Probiotics in foods not containing milk or milk constituents, with special reference to *Lactobacillus plantarum* 299v1–3

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ABSTRACT Lactic acid fermentation is the simplest and safest way of preserving food and has probably always been used by humans. Species such as *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, *Lactobacillus acidophilus*, and *Lactobacillus salivarius* are common in the human mucosa, from the mouth to the rectum. In food, *L. paracasei* and *L. rhamnosus* are usually associated with dairy products whereas *L. plantarum* is found in fermented foods of plant origin. A probiotic food product containing no milk constituent was launched in Sweden in 1994. The product is a lactic acid fermented oatmeal gruel that is mixed in a fruit drink. It contains 5 × 10^10 colony-forming units of *L. plantarum* 299v/L. The strain *L. plantarum* 299v originates from the human intestinal mucosa and has been shown in rats to decrease translocation, improve mucosal status, improve liver status, improve the immunologic status of the mucosa, and reduce mucosal inflammation. In humans, *L. plantarum* 299v can increase the concentration of carboxylic acids in feces and decrease abdominal bloating in patients with irritable bowel disease. It can also decrease fibrinogen concentrations in blood. Should probiotics be administrated through foods, the probiotic organism must remain vigorous in the food until consumption and the food must remain palatable, ie, the food carrier and the organism must suit each other. *L. plantarum* 299v not only affects the bacterial flora of the intestinal mucosa but may also regulate the host’s immunologic defense. The mechanisms involved need to be clarified. *Am J Clin Nutr* 2001;73(suppl):380S–5S.

KEY WORDS *Lactobacillus plantarum*, probiotic, nondairy food, fermented food, lactic acid fermentation

BACKGROUND The idea that lactic acid bacteria prevent intestinal disorders and diseases is nearly as old as the science of microbiology. The first formula for deliberate administration of live lactic acid bacteria was a sour milk product (a yogurt) based on a culture called *la Lactobacilline* isolated by Metchnikoff (the French spelling of his Russian name Ilja Metnjikov). The product was launched in Paris at the beginning of the 20th century (1). The culture consisted of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. Ever since this successful introduction, the incorporation of probiotic bacteria in foods has been focused on milk-based products. For example, in the 1930s, *Lactobacillus acidophilus* was incorporated into milk (2) because the traditional yogurt culture (ie, *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*) did not survive well in the human gastrointestinal system. However, although *L. acidophilus* is a frequently used probiotic, these bacteria do not multiply well in milk.

Even though many traditional lactic acid fermented foods besides milk-based ones are available, for one reason or another, these are rarely considered as carriers for probiotic bacteria. Examples of traditional, non-milk-based foods containing high concentrations of lactobacilli are brined olives, salted gherkins, and sauerkraut, ie, lactic acid fermented plant materials. Another type of plant-based, traditional, and well-established lactic acid fermented product is sourdough, even though these lactobacilli are not consumed alive because the product is baked before consumption. However, some cereal-based, lactic acid fermented products are eaten without heat treatment after fermentation, eg, the Tanzanian beverage *togwa* (Figure 1). *Togwa* is often made from sorghum or maize and is consumed regularly by young children (6–60 mo of age). It was shown to decrease the occurrence of enteropathogens in rectal swabs (4) and to improve the barrier function of the intestinal mucosa in children aged 6–25 mo with acute diarrhea (5).

Lactic acid fermented foods have made up a significant portion of food intake by humans for a long time and still do in many developing countries, eg, in Africa. Lactic acid fermentation is the simplest and often the safest way of preserving food, and before the Industrial Revolution, lactic acid fermentation was used just as much in Europe as it still is in Africa. In fact, there are archaeologic indications that humankind has always used this technique (6, 7; Figure 2). In this way, humans consumed large numbers of live lactic acid bacteria, and presumably those associated with plant material were consumed before those associated with milk. Thus, it may well be that the human gastrointestinal tract evolved to adapt to a more or less daily supply of live lactic acid bacteria. This supply ceased in industrialized countries during the 20th century, which may have led to gastrointestinal problems, perhaps even immunologically dependent ones.

