarum* 299v abolished this increase in permeability (Mangell *et al.* 2002).

Many of the intestinal bacteria that translocate in the rats with liver failure will end up in the liver which will enhance the inflammation of the liver and the condition of the liver will worsen. This deterioration can be measured by the concentration of liver enzymes in the blood. In the liver failure model, it was shown that pre-treatment with *L. plantarum* 299v decreased the concentration of the liver enzymes, aspartate-transaminase and alanine-transaminase in the blood, indicating that the liver status was improved by the treatment (Adawi *et al.* 1997; Kasravi *et al.* 1997; Adawi *et al.* 1999b).

The preventive effect of *L. plantarum* 299v on translocation has also been seen in other experimental rat-models. *L. plantarum* 299v significantly reduced the translocation in rats with enterocolitis, induced by Methotrexate (Mao *et al.* 1996a). In this model, the mucosa is inflamed and damaged in contrast to the liver failure model, where the mucosa is unaffected. The lactobacilli administration to the enterocolitis rats mitigated the mucosal injuries induced by the chemotherapy (Mao *et al.* 1996a). A decreased translocation has also been observed by treatment with *L. plantarum* 299v in an experimental rat model with pancreatitis (Mangiante *et al.* 2001), and in a DSS-induced colitis model, also in rat (Osman *et al.* 2004).

There can be several explanations as to how *L. plantarum* 299v can improve the mucosa status and decrease the translocation rate. One is the traditional probiotic effect, that the administered probiotic strain counteracts adverse bacteria. These aggressive, adverse bacteria can induce and maintain an inflammation, and they may be especially suited for translocation and are capable of fighting off the host's immunological defence. It is also possible that the probiotic strain not only counteracts adverse components of the flora, it might also stimulate beneficial components that are part of the resident flora. In fact, the domination of resident intestinal lactobacilli of rats increased after treatment with *L. plantarum* 299v (Wang *et al.* 2001). This was also indicated in humans when the amount of propionic acid in faeces increased after consumption of *L. plantarum* 299v, since propionic acid is not produced by 299v (Johansson *et al.* 1998). However, the improved barrier effect of the mucosa can also be due to an immunomodulation (see below) and to a stimulation of the mucin production of the human mucosa cells.

In a prospective randomised controlled study on patients undergoing elective abdominal surgery it was shown that the concentration of IgM at the mucosal surface in specimens of normal small bowel was increased in the control group while it was constant in the patients given *L. plantarum* 299v prior to surgery (Woodcock *et al.* 2004). An increase in IgM may be an indication of bacterial translocation (Woodcock *et al.* 2001; Woodcock *et al.* 2004).

Risk-factors for coronary artery disease

L. plantarum 299v in ProViva has been shown to decrease different risk factors for coronary artery diseases in individuals at risk. In a small randomized, placebo controlled and double blind study on men with slightly elevated cholesterol levels, it was shown that

the concentrations of total cholesterol and of LDL-cholesterol were decreased after consumption of *L. plantarum* 299v in ProViva rosehip (Bukowska *et al.* 1998). The study included 30 individuals divided into two groups, where the treatment group consumed 200 ml fruit drink (rose hip), containing 5×10^7 CFU per ml, for 6 weeks and the placebo group consumed fruit drink without lactobacilli. The fall in cholesterol level was small but statistically significant (Bukowska *et al.* 1998). However, even more surprising, it was shown in the same study that the fibrinogen level of the serum also was decreased significantly ($P < 0.001$), representing a reduction of 13.5% (Bukowska *et al.* 1998). Fibrinogen is an acute phase protein that reflects the inflammatory status of the individual, and also is an independent risk factor for coronary artery disease (Kannel *et al.* 1987).

In a subsequent, placebo controlled randomized double blind study, with thirty-eight healthy smokers, it was shown that the consumption of 400 ml ProViva rosehip daily for six weeks not only significantly decreased the level of fibrinogen, but also F₂-isoprostans and IL-6 which are other inflammatory markers (Naruszewicz *et al.* 2002). Moreover, *L. plantarum* 299v in the ProViva also positively affected the systolic blood pressure, and the insulin and leptin response (Naruszewicz *et al.* 2002).

