

Lactobacillus plantarum 299

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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 in 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 in 2002, it was shown in meta-analyses of published clinical trials that different kinds 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 weakened their immunological defence. The different kinds 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

they all are included in the order of *Lactobacillales*.

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 14917^T (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 (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 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-*kappa*B-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 strain *Lactobacillus plantarum* 299

L. plantarum strain 299 (= DSM 6595) (Molin *et al.* 1993; Johansson *et al.* 1993) is included in a genetic subgroup within the species *L. plantarum*

(Johansson *et al.* 1995a) where the members mostly originate from the 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 depends on a mannose-binding adherence mechanism (Adlerberth *et al.* 1996; Ahrné *et al.* 1998). Moreover, *L. plantarum* strains of this particular genomic subtype frequently dominate the total *Lactobacillus* flora of healthy individuals, both on oral and rectal mucosa (Molin *et al.* 1993; Ahrné *et al.* 1998). A mannose-adhesion encoding gene in *L. plantarum* has been identified Pretzer *et al.* (2005).

L. plantarum 299 has been isolated from healthy human intestinal mucosa (Molin *et al.* 1993; Johansson *et al.* 1993). The two closely related strains *L. plantarum* 299 and *L. plantarum* 299v (=DSM 9843) can be defined and identified by restriction endonuclease analysis (REA) of total chromosomal DNA by the use of relatively frequently cutting restriction endonuclease enzymes such as *EcoRI* and *ClaI*, and the fragment pattern can be visualised by traditional agarose gel electrophoresis (Johansson *et al.* 1995a). This method was successfully used for strain-definition and identification of isolates of *L. plantarum* 299 from mucosal biopsies, obtained in an administration study in humans (Johansson *et al.* 1993). The strain was re-isolated from mucosal biopsies taken from jejunum and rectum after oral administration of the strain (Johansson *et al.* 1993). In some individuals, *L. plantarum* 299 could be found as a dominating part of the mucosal lactobacilli-flora even 11 days after the end of administration (Johansson *et al.* 1993).

L. plantarum 299 contains four plasmids of the size 4, 9, 15 and 21 Mda (Johansson *et al.* 1995c). 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 *EcoR1* and five bands after cleavage with *HindIII* (Johansson *et al.* 1995c).

When the genome of *L. plantarum* 299 was compared with 19 other *L. plantarum* strains by microarrays, containing a subset of small genomic fragments of the strain *L. plantarum* WCFS1 (Molenaar *et al.* 2005), *L. plantarum* 299 was shown to be genomic different from all the tested strains, but was closest related to the strain, *L. plantarum* 299v (=DSM 9843) (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 glycolytic enzymes glyceraldehydes 3-phosphate dehydrogenase (GAPDH) and enolase (ENO) are normally regarded as intracellular but they have been isolated from outer cell surface of *L. plantarum* 299 (Glenting *et al.* 2013). When the adhesive properties of these two enzymes were characterized, it was demonstrated that both have a highly specific binding to plasmogen and

fibronectin while GAPDH but not ENO also showed weak binding to mucin. Furthermore, a pH-dependant and specific binding to Caco-2 cells was found for both enzymes (Glenting *et al.* 2013).

Health effects

Suppression of pathogens in humans

The strain *L. plantarum*, 299 (=DSM 6595) that survives the passage through the human digestive tract (Johansson *et al.* 1993), have been shown *in vitro* to possess anti-microbial activity against pathogenic or opportunistic pathogenic species such as *Listeria monocytogenes*, *Bacillus cereus*, *Escherichia coli*, *Shigella flexneri*, *Yersinia enterocolitica*, *Citrobacter freundii*, *Enterobacter cloacae* and *Enterococcus faecalis* (Jacobsen *et al.* 1999). Furthermore, when healthy volunteers consumed a mixture of lactobacilli strains, including *L. plantarum* 299, the level of lactobacilli in the intestine increased, and there was also a decrease in the viable count of Gram-negative anaerobes, *Enterobacteriaceae* and sulphite-reducing clostridia (Johansson *et al.* 1993).

In a randomised clinical trial where *L. plantarum* 299 was given as supplement to enteral nutrition in patients with acute pancreatitis, the strain was efficiently reducing pancreatic sepsis and number of surgical interventions (Olah *et al.* 2002). Furthermore, it was indicated that *L. plantarum* 299 decrease the time that patients which had undergone major abdominal surgery needed antibiotics (Rayes *et al.* 2002a), and treatment with *L. plantarum* 299 reduced postoperative infections in liver transplant recipients (Rayes *et al.* 2002b).

