
Lactobacillus plantarum 299v

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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 million 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 immunologically dependant ones.

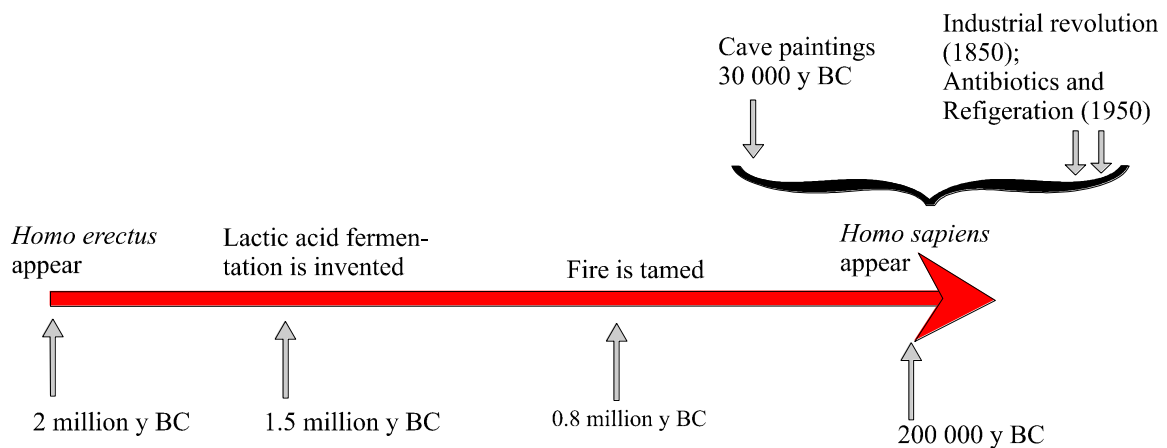


Figure 1. A suggested time scale for human developments.

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 today used more generally for describing live bacteria that exercise health beneficial effects after ingestion.

The hierarchy

Lactic acid bacteria

The organisms performing the conversion of carbohydrates to carboxylic acids, mainly lactic acid, are by tradition called lactic acid bacteria (LAB). Food microbiologists used the term early, and 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 and applied to those bacteria that are harmless to both food quality and human health, and that occurs spontaneously multiplying 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 different bacterial groups. How many genera and species that should be included in LAB depend 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 more restricted becomes the types of bacteria to be accepted in the fermentation. In a product of poorer quality, all types of unwanted organisms can be present in high numbers also in the final product. The only absolute condition for the organisms involved in lactic acid fermentation of a food product must be that the bacteria mainly produce lactic acid and that they are harmless to consume in high numbers, even for consumers with underlying sicknesses that weaken their immunological defence. Lactic acid producing bacteria frequently occurring in high numbers in traditional and spontaneously fermenting lactic acid fermented foods belong to genera as *Lactobacillus*, *Pediococcus*, *Weissella*, *Leuconostoc*, *Oenococcus*, *Lactococcus*, and the species *Streptococcus thermophilus* (and 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 the 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 (division) of *Firmicutes*.

The species, *Lactobacillus plantarum*

L. plantarum is one 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't ferment gluconate or pentoses, while Group II also ferments hexoses to lactic acid but is additionally able to ferment pentoses and/or gluconate. Group III ferments 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. This indicates abilities to adapt to many

different conditions (Kleerebezem *et al.* 2003).

2) *L. plantarum* can 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), points 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; Plengvidhya *et al.* 2007), 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). Finally, *L. plantarum* frequently occurs on the human gastro intestinal mucosa, from the mouth to the rectum (Molin *et al.* 1993; Ahrné *et al.* 1998).

In order to get a clue how humans acquire immune tolerance against harmless food associated bacteria, van Baarlen *et al.* (2009) studied the stimulating effect of *Lactobacillus plantarum* (strain, WCFS1) on the immune system of adult, healthy volunteers in a randomized double-blind placebo-controlled cross-over study. The subjects ingested either living or heat-killed *L. plantarum*. The expression profiles of biopsies from the intestinal duodenal mucosa were analyzed using whole-genome microarrays and by biological pathway reconstructions. The expression profiles displayed differences in modulation of NF- κ B-dependent pathways, notably after consumption of living *L. plantarum*. In other words, mucosal gene expression patterns and cellular pathways that correlated with the establishment of immune tolerance were revealed (van Baarlen *et al.* 2009). This demonstrates a close relationship between *L. plantarum* and the immune affected physiology of humans.

