Lactobacillus plantarum HEAL19

CONTENT

Consumption of live lactic acid bacteria (probiotics) – p. 2

Functional groups and scientifically based taxa – p. 3
   Lactic acid bacteria – p. 3
   The species Lactobacillus plantarum – p. 4
   The bacterial strain Lactobacillus plantarum HEAL19 – p. 5

Tannin degradation – p. 7

Health effects – p. 8
   Animal experimental models – p. 8
      Fermentation in the gut – p. 8
      Body weight – p. 8
      Blood pressure – p. 9
      Insulin resistance – p. 9
      Colitis – p. 9
      Liver injury – p. 10
      Multiple sclerosis – p. 10
      Oxidative stress – p. 10
      Interaction with green tea – p. 11
      Protection from cortical bone loss – p. 11

   Human trials – p. 12
      Recovery after oral administration – p. 12
      Incorporated in a health promoting diet – p. 12
      Phagocytic activity – p. 12

References – p. 13
Consumption of live lactic acid bacteria

Consumption of live lactic acid bacteria (LAB), included in fermented foods, has been a regular part of the human food intake for a long time. In fact, there are archaeological signs that humankind has used this technique from the beginning of time: it was presumably invented 1.5 million years ago by the early humanoids (Leakey 1993; Leakey 1995). Thus, humans have in this way consumed large numbers of live LAB throughout their entire history.

Fermentation is the simplest and often the safest way to preserve food, and before the Industrial Revolution, fermentation was applied just as much in Europe as it still is in many rural areas of the World. Thus, it could very well be that the human digestive tract evolved to adapt to a more or less daily supply of live LAB. This supply of live LAB ceased in many industrialized countries during the twentieth century, which eventually may have led to increased frequency of gastro-intestinal (GI) and immunological dysfunctions in urbanised humans.

When beneficial effects of certain types of live bacteria have been discussed, these types of bacteria have been gradually called “probiotics”. The original concept of probiotics implies that the balance between beneficial and harmful bacteria in the microbiota of the GI-tract can be positively affected by eating the right type of live microorganisms (Parker 1974; Fuller 1989). However, the concept of probiotics is today used more generally for describing live bacteria that after ingestion, exercise health beneficial effects beyond conventional nutrition. It is presupposed that these health beneficial effects have been scientifically proved.
Functional groups and taxonomically based taxa

Lactic acid bacteria

The bacteria performing the conversion of carbohydrates to carboxylic acids, mainly lactic acid in traditional fermented foods, are 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, unaware of the fact that LAB is not forming a systematically defined group based on evolutionary relationships; instead it can be regarded as a functional group used by food microbiologists, aiming at those bacteria that occur and multiply spontaneously in traditional lactic acid fermented foods. Furthermore, it is understood that LAB are harmless to human health. Already 2002, it was shown in meta-analyses of published clinical trials that different kind of LAB 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 taxonomically based groups (taxa). The only absolute condition for organisms involved in lactic acid fermentation of food 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 may have weaken their immunological defence. The different kind of lactic acid producing bacteria frequently occurring in high numbers in traditional, spontaneously fermented foods belong to genera as Lactobacillus, Pediococcus, Weissella, Leuconostoc, Oenococcus, Lactococcus, and the species Streptococcus thermophilus (and similar species).

The genera Lactobacillus and Pediococcus belong to the family Lactobacillaceae which also includes the relatively new genera Paralactobacillus and Sharpea. They can all be included in the trivial expression “lactobacilli”.

Leuconostoc, Weissella and Oenococcus belong to the family Leuconostocaceae together with the genus Fructobacillus.

Lactococcus and S. thermophilus have from the phylogenetic point of view relatively little in common with Lactobacillaceae and Leuconostocaceae even if
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 and subspecies. By tradition, the *Lactobacillus* spp. have been divided into three functional groups depending on their fermentation abilities: the obligate homofermentatives (Group I), the facultative heterofermentatives (Group II) and the obligate 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 14917T (Kandler and Weiss 1986).