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LACTOBACILLI IN THE HUMAN INTESTINAL MUCOSA

Lactobacilli belong to the normal mucosal flora of the human mouth (11, 12) and intestine (13–15). L. acidophilus is frequently referred to as being dominant in the gastrointestinal tract (13, 16), but unreliable identification methods were used in most older studies. A numerical study of phenotypic features showed that the most common lactobacilli taxa in intestinal mucosal biopsies are Lactobacillus rhamnosus, L. acidophilus-like, and Lactobacillus salivarius (17). In this study, L. acidophilus could not be distinguished from Lactobacillus crispatus, Lactobacillus jensenii, or Lactobacillus gasseri. The individuals studied were seeking medical advice for gastrointestinal complaints or were surgery patients. Thus, although one-half of the subjects were considered to have healthy mucosae, they may not have had undisturbed intestinal tracts. In a more recent study in which the phenotype analysis was supplemented by an examination of genetic traits, the most common lactobacilli in the mucosa of healthy subjects were Lactobacillus plantarum, L. rhamnosus, and Lactobacillus paracasei subsp. paracasei, which were isolated from 52%, 26%, and 17% of the individuals, respectively (both from rectal and oral mucosae; 18).

The occurrence of L. paracasei and L. rhamnosus is otherwise mostly reported in association with consumption of dairy products. On the other hand, L. plantarum is frequently associated with lactic acid fermented foods of plant origin, eg, brined olives (19), sauerkraut (20), salted gherkins (21), sourdough (22), Nigerian ogi [made with maize or sorghum (23)], Ethiopian kocho [made with starch from Ensete ventricosum (24, 25)], and Ethiopian sourdough made from tef [Eragrostis tef (25, 26)]. Thus, individuals consuming lactic acid fermented products of plant origin also consume large amounts of L. plantarum. This is interesting with respect to the high prevalence of L. plantarum in the human intestinal mucosa (18). Furthermore, it was shown that 2 strains of L. plantarum (strain 299v or DSM 6595 and strain 299v or DSM 9843) can become established in the intestinal mucosa after ingestion by healthy volunteers (27). L. plantarum 299v was isolated in high numbers from jejunal and rectal biopsies as late as 11 d after administration had ceased in 11 of 13 individuals (27). This strain and most L. plantarum strains of intestinal origin can adhere to cell lines of intestinal origin because they possess a mannose binding adhesin (18, 28).

LACTOBACILLUS PLANTARUM 299V IN LACTIC ACID FERMENTED OATMEAL GRUEL

Probiotic product

A new probiotic functional food was launched in Sweden in 1994 and to my knowledge this is the first probiotic food that does not contain milk or milk constituents (ProViva: Skane Dairy, Malmö, Sweden). The active component is a lactic acid fermented oatmeal gruel that was originally developed as a new concept for enteral (nasogastric) feeding (29). The production process for the enteral feeding formula is outlined in Figure 3. Malted barely is added to enhance the liquefaction of the product and L. plantarum 299v carries out the fermentation. The final product contains $1 \times 10^{12}$ colony-forming units (CFU) of L. plantarum/L. This formula is used as the active ingredient in the food product in which 5% of the oatmeal gruel is mixed with a fruit drink (rose hip, strawberry, blueberry, black currant, or tropical fruits). The consumer product contains $5 \times 10^{10}$ CFU L. plantarum 299v/L. The bacteria are highly resistant to the low pH of the fruit drink (pH <2.8–3.4, depending on the type of fruit drink) and can be stored in a refrigerator for ≥1 mo without loss of viability.

Effects on intestinal microflora

When healthy volunteers consume lactobacilli that can become established in the mucosa, the concentration of lactobacilli in the intestinal mucosa tends to increase and the concentration of Gram-negative anaerobes, Enterobacteriaceae, and sulfite-reducing clostridia tends to decrease (27). The inhibitory effect of L. plantarum 299v on Enterobacteriaceae and Gram-negative anaerobes in the mucosa was shown in vivo in rat models simulating severe clinical conditions (30, 31).

