Lactobacillus plantarum—survival, functional and potential probiotic properties in the human intestinal tract

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Abstract

Lactobacillus plantarum is a versatile lactic acid bacterium that is encountered in a range of environmental niches, has a proven ability to survive gastric transit, and can colonize the intestinal tract of human and other mammals. Several studies describe the effects of L. plantarum consumption on human physiology. The availability of the complete genome sequence of L. plantarum WCFS1 makes it a suitable model to explore the molecular mechanisms underlying the targeted intestinal properties of this species. An increasing number of studies address the development of L. plantarum into an ingestible living vaccine. Furthermore, studies are emerging to determine the activity of L. plantarum in the human intestinal tract. This review discusses the studies of the safety and survival of L. plantarum in the human intestinal tract, the effects of this bacterium on the host and it provides an overview of the molecular studies addressing the activity of L. plantarum in the human gut environment.

Keywords: Lactobacillus plantarum; Human intestinal tract; Molecular analysis; Physiological effects; Living vaccine

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1. Introduction

Lactic acid bacteria are Gram-positive, non-spore forming, fermentative bacteria that grow anaerobically, and are traditionally applied in the conservation of a variety of fermented food products (Holzapfel, Haberer,
The largest group of lactic acid bacteria belong to the genus of *Lactobacillus* that comprises more than 50 different species (Stiles & Holzapfel, 1997; Tannock, 2004). In many cases, these lactobacilli are also used as starter cultures in industrial and artisanal food fermentation since they contribute to the conservation, flavor, and texture of the fermented foods. While the fermentative conversion of sugars present in the raw materials into lactic acid is their main function, production of anti-microbial peptides, exopolysaccharides and a variety of other metabolites are other important properties (Ross, Morgan, & Hill, 2002; Tamime, 2002). In addition, *Lactobacillus* species are found in the gut of humans and other animals, while their numbers may vary with the animal species, the age of the host, or the location within the gut. However, only a few *Lactobacillus* species contain representatives that are both involved in traditional and industrial food fermentations and reside in the human gut. Those include *L. crispatus*, *L. gasseri*, and *L. plantarum* (Cataloluk & Gogebakan, 2004) of which the phylogenetic position is depicted in Fig. 1. In this review, we will focus on *L. plantarum*.

*L. plantarum* is a versatile lactic acid bacterium, that is encountered in a range of environmental niches including dairy, meat and many vegetable fermentations (Table 1). Moreover, it is commonly found in the human gastrointestinal-tract (GI-tract) as described below. Furthermore, *L. plantarum* can be involved in spoilage of foods, such as meat (Borcha, Kant-Muermansb, & Blixta, 1997), wine (Beneduce, Spano, Vernile, Tarantino, & Massa, 2004) or orange juice (Alwazeer, Cachon, & Divies, 2002). Recently, the complete genome sequence of *L. plantarum* WCFS1, a single colony isolate of *L. plantarum* NCIMB 8826 from human saliva, has been determined and annotated (Kleerebezem et al., 2003). This analysis confirmed that *L. plantarum* has the coding capacity for the uptake and utilization of many different sugars, uptake of peptides, and formation of most amino acids. The large number of surface-anchored proteins suggests that *L. plantarum* has the potential to associate with many different surfaces and potential substrates for growth. In addition, the relatively high number of genes encoding regulatory functions indicated the ability to adapt to many different conditions. All together this reflects the potential of *L. plantarum* to grow in a large range of environmental niches. The large number of genes encoding surface proteins (217 predicted proteins) could function in recognition of or binding to certain components in the environment, since several of those genes show homology to proteins with predicted functions like mucus-binding, aggregation-promoting, and intercellular adhesion (Kleerebezem et al., 2003). A DNA-micro-array based comparison between 20 strains of *L. plantarum* showed absence or presence of different DNA regions. The main differences could be found in transferable regions like prophages and IS-elements, but also in other regions that are predicted to encode for

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**Table 1**

<table>
<thead>
<tr>
<th>Raw material</th>
<th>Product</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant products</td>
<td>Olives</td>
<td>(Duran Quintana, Garcia Garcia, &amp; Garrido Fernandez, 1999; Randazzo, Restuccia, Romano, &amp; Caggia, 2004)</td>
</tr>
<tr>
<td></td>
<td>Cocoa beans</td>
<td>(Ardhana &amp; Fleet, 2003)</td>
</tr>
<tr>
<td></td>
<td>Cassava</td>
<td>(Lei, Amoa-Awua, &amp; Brimer, 1999)</td>
</tr>
<tr>
<td></td>
<td>Suerkrat</td>
<td>(Stamer, 1983)</td>
</tr>
<tr>
<td></td>
<td>Togwa</td>
<td>(Kingamkono et al., 1999)</td>
</tr>
<tr>
<td></td>
<td>Wine</td>
<td>(Spano, Chieppa, Beneduce, &amp; Massa, 2004)</td>
</tr>
<tr>
<td>Milk products</td>
<td>Stilton cheese</td>
<td>(Ercolini, Hill, &amp; Dodd, 2003)</td>
</tr>
<tr>
<td></td>
<td>Traditional feta cheese</td>
<td>(Manolopoulou et al., 2003)</td>
</tr>
<tr>
<td></td>
<td>Ricotta forte cheese</td>
<td>(Baruzzi, Morea, Matarante, &amp; Cocconcelli, 2000)</td>
</tr>
<tr>
<td>Meat products</td>
<td>Fermented dry sausage</td>
<td>(Cocolin, Manzano, Cantoni, &amp; Comi, 2000; Enan, el-Essawy, Uyttendaele, &amp; Debever, 1996; Gevers, Danielsen, Huys, &amp; Swings, 2003)</td>
</tr>
<tr>
<td></td>
<td>Fermented Italian sausage</td>
<td>(Cocolin et al., 2000)</td>
</tr>
</tbody>
</table>
example the production of plantaricin, non-ribosomal peptides or exopolysaccharides. Very high levels of strain specific variation are encountered in a 200-kb region encoding mainly genes in sugar metabolism, which was probably acquired by horizontal gene transfer, and may represent a lifestyle adaptation island (Kleerebezem et al., 2003; Molenaar et al., 2005; Siezen, van Enckevort, Kleerebezem, & Teusink, 2004).