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

1) *L. plantarum* has a relatively large genome in comparison with many other *Lactobacillus* spp. This indicates an adaptive ability for 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 free radicals to hydrogen peroxide (H₂O₂; Archibald and Fridovich 1981a). The produced H₂O₂ can then be converted to oxygen (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 final pH usually is below 4.0, and also can survive the passage through the acid conditions of the human stomach (Johansson *et al.* 1993), points to the 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 and multiply spontaneously to high numbers in most lactic acid fermented foods, especially when the foods are based on plant material, for example, in brined olives (Fernández Gonzalez et al. 1993), capers (capber 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 1985; 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). L. plantarum also occurs in grape juice and wine (Vaquero et al. 2004). Thus, it is obvious that individuals consuming traditionally fermented products of plant origin that haven’t been heat-treated also consume large amounts of live L. plantarum. Not surprisingly, L. plantarum frequently occurs in the human GI-tract, from the mouth to the rectum (Molin et al. 1993; Ahrné et al. 1998).

In order to get an idea 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 live or heat-killed L. plantarum. The expression profiles in biopsies taken 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-kappaB-dependent pathways, notably after consumption of live L. plantarum. In other words, it was seen that the mucosal gene expression patterns and cellular pathways correlated with the establishment of immune tolerance after consumption of live L. plantarum (van Baarlen et al. 2009). This demonstrates a close relationship between L. plantarum and the immune-affected physiology of humans.

Furthermore, genotyping of twenty different strains of L. plantarum from various sources have been assessed by microarrays containing a subset of small genomic fragments from 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 HEAL19

L. plantarum HEAL19 (= DSM 15313 = 52A) has been isolated from the gastro-intestinal (GI) mucosa of a healthy human. HEAL stands for “Human Eatable Abdominal Lactobacillus” and DSM stands for Deutsche Sammlung
von Mikroorganismen. The latter is the label for the international culture collection, German collection of microorganisms and cell cultures [http://www.dsmz.de].

*L. plantarum* strain HEAL19 can be defined and identified by restriction endonuclease analysis (REA) of total chromosomal DNA, with the use of relatively frequently cutting restriction enzymes such as *Eco*RI and *Cla*I, and traditional agarose gel electrophoresis (Johansson *et al.* 1995b). *L. plantarum* HEAL19 can in this way easily be separated from other *L. plantarum* strains, e.g. *L. plantarum* 299: *L. plantarum* 299v, *L. plantarum* HEAL9 and *L. plantarum* HEAL99.

*L. plantarum* HEAL19 has been selected on the basis of its high tannase activity and the pronounced ability to attach to human mucosa cells *in vitro*. *L. plantarum* HEAL19 has also a strong tendency of auto-agglutination.

In comparison with the well known probiotic strain *L. plantarum* 299v (=DSM 9843), *L. plantarum* HEAL19 have higher cell-binding capacity *in vitro* and higher tannase activity, but it lack the mannose-sensitive adhesion of *L. plantarum* 299v (Rask *et al.* 2013).
Tannin degradation

Polyphenols or phenolics are molecules with an aromatic ring bearing one or more hydroxyl groups. Tannins are polyphenols and they are known as anti-nutrients, i.e. they decrease the efficiency of the body to convert digested nutrients to new body constituents. Traditionally, tannins have been defined as water-soluble phenolics that can precipitate proteins from aqueous solution. There are two classes of tannins, the hydrolysable tannins, deriving from gallic acid and ellagic acid, and the condensed tannins, i.e. proanthocyanidins, which are oligomers and polymers of flavanols.

Tannins and other polyphenols are secondary metabolites of plants and more than 8 000 different phenolics isolated from plants have been described (Bravo 1998; Ross and Kasum 2002). Phenolics are involved in plant growth and reproduction and provide protection for the plant from ultraviolet radiation, oxidative stress and pathogens (Ross and Kasum, 2002). Tannins inhibit the growth of a number of micro-organisms and are often resistant to microbial degradation (Chung et al. 1998). Moulds and yeasts and some aerobic bacteria are usually best fitted to degrade tannins but also anaerobic degradation occurs, e.g. in the intestinal tract (Bhat et al. 1998). The anaerobic breakdown products of polyphenols in the digestive tract can have health beneficial effects (Bhat et al. 1998). Such breakdown compounds are, for example, derivates of phenylpropionic or phenylacetic acids (Bhat et al. 1998). When absorbed in the digestive tract, these phenolic compounds can have anti-inflammatory effects. Polyphenols can also have antimicrobial capacities in the digestive tract but the susceptibility differs between different types of microorganisms and between different phenolics.