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 dependent on a mechanism for mannose-binding adherence (Adlerberth *et al.* 1996; Ahrné *et al.* 1998). A mannose adhesin coding gene in *L. plantarum* has been identified Pretzer *et al.* (2005).

Moreover, *L. plantarum* strains of this particular genomic subtype frequently dominate the total *Lactobacillus* flora of healthy individuals, both on oral and rectal 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 human HT-29 colonic epithelial cell line (McCracken *et al.* 2002), and also it has been shown to be important for the ability of the *L. plantarum* 299v to decrease translocation in a septic rat model (see below).

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 the fragment pattern can be visualised by 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).

Moreover, the *in vivo* gene expression of *L. plantarum* 299v in the human gut has been demonstrated (de Vries *et al.* 2006). Prior to surgery, three patients diagnosed with colon cancer ingested *L. plantarum* 299v (10^{11} CFU per d) for one week. Total RNA was isolated from the mucosa of surgically removed intestinal segments of tissue, and hybridized to a DNA microarray comprising clones covering the *L. plantarum* genome. The presence of living *L. plantarum* 299v on the mucosa was confirmed, and the ingested *L. plantarum* 299v cells were metabolically active in all subjects as demonstrated by the detection of about 10% expressed genes by the DNA microarrays (de Vries *et al.* 2006).

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 *Eco*R1 and five bands after cleavage with *Hind*III (Johansson *et al.* 1995d).

When the genome of *L. plantarum* 299v was compared with that of 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 Swedish functional food product with the brand name ProViva[®] (Molin 1995; Molin and Ahrné 1999; Molin 2001; Molin 2003; Molin 2007; Molin 2008). ProViva[®] is primarily a fruit-beverage that 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 public company, Probi AB (Lund, Sweden). In the USA products of the same concept are marketing by NextFoods under the brand name GoodBelly.

The lactic acid fermented component in the drink ProViva is an oatmeal beverage that has been fermented with *L. plantarum* 299v. See Figure 2. The lactic acid fermentation produces about 1×10^9 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.

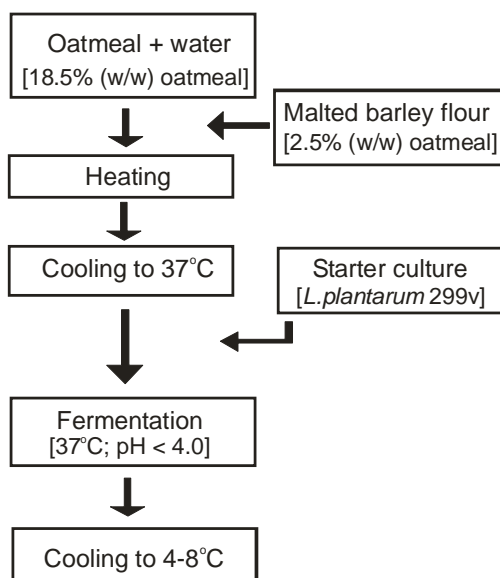


Figure 2. Flow scheme of the production of lactic acid fermented oatmeal beverage to be used, for example, in ProViva.

The process to produce the lactic acid fermented oatmeal beverage is patented (Figure 2; Molin *et al.* 1991a). The viscosity of the products is lowered by a supplement of malt flour (malted barley) 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 (18.5%; w/w) 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).

L. plantarum 299v is marketed internationally as probiotics in the product category “Food supplements”, i.e. in tablets, capsules or sachets by different companies in different countries.

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 microorganisms (Parker 1974; Fuller 1989). *L. plantarum* 299v is after oral administration to humans found in high numbers on the rectal mucosa (Nobaek *et al.* 2000) and in faeces (Johansson *et al.* 1998; Nobaek *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; Goossens *et al.* 2006a; Goossens *et al.* 2006c).

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).