According to the definition of the World Health Organization, probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host (Gilliland, Morelli, & Reid, 2001). Recommended properties for a probiotic microbe include survival of the gut, persistence in the host, and proven safety for human consumption (Charteris, Kelly, Morelli, survival of the gut, persistence in the host, and proven safety for human consumption (Charteris, Kelly, Morelli, & Reid, 2001). Recommended properties for a probiotic microbe include survival of the gut, persistence in the host, and proven safety for human consumption (Charteris, Kelly, Morelli, & Reid, 2001).

<table>
<thead>
<tr>
<th>Administration</th>
<th>Product</th>
<th>Website</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsule</td>
<td>IFlora Acidophilus Formula</td>
<td><a href="http://sedonalabs.com/products/iflora.html">http://sedonalabs.com/products/iflora.html</a></td>
</tr>
<tr>
<td></td>
<td>Probiotic Eleven</td>
<td><a href="http://www.greatthestherbsonearth.com/nsp/probiotic_eleven.htm">http://www.greatthestherbsonearth.com/nsp/probiotic_eleven.htm</a></td>
</tr>
<tr>
<td></td>
<td>Living Vitamin C caps</td>
<td><a href="http://www.monstermarketplace.com/SearchByCategory/Product/1425/Landing/457/">http://www.monstermarketplace.com/SearchByCategory/Product/1425/Landing/457/</a></td>
</tr>
<tr>
<td></td>
<td></td>
<td>LivingVitaminCCAPS90:266:27:82</td>
</tr>
<tr>
<td>Fruit drink</td>
<td>Probiotic Eleven</td>
<td><a href="http://www.probiotic.co.uk">http://www.probiotic.co.uk</a>; <a href="http://www.probiotic.se">http://www.probiotic.se</a></td>
</tr>
<tr>
<td>Drink</td>
<td>Lactovitale</td>
<td><a href="http://www.filipinovegetarianrecipe.bravehost.com/lactobacillus_plantarum/">http://www.filipinovegetarianrecipe.bravehost.com/lactobacillus_plantarum/</a></td>
</tr>
<tr>
<td></td>
<td></td>
<td>intro_lacto_pafi.htm</td>
</tr>
</tbody>
</table>

Table 2

L. plantarum in health products as found by the 10 highest hits in Google (www.Google.com; Lactobacillus plantarum health products)

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L. plantarum in health products as found by the 10 highest hits in Google (www.Google.com; Lactobacillus plantarum health products)

3. Survival in the human GI-tract

L. plantarum has a long history of natural occurrence and safe use in a variety of food products including its well-known use in large numbers in sauerkraut and olive preparations (Table 1). Among the hundreds of reports on its safe use, there are only limited reports suggesting that some L. plantarum strains may be involved in infection. One of these concerns the isolation of L. plantarum from infective endocarditis. Those strains could in vitro coagulate blood by aggregation of human platelets, a possible pathogenic trait because of the danger of blood clotting (Harty, Oakey, Patrikakis, Hume, & Knox, 1994). However, this in vitro trait may not be reflecting the in vivo danger, since a large number of lactic acid bacteria appear to share this property. Moreover, the identification of the species was based on only a limited number of tests. Recent investigations showed that the human saliva isolate L. plantarum NCIMB 8826 did not induce macroscopic or histological inflammation or abnormal translocation through the intestinal barrier in mice (Pavan, Desreumaux, & Mercenier, 2003). On the contrary, total translocation of endogenous microbiota was reduced in mice suffering from colitis that were fed L. plantarum NCIMB 8826 (Pavan et al., 2003). After an intravenous injection of Sprague-Dawley rats with 10^⁸ cfu of L. plantarum 299v, this organism could not be recovered from the heart and blood, when the rats were sacrificed 96 h after injection. So even if the intestinal barrier was crossed, no infection took place, showing the apparent safety of the organism (Adawi, Molin, Ahrne, & Jeppsson, 2002). Moreover, a recent post-market surveillance study showed that L. plantarum is not found in bacteremia cases (Salminen et al., 2002). Finally, various clinical studies, as described below, underline the safe use of L. plantarum in humans.