*L. plantarum* possesses the enzyme tannin acylhydrolase (tannase) which enable *L. plantarum* to degrade hydrolyzable tannins and tannic acid, thereby producing gallic acid and the antioxidant pyrogallol (Osawa et al, 2000; Vaquero et al. 2004). Hydrolysable tannins are composed of esters of gallic acid or ellagic acid with a sugar moiety, usually glucose, and may be hydrolysed into monomeric products.

*L. plantarum* also have phenolic acid decarboxylase enzymes that can transform phenolic acids into their vinyl derivates and phenylpropionic acids (Barthelmebs et al, 2000; Barthelmebs et al. 2001; Rodríguez et al, 2009).
Health effects

Animal experimental models

Fermentation in the gut

*L. plantarum* HEAL19 together with *Lactobacillus crispatus* DSM 16743 and *L. gasseri* DSM16737, and with or without blueberry husks, were given to healthy rats in metabolic cages (Bränning *et al.* 2009). The dietary fibres of blueberry husks were fermented to 61% in colon, and the elevated faecal excretion of fibre and protein contributed to a high faecal bulking capacity. The supplement of the mixture of *Lactobacillus* strains lowered the total caecal amount of carboxylic acids when added to blueberry husks, while the concentration of propionic acid increased. Administarting the *Lactobacillus* supplement resulted in an increase in the median viable counts of lactobacilli. *L. plantarum* HEAL19 was recovered from the caecal mucosa in rats fed the *Lactobacillus* strains, while *L. crispatus* DSM 16743 and *L. gasseri* DSM 16737 were left undetected. Thus, as it seems *L. plantarum* HEAL19 became the far most dominant *Lactobacillus* strain in the rat gut after administration (Bränning *et al.* 2009).

Rats fed blackcurrant in the diet got a higher caecal pool of short-chain fatty acids than rats fed blackberries or raspberries (Jacobsdottir *et al.* 2013). Furthermore, anthocyanins were detected in the urine after consumption of blackcurrants but not from the other berries. When *L. plantarum* HEAL19 was added to the blackcurrant supplemented diet, the total amount of short-chain fatty-acids in distal colon increased, and no anthocyanins were any longer detected in the urine (Jacobsdottir *et al.* 2013). It seems as *L. plantarum* HEAL19 enhanced the rat’s capability to metabolize anthochyanins.

Body weight

Long-term effects of a high-energy-dense diet on weight gain, fattening and the gut microbiota have been followed in rats. Since the mother’s dietary habits can influence offspring physiology, dietary regimens started with the dams at pregnancy and throughout lactation and continued with the offspring for six months (Karlsson *et al.* 2011). When the high-energy-dense diet throughout the period had been supplemented with live *L. plantarum* HEAL19 the weight gain was reduced, and there was a tendency towards a more diverse GI-
microbiota (Karlsson et al. 2011).

**Blood pressure**

With the aim to examine the anti-hypertensive capacity of two food supplements, combining fermented blueberries and *Lactobacillus plantarum* DSM 15313, rats were divided into groups of nine each (Lazou Ahrén et al. 2014). Some of the groups were given NG-nitro-L-arginine methyl ester (L-NAME) in the drinking water to induce a hypertensive state, and some were untreated (healthy rats). Two fermented blueberry products with *L. plantarum* HEAL19 and different content of phenolic acids were tested, and each rat received 2 g products per day for 4 weeks. Healthy rats consuming the fermented blueberries with *L. plantarum* HEAL19 showed a significant reduction in blood pressure, and a change in gut microbiota. In rats with L-NAME induced hypertension, there was a significant reduction of the blood pressure after two weeks. In conclusion, blueberries fermented with *L. plantarum* HEAL19 possessed anti-hypertensive properties (Lazou Ahrén et al. 2014).