Irritable Bowel Syndrome (IBS)

Irritable bowel syndrome (IBS) is common, but its cause is unknown. It is not a single condition, but rather a collection of disorders causing similar symptoms of abdominal pain, diarrhoea, constipation or variability of bowel habit. The absence of strict pathogenic features has made IBS a disease without a proper diagnosis. Attempts have been made to develop criteria for a positive diagnosis of IBS (Manning *et al.* 1978; Thompson *et al.* 1992). 20-50% of patients coming to gastroenterology clinics are suffering from IBS, even if most patients with IBS do not seek medical care (Maxvell *et al.* 1997). IBS is a chronic relapsing condition that perhaps occurs in most adults at some point in their lives. Symptoms begin before age 35 in 50% of patients, and 40% of patients are aged 35-50 (Maxvell *et al.* 1997). IBS was found in 18% of the adult population in the Bristol area in the UK (Heaton *et al.* 1992).

L. plantarum 299v in the fruit drink ProViva (rose hip) was administered to patients with IBS in two, double blinded, placebo controlled studies, one in Poland (Niedzielin *et al.* 2001) and one in Sweden (Nobaek *et al.* 2000). In both studies the patients were divided into two groups, one was given *L. plantarum* 299v and the other a similar rosehip drink without *L. plantarum* 299v (placebo). In the Swedish study, patients with slight to moderate symptoms, mainly bloating and pain, were included (Nobaek *et al.* 2000) while the Polish study required patients that besides bloating and pain also had problems with irregularity in defecation and stool consistency (Niedzielin *et al.* 2001). The results of the Polish study were that the magnitude of several of the experienced IBS symptoms decreased in the *L. plantarum* group, and a higher proportion of the patients were free from their symptoms in the treatment group than in the placebo group (Niedzielin *et al.* 2001). In the Swedish study, *L. plantarum* 299v significantly decreased the subjectively experienced bloating during the treatment period (Nobaek *et al.* 2000). Pain was also significantly reduced in both the treatment-group and in the placebo-group, but the decrease was more rapid and more pronounced in the *L. plantarum* group. Twelve months after the treatment, the patients given *L. plantarum* 299v in the study, still experienced a

better overall gastrointestinal function than the patients that had gotten the placebo (Nobaek *et al.* 2000).

The bloating and pain experienced by IBS-patient might be due to abnormal colonic fermentation giving rise to an excess of gas production, especially of hydrogen (King *et al.* 1998). In a small randomised placebo controlled study on *L. plantarum* 299v in ProViva, the gas production and composition was measured after 4 weeks consumption and no difference were seen between the placebo and the treatment group (Sen *et al.* 2002). However, if the patients were provoked by consuming 20 g lactulose, the hydrogen in the breath was significantly decreased in the group treated with *L. plantarum* 299v. Thus, the intestinal microflora must have been changed in some way. It should be pointed out that the study of Sen *et al.* (2002) unfortunately was performed with an invalid cross-over design that disfavoured differences between the groups. Furthermore, the six patients in the treatment group were only consuming 125 ml ProViva per day (5×10^7 CFU per ml) which is a relatively small dose. For example, in the study of Nobaek *et al.* (2000), the patients consumed 400 ml ProViva per day.

Inflammatory Bowel Disease (IBD)

Inflammatory bowel disease (IBD) is a chronic inflammation along the GI tract. It can be limited to the large bowel (ulcerative colitis) or it can be situated anywhere along the GI tract (Crohn's disease). Ulcerative colitis is a relatively superficial ulcerative inflammation, while Crohn's disease is a transmural, granulomatous inflammation. IBD is thought to be due to an abnormal and uncontrolled immune response to normally occurring constituents of the intestine. The etiology of IBD is unknown. Microbial agents appear to be involved in the pathogenesis of IBD and intestinal bacteria seem to be an important factor in their development and chronicity (Ardizzone *et al.* 1999; Campieri and Gionchetti 2001; Schultz and Sartor 2000). In these conditions there is a complex interaction of bacteria, mucosa and immune system but this interaction is far from clear (Campieri and Gionchetti 2001).