3. Survival in the human GI-tract

After oral ingestion, bacteria encounter a number of human defence systems that are associated with secretions. These include high concentrations of mucins that cover the gut, gastric acid inducing a low pH in the stomach, and bile salts secreted into the luminal content in the proximal small intestine (Ouwehand, Derrien, de Vos, Tiihonen, & Rautonen, 2005). L. plantarum WCFS1 does not utilize mucin, although it is predicted to encode mucin-binding proteins (Kleerebezem et al., 2003). In addition, different
strains of *L. plantarum* were found to show a high tolerance to the consecutive exposure to hydrochloric acid (pH 2.0) and bile salts. This was observed both for strains isolated from intestinal samples and for those isolated from fermented foods (Haller et al., 2001). Of the *L. plantarum* cells 0.003–10% survived those conditions compared to no survival and very limited survival (0–0.001%) for *L. sakei* and *L. paracasei*, respectively (Haller et al., 2001). *L. plantarum* NCIMB 8826 also displayed high survival in vivo following human ingestion (Vesa, Pochart, & Marteau, 2000). Survival was tested using intestinal intubation of the ileum after a single dose of $10^8$ cells was given to healthy volunteers. Direct plating showed 7±2% survival in the human ileum, which is possibly a relevant location in terms of specific gut properties of this bacterium. In contrast, *Lactococcus lactis* showed only 1±0.8% survival and for *L. fermentum* 0.5±0.5% survival was determined in this experiment. Survival of *L. plantarum* NCIMB 8826 up to 25% compared to an inert marker was reached in the faeces after 1 week of daily ingestion. During this study, the strain used did not persist in these subjects, as the transit time of *L. plantarum* was the same as for an inert marker (Vesa et al., 2000). However, 11 days after the end of the administration which consisted of daily simultaneous intake for 10 days of different strains of lactobacilli with $5 \times 10^8$ cfu per strain, colonization with *L. plantarum* 299 and 299v was found in the jejunum and rectum of 85% of healthy volunteers indicating that colonization can be person-dependent (Johansson et al., 1993). In this study, colonization with *L. plantarum* was considerably better than with *L. salivarius, L. reuteri, L. gasseri, L. acidophilus, L. casei*, and *L. agilis*, because two thirds of the strains recovered using plate-counting and confirmed with API-50CH system and restriction analysis belonged to *L. plantarum* and a mix of the other administered species accounted only for one third of the recovered strains (Johansson et al., 1993).

As a common indigenous bacterium *L. plantarum* could be isolated from 1 out of 20 of the ileum and colonic samples using plate counting and phenotypical characterization of both healthy and diseased persons (Molin et al., 1993). Nevertheless, *L. plantarum* could be isolated from the mouth or rectum of the majority of healthy subjects tested (Ahrne et al., 1998), indicating that the presence of *L. plantarum* differs per sampling location and is a common gut bacterium. If no *L. plantarum* was consumed, the numbers of this organism in faeces were too low to be detected, which corresponds to a viable count of less than $3.2 \times 10^6$ cfu g$^{-1}$ (Johansson et al., 1998). Two thirds of the isolates from mouth and rectum showed mannose-inhibited adherence to the human colonic cell line HT-29 suggesting the possibility of permanent colonization. This feature was infrequently found for other lactobacilli isolated from the gut (Ahrne et al., 1998). Different strains of *L. plantarum*, including 299v, indeed showed the capacity to adhere to human cells in a mannose-inhibited manner that is indicative of binding to a mannosylated cell-bound receptor (Adlerberth et al., 1996). Competition for those receptors between *L. plantarum* and pathogenic bacteria, including *Escherichia coli*, reduces adherence to the human cells of the latter and in this manner may protect the host from infection. By combining information from genome-wide array-based genotyping of different *L. plantarum* strains with specific inactivation studies, the mannose-adhesion gene was identified (Molenaar et al., 2005; Pretzer et al., 2004). This gene is one of the first genes to be identified that is associated with a definite probiotic effect. It is predicted to encode a large (>1000 residues) cell envelope-located protein. Its identification allows for detailed studies with deletion and overproduction strains that will allow for the construction or identification of strains that effectively exclude pathogens that contain type I fimbriae (Pretzer et al., 2004).

### 4. Effects of *L. plantarum* in healthy volunteers

Due to their abundance, easy growth characteristics, and human origin, various *L. plantarum* strains have been tested for health effects. Different effects were observed following consumption of significant amounts of living *L. plantarum* cells in healthy subjects (Table 3). A significant increase in the total faecal concentration of carboxylic acids (from 83 to 113 mmol g$^{-1}$ wet faeces), acetic acids (from 48 to 64 mmol g$^{-1}$ wet faeces) and propionic acid (from 11 to 17 mmol g$^{-1}$ wet faeces) was found in a study in which the subjects consumed *L. plantarum* 299v (= DSM 9843) daily for 3 weeks (Johansson et al., 1998). This effect is probably due to an effect of *L. plantarum* on specific colonic bacteria, because *L. plantarum* produces mainly lactic acid instead of acetic acid and lacks several of the genes encoding enzymes for the production of propionic acid. Together with an increase in stool volume, a decrease in flatulation and slightly softer stools, those results indicate an altered fermentation in the colon (Johansson et al., 1998).

