Probiotic Lactic Acid Bacteria: A Review

Emiliano J. Quinto1*, Pilar Jiménez1, Irma Caro1, Jesús Tejero1, Javier Mateo2, Tomás Girbés1

1Department of Food Science and Nutrition, Faculty of Medicine and CINAD (Centro de Investigación en Nutrición, Alimentación y Dietética; Edificio Lucia), University of Valladolid, Valladolid, Spain
2Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, University of León, León, Spain

Email: *equinto@ped.uva.es

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Abstract

Lactic acid bacteria (LAB) play a critical role in food production and health maintenance. There is an increasing interest in these species to reveal the many possible health benefits associated with them. The actions of LAB are species and strain specific, and depend on the amount of bacteria available in the gastrointestinal tract. Consumers are very concerned of chemical preservatives and processed foods. However, products with or processed with LAB are accepted as a natural way to preserve food and promote health. This paper aimed to review the recent data in regard to the role of probiotic LAB in the preservation of foods, in the immunomodulation in the gastrointestinal tract, and in its health benefits.

Keywords

Lactic Acid Bacteria, Probiotic, Gastrointestinal Tract, Health

1. Introduction

Lactic acid bacteria (LAB) are a group of Gram-positive, non-sporulating, anaerobic or facultative aerobic cocci or rods, which produce lactic acid as one of the main fermentation products of the metabolism of carbohydrates [1]. The monograph published by Orla-Jensen [2] is the base of the present classification of lactic acid bacteria (LAB) using the following criteria: cellular morphology, mode of glucose fermentation, range of growth temperature, and sugar utilization patterns. Four genera were recognized as LAB: Lactobacillus, Leuconostoc, Pediococcus, and Streptococcus. Molecular biological methods have increased the number of genera included in this group [3]. The current taxonomic classification includes the LAB group in the phylum Firmicutes, class Bacilli, and order Lactobacillales. The different families and genera can be search in the NCBI Taxonomy Browser

*Corresponding author.

2. Niche-Specific Adaptation: The Intestinal Environment

The hypothetical first niche of the ancestral LAB is considered soil and plants and, subsequently, the gut of herbivorous animals [6]. The mammalian intestine is colonized by 100 trillion of microorganisms (called “microbiota”) that are essential for health [7] [8]. The transition from soil and plants to the animal gut has three areas of genomic adaptation [9]: resistance to host barriers, adhesion to intestinal cells, and fermentation of some substrates in the gut. The membrane lipid composition is affected by low pH and bile salts. Microarray analysis has shown the expression of a glyceralphosphatase in Lactobacillus reuteri after an acid shock, and an increase in the sensitivity to acid in Lactobacillus acidophilus [10]. Extracellular lipopolysaccharides (LPS) also play a role in the resistance, but it is unclear [6]. The adhesion of LAB to intestinal cells is associated with the peristaltic flow, a good adherence capacity and the presence of mucus to protect and lubricate the epithelial surfaces [11]. Resistant intestinal bacteria are able to inhibit the adherence of pathogenic bacteria to intestinal epithelial cells as a result of their ability to increase the production of intestinal mucus [12]. Lactobacillus plantarum increases the levels of expression of the mRNA of some mucus, inhibiting the cell attachment of enteropathogenic Escherichia coli [13] [14]. LAB have access to simple sugars and complex carbohydrates, so bacteria with genes involved in its degradation are probably in better condition to multiply in the gut [6].

The resident gastrointestinal microbiota provide a microbial barrier against microbial pathogens [12]. Lactobacillus and Bifidobacterium spp. of human intestinal origin produce antimicrobial substances that are active in vitro and in vivo against enteropathogenic microorganisms involved in diarrhea [15]; both genera have the capacity for interfering with or block the pathogenic process of enteric bacterial pathogens [12]. Strains of Lactobacillus acidophilus, L. johnsonii, L. rhamnosus, L. casei, L. acidophilus, and L. rhamnosus interfere with a wide range of pathogens, such as enteropathogenic Escherichia coli, enterohemorrhagic E. coli, Listeria monocytogenes, Salmonella enterica serovar Typhimurium, and S. flexneri [16]-[22]. Blocking the process of pathogenicity of enteric pathogens is carried out without affecting their viability, such as the inhibition of Salmonella spp, S. flexneri, and L. monocytogenes by E. coli strain Nissle 1917 [23], and the blocking of E. coli LF82 adherent-invasion in the Crohn’s disease [24].