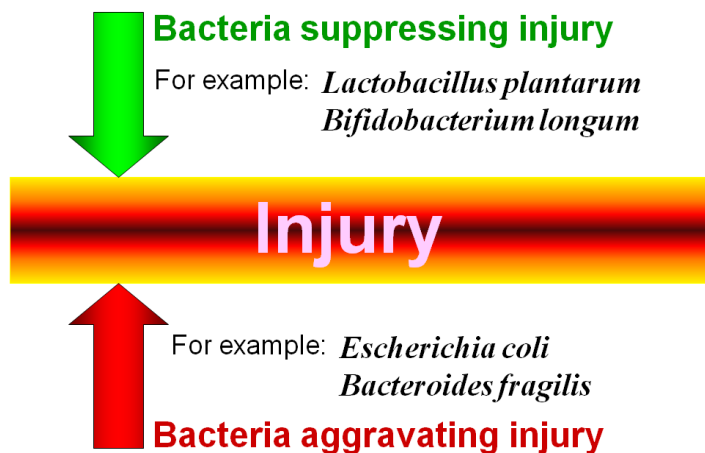


Figure 3. There are bacteria that suppress an injury or inflammation in the GI-tract and there are those that aggravate the injury.

Even if the definition of probiotics today is used in a broader sense; probiotics are the same as live microorganisms with beneficial health effects when administrated to animals and humans; the original concept of counteracting deleterious bacteria in the GI-tract still remains, and a 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 bacterial flora of the human GI tract 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, and they synthesise lipopolysccarides (LPS; also called endotoxins) that become associated to their cell walls. LPS have strong proinflammatory effect.

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 bacterial 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 affect the colonic environment.

Antagonistic effects against adverse bacteria

L. plantarum 299v 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), and relatively strong antagonistic properties against *Salmonella enterica* subsp. *enterica* (Hütt *et al.* 2006), and more intermediate antagonistic activity against *Helicobacter pylori* (Hütt *et al.* 2006).

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).

Enterobacteriaceae is a 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. 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* (included in the family *Enterobacteriaceae*) adhesion 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). It has also been shown that increased levels of *E. coli* in pregnant rats resulted in pups with increased systemic inflammation (Fåk *et al.* 2008a). No such negative effects were seen when the rat mothers were administered *L. plantarum* 299v, instead it improved the gut maturation of the pups (Fåk *et al.* 2008b). When pups were treated directly at different ages, it was indicated that the exposure with *L. plantarum* 299v affected the gut function, e.g. the gut permeability for macromolecules was reduced with age (Fåk *et al.* 2008c), i.e. the effect was age related and the youngest tested rats responded most (Fåk *et al.* 2008c).

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 lipopolysaccharides (LPS) 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 inhibitory effect of *L. plantarum* 299v against *Bacteroides* was shown in a placebo controlled study in patients with inactive ulcerative colitis (Goossens *et al.* 2006b).

Sulphite-reducing clostridia includes species with toxin producing capacity, and sulphite-reducing clostridia generally produce hydrogen sulphide (H₂S) that is a gentoxic agent in concentrations as low as 250 μ mol/litre which is in agreement to that found in the human colon (Attene-Ramos *et al.* 2006). The well known pathogens, *Clostridium perfringens* and *Clostridium difficile* belongs to *Clostridium* cluster I and *Clostridium* cluster XI of Collins, respectively. *L. plantarum* 299v has been found to reduce recurrence of *Clostridium difficile*-associated disease, i.e. enteral administration of *L. plantarum* 299v to critically ill patients treated with antibiotics reduced colonisation with *C. difficile* (Klarin *et al.* 2008).

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 enteropathogens such as *Campylobacter*, enteropathogenic *Escherichia coli*, *Salmonella* and *Shigella* was evaluated. The proportion of children with isolated faecal enteropathogens decreased significantly (P<0.001) during the study period (Kingamkono *et al.* 1999).

Effects on the GI-environment

The ingestion of probiotics can positively alter the bacterial flora of the GI-tract as has been seen by the decreased plate counts of *Enterobacteriaceae* and sulphite reducing clostridia after ingestion of lactobacilli (Johansson *et al.* 1993). Furthermore, 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). 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 especially propionic acid must be regarded as beneficial from a health-perspective. Propionic acid is 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 bacteria in the GI tract decreased.