A significant decrease in anaerobic bacterial counts (from $4.0 \times 10^6$ to $1.0 \times 10^6$ cfu g$^{-1}$ of mucosa) and Gram-negative anaerobic counts (from $1.0 \times 10^6$ to $7.9 \times 10^4$ cfu g$^{-1}$ of mucosa) in the rectum of healthy volunteers was found to occur with a daily intake of an oatmeal soup containing different strains of lactobacilli (Johansson et al., 1993). The *Lactobacillus* strains that were mainly recovered at 1 and 11 days after the end of the trial in both the jejunum and the rectum were *L. plantarum* 299 and 299v, two very closely related strains. *L. agilis* was also recovered in high amounts after 1 day, but this was drastically reduced 11 days after ending the administration. The two *L. plantarum* strains showed survival of passage as well as a prolonged retention, indicating colonization in the GI tract. The reduction in Gram-negative anaerobic bacterial counts was only found 11 days after the end of the trial, suggesting that this effect takes place after a period of establishment of the lactobacilli (Johansson et al., 1993). Similar reductions in Gram-negative anaerobic bacteria were obtained following *L. plantarum* E98 addition to a simulator of the human...
Table 3  
Influences of *L. plantarum* on animal models, healthy volunteers and patients as assessed by in vitro and in vivo studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Host</th>
<th>Dose* (strain)</th>
<th>Subjects</th>
<th>Intake</th>
<th>Effect</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Healthy subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Johansson et al. (1998)</td>
<td>Human</td>
<td>2.0 × 10^{10} (1)</td>
<td>26</td>
<td>3 weeks</td>
<td>Increased short-chain fatty acid content of faeces</td>
<td>Randomised placebo controlled double-blind study</td>
</tr>
<tr>
<td>Johansson et al. (1993)</td>
<td>Human</td>
<td>5.0 × 10^8 (1, 2)</td>
<td>13</td>
<td>10 days</td>
<td>Changing microbiota in ileum and rectum; Dominant recovery of <em>L. plantarum</em> (2/3 of recovered strains)</td>
<td>Mixed use of different strains; Unclear cause-effect relation</td>
</tr>
<tr>
<td>McNaught et al. (2002)</td>
<td>Human</td>
<td>2.5 × 10^{10} (1)</td>
<td>129</td>
<td>Differed</td>
<td>No effect on post-operative wound infection</td>
<td>Study not blinded and intake may differ considerably between patients</td>
</tr>
<tr>
<td>Kingamkono et al. (1999)</td>
<td>Human</td>
<td>Unknown (1)</td>
<td>151</td>
<td>13 days</td>
<td>Up to six times reduction in carriage of faecal enterobacteriaceae</td>
<td>Placebo controlled study with large number (151) of subjects, but no dose specified</td>
</tr>
<tr>
<td>Bukowska et al. (1998)</td>
<td>Human</td>
<td>10^{10} (1)</td>
<td>30</td>
<td>6 weeks</td>
<td>Reduction in LDL-cholesterol (9.6%) and fibrinogen (13.5%)</td>
<td>Randomised placebo controlled double-blind study with male subjects and questionable proposed mode of action</td>
</tr>
<tr>
<td>Naruszewicz et al. (2002)</td>
<td>Human</td>
<td>2.0 × 10^{10} (1)</td>
<td>36</td>
<td>6 weeks</td>
<td>Reduction in LDL-cholesterol (11.7%) and fibrinogen (21.0%)</td>
<td>Randomised placebo controlled double-blind study</td>
</tr>
<tr>
<td><strong>Clinical trials</strong></td>
<td></td>
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<tr>
<td>Cunningham-Rundles et al. (2000)</td>
<td>Children exposed to HIV</td>
<td>Unknown (1)</td>
<td>18</td>
<td>1 month</td>
<td>Improved natural immune response</td>
<td>Placebo controlled double-blind study with unclear experimental set-up and low number of subjects</td>
</tr>
<tr>
<td>Wullt et al. (2003)</td>
<td>Human</td>
<td>5.0 × 10^{10} (1)</td>
<td>20</td>
<td>38 days</td>
<td>1/3 reduction in recurrence of <em>Clostridium difficile</em>-associated diarrhoea</td>
<td>Placebo controlled double-blind study with low number of subjects; significance unclear</td>
</tr>
<tr>
<td>Nobaek et al. (2000)</td>
<td>Human</td>
<td>2.0 × 10^{10} (1)</td>
<td>60</td>
<td>4 weeks</td>
<td>Reduction in symptoms IBS</td>
<td>Randomised placebo controlled double-blind study with subjects recording their own symptoms</td>
</tr>
<tr>
<td>Niedzielin et al. (2001)</td>
<td>Human</td>
<td>2.0 × 10^{10} (1)</td>
<td>40</td>
<td>4 weeks</td>
<td>Reduction in symptoms IBS</td>
<td>Randomised placebo controlled double-blind study with mainly female subjects and gastroenterologists and patients recording symptoms</td>
</tr>
<tr>
<td>Sen et al. (2002)</td>
<td>Human</td>
<td>6.3 × 10^9 (1)</td>
<td>12</td>
<td>4 weeks</td>
<td>No reduction in symptoms IBS</td>
<td>Randomised double-blind crossover study with gastroenterologists and patients recording symptoms; low dose may reduce effects; low number of patients</td>
</tr>
<tr>
<td><strong>In vitro studies</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Alander et al. (1998)</td>
<td>SHIME (GI-tract model)</td>
<td>1.4 × 10^9</td>
<td>7 days</td>
<td></td>
<td>Decrease enterobacteriaceae and clostridia and slight increase enterococci</td>
<td>Experiment done only once</td>
</tr>
<tr>
<td>McCracken et al. (2002)</td>
<td>Human cell line HT-29</td>
<td>1 cfu/1000 cells (1)</td>
<td></td>
<td>3 h</td>
<td>Increase of IL-8 mRNA in epithelial cells and down regulation of IL-8 secretion</td>
<td><em>L. plantarum</em>/host-cell ratio rather unfavourable</td>
</tr>
<tr>
<td>Michail and Abernathy (2003)</td>
<td>T-84 cell line</td>
<td>10^7, 10^8, and 10^9 (1)</td>
<td></td>
<td>2.5 h</td>
<td>Inhibition of EPEC induced neutrophil migration</td>
<td><em>L. plantarum</em>/host-cell ratio unknown and only 10^9 cells induced effect</td>
</tr>
<tr>
<td>Mack et al. (1999)</td>
<td>Human cell line HT-29</td>
<td>10^5, 10^7, 10^8 and 10^9 (1)</td>
<td></td>
<td></td>
<td>Inhibition of enteropathogenic <em>E. coli</em> adherence</td>
<td><em>L. plantarum</em>/host-cell ratio unknown and more induced effect</td>
</tr>
<tr>
<td><strong>Animal models</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perdigon et al. (1999)</td>
<td>Mice</td>
<td>10^9 (3)</td>
<td>20–24</td>
<td>2, 5 or 7 days</td>
<td>Increase in specific and unspecific immunity</td>
<td>Effects were dependent on intake</td>
</tr>
</tbody>
</table>
Table 3 (continued)