3. Biopreservation of Food

Consumers are very concerned of chemical preservatives and processed foods, but they accept easily LAB as a natural way to preserve food and promote their health [25]. Bacteriocins are ribosomally synthesized small proteins produced by LAB that inhibit the growth of spoilage and pathogen bacteria in foods; moreover, bacteriocinogenic LAB are linked and are used as starter cultures in food processing [26]. Bacteriocins have been classified into four major groups [27]. Group I, also known as lantibiotics, has nisin as the first and best known bacteriocin. Group II is a large group of small heat-stable proteins subdivided into three groups [25]: i) subgroup IIA, bacteriocins active against Listeria monocytogenes, and pediocin PA-1, sakacins A and P, leucocin A, bavaricin MN, and curvacin A are members of this group; ii) subgroup IIB require two different peptides for activity, and lactococcins G and M, and lactacin F are members of it; and iii) subgroup IIC, such as lactacin B, require reduced cysteines for activity. Into group III are classified the larger heat-labile proteins, such as helveticins J and V, and lactacins A and B. Leuconocin S, lactocin 27, and pediocin SJ-1 have lipid or carbohydrate moieties and are classified into group I.V. Yang et al. [28] classified the gram-positive bacteriocins into three classes: Class I (modified peptides, lantibiotics), Class II (unmodified peptides, non-lanthionine), and Class III (large proteins, heat unstable). Cotter et al. [29] subdivided Class II into five sub-classes.

Only the bacteriocin nisin is commercially available for addition in pure form. It is added to milk, cheese, and dairy products, canned foods, mayonnaise, and baby foods [30]. Bacteriocinogenic cultures can also be added to the non-fermented foods or to fermented foods as starter cultures.

Due to the sensitivity of bacteriocins to some proteases, harmless bacteriocins are possibly digested [31] [32]. Thus, bacteriocins are considered as basically safe food additives after intake by the gastrointestinal system [28].

4. Immunity Stimulation

External microorganisms can penetrate the gut wall by translocation through the epithelial layer or through Peyer’s patches. Indigenous intestinal bacteria including lactobacilli are able to cross the intestinal mucous layer
and they can survive in the spleen or in other organs for many days where they stimulate phagocytic activity [33]. The thickness and physical state of the intestinal mucus layer [34] [35] and its response to orally consumed lactobacilli [36] [37] are important in the immune response.

Lactobacilli can elicit innate and adaptive immune response in the host via binding to specific receptors on immune cells and other tissues such as the intestinal epithelium [38]. Those receptors induce the production of cytokines, chemokines and other innate effectors: naïve T cells, regulatory T cells, and the activation of dendritic cells DC and macrophages [38]-[42]. Different strains and species of lactobacilli induce different effectors, which can modulate the immune response in different ways [38].

New approaches are being used to find immunomodulatory components of LAB. The comparative genomic analysis to identify loci linked to certain phenotypes has been done with Lactobacillus spp. [43]-[46]. However, a major gap in the knowledge about the mechanisms of immunomodulation by probiotic cells is their fate in vivo [38] [47]. Numerous probiotic studies with different strains of Lactobacillus have been performed in humans and murine models with negative/inconclusive or positive results. Positive results have been obtained treating acute infectious diarrhoea [48] or some allergic diseases such as the prevention of atopic eczema or dermatitis [49] [50]. These probiotics can increase the immunogenicity of orally administered vaccines such as rotavirus [51], polio [52], cholera [53] and influenza [54].

5. Health Benefits

The target of probiotic food products is to have up to $10^7$ CFU/g at the end of their shelf life [55]; but probiotic LAB must endure some stresses to ensure they reach the adequate numbers in the target location to elicit their effect. The human gastrointestinal tract contains up to $10^{13} - 10^{14}$ cells [56]. It is a complex ecosystem combining the gastrointestinal epithelium, immune cells and resident microbiota [12] [57]. Simon and Gorbach [58] estimated a generation or doubling time between 1 and 4 per day.

The three major sections of the human gastrointestinal tract are the stomach, the small intestine, and the large intestine. Every section has its own distinct microbiota [56] [58]-[60]. The stomach is primarily inhabited by aerobic gram-positive microorganisms ($<10^3$ CFU/g). The small intestine is inhabited by the genera Lactobacillus, Bifidobacterium, Bacteroides, and Streptococcus ($10^3 - 10^8$ CFU/g). And the large intestine is populated by the genera Bacteroides, Fusobacterium, Lactobacillus, Bifidobacterium, and Eubacterium in large numbers ($10^{11} - 10^{12}$ CFU/g).

There are many reports of the probiotic effect of LAB [61]. Some species involved are Lactobacillus acidophilus, L. casei, L. johnsonii, L. fermentum, L. rhamnosus, L. plantarum, L. reuteri, L. salivarius, L. paracasei, L. delbrueckii subsp. bulgaricus, Saccharomyces boulardii, Streptococcus thermophilus, Bifidobacterium lactis, B. longum, and B. breve. Probiotics presumably exert a dual effect, preventing/decreasing the intestinal colonization with pathogen microorganisms [62], or interacting with the gut-associated lymphoid tissue (GALT) to prevent inflammatory responses and promote a state of tolerance to themselves and possibly to foods [63].

