

Lactic Acid Bacteria: Benefits, Selection Criteria and Probiotic Potential in Fermented Food

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Abstract

Probiotics have been defined a number of times. Presently the most common definition is that from the FAO/WHO which states that probiotics are "live microorganisms that, administered in adequate amounts, confer a health benefit on the host." One of the most significant groups of probiotic organisms are the lactic acid bacteria, commonly used in fermented dairy products. There is an increase in interest in these species as research is beginning to reveal the many possible health benefits associated with lactic acid bacteria. The difficulty in identifying and classifying strains has complicated research, since benefits may only be relevant to particular strains. Nevertheless, lactic acid bacteria have a number of well-established and potential benefits. They can improve lactose digestion, play a role in preventing and treating diarrhea and act on the immune system, helping the body to resist and fight infection. More work needs to be done to authenticate the role lactic acid bacteria might play in anti-tumor effects, hyper cholesterol effects, preventing urogenital infections, alleviating constipation and treating food allergy.

Keywords: Lactic acid bacteria; Probiotics; Election criteria; Benefits

Introduction

Kollath [1] and Vergin [2] were perhaps the first to commence the term probiotic [3], while a beneficial association of lactic acid bacteria (LAB) with the human host was already recommended in Biblical times and by Metchnikoff [4]. The latter considered the longevity of Bulgarian peasants to be related to their high intake of fermented milk products, as he considered gut microbes detrimental rather than beneficial to human health [5]. While documentation of said longevity is inadequate, recent large studies support at least the ability of regular intake of fermented foods to reduce the incidence of serious disease [6,7]. In this perspective the LAB and their production of lactic acid as a result of sugar metabolism were suggested to be health promoting agents. Originally defined as microorganisms promoting the growth of other microbes [8], probiotics have been re-defined a number of times. Fuller [9] defined a probiotic as 'a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance. Havenaar, et al. [10] defined probiotics as 'mono- or mixed cultures of live microorganisms which, when applied to animal or man, beneficially affect the host by improving the property of the indigenous flora', while in relation to food, probiotics were considered as 'viable preparations in foods or dietary supplements to improve the health of humans and animals' [11]. The Federal Institute for Risk Assessment defined probiotics as 'specific live microorganisms which reach the intestinal tract in active form and in sufficient numbers to positively affect the health of the host' [12]. Presently the most common definition is that from the FAO/WHO which states that probiotics are 'live microorganisms that, administered in adequate amounts, confer a health benefit on the host' [13].

Immense attention is presently addressed to probiotics, prebiotics, or their combined use as synbiotics, to improve human health in natural ways. Among the many-sided benefits of probiotics is the

maintenance of healthy gut microflora, which may provide protection against gastrointestinal disorders, together with gastrointestinal infections and inflammatory bowel diseases. Also it was suggested that the efficiency of probiotics to offer a proper alternative to the use of antibiotics in the treatment of enteric infection [14] or to reduce the symptoms of antibiotic-associated diarrhea [15]. Probiotic bacterial cultures modulate the growth of intestinal microbiota, suppress potentially harmful bacteria, and reinforce the body's natural defense mechanisms [16-18]. In addition to human health benefits, probiotics can also improve various aspects of growth and performance in livestock and poultry, as well as control undesirable microorganisms in food animals [19]. Although it has extensively been proven that dairy fermented products are the best matrices to deliver probiotics, there is growing evidence on the possibility to obtain probiotic foods from nondairy matrices. Several raw materials like cereals, fruits, and vegetables have recently been investigated to determine their suitability to design new, nondairy probiotic foods [20]. Today, a variety of microorganisms, typically lactic acid bacteria (LAB) and bifidobacteria, have been evaluated for their probiotic potential and are applied as adjunct cultures in various types of food products or in therapeutic preparations [21]. Therefore, the following sections will be devoted to general features, taxonomy, role in fermented food, their health benefits, selection criteria and their mechanism of action in food and human health of this important group of lactic acid bacteria.

Taxonomy of Lactic Acid Bacteria

LAB represents a ubiquitous and heterogeneous species with common feature of lactic acid production. Taxonomically, LAB species are found in two distinct phyla, namely Firmicutes and Actinobacteria. Within the Firmicutes phylum, LAB belongs to the Lactobacillales order and includes the following genera: *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Enterococcus*, *Tetragenococcus*, *Aerococcus*, *Carnobacterium*, *Weissella* [22-24],

Alloicoccus, *Symbiobacterium* and *Vagococcus* which are all low guanine-cytosine (GC) content organisms (31-49%). Within the Actinobacteria phylum, LAB belongs to the Atopobium and Bifidobacterium genera, with a GC content of 36-46% and 58-61%, respectively. More generally, however, the term "LAB" does not reflect a phyletic class, but rather the metabolic capabilities of this heterogeneous bacterial group, the most important of which is the capacity to ferment sugars primarily into lactic acid. LAB are also characterized by being Gram positive, catalase negative, non-sporulating organisms that are devoid of cytochromes and of nonaerobic habit but are aero tolerant, fastidious, non-motile, acid tolerant and strictly fermentative; lactic acid is the major end product of sugar fermentation [22,24,25].

LAB is widely used in numerous industrial applications, ranging from starter cultures in the fermented food industry to probiotics in dietary supplements, and as bioconversion agents. Due to the limited biosynthetic abilities and their high requirements in terms of carbon and nitrogen sources, the natural habitat of LAB is represented by nutritionally rich environments. LAB are generally associated with plant and animal raw materials, and the corresponding fermented food products, including dairy, meat, vegetable, and cereal plant environments, where fermentation can occur. Some species also occur in the respiratory, the intestinal and genital tracts of humans and animals. The ability to colonize such a variety of habitats is a direct consequence of the wide metabolic versatility of this group of bacteria. Thus, it is not unexpected that LAB has been used for decades in food preservation, leading to their widespread human consumption, and that they have a "Generally Recognized as Safe (GRAS)" status [23].