<table>
<thead>
<tr>
<th>Study</th>
<th>Host</th>
<th>Dose (strain)</th>
<th>Subjects</th>
<th>Intake</th>
<th>Effect</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mangell et al. (2002)</td>
<td>Rats</td>
<td>Differed per group (1)</td>
<td>25</td>
<td>1 week</td>
<td>Inhibition of E. coli-induced intestinal permeability</td>
<td>Intake of drinking water containing L. plantarum was unknown</td>
</tr>
<tr>
<td>Liu et al. (2001)</td>
<td>Rats</td>
<td>2 × 10⁶ (1)</td>
<td>72</td>
<td>22 days</td>
<td>Reduction in side effects of external radiation on colon anastomotic healing</td>
<td>Placebo controlled study</td>
</tr>
</tbody>
</table>

CEFU per day unless stated otherwise; strain 1 L. plantarum 299v; strain 2 L. plantarum 299; strain 3 L. plantarum CRL 936.

intestinal microbial ecosystem (SHIME) (Alander et al., 1999). A reduction in Gram-negative anaerobic bacteria is considered to be advantageous from a medical perspective, since these bacteria are frequently isolated from infected sites after intestinal surgery (Nichols & Smith, 1994). In several healthy subjects who daily consumed an oatmeal soup containing different strains of lactobacilli also a reduction in Enterobacteriaceae (at least 1000-fold), or sulfite-reducing clostridia (10–100-fold) could be observed (Johansson et al., 1993). A significant (more than 6-fold) decrease in the carriage of enteropathogenic bacteria was also found in healthy children less than 5 years old that daily consumed an L. plantarum 299v fermented cereal gruel known as togwa. At the start of the experiment 38.9% of the children had enteropathogenic bacteria detected in rectal swabs, which declined to 6.1% and 6.9% after 1 and 2 weeks of togwa, respectively. The effects were still detected 2 weeks after the consumption of togwa with only 6.5% of the children carrying enteropathogens. Several mechanisms have been proposed and included a lower transmission of enteropathogens through eating the pathogen-free togwa, an inhibitory effect in the gut through production of inhibitory factors, or a competition for specific niches and nutrients (Kingamkono, Sjogren, & Svanberg, 1999).

In a study with subjects with moderately elevated cholesterol, it has been shown that consumption of L. plantarum 299v could reduce both the LDL-cholesterol and fibrinogen levels in the blood significantly, with 9.6% (p < 0.01) and 13.5% (p < 0.001), respectively (Bukowska, Pieczul-Mroz, Jastrzebska, Chelstowski, & Naruszewicz, 1998). In a study on heavy smokers, which were given twice that dose of L. plantarum a more pronounced effect was seen, and an 11.7% decrease in LDL-cholesterol and 21% in fibrinogen was observed. Both LDL-cholesterol and fibrinogen are independent risk factors for coronary artery disease. The suggested mechanism is an anti-inflammatory action of the propionic acid production derived by L. plantarum. This is also found for ibuprofen, a derivative of propionic acid (Naruszewicz, Johansson, Zapolska-Downar, & Bukowska, 2002). Consumption of L. plantarum indeed showed a significant increase in propionic acid in the faeces of healthy volunteers, probably by influencing other colonic bacteria (Johansson et al., 1998).