Ventilator-associated pneumonia in critically ill patients is usually caused by aspiration of pathogenic bacteria from the oropharynx. Oral decontamination with chlorhexidine has been used as prophylaxis against this complication. With this background, fifty critically ill patients on mechanical ventilation were randomised to either oral mechanical cleansing followed by washing with 0.1% chlorhexidine solution or to the same cleansing procedure followed by oral application of an emulsion of *L. plantarum* 299 instead of the chlorhexidine treatment (Klarin *et al.* 2008). *L. plantarum* 299 was recovered from the oropharynx of all patients treated with *L. plantarum* 299. Furthermore, potentially pathogenic bacteria, absent at the time of inclusion, were identified in oropharyngeal samples from eight of the patients treated with *L. plantarum* 299 while the corresponding number of patients treated with chlorhexidine was 13 (p = 0.13). Hence, no difference in disinfection capacity was found between the treatment with *L. plantarum* 299 and that with chlorhexidine (Klarin *et al.* 2008).

Intestinal mucosal status and reduced translocation

Animal models

The effect of *L. plantarum* 299 on the mucosal status and barrier function has been studied in rat models. 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, for example, be studied in rats with an acute liver injury, induced by an injection with D-galactose-amine which causes a severe liver inflammation (Kasravi *et al.* 1996a; Kasravi *et al.* 1996b). Twenty-four hours after the onset of the liver injury, translocating bacteria can be found in organs such as the liver and spleen, and in the portal and arterial blood. The liver injury does not directly affect the intestinal mucosa but the immunological defence of the animal is severely weakened, which allows the translocating bacteria to travel beyond the mesenteric lymph-nodes and the liver. However, by pre-treating of the animals with *L. plantarum* 299, the translocation was significantly decreased (Adawi *et al.* 1999).

Many of the intestinal bacteria that translocate in the rats with liver injury will also end up in the liver which will enhance the liver inflammation 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 injury model, it was shown that pre-treatment with *L. plantarum* 299 decreased the concentration of the liver enzymes, aspartate-transaminase (AST) and alanine-transaminase (ALT) in the blood, indicating that the liver status was improved by the treatment (Adawi *et al.* 1999).

The preventive effect of *L. plantarum* 299 on translocation has also been seen in other experimental rat models. *L. plantarum* 299 significantly reduced the translocation in rats with enterocolitis, induced by Methotrexate (Mao *et al.* 1997). 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.* 1997). Also, *L. plantarum* 299 have been shown to reduce intestinal permeability in experimental biliary obstruction (White *et al.* 2006). Thus, it was concluded that *L. plantarum* 299 reduces intestinal hyperpermeability associated with experimental biliary obstruction (White *et al.* 2006).

Human trial

In an randomised clinical trial where *L. plantarum* 299 was given as supplement to early enteral nutrition in patients with acute pancreatitis, it

was shown that this strain was effective in reducing pancreatic sepsis and the treatment reduced the number of surgical interventions (Olah *et al.* 2002).

Suppression of inflammation

L. plantarum 299 can *in vitro* reduce cytokine production from colonic epithelial cell monolayers following exposure to enteric pathogens (Pathmakanthan *et al.* 1999).

L. plantarum 299 increased IL-10 synthesis and secretion in macrophages and T-cells derived from inflamed colon in patients with ulcerative colitis (Pathmakanthan *et al.* 2004). In contrast, no significant increase in IL-1 β , TNF- α or IFN- γ was seen in cells derived from active or inflamed mucosa when exposed to *L. plantarum* 299. It was suggested that this could “provide a mechanism through which probiotic bacteria ameliorate inappropriate inflammation and induce tolerance” (Pathmakanthan *et al.* 2004).

Safety

The safety of consuming high numbers of live bacteria has now and then been questioned, and there are reports that *Lactobacillus* spp., including *L. plantarum* strains, have been isolated from diseased sites in patients (Aguirre and Collins 1993). However, the potential of *Lactobacillus* spp. to cause infections has been assessed in Finland by studying the prevalence of bacteraemia due to *Lactobacillus* spp. during a 4 year period (Saxelin *et al.* 1996). It was concluded 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.* 1995b; Moorthy and Mathew 1998) and that these products in the public mind, all over the world, have a reputation of being safe and wholesome, indicates that live *L. plantarum* can safely be consumed. This becomes especially obvious if the long historical tradition of the lactic acid fermented foods is taken into account.

Intensive care unit (ICU) patients with expected mechanical ventilation ≥ 48 h and/or expected ICU stay ≥ 72 h received *L. plantarum* 299 two times daily (Oudhuis *et al.* 2010). The 130 critically ill patients receiving *L. plantarum* 299 did not show increased ICU mortality or mortality at day 28 compared with 124 patients receiving selective decontamination of the digestive tract with antibiotics (Oudhuis *et al.* 2010).

L. plantarum 299 has been evaluated in the EU funded PROSAFE project (Vankerckhoven *et al.* 2008), and the identity of the strain was confirmed and no acquired antibiotic resistance could be detected (PRO SAFE report on strain *Lactobacillus plantarum* 299).

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