Inflammation and the potential of *L. plantarum* 299v to counteract the inflammation has been studied in different animal models. In rats with enterocolitis induced by Methotrexate, administration with *L. plantarum* 299v mitigated the mucosal injuries induced by the chemotherapy (Mao *et al.* 1996a). Furthermore, inflammation in the intestinal mucosa of rats after radiation was decreased by administration of *L. plantarum* 299v in fermented oatmeal beverage (Liu *et al.* 2001).

In a study using interleukin-10 deficient mice in germ-free and specific pathogen-free (SPF) environments, *L. plantarum* 299v was able to attenuate the established colitis when the bacterium had colonized the gastrointestinal tract of the mouse before the mouse was transferred to the SPF environment (Schultz *et al.* 1998; Schultz and Sartor 2000; Schultz *et al.* 2002). It was also demonstrated that a mono-association with *L. plantarum* 299v (i.e. *L. plantarum* 299v was the only bacterium in the animal) did not induce colitis but only initiated a very mild immune response. Shultz *et al.* (2002) concluded "these results demonstrated that *L. plantarum* can attenuate immune-mediated colitis and suggest a potential therapeutic role for this agent in clinical inflammatory bowel diseases". It has also been shown that *L. plantarum* 299v have a more active role than the probiotic strain

Lactobacillus rhamnosus GG in preventing the onset of colitis in gnotobiotic IL-10 mice on an inbreed 129SvEv background colonized with SPF bacteria (Veltkamp *et al.* 1999).

In DSS-induced colitis in rat, *L. plantarum* 299v decreased the Disease activity index (DAI), i.e. the severity of the colitis (Osman *et al.* 2004). Dextran sulphate sodium (DSS) is given in the drinking water and after 5 days the animal develop colitis. The DSS-induced lesions and the location of the lesions (mainly the left colon) have resemblances to ulcerative colitis in humans.

Immune modulation

Expression of cytokines in cells, in vitro

The cytokine response of human peripheral blood mononuclear cells differs between different *Lactobacillus* spp. It has been shown that different strains of *L. plantarum* of intestinal origin are able to induce the production of the cytokines IL-12 and IL-10 from blood mononuclear cells (Hessle *et al.* 1999). Compared to *E. coli*, less IL-10 was produced but considerably more IL-12 was produced. In the same study, *L. paracasei* induced the production of a higher proportion of IL-12, and *L. rhamnosus* induced a higher proportion of IL-10. The response of the mononuclear cells was more balanced in respect to IL-10 and IL-12 production when they were exposed to *L. plantarum*, than to the other two *Lactobacillus* spp. (Hessle *et al.* 1999).

The cytokine response of bone marrow-derived, murine, dendritic cells when exposed to different probiotic strains of *Lactobacillus* have also been shown to vary (Christensen *et al.* 2002). Substantial differences could be seen between strains in their capacity to induce IL-12 and TNF- α production in dendritic cells. The ranking among the tested strains was as follows: *L. casei* subsp. *alactus* CHCC3137 >> *L. plantarum* Lb1 > *L. fermentum* Lb20 > *L. johnsonii* La1 > *L. plantarum* 299v >> *L. reuteri* DSM 12246 (Christensen *et al.* 2002). Similar but less pronounced differences were observed among the test strains in the induction of IL-6 and IL-10.

The ability of the proinflammatory cytokine tumour necrosis factor, TNF- α to influence epithelial IL-8 responses to *L. plantarum* 299v has been analysed in HT-29 colonic epithelial cell line (McCracken *et al.* 2002). The results showed that TNF- α sensitises HT-29 cells to *L. plantarum* 299v and the IL-8 mRNA expression was increased above levels induced by TNF- α alone. However, even if the expression had been increased, the IL-8 secretion was most unexpectedly decreased in the HT-29 cells that had been exposed to *L. plantarum* 299v. This means that even if *L. plantarum* 299v sensitises the HT-29 cells, the bacteria exert a protective effect by down regulating IL-8 secretion (IL-8 is a strongly proinflammatory cytokine) (McCracken *et al.* 2002). In a way, this gives an explanation to the paradox that *L. plantarum* 299v is able to both up regulate the immunological response and exercise an anti-inflammatory effect.