5. Effects of L. plantarum on disease

A variety of clinical trials have been reported aimed at demonstrating an effect of consumption of L. plantarum on different diseases (Table 3). Considerable attention has been given to immune stimulation by L. plantarum strains. In one study it was shown that L. plantarum 299v was able to colonize children congenitally exposed to the human immunodeficiency virus (HIV) and preliminary results showed an increase in weight and specific immune response of those children (Cunningham-Rundles et al., 2000). However, the experimental setup of this trial was unclear and only a low number of subjects participated. Stimulation of the immune response was also suggested by the findings that the proinflammatory cytokine TNF-alpha can sensitize HT-29 epithelial cells to viable L. plantarum 299v cells (McCracken et al., 2002). While TNF-alpha exposure resulted in increasing production of the mRNA for the inflammatory protein, interleukin-8 (IL-8), the addition of L. plantarum could exert a protective effect by down-regulating IL-8 secretion from the cells. However, the mechanism behind this is not yet known (McCracken et al., 2002). The mechanism for immune stimulation by L. plantarum was addressed in animal experiments to verify in vivo activity. A possible mechanism of stimulation of the immune system via oral administration of L. plantarum CRL 936 in mice was through stimulation of the M-cells at the Peyers patches, which both increased specific immunity by IgA⁺ cells in both the intestine and in the bronchus, and increased unspecific immunity by inducing the level of the CD4⁺ T cells (Perdigon, Vintini, Alvarez, Medina, & Medici, 1999).

Various studies showed a protective effect of L. plantarum against intestinal infections (Table 3). In a small double-blind, placebo-controlled trial, Clostridium difficile-associated diarrhea was found to be less recurrent in a small group of patients if antibiotics were administered in combination with L. plantarum 299v (4 of 11 patients instead of 6 of 9 patients in the placebo group) (Wullt, Johansson Hagslätt, & Odenholt, 2003). A protective effect against E. coli-induced intestinal permeability in rats after mixing their drinking water with a fermented oatmeal drink, containing 10⁸ CFU mL⁻¹ of L. plantarum 299v for 1 week was also observed using Ussing chambers.
In contrast to the untreated group, *E. coli* did not negatively influence the permeability of the intestinal cell wall of rats that received *L. plantarum* (Mangell et al., 2002). Pre-incubation of an intestinal epithelial T-84 monolayer with *L. plantarum*, showed a reduced attachment of enteropathogenic *E. coli* and reduction in inflammatory factors, like neutrophil migration (Michail & Abernathy, 2003). However, short-term addition of *L. plantarum* did not reduce the effect of *E. coli* in similar experiments on the intestinal cells (Mangell et al., 2002; Michail & Abernathy, 2003). A possible mechanism is competitive exclusion between *L. plantarum* and *E. coli* via the mannose adhesin that can be involved in competition for the mannose-specific binding sites as described earlier (Pretzer et al., 2004). Another possible mechanism could involve the induction of the expression of the MUC2 and MUC3 genes in HT-29 cells and subsequently stimulating the mucin production, which could inhibit adherence of *E. coli*. This effect is induced by a component secreted in the medium, since the cell-free supernatant of *L. plantarum* 299v showed the same effect as whole cultures (Mack, Michail, Wei, McDougall, & Hollingsworth, 1999). A candidate for this effect is the product of the sdr gene, that is predicted to be a 3360-residue protein with a nearly perfect SD (Ser-Asp)-repeat. It has been suggested that glycosyltransferases could make O-linked glycosylations on the serines, producing mucin like structures, which may interact with the host cell mucins (Kleerebezem et al., 2003). This interaction may influence the expression of the MUC genes. The effect of *L. plantarum* on *C. difficile* infection may have a similar mechanism involving stimulation of mucin production or another form of mannose binding.

The pathogenic mechanisms of irritable bowel syndrome (IBS) are not known yet but a major factor has been implied to be disturbance of the intestinal bacterial microbiota (Nobaek, Johansson, Molin, Ahrne, & Jeppsson, 2000). This factor is known to be affected by the consumption of *L. plantarum*, but only few and contradicting studies have been reported with individuals suffering from IBS (Table 3). A reduction in abdominal bloating and pain of subjects suffering from IBS was found in both a Polish and a Swedish study after daily administration of 400 ml of a rose-hip drink containing *L. plantarum* 299v for 4 weeks. In addition, flatulence was rapidly and significantly reduced and abdominal pain was less (Niedzielin, 2001; Nobaek et al., 2000). However, a subsequent study did not find any effect of the daily administration of 125 mL of this rose-hip drink containing *L. plantarum*, possibly due to the lower numbers of *L. plantarum* used or due to the low number of patients tested (Sen et al., 2002).