**Insulin resistance**

With the objective to evaluate the metabolic effects of oral supplement with *L. plantarum* HEAL19 to high-fat diet, mice were fed high-fat diet for 20 weeks, after which the high-fat diet had induced an insulin-resistant state (Andersson et al. 2010). Fasting plasma glucose levels were lower in mice fed *L. plantarum* HEAL19, whereas insulin and lipids were unchanged in the control. An oral glucose tolerance test showed that mice fed *L. plantarum* HEAL19 had a significantly lower insulin release compared to control mice, although the rate of glucose clearance did not differ. It was suggested that *L. plantarum* HEAL19 has anti-diabetic properties when fed together with a high-fat diet (Andersson et al. 2010).

**Colitis**

Treatment with *L. plantarum* HEAL19 attenuated the severity of Dextran Sulphate Sodium (DSS) induced colitis (Osman et al. 2008). Disease activity index was significantly lower in treated rats compared to a colitis control. Also the inflammatory marker myeloperoxidase (MPO) and the bacterial translocation to the liver and the mesenteric lymph-nods decreased (Osman et al. 2008). Orally administrated *L. plantarum* HEAL19 could be found in high numbers in caecum also when the animals had eaten large doses of blueberry (Osman et al. 2008).

*L. plantarum* HEAL19 together with *L. gasseri* DSM16737 and *Bifidobacterium infantis* DSM 16737, and with or without blueberry husks,
were given to rats subjected to long-term, cyclic treatment with dextran sulphate sodium (DSS) (Håkansson et al. 2010). The probiotic mixture decreased faecal viable count of Enterobacteriaceae, mitigated hepatic injuries by decreasing parenchymal infiltration an incidence of stasis and translocation (Håkansson et al. 2010). However, the contribution of each individual strain was not be evaluated.

**Liver injury**

Pre-treatment with *L. plantarum* HEAL19 in a rat liver injury model mitigated the liver injury (decreased levels of bilirubin in the blood) and inflammation (decreased TNF-alfa, IL-1beta and myeloperoxidase, and increased glutathione values in the liver) (Osman et al. 2007). Also the translocation of gut bacteria to the liver and the mesenteric lymph nodes was decreased by the pre-treatment with *L. plantarum* HEAL19 (Osman et al. 2007).

In a mixture of probiotics, *L. plantarum* HEAL19 was used together with *L. gasseri* DSM 16737 and *B. infantis* DSM 15158, and given to rats subjected to a long term, cyclic treatment with DSS (Håkansson et al. 2010). The probiotics mitigated hepatic injuries by decreasing parenchymal infiltration and incidence of stasis and translocation. However, the contribution of each individual strain was not evaluated (Håkansson et al. 2010).

*L. plantarum* HEAL19 together with fermented blueberries showed a protective effect on liver cells in a hypertensive rat-model where increasing blood pressure is induced by L-NAME (Xu et al. 2013).

**Multiple sclerosis**

Multiple sclerosis (MS) is a Th1 cell-mediated chronic inflammatory disease of the central nervous system. Treatment with *L. plantarum* HEAL19 in a mouse model for experimental autoimmune encephalomyelitis (EAE), mimicking MS, prevented and delayed the onset of the clinical signs of EAE compared to control mice (Lavasani et al. 2010). In contrast, treatment with *Lactobacillus paracasei* PCC 101 or *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 20081 had no effect on the disease development. *L. plantarum* HEAL19 increased serum levels of IL-27 (Lavasani et al. 2010).