Experimental models in rat

The sub-normal levels of secretory IgA-antibodies in the intestines of rats with Methotrexate-induced enterocolitis were increased, and approached a more normal level, after the administration of *L. plantarum* 299v. Also the level of CD4 and CD8

lymphocytes in the intestinal *lamina propria* increased to more normal levels, after treatment with *L. plantarum* 299v (Mao *et al.* 1996b).

The levels of total serum IgA antibodies increased, and the IgA and IgM antibody levels against *Escherichia coli*, were marginally higher in gnotobiotic rats colonized with *E. coli* together with *L. plantarum* 299v, compared with rats that only were colonized with *E. coli* (Herías *et al.* 1999). The group treated with *L. plantarum* 299v also showed a significantly increased density of CD25-positive cells in *lamina propria*, and displayed by a decreased proliferative spleen cell response after stimulation with ConA one week after colonization. The results indicated that *L. plantarum* 299v can modulate a response to antigens presented via the gut (Herías *et al.* 1999).

Immune response in HIV positive children

Children congenitally exposed to human immuno-deficiency virus (HIV) have received *L. plantarum* 299v in a fermented oatmeal beverage (freeze dried), in a pilot-study. The results suggested that *L. plantarum* 299v elicits specific systemic immune responses after oral supplementation (Cunningham-Rundles *et al.* 2000; Cunningham-Rundles *et al.* 2002).

Attenuation of the systemic inflammatory response in critically ill patients

One hundred and three critically ill patients were randomised to receive an oral preparation containing *L. plantarum* 299v (ProViva, Strawberry) in addition to conventional therapy (treatment group, n=52) or conventional therapy alone (control group, n=51) (McNaught *et al.* 2005). On day 15, serum IL-6 levels were significantly lower in the treatment group compared to controls (McNaught *et al.* 2005). IL-6 is a cytokine produced by many cell types, including lymphocytes, fibroblasts and monocytes. It has a variety of systemic effects including activation of B and T lymphocytes and induction of acute phase protein production in the liver. IL-6 appears to be a good indicator of activation of the cytokine cascade and predicts subsequent organ dysfunction and mortality (Blackwell and Christman, 1996). Thus, the enteral administration of *L. plantarum* 299v to critically ill patients was associated with a late attenuation of the systemic inflammatory response (McNaught *et al.* 2005). This was associated with a change in EndoCAB levels in the patients administered *L. plantarum* 299v, indicating a decreased endotoxin exposure (McNaught *et al.* 2005).

***Clostridium difficile* associated diarrhoea.**

Recurrent *Clostridium difficile* associated diarrhoea is a serious condition that often requires prolonged treatment with antibiotics, but these treatments often fail to prevent further recurrences. In a double-blind, placebo-controlled trial the ability of *L. plantarum* 299v to prevent recurrent episodes of *Clostridium difficile* associated diarrhoea was tried (Wullt *et al.* 2003). Recurrence of clinical symptoms was seen in 4 of 11 patients who received metronidazole in combination with *L. plantarum* 299v and in 6 of 9 treated with metronidazole in combination with placebo. The investigation was limited to 21 patients, and the results were not statistically significant. Nevertheless, there was a tendency towards fewer recurrences in the lactobacillus group in comparison with the placebo group

and this tendency was maintained for additional 3 months after the study period, as indicated by telephone follow-ups. To reach statistical significance with a power 80%, 40 patients must be included in each arm. The study encourages the performance of large multicentre studies (Wullt *et al.* 2003).

Antioxidative capacity in serum

Rest and food are important for the recovery of the body after physiological stress, training and other pressures. Oxidative stress could otherwise give rise to reactive oxygen species (ROS) that cause damage to body tissue. Antioxidants protect the body against damage, and foods with high content of antioxidants are believed to have preventive effect on different diseases such as arteriosclerosis and cancer. It was shown in a placebo-controlled trial on healthy volunteers that a drink containing a mixture of antioxidants and *L. plantarum* 299v (ProViva Active[®], Skånemejerier, Malmö) increased the total plasma antioxidant capacity together with the content of selenium and selenoprotein P in serum (Önning *et al.* 2003). However, the eventual role of *L. plantarum* 299v in these effects was not addressed in the study. The total load of lactobacilli in faeces increased in the treatment group (Önning *et al.* 2003).