Colorectal cancer is mainly treated by surgical resection and in some cases is followed by external radiation to avoid recurrence. To reduce complications, like diarrhoea, retarded healing, and mucosal atrophy, after this treatment enteral feeding of *L. plantarum* 299v in rats was examined and found to reduce inflammatory reactions and increase healing of the wound (Liu et al., 2001) (Table 3). However, patients who were scheduled for intestinal surgery were asked to consume *L. plantarum* a week prior to the intervention. In comparison with the untreated control group no protection from wound infections were observed after intestinal surgery (McNaught, Woodcock, MacFie, & Mitchell, 2002). However, the study was not blinded and *L. plantarum* intake may have differed considerably between patients.

6. *L. plantarum* as a living vaccine

The potential of living vaccines to deliver heterologous antigens to the mucosal immune system offers a number of advantages over traditional vaccination, such as non-invasiveness and the possibility to induce both a systemic and mucosal immune response (Grangette et al., 2002). This has been studied using *L. plantarum* NCIMB 8826 as a model. To determine the most appropriate manner for immunization of the host by *L. plantarum*, green fluorescent protein (GFP) was used to tag the bacterium (Geoffroy et al., 2000). Fluorescent bacteria could be detected in the lumen mainly associated with the mucus after administration by intragastric gavage in mice. However, the *L. plantarum* cells were not found to be associated with the Peyer’s patches, which may be caused by the low amount of cells administered. After nasal administration, *L. plantarum* could be visualized in the macrophages, indicating the suitability of this immunization path (Geoffroy et al., 2000). Another study using a non-toxic C fragment of tetanus toxin-producing *L. plantarum* strain for immunization of mice, indicated that both the intragastric and nasal route of administration were appropriate (Reveneau, Geoffroy, Loacht, Chagnaud, & Mercenier, 2002). The immune response triggered by *L. plantarum* cells excreting antigens or delivering these on the cell surface was more efficient than with antigens present in the cytoplasm (Reveneau et al., 2002). *L. plantarum* has a higher immunization capacity than *L. lactis*, which may indicate that persistence plays a role (Grangette et al., 2002). Cell wall mutants, lacking the alanine racemase gene (*ahr*), were as persistent in the murine gut as their wild type counterparts. However, the depletion of α-alanine in the medium showed increased membrane permeability. In addition, the cell wall mutants were far more immunogenic than the wild type cells (Grangette et al., 2004). An *L. plantarum* strain, encoding the peptide 111-139 of Der p 1 of the house dust mite under control of the constitutive lactate dehydrogenase (*ldh*)-promoter of *L. casei*, could be used to inhibit house dust mite-specific T-cell responses in mice, indicating a possible treatment of allergic disorders (Kruisselbrink, Heijne Den Bak-Glashouwer, Havenith, Thole, & Janssen, 2001). In conclusion, a variety of studies exploit the capacity of *L. plantarum* to secrete bioactive molecules that evoke an immune response. In general, *L. plantarum* is preferred
above other food-grade lactic acid bacteria based on its convenience in production, high-level genetic accessibility and performance in the GI-tract.

7. The effect of conditions resembling the gut on \( L. \) plantarum

Even though much is known about the effect of \( L. \) plantarum on the physical condition of the host, limited knowledge is available on the reaction of the bacterium to the intestinal conditions it encounters after consumption. The determination of the complete genome sequence has expanded considerably our understanding of the possible functions of \( L. \) plantarum. A combination of this information with the knowledge of the behavior of \( L. \) plantarum in the gut could give a broader insight in the mechanisms behind the health promoting properties and possible further functions of this bacterium (de Vos, Bron, & Kleerebezem, 2004).

Several in vitro studies have been performed to detect the genes that are switched on under the conditions typical in the gut. Cloning random fragments of the \( L. \) plantarum WCFS1 genome upstream of a promoterless alanine racemase \((\text{alr})\) gene of \( L. \) lactis in a low-copy-number plasmid vector resulted in a plasmid library with 98% coverage of the genome (Bron, Hoffer, Van Swam, Vos, & Kleerebezem, 2004). The plasmid library was introduced into an \( L. \) plantarum \( \text{Δalr} \) strain and screened for clones that could complement the D-alanine auxotroph phenotype in the presence of 0.8 M NaCl. Eight clones were detected that showed significant higher \( \text{alr} \) production and found to contain \( L. \) plantarum promoters preceding genes coding for different functions such as an integral membrane protein, glyceralate kinase, permease, short chain dehydrogenase, and different hypothetical proteins. Four of the promoters contained the same conserved motive, which is not found further on the chromosome, indicating a specific regulation of their genes (Bron, Hoffer et al., 2004). A more elaborate screen using the same \( \text{alr} \)-complementation approach on 0.1% porcine bile showed induction of 31 genes, including 11 membrane- and cell-wall-associated functions, five functions involved in redox reactions and also five regulatory factors (Bron, Molenaar, Vos, & Kleerebezem, 2004). Another in vitro approach exploited the use of DNA micro-arrays and compared \( L. \) plantarum WCFS1 grown on MRS agar with or without 0.1% porcine bile salts (Bron, Hoffer et al., 2004). This global screening approach showed up regulation by bile of stress proteins, cell-envelope located proteins, and proteins involved in redox reactions (Bron, Molenaar et al., 2004). All three in vitro studies showed alterations in the cell wall, presumably to protect the cell from the harsh conditions. This is also evident from the observed altered cell morphology when \( L. \) plantarum is exposed to bile acids (Bron, Hoffer et al., 2004). The common upregulation of genes involved in redox reactions may point to different metabolic reactions under intestinal conditions. While several regulatory genes are also affected in these in vitro studies, it remains to be seen whether specific regulatory circuits are operating under intestinal conditions. Moreover, it is difficult to extrapolate data obtained under in vitro conditions to those that are really met in the intestine. Hence, approaches to study \( L. \) plantarum gene expression in the intestine itself are essential as discussed below.

