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Lactobacillus plantarum 299

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 humans have used this technique from the beginning of time. Presumably, it was 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 most probably 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 dysfunction.

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 gastro-intestinal (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 species *Lactobacillus plantarum*

In foods

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). *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 both lactic acid and acetic acid. *L. plantarum* can also 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).

Some characteristics

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

1) *L. plantarum* has a relatively large genome. This indicates adoption abilities 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_2O_2 (Archibald and Fridovich 1981a). The produced H_2O_2 can then be converted to O_2 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 often 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).

Immune modulation

The cytokine response *in vitro* 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).

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.

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 adhesinen coding gene *in L. plantarum* has been identified Pretzer *et al.* (2005).

L. plantarum 299 that has been isolated from healthy human intestinal mucosa (Molin *et al*. 1993; Johansson *et al*. 1993), has been granted patent in Europe and USA amongst others (possessor of all rights are Probi AB, Lund, Sweden). The two closely related strains *L. plantarum* 299 and *L. plantarum* 299v (=DSM 9843) can be defined and identified by restriction endonculease analysis (REA) of total chromosomal DNA by the use of relatively frequently cutting restriction endonuclease enzymes such as *Eco*RI and *Cla*I, 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 *Eco*R1 and five bands after cleavage with *Hind*III (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. plantraum* 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).

Beneficial health effects

Suppression of pathogenes

The strain *L. plantarum*, 299 (=DSM 6595) that have been shown to survive the passage through the human GI tract (Johansson *et al*. 1993), have also been shown *in vitro* to possess anti-microbial activity against potentially 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 level of Gram-negative anaerobes, *Enterobacteriaceae* and sulphite-reducing clostridia (Johansson *et al*. 1993).

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

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 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 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, it was found that potentially pathogenic bacteria, absent at the time of inclusion, were identified in oropharyngeal samples from eight of the patients treated with *L. plantarum* 299 and 13 of those treated with chlorhexidine ($p = 0.13$). 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 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 (DSM 6595), the translocation was significantly decreased (Adawi *et al*. 1999).

Many of the intestinal bacteria that translocate in the rats with liver failure will end up in the liver which will enhance the 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 failure model, it was shown that pre-treatment with *L. plantarum* 299 decreased the concentration of the liver enzymes, asparate-transaminase and alanine-transaminase 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 models in rats. *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).

There can be several explanations as to how *L. plantarum* can improve the mucous status and decrease the translocation rate. One is the traditional probiotic effect, that the administrated probiotic strain counteracts adverse bacteria. These aggressive, adverse bacteria can induce and maintain inflammation, and they may be especially suited for translocation and are capable of fighting off the host's immunological defence. It is also possible that the probiotic strain not only counteracts adverse components of the flora, it might also stimulate beneficial components that are part of the resident flora. However, the improved barrier effect of the mucosa can also be due to an immune modulation and/or to a stimulation of the mucin production of the human epithelial cells.

Human trial

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Safety Aspects

The safety of consuming high numbers of live bacteria has been questioned, and there are reports that *Lactobacillus* spp., including *L. plantarum* strains, have been isolated from diseased sites in patients (Aguirre and Collins 1993). However, the potential of *Lactobacillus* spp. to cause infections has been assessed in Finland by studying the prevalence of bactermia 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, strongly 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.

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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