The beneficial effects of probiotics are often disparate and strain-specific [64]. Some species conferred beneficial effects, such as the treatment of acute diarrhoea associated with rotavirus [51], ulcerative colitis [65] [66], Clostridium difficile-associated diarrhoea [67], and Helicobacter pylori infection [68] [69].

Some studies reported preventive effects, such as the prevention of antibiotic-associated diarrhoea in children [70], and improvement in lactose digestion [71]. Other effects are still under investigation [64]: liver disease, allergy, or AIDS.

Lucas and Cole [72] reported a decrease of necrotizing enterocolitis in preterm infants if Lactobacillus and Bifidobacterium colonize the intestine, or if breast milk rather than formula is used. Gewolb et al. [73] reported that premature infants delivered by caesarian section did not suffer the normal process by which LAB are ingested via vaginal birth and propagated by the mother’s milk, allowing pathogens to establish within the premature intestine [74]. Hoyos [75] and Caplan and Jilling [76] reported that bifidobacteria reduced endotoxemia. The strongest evidence of a beneficial effect of probiotics has been reported for Lactobacillus rhamnosus GG and Bifidobacterium lactis BB-12 for prevention, and L. reuteri SD2222 for treatment [74]. Rohan and Wainwright [77] highlights the benefits of probiotic administration to babies < 1500 g, in the reduction of the incidence and severity of necrotizing enterocolitis. There is no consistency in the type or combination of probiotics used. In different studies where only one probiotic strain was used there was not significant statistical change in the incidence of necrotizing enterocolitis; but when two or more probiotic strains were used in combination, a decrease in the incidence was observed [77].
Korterink et al. [78] performed a systematic meta-analysis for the use of probiotics for childhood functional gastrointestinal disorders (abdominal pain, vomiting and aerophagia), which cannot be explained in terms of structural or biochemical abnormalities. The use of Lactobacillus rhamnosus GG, L. reuteri DSM 17 938 and L. reuteri VSL#3 significantly increases treatment success in children with abdominal pain related with functional gastrointestinal disorders. In addition, L. rhamnosus GG and L. reuteri DSM 17 938 significantly decrease the intensity of abdominal pain. Similarly, de Silva et al. [79] performed a systematic analysis of ways to prevent the development of food allergy in children and adults. Among several variables, probiotics did not seem to be protective in infants at high or normal risk. Redman et al. [80] carried out a study about the efficacy and safety of probiotic use in immunocompromised cancer patients, as case reports identified a Lactobacillus strain used in probiotic therapy to be involved with bacteremia [81] and sepsis [82]. The study demonstrated that there is currently insufficient evidence to claim that probiotics are effective and safe in people with cancer. In fact, meta-analyses found that probiotics significantly reduced the incidence of CTC grade ≥ 2 diarrhoea (Common Terminology Criteria for Adverse Events, CTC, National Cancer Institute Common Toxicity Criteria, USA) [83], may reduce the incidence of CTC grade ≥ 3 diarrhoea, may reduce the frequency of bowel movements, but most of the evidence is not clinically convincing, and they may be a rare cause of sepsis [80]. Ebel et al. [64] studied the impact of probiotics on risk factors for cardiovascular disease, such as medical disorders (obesity, diabetes, hypertension, and hypercholesterolemia) and metabolic disturbances (hyperhomocysteinemia and oxidative stress). Probiotics showed positive effects on the in vitro and in vivo studies considered for the study. These authors stated that those effects were most notably due to their anti-inflammatory properties or their enzymatic capacities. Moreover, no studies to date have directly addressed the impact of probiotics on risk factors for cardiovascular diseases, which include aneurysm, angina, atherosclerosis, cerebrovascular accident, cerebrovascular disease, congestive heart failure, coronary artery disease, myocardial infarction, and peripheral vascular disease [64].

Finally, LAB are good candidates to develop novel oral vectors for mucosal delivery strategies, constituting attractive alternatives to attenuated pathogens. Bermúdez-Humarán et al. [84] studied the development of genetic tools for Lactococcus lactis for the heterologous expression of therapeutic proteins such as antigens, cytokines and enzymes. These authors obtained good results against human papillomavirus type 16 (HPV-16), to prevent a bovine b-lactoglobulin (BLG)-allergic reaction, and to regulate body weight and food consumption. The use of L. lactis to deliver DNA at the mucosal level has been also developed [84], modulating the host immune response.