It was shown in patients, with cardiovascular disease, that daily consumption of high amounts of *L. plantarum* 299v increased the diversity of the colonic microbiota, i.e. administration of a single-strain probiotic increased the bacterial diversity in the gut (Karlsson *et al.* 2010). The results suggest that administration of *L. plantarum* 299v might be a strategy to favour a diverse intestinal microbiota, which presumably is favourable for the condition of the mucosa, and a healthy mucosa decrease the risk of translocating so called “pathogen-associated molecular patterns” that negatively affects atherosclerosis.

Intestinal mucosal status and reduced translocation

Liver failure

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 liver injury (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 animals with *L. plantarum* 299v, the translocation was 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 also 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 that is translocating in rats with liver failure (Wang *et al.* 2001). In rats that had not received any probiotic 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 did not only decrease the rate of translocation to the liver, but no bacteria whatsoever 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 did not only decrease the rate of translocation, it obviously had a controlling impact on the intestinal microflora and enhanced the domination of *Lactobacillus*. It can be noted that *L. plantarum* 299v never was found in extra-intestinal sites in spite of the large pre-treatment doses (Wang *et al.* 2001).

Many of the intestinal bacteria that translocate in 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).

Mucosal status

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). Furthermore, maternal consumption of *L. plantarum* 299v affected gastrointestinal growth and function in suckling rats (Fåk *et al.*, 2008b). The small intestine, pancreas and liver weighed more in 14d-old pups born from dams exposed to *L. plantarum* 299v than in the control pups from dams given only water, and the *L. plantarum* 299v pups showed decreased gut permeability (Fåk *et al.*, 2008b). Also direct exposure of pups resulted in decreased gut permeability (Fåk *et al.*, 2008c).

Translocation in different *in vivo* models

The preventive effect of *L. plantarum* 299v on translocation has also been seen in many different experimental *in vivo* models.

Pre-treatment of rats with *L. plantarum* 299v in the drinking water for one 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).

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 was also observed by treatment with *L. plantarum* 299v in an experimental rat model with **pancreatitis** (Mangiante *et al.* 2001), in a **DSS** (dextran sulphate sodium) **induced** colitis model in rat (Osman *et al.* 2004), and in a **septic** rat model (Mangell *et al.* 2006).

Protective mechanisms

There can be several explanations as to how *L. plantarum* 299v 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 has been shown in septic rat that the mannose sensitive adhesion-ability of *L. plantarum* 299v was important for the translocation-blocking capability (Mangell *et al.* 2006). 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). Furthermore, the overall bacterial diversity of the gut flora in humans has been shown to increase after administration of *L. plantarum* 299v (Karlsson *et al.* 2010).

An improved barrier effect of the mucosa can also be a result of a beneficial immunomodulation (see below) and/or to a stimulation of the mucin production of the human mucosa cells.

Translocation in humans

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). Furthermore, it was shown in patients in intensive care that *L. plantarum* 299v has a positive impact on the gut barrier (Klarin *et al.* 2008).

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 did not only significantly decrease 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).

Sixteen males, with atherosclerotic plaque on the carotid wall, were randomly selected from a larger cohort and included in this double blind, placebo controlled study. It was shown that the consumption of *L. plantarum* 299v increased the bacterial diversity of the gut (Karlsson *et al.* 2010). The administration of *L. plantarum* 299v might be favourable for the condition of the mucosa, and a healthy mucosa decrease the risk of translocation; translocation affects atherosclerosis negatively.

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).

The effects of *L. plantarum* 299v have been studied in a murine IBS model where the intestinal dysfunction was created by rectal administration of 1% allyl isothiocyanate (oil of mustard) in 30% ethanol (Waugh *et al.* 2009). *L. plantarum* 299v was gavaged for up to 28 days, beginning either 7 days before (pretreatment) or 8 days after oil of mustard administration (post-treatment). *L. plantarum* 299v reduced inflammation and normalized intestinal transit rates in the mice (Waugh *et al.* 2009).