**Oxidative stress**

Ischemia-reperfusion (I/R) in the intestines is an inflammatory condition which activates leuocytes and reactive oxygen species (ROS) and leads to lipid peroxidation and DNA damage. Fruits rich in polyphenols may act as antioxidants and prevent lipid peroxidation, and *L. plantarum* may improve...
the microbial status in the intestines and increase the metabolic activity towards polyphenolic degradation. In an I/R-model in mice it was seen that bilberry (*Vaccinium myrtillus*) alone or in combination with *L. plantarum* HEAL19 decreased lipid peroxidation (with malondialdehyde as marker; Jakesevic *et al.* 2011)

**Interaction with green tea**

Green tea is rich in polyphenols, and *L. plantarum* HEAL19 has the ability to metabolize phenolics. Mice were for 22 weeks fed a high-fat diet with or without a supplement of 4% green tea powder, and given *L. plantarum* HEAL19 through the drinking water, alone or together with the supplement of green tea (Axling *et al.* 2012). The gut microbiota was examined together with different parameters related to obesity, glucose tolerance, lipid metabolism, hepatic steatosis and inflammation. *L. plantarum* HEAL19 and green tea powder in combination increased the load of lactobacilli in both the small intestine and in caecum, but it also increased the diversity of the gut microbiota. Furthermore, the combination of *L. plantarum* HEAL19 and green tea mitigated inflammation induced by the high fat diet and up-regulated genes regulating cholesterol synthesis (Axling *et al.* 2013). The results are in support of the hypothesis that a tannase active strain of *L. plantarum* consumed together with food rich in phenolics can impose health promoting effect above that achieved with each individual component.

**Protection from cortical bone loss**

With the aim to determine if probiotics can protect from ovariectomy-induced bone loss, mice were given a mixture of *Lactobacillus paracasei* DSM13434, *L. plantarum* HEAL9 and *L. plantarum* HEAL19 in the drinking water for 6 weeks, starting 2 weeks prior before ovariectomy (Ohlsson *et al.* 2014). It was found that the probiotic mixture decreased the serum levels of the resorption marker C-terminal telopeptides and the urinary fractional excretion of calcium after ovariectomy, and reduced the expression of the two inflammatory cytokines, TNF-α and IL-1β, and increased the expression of osteoprotegerin (OPG) which is a potent inhibitor of osteoclastogenesis, in cortical bone. The probiotic treatment also improved the frequency of regulatory T cells in bone marrow (Ohlsson *et al.* 2014). The authors conclude that “treatment with the probiotic mixture prevents ovariectomy-induced cortical bone loss”, and the findings “indicate that the probiotic treatment alter the immune status in bone resulting in attenuated bone resorption” (Ohlsson *et al.* 2014). The effect of only *L. plantarum* HEAL19 without the other strains was not tested.
Human trials

Recovery after oral administration

In an administration study where 10 healthy women were orally administered the strain HEAL19 together with eleven other Lactobacillus strains suspended in an oat-milk/blueberry drink for 10 days (HEAL19 was given in a daily dose of $10^9$ CFU) (Vásquez et al. 2005). L. plantarum HEAL19 was isolated from faeces of seven of the volunteers after 10 days of administration and in vagina from three of the volunteers (Vásquez et al. 2005).

Incorporated in a health promoting diet

With the aim to demonstrate dietary effects on cardio-metabolic risk factors in overweight healthy individuals, 44 volunteers participated in a randomized crossover intervention comparing a multifunctional diet (low glycemic impact meals, antioxidant-rich foods, oily fish, viscous dietary fibres, soybean and whole barley kernel products, almonds, stanols and L. plantarum HEAL19) with a control diet without the active components (Tovar et al. 2012). While the control diet did not modify the metabolic measurements, the active diet lowered total serum cholesterol, LDL-cholesterol, triglycerides, LDL/HDL, apoB/apoA, HbA1C, hs-CRP and systolic blood pressure (Tovar et al. 2012).

Phagocytic activity

In a blinded, placebo-controlled trial, the effect of a daily intake of L. plantarum HEAL19 for two weeks on the innate and acquired immune system was investigated in healthy volunteers (Rask et al. 2013). Blood lymphocyte subsets were quantified by flow cytometry and the expression of activation and memory markers was determined. The strain was also examined for its capacity to be phagocytosed by human peripheral blood mononuclear cells. The phagocytic activity of granulocytes towards Escherichia coli was increased following intake of L. plantarum HEAL19, but no effect was seen on any of the other examined parameters (Rask et al. 2013).
References


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