Gram-negative anaerobes are considered noxious because they are often involved in secondary infections after abdominal surgery (32–34). Furthermore, Gram-negative bacteria always contain endotoxins and they initiate, even in small numbers, violent inflammatory reactions. Gram-negative anaerobes are also held responsible for the production of carcinogenic substances in the intestine (35, 36). The sulfite-reducing clostridia can contain strains that produce toxin. They also produce hydrogen sulfide, which has a general toxicity. Furthermore, clostridia can produce carcinogenic substances in the intestine (35). Enterobacteriaceae is a genetically close family and includes several pathogens. Even normally nonpathogenic Enterobacteriaceae taxa have a pathogenic potential in situations in which the immunologic defense of the host is impaired.

L. plantarum 299v, in the fruit drink supplemented with lactic acid fermented oatmeal gruel, was shown to increase the concentration of carboxylic acids in feces of healthy volunteers (mainly acetic acid and propionic acid; 37). These short-chain fatty acids in the colon are an energy source for the mucosal cells, ie, an increased concentration of short-chain fatty acids in the lumen can be beneficial for the status of the mucosa.

This observed increase in the content of short-chain fatty acids may have been due to a changed composition of the colonic microflora. That is, L. plantarum may support an increased
number of bacterial taxa able to produce acetic acid and propionic acid or suppress taxa that catabolize these compounds. A supplementary explanation might be that *L. plantarum* 299v increases the amount of mucus in the colon because *L. plantarum* 299v was shown to stimulate epithelial mucus production in vitro (38). Thus, the increased amount of mucus may lead to a higher amount of fermentable material in the colon, material that can be converted to short-chain fatty acids.

**Experimental studies in rats**

It was shown in experimental studies in rats that *L. plantarum* 299v decreases translocation of intestinal bacteria (30, 31, 39, 40), 2) competes with *Enterobacteriaceae* in the intestinal mucosa (30, 31, 41), 3) improves the condition of the intestinal mucosa (30, 39, 40), 4) improves liver status during liver injury (31, 39, 40), 5) improves the immunologic status of the mucosa (42), 6) decreases inflammation in the mucosa (30), and 7) modulates responsiveness to antigens presented via the gut (41).

**Translocation**

Translocation, ie, the passage of viable bacteria through the epithelial mucosa into the lamina propria and then to the mesenteric lymph nodes and possibly other tissues (43), can be reduced in rats with acute liver injury induced by N-galactosamine (44, 45) by the administration of lactobacilli (31, 39, 40). Several lactobacilli strains have been tested in the liver failure model (eg, 2 strains of *Lactobacillus reuteri* and one of *Lactobacillus fer-

mentum*) and have been shown to decrease the translocation rate. However, *L. plantarum* 299v was the most effective of the tested strains (31). *L. plantarum* 299v significantly reduced translocation in rats with enterocolitis (induced by methotrexate). The lactobacilli administration mitigated the mucosal injuries induced by the chemotherapy (30).

**Competition with Enterobacteriaceae**

*L. plantarum* 299v decreased the counts of viable *Enterobacteriaceae* in the intestinal mucosa of rats subjected to liver injury (31) and of rats with enterocolitis (42). It was also shown that the colonization of *L. plantarum* 299v competes with that of *Escherichia coli* in gnotobiotic rats (41).

**Mucosal status**

The status of the intestinal mucosa, evaluated as the content of protein or the content of ribosomal RNA and DNA, was improved in rats with acute liver injury (39, 40) and in rats with enterocolitis (30) when the rats were treated with *L. plantarum* 299v. Also, the permeability of the mucosa to EDTA decreased with *L. plantarum* 299v treatment (30).