Ischaemia/reperfusion (I/R) of the colon is an inflammatory condition leading to tissue injury where reactive oxygen species play a central role. In an I/R-model in mouse the antioxidative activity of probiotics and other antioxidants can be evaluated *in vivo*. The combination of *L. plantarum* 299v and Rose hip which is rich in biologically active polyphenols with antioxidative properties (which may be important in prevention of lipid peroxidation) was studied in the I/R-model (Håkansson *et al.* 2005). *L. plantarum* 299v possesses enzymatic activity towards polyphenols (tannins) which can split up the tannins to flavonoids and thus increase the antioxidative capacity of Rose hip. It was shown that administration of rose hip and *L. plantarum* 299v, together, significantly decreased lipid peroxidation (the content of malondialdehyde [MDA] was taken as an index of lipid peroxidation) in caecum tissue and viable count of *Enterobacteriaceae* in caecum stool. A positive correlation between MDA levels and *Enterobacteriaceae* counts was found. The results support a synergistic/additive role of rose hip and *L. plantarum* in reducing lipid peroxidation (Håkansson *et al.* 2005).

SAFETY ASPECTS

The safety of consuming high numbers of live bacteria has been questioned, and there are reports that *Lactobacillus* spp., including *L. plantarum* strains, have been isolated from diseased sites in patients (Aguirre and Collins 1993). However, the potential of *Lactobacillus* spp. to cause serious infections has been assessed by studying the prevalence of bacteremia due to *Lactobacillus* spp. during a 4 year period, which indicated that the pathogenic potential of *Lactobacillus* spp. is low (Saxelin *et al.* 1996).

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; Fernández Gonzalez *et al.* 1993; McDonald *et al.* 1993; Lönnér and Ahrné 1995; Johansson *et al.* 1995c; Moorthy and Mathew 1998) and that these products in the

public mind, all over the world, have a reputation of being safe and wholesome, strongly indicates that live *L. plantarum* can be safely consumed. This becomes especially obvious if the long historical tradition of the lactic acid fermented foods is taken into account. However, in the case of the *L. plantarum* 299v, the safety has been more directly confirmed in a series of different studies.

L. plantarum 299v has been given in a daily dose of 10^{10} CFU to two patients with small bowel bacterial overgrowth in short bowel syndrome (with D-lactic acidosis; Vanderhoof *et al.* 1998). No negative effects of the *L. plantarum* 299v administration were noted. Instead, it was concluded for the whole case study, including six patients, that: “Preliminary experience with probiotics to change the flora to nonpathogenic organisms is promising and may demonstrate greater effectiveness and results in fewer long-term complications” (Vanderhoof *et al.* 1998).

L. plantarum 299v has been given in doses of 2×10^{10} CFU per day to 64 patients undergoing elective major abdominal surgery for at least a week preoperatively and in the postoperative period, without any negative signs, e.g. increased translocation due to the increased bacterial load (McNaught *et al.* 2002).

L. plantarum 299v has been given in high doses to immune-compromised children with HIV, for extended time periods, without any adverse effects (Cunningham-Rundles *et al.* 2000; Cunningham-Rundles *et al.* 2002).

L. plantarum 299v has been given, to critical ill patient in the intensive care without any adverse effects (Klarin *et al.* 2005; McNaught *et al.* 2005). Eventual bacteremia (bacteria in the blood) was followed by Klarin *et al.* (2005). *L. plantarum* 299v was never found in the blood.

The risk of endocarditis has been tested in an experimental rat model (Adawi *et al.* 2002). A catheter was passed down the right common carotid artery into the lumen of the left ventricle. The catheter was tied in place and the neck incision was closed. After 48 hours, 10^8 CFU of *L. plantarum* 299v was injected (0.5 ml of bacterial suspension) through the tail vein. Four days after the injection of the *L. plantarum* strain, the rats were sacrificed and the blood, heart tissue and catheter were sampled for bacteria. No *L. plantarum* 299v could be found in any of the sample sites (Adawi *et al.* 2002). Thus, even with this animal model, using a very unusual and challenging situation where a high dose of the bacteria is injected directly into the blood stream of an animal with an implant of artificial material in the artery and heart, the *L. plantarum* strain was removed from the system before causing any damage. Thus, *L. plantarum* 299v is perfectly safe for consumption of all categories of consumers, also severely ill ones.

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