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# *Lactobacillus plantarum* HEAL9

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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 indications 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). 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 to immunological dysfunctions.

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 micro-organisms (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 species *Lactobacillus plantarum*

*L. plantarum* is a so called facultatively heterofermentative *Lactobacillus*, i.e. *L. plantarum* ferment hexoses exclusively to lactic acid, but can also ferment pentoses and/or gluconate, and then producing lactic and acetic acid. Furthermore, *L. plantarum* can ferment malic acid to lactic acid and carbon dioxide, and citric acid to diacetyl, acetoin and carbon dioxide. 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

H<sub>2</sub>O<sub>2</sub> (Archibald and Fridovich 1981a). The produced H<sub>2</sub>O<sub>2</sub> can then be converted to O<sub>2</sub> 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). 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).

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).

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- $\kappa$ 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.

## Tannin degradation

Traditional lactic acid fermented foods where *L. plantarum* spontaneously grow to high

number often contain high amounts of tannins. Tannins, defined as water-soluble phenolic products that can precipitate proteins from aqueous solution, are naturally occurring compounds. 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 inhibit the growth of a number of microorganisms 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).

Tannins are known as anti-nutrients, i.e. they decrease the efficiency of the body to convert digested nutrients to new body constituents. However, also health beneficial effects of tannins have been reported, e.g. anti-carcinogenic effects, ability to reduce blood pressure and to modulate immune-responses. These effects might be due to the anti-oxidative properties of tannins (Chung *et al.* 1998). An efficient antioxidative compound with reported anti-cancerogenic properties is ellagic acid. Another type of tannin with exceptional high anti-oxidative capacity is proanthocyanidins, present in for example grapes and olives. Thus, tannins present in varying concentrations in plant derived foods have profound effects on human health. In contrast, it is not advisable to ingest large quantities of tannins as they may be involved in cancer formation and anti-nutrition activity, but the intake of small quantity of the correct kind of tannin may be beneficial to human health by affecting the metabolic enzymes, immune-modulation or other functions (Chung *et al.* 1998). However, also the anaerobic breakdown products from many tannins, as produced in the intestinal tract, can generate compounds with health beneficial effects (Bhat *et al.* 1998). Such breakdown compounds are, for example, derivatives of phenylpropionic or phenylacetic acids (Bhat *et al.* 1998). When absorbed in the GI-tract these compounds have an anti-inflammatory effect. These compounds together with other breakdown products from tannins have also a wide range antimicrobial effect in the GI-tract, suppressing unwanted bacteria.

*L. plantarum* are also able to metabolise phenolic acids (Barthelmebs *et al.* 2000; Barthelmebs *et al.* 2001). Thus when these bacteria are growing in environments where there are tannins, they have the relatively unique abilities to break-up tannins and to metabolise the phenolic acids.

## The bacterial strain, *Lactobacillus plantarum* HEAL9

*L. plantarum* HEAL9 (= DSM 15312 = 39D) has been isolated from the gastro-intestinal mucosa of a healthy human. HEAL stands for “Human Eatable Abdominal *Lactobacillus*” and DSM (= Deutsche Sammlung von Mikroorganismen) is the label for the international culture collection, German collection of microorganisms and cell cultures [<http://www.dsmz.de>].

*L. plantarum* HEAL9 have been chosen on the basis of its tannase activity and pronounced ability to attach to human mucosa cells *in vitro*. *L. plantarum* HEAL9 has an adhesion that is dependent on a mannose-binding, adherence mechanism (Adlerberth *et al.* 1996; Ahrné *et al.* 1998). In comparison with the well known probiotic strain *L. plantarum* 299v (=DSM 9843), the HEAL9 strain had a higher cell-binding capacity and tannase activity.

## Animal experimental model

### Multiple sclerosis

Multiple sclerosis (MS) is a Th1 cell-mediated chronic inflammatory disease of the central nervous system. Treatment with *L. plantarum* HEAL9 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). Treatment with *Lactobacillus paracasei* PCC 101 (PCC = Probi Culture Collection) or *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 20081 had no effect on the disease development (Lavasani *et al.* 2010). *L. plantarum* HEAL9 induced CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> regulatory T cells in mesenteric lymph nodes and enhanced production of serum TGF-*beta*1 (Lavasani *et al.* 2010).

### Human trial

In an administration study where 10 healthy women were orally administered HEAL9 (together with eleven other *Lactobacillus* strains) suspended in an oat-milk/blueberry drink for 10 days (HEAL9 was given in daily doses of 10<sup>9</sup> CFU) (Vásquez *et al.* 2005). *L. plantarum* HEAL9 (and/or the closely related strain HEAL99) were found in faeces of four of the volunteers and in vagina from two of the volunteers (Vásquez *et al.* 2005).

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