**Liver status**

The liver status, as measured by the concentration of liver enzymes in the blood, of rats with acute liver injury was improved by the administration of *L. plantarum* 299v before the onset of the injury (31, 39, 40).

**Immunologic status**

The subnormal concentration of secretory immunoglobulin A (IgA) antibodies in the intestine of rats with enterocolitis (induced by methotrexate) was increased and approached a more normal level by the administration of *L. plantarum* 299v (42). Also, concentrations of CD4+ and CD8+ lymphocytes in the intestinal lamina propria increased to a more normal level by treatment with *L. plantarum* 299v (42).

The concentrations of total serum IgA antibodies increased and the IgA and IgM antibody concentrations against *E. coli* were marginally higher in gnotobiotic rats colonized with *E. coli* together with *L. plantarum* 299v than in rats colonized only with *E. coli* (41). The group treated with *L. plantarum* 299v also showed a significantly increased density of CD25+ cells in the lamina propria and displayed a decreased proliferative spleen cell response after stimulation with concanavalin A 1 wk after the colonization. The results indicated that *L. plantarum* 299v can modulate responsiveness to antigens presented via the gut.

**Inflammation**

Inflammation measured as myeloperoxidase activity decreased in the mucosae of rats with experimentally induced enterocolitis after treatment with *L. plantarum* 299v (30).

**Clinical trials**

**Irritable bowel syndrome**

A probiotic rose hip fruit drink supplemented with lactic acid fermented oatmeal gruel containing *L. plantarum* 299v was administrated to patients with irritable bowel syndrome in a double-blind, placebo-controlled study (46). The patients were divided into 2 groups: 1 group was given the product containing *L. plantarum* 299v (25 patients) and 1 was given a similar fruit drink (25 patients). The patients were treated over a period of 4 wk with a double-blind administration of the product. The results indicated that the probiotic drink containing *L. plantarum* 299v can be effective in the treatment of irritable bowel syndrome.
The active product contained 5 studies of the more milk-oriented in the results of the studies reviewed here and in several other

CONCLUSIONS

than in the placebo group. Pain was significantly reduced in both the treatment group and the placebo group, although the decrease was more rapid and more pronounced in the treatment group. Twelve months after treatment, the treatment group was still experiencing better overall gastrointestinal function than was the placebo group.

Fibrinogen and cholesterol

* L. plantarum* 299v administered in a probiotic rose hip fruit drink supplemented with lactic acid fermented oatmeal gruel for 6 wk was also shown to significantly reduce fibrinogen and LDL-cholesterol concentrations in serum of Polish men with moderately elevated cholesterol (47). The study was performed in 30 subjects who were randomly divided into 2 groups in a double-blind study with placebo. The placebo was a rose hip drink without *L. plantarum* 299v (placebo; 27 patients). The patients consumed the products daily for 4 wk (400 mL product/d; the active product contained 5 × 10^{10} CFU/L). Abdominal bloating was significantly lower in the treatment group than in the placebo group. Pain was significantly reduced in both the treatment group and the placebo group, although the decrease was more rapid and more pronounced in the treatment group. Twelve months after treatment, the treatment group was still experiencing better overall gastrointestinal function than was the placebo group.

L. plantarum* 299v performs well in both the food system (the supplemented fruit drink) and in the human intestine. A question that remains to be answered, however, is what mechanisms are involved in this strain’s intestinal and physiologic effects. It is known that the strain is adapted to establishment in the human intestinal mucosa (27) and that it possesses a specific binding mechanism for adhesion to epithelial cells (28). The strain also seems to counteract groups of adverse bacteria. This effect by itself may explain the decreased translocation and improved condition of the mucosa after *L. plantarum* 299v treatment. Furthermore, the influence of this probiotic strain on the composition of the intestinal flora is indicated indirectly by the changes in the concentration of short-chain fatty acids in feces (37). However, it is also possible that mechanisms are involved other than solely competition with different fractions of the intestinal flora, eg, the strain may regulate the host’s immunologic defenses.

REFERENCES