6. Enumeration of Probiotic Strains

Standardized methods are available for a limited number of species in certain dairy products, such as publications from the International Organization of Standardization (ISO) regarding enumeration standards for Lactobacillus acidophilus (ISO 20128/IDF 192:2006) and Bifidobacterium (ISO 29981/IDF 220:2010) [85].

No single culture-based methodology is applicable to all probiotic microorganisms [85], because of the variability among species or strains. Moreover, the range of selective media available to identify and enumerate probiotic strains is relatively limited. There is not a single medium or technique applicable to strain isolation of all the microorganisms with probiotic interest.

In recent years new culture-independent methods have been used to accurately enumerate probiotic strains based on viability. These techniques either use dyes to differentiate live and dead cells, measure the cell membrane integrity, or characterize some aspect of metabolic activity (synthesis of nucleic acids, or respiration). Confocal scanning laser microscopy increases de sensitivity and enable the observation of subsurface structures of foods in situ [85]-[87]. The use of this technique in combination with the quantitative polymerase chain reaction (qPCR) has been used for the enumeration of probiotic strains in cheese [88] [89]. Ethidium monoazide-PCR and propidium monoazide-PCR are emerging techniques that limit enumeration to live cells and can also be referred to as viability-PCR (vPCR) [90] [91].

Cell sorting methods (Coulter counters and flow cytometry) were originally developed for counting red blood cells, but they have been upgraded and adapted to analyze much smaller cells such as bacteria [85]. Flow cytometry allows the study of a large number of cells at a time, recording, for each event (bacteria) several parameters. Different fluorescent probes can be applied to examine the physiological characteristics of probiotic living cells, such cell membrane integrity, intracellular enzyme activity, cytoplasmic pH, and membrane potential [92]. Limitations and new challenges in probiotic enumeration and identification can be found in Davis [85].
7. Antibiotic Resistance

FAO/WHO [93] outlined the problem of antibiotic resistance genes in probiotic strains. The capacity of probiotics to transfer antibiotic resistance genes is one of the most important parameters for their selection [94], and their use should not be allowed [95].

When probiotics enter the gut, they interact with the native microbiota and gene transfer can occur, contributing to the transfer of antibiotic resistance genes to commensal or pathogen bacteria present in the gastrointestinal tract. Fukao and Yajima [96] reviewed the principal resistance genes in probiotic lactobacilli: \( \text{tet} \), \( \text{erm} \), or \( \text{cat} \) genes coding for tetracycline, erythromycin, or chloramphenicol resistances, respectively. D’Aimmo et al. [95] and Patel et al. [97] described other antibiotic resistance genes, such as \( \text{str} \) for streptomycin and \( \text{vanA} \) for vancomycin.

The identification of antibiotic resistances in probiotic strains could be done following phenotypic and genotypic methods: i) the determination of the minimum inhibitory concentration (MIC) of the most relevant antibiotics for each bacteria strain [98]; and ii) the use of PCR-based techniques and microarray analysis [99]-[101].

8. Conclusions and Future Perspectives

The expectations of probiotic bacteria have become the most demanding for any bacterial group. Probiotics have become a very important element to everyday health food products, and their global market is estimated above US$28.8 billion by 2015 [59]. Consumers are very concerned of chemical preservatives and processed foods, even though it provides a grade of safety and food diversity never seen before. However, consumers accept easily LAB as a natural way to preserve food and promote their health. In the last decade the interest in bacteriocins produced by LAB has increased dramatically [26]. Many bacteriocins inhibit the growth of spoilage and pathogen bacteria in foods; moreover, bacteriocinogenic LAB are linked and are used as starter cultures in food processing. However, there are many different kinds of pathogens in nature, so the specific use of a particular bacteriocin cannot eliminate all bacterial pathogens. In recent years, the increased number of multi-drug resistant pathogens has become a serious problem. The development of a new generation of antimicrobial agents is a difficult task. Biotechnological methods are been applied to create new or multi-functional bacteriocins, so they could be widely used in food, animal husbandry, and medicine.

Both traditional cell culture methods, as well as the alternative techniques (direct imaging and visual enumeration, nucleic acid-based enumeration methods, and flow cytometry and cell sorting), offer advantages and limitations for enumerating probiotic microorganisms [85]. The new methods and techniques show considerable promise for quantifying live microorganisms in different metabolic states. But the probiotic efficacy cannot be predicted solely on the basis of viable cells. Salminen et al. [102] reported that cell wall components from brood dead and living cells contributed to the probiotic efficacy.

Very few microorganisms have been subjected to thorough in vitro studies confirming their specific health-promoting activity, and even fewer have been subsequently subjected to and passed the appropriate human trials [103]. Additionally, probiotics can be dangerous, as they have been linked to an increase in mortality rate if administered to severely immunocompromised patients [104]. Subsequent studies are needed to evaluate the health-promoting activity of probiotic bacteria.

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