8. The effect of the gut on \( L. \) plantarum

Genes of \( L. \) plantarum WCFS1 specifically switched on in the gut of mice have been determined using a resolvase-based in vivo expression technology (R-IVET) (Bron, Grangette, Mercenier, de Vos, & Kleerebezem, 2004). These include sugar-related functions, acquisition and synthesis of amino acids, nucleotides, cofactors and vitamins (Fig. 2). Also stress-related functions were found to be specifically expressed and reflecting the harsh conditions of the gut (Bron, Grangette et al., 2004). Deletion mutants of those genes demonstrated a reduced survival of the GI-tract of mice indicating the importance of those genes for survival in those conditions (Bron, Meijer, Bongers, Vos, & Kleerebezem, 2004). Interestingly, the \( L. \) plantarum genes found to be induced in the GI-tract demonstrate a large overlap to the induced genes of pathogens under the same conditions (Bron, Grangette et al., 2004). To validate the R-IVET strategy, the intestinal expression of several of these genes, including several cell surface proteins, transporters, including a cellobiose PTS gene and a copper transporting ATPase gene, and genes involved in sugar metabolism, including an alcohol dehydrogenase gene and a ramnosidase gene, were analyzed by a quantitative reverse transcriptase PCR approach (Marco, M., personal communication, 2005, NIZO Food Research, Ede, The Netherlands). Insight into the promoters that are switched on in the gut can be used to construct delivery systems aimed to produce at an

![Fig. 2. Functional classes of genes of \( L. \) plantarum identified as in vivo induced \((\text{ivii})\) in the gut, using R-IVET screening in a murine model (Bron, Grangette et al., 2004).](image)
appropriate intestinal location enzymes, antigens or other therapeutic proteins (Hannify et al., 2004). In addition, the presentation of antigens to the immune system can be improved by placing a lytic cassette under control of an intestine-specific promoter, inducing intestinal lysis of the cells (Hannify et al., 2004).

While model animals such as mice are ideal systems for formulating hypotheses on microbial activity, the real answers come from experiments in human systems. Hence, various approaches have been proposed to realize this (de Vos et al., 2004). In a recent study, DNA micro-arrays were used to monitor gene expression of \textit{L. plantarum} in surgically removed intestinal segments of potential colon cancer patients who prior to surgery ingested a fermented oatmeal drink with \textit{L. plantarum} 299v (10^{11} viable cells daily) or a placebo for 1 week. Specific expression was observed of genes encoding sugar uptake and metabolism, amino acid biosynthesis, as well as cell division and stress-related genes. These indicated survival, metabolic activity, and even growth of \textit{L. plantarum} more or less attached to the human gut wall. In combination with clinical studies, this approach is a powerful and high-throughput tool to provide insight and new perspectives on in vivo host-microbe interactions (unpublished data). Even though the approaches for investigating differential gene expression in the GI-tract were different, as well as the hosts, mouse versus human, a substantial 46% of the genes revealed by R-IVET were expressed in the microarray experiments. Those genes were mainly involved in nutrient acquisition and synthesis, stress, and extracellular functions. This indicates that the GI-tract conditions of mouse and man may have similar effects on global gene expression of \textit{L. plantarum} in the GI-tract.

9. Conclusions

\textit{L. plantarum} has a proven ability to survive gastric transit and colonize the gut, with an apparent safety to the consumer. Many studies describe the physiological effects of consumption of \textit{L. plantarum} on humans. However, there is a great variability in the experimental setup and quality of the studies. In some studies, consumption of \textit{L. plantarum} showed, amongst other effects, reduction in carriage of faecal enterobacteriacea, reduction of certain risks factors for coronary artery diseases, and a dose-dependent reduction in the symptoms of IBS. The development of \textit{L. plantarum} as a living vaccine offers a large range of therapeutic possibilities. Studying mechanisms for targeted gastrointestinal properties, like competitive adherence of \textit{L. plantarum} to mannose-specific receptors or reduction in pathogenicity by induction of the human mucin genes, is a relatively new field of investigation. The availability of the complete genome sequence of \textit{L. plantarum} WCFS1 makes it a suitable model to study with molecular approaches such as promoter screens, R-IVET, and DNA micro-arrays. This is expected to contribute to unravelling more mechanisms behind the targeted gastrointestinal properties and possible further functions of \textit{L. plantarum}. In addition, these molecular strategies will reveal targets for genetic screening for culture collections aiming to select strains with predictable in situ functional properties. All this will lead to a second generation of probiotics with a scientifically proven basis for the health effect they provide.

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References