L. plantarum 299v in the fruit drink ProViva (rosehip; fruits from roses) 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 became free from 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 drunk 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. However, no difference was seen between the placebo and the treatment group (Sen *et al.* 2002). On the other hand, if the patients were provoked by consuming 20 g lactulose, the hydrogen in the breath was significantly decreased in the group pretreated 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) was performed with a cross-over design that might disfavour differences between the groups.

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 development and chronicity (Ardizzone *et al.* 1999; Campieri and Gionchetti 2001; Schutlz and Sartor 2000). Under these conditions, there are a complex interaction of bacteria, mucosa and immune system but this interaction is far from clear (Campieri and Gionchetti 2001).

The potential of *L. plantarum* 299v to counteract the inflammation have 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; Schutlz 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 inbred 129SvEv background colonized with SPF bacteria (Veltkamp *et al.* 1999).

In DSS (dextran sulphate sodium) induced colitis in rat, *L. plantarum* 299v decreased the Disease Activity Index (DAI), i.e. the severity of the colitis (Osman *et al.* 2004). DSS is given in the drinking water and after 5 days the animal develops 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 *in vivo* models

The subnormal 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).

Critically ill patients are often treated with antibiotics and are at high risk of developing *Clostridium difficile*-associated disease (CDAD). The intensive care unit (ICU) patients were investigated regarding the impact of *L. plantarum* 299v on *C. difficile* colonisation, and it was shown that enteral administration of *L. plantarum* 299v to critically ill patients treated with antibiotics reduced colonisation with *C. difficile* (Klarin *et al.* 2008).

Antibiotic associated diarrhoea

Diarrhoea is a frequently occurring side effect of antibiotic therapy. Antibiotic treated, hospitalised patients were receiving *L. plantarum* 299v in a fruit drink (treatment product) or a fruit drink without probiotics (placebo): The overall risk of developing loose or watery stools was significantly reduced among patients receiving *L. plantarum* 299v, and so was the development of nausea (Lönnermark *et al.* 2010). The results indicate that intake of *L. plantarum* 299v can have a preventive effect on gastrointestinal symptoms during antibiotic treatment.

Antioxidative capacity in serum

Effects in *in vivo* model

Foods 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 can cause damage to body tissue. Antioxidants protect the body against damage from ROS, and foods with high content of antioxidants are believed to have preventive effect on different diseases such as arteriosclerosis and cancer.

Ischemia/reperfusion (I/R) of the colon is an inflammatory condition leading to tissue injury where ROS 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 rosehip 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.* 2006). *L. plantarum* 299v possesses enzymatic activity towards polyphenols (tannins) and can split up the tannins to flavonoids and thus increase the antioxidative capacity of the phenolics in rosehip. It was shown that administration of rosehip 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. Also the number (viable count) of *Enterobacteriaceae* in caecum stool was decreased. A positive correlation between MDA levels and *Enterobacteriaceae* counts was found. The results support a synergistic or additive role of rosehip and *L. plantarum* in reducing lipid peroxidation (Håkansson *et al.* 2006).

Effects in humans

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 separately in the study. The total load of lactobacilli in faeces increased in the treatment group (Önning *et al.* 2003).

Safety aspects

The safety of consuming high numbers of live bacteria has been addressed, and there are reports that *Lactobacillus* spp., including *L. plantarum* strains, have been isolated from diseased sites of patients (Aguirre and Collins 1993). However, the potential of *Lactobacillus* spp. to cause serious infections is low and this has been shown by studying the prevalence of bacteremia due to *Lactobacillus* spp. during a 4 year period in Finland, 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; Lönner 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 (see below):

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 bacteraemia (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.

It has been stated in “Biosafety assessment of probiotics used for human consumption: recommendations from the EU-PROSAFE project” that “for *in vivo* assessment of safety by investigating strain pathogenicity in animal models, the rat endocarditis model appeared to be the most reliable model tested in the PROSAFE project” (Vankerckhoven *et al.* 2008). *L. plantarum* 299v has been evaluated in the EU funded PROSAFE project: The identity of the strain was confirmed and no acquired antibiotic resistance could be detected (PRO SAFE report on strain *Lactobacillus plantarum* 299v).

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