Role of Probiotic LAB in Fermented Food

Fermented foods are the main vehicle of administration of probiotic organisms and, among them; dairy products are by far the most important vehicles for the delivery of LAB probiotics. In recent years, there has been a remarkable increase in the number of dairy products including LAB probiotics (e.g. pasteurized milk, fermented milks, ice cream, and several cheese varieties). However, there is an increasing consumer demand for nondairy-based probiotic food and probiotics are being incorporated into drinks or marketed as dietary supplements in the form of tablets and freeze-dried preparations. Recently, several raw materials have been extensively investigated for their suitability to carry and deliver probiotics. In particular, cereals seem good substrates to develop new probiotic foods [20].

Most probiotic foods available today are milk based, but consumer's preference today lie more with botanical dietary supplements, which are either free from or have minimal cholesterol content. The above fact is highlighted by the trend in the U.S. functional food market, which is developing in a different fashion from that seen in Europe, with its functional food sector more broadly defined as nutraceuticals and consumer interest tending to lie more with botanical dietary supplements rather than fortification of foodstuffs. This trend however, is changing, as interest in immunity, cancer and heart health grows. Also, the market for functional foods is in its infancy in many countries; however, product innovation throughout a number of sectors, such as drinks, bakery and probiotics, is evident [26].

Probiotic products are usually marketed in the form of fermented milks and yoghurts; however, with an increase in the consumer vegetarianism throughout the developed countries, there is also a demand for the vegetarian probiotic products. Furthermore, lactose intolerance and the cholesterol content are two major drawbacks related to the fermented dairy products [27,28]. There are a wide

variety of traditional non-dairy fermented beverages produced around the world. Much of them are non-alcoholic beverages manufactured with cereals as principal raw material. The nondairy probiotic beverages may be made from a variety of raw materials, such as cereals, millets, legumes, fruits and vegetables.

Cereal grains are an important source of protein, carbohydrates, vitamins, minerals and fiber for people all over the world, and can be used as sources of non-digestible carbohydrates that besides promoting several beneficial physiological effects can also selectively stimulate the growth of *Lactobacilli* and *Bifidobacteria* present in the colon, there by acting as prebiotics. Cereals contain water soluble fiber (such as β -glucan and arabinoxylan), oligosaccharides (such as galacto- and fructo oligosaccharides) and resistant starch, and thus have been suggested to fulfill the prebiotic concept [29]. Strains of *Lactobacillus* have been recognized as complex microorganisms that require fermentable carbohydrates, amino acids, B vitamins, nucleic acids and minerals to grow and therefore fermentation of cereals may represent a cheap way to obtain a rich substrate that sustains the growth of beneficial microorganisms. A multitude of non-dairy fermented cereal products has been created throughout history for human nutrition, but only recently probiotic characteristics of microorganisms involved in traditional fermented cereal foods have been reported. Microflora identification of Bulgarian boza shows that it mainly consists of yeasts and LAB, in an average LAB/yeast ratio of 2.4. The LAB isolated were *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Lactobacillus coprophilus*, *Leuconostoc reffinolactis*, *Leuconostoc mesenteroides* and *Lactobacillus brevis*. The yeasts isolated were *Saccharomyces cerevisiae*, *Candida tropicalis*, *Candida glabrata*, *Geotrichum penicillatum* and *Geotrichum candidum* [30].

Bushera is a traditional beverage prepared in the Western highlands of Uganda, consumed by both the young children and the adults. The sorghum, or millet flour from the germinated sorghum and millet grains is mixed with the boiling water and left to cool to ambient temperature. The LAB isolated from Bushera comprised of five genera, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Enterococcus* and *Streptococcus*. *Lactobacillus brevis* was more frequently isolated than other species. Mahewu is a sour beverage made from the maize porridge, which is mixed with the water. The sorghum, millet malt, or wheat flour is then added and left to ferment. It is consumed in Africa and some Arabian Gulf countries. The predominant microorganism found in African mahewu is *Lactococcus lactis* subsp [30]. Pozol a refreshing beverage, consumed in the Southeastern Mexico, is made by cooking maize in an approximately 1% (w/v) lime solution, washing with water, grinding to make a dough known as nixtamal, shaping into balls, wrapping in banana leaves and leaving to ferment at ambient temperature for 0.5-4 days. The fermented dough is suspended in the water and drunk. Some fibrous components are not completely solubilized by nixtamalization and sediment is present in the beverage when the dough is suspended in the water [31]. LAB can rarely convert starch into lactic acid, however, some strains of *Lactobacillus* and *Streptococcus* [32], for example, *Lactobacillus plantarum* A isolated by Giraud et al. [33] showed extracellular amylase activity. Sour cassava starch is obtained by a natural fermentation and this product is largely appreciated in Africa, South America and other developing countries. Santos [34] developed a probiotic beverage with the fermented cassava flour using mixed culture of *Lactobacillus plantarum*, which were amyolytic strains of *Lactobacillus casei* Shirota and *Lactobacillus acidophilus*. Angelov, et al. [35] produced a symbiotic functional drink from the oats by combining a probiotic starter culture and whole-grain oat substrate. The oats and barley are the cereals with highest content of β -glucan, recognized as the main functional component of the cereal fibers. Soymilk is suitable for the growth of the LAB especially

Bifidobacteria [36]. Several studies have mentioned the production and use of the fermented soymilk drinks as probiotic, mainly soybean yogurt, which further can be supplemented with oligo fructose and inulin [37].

Despite potential sensory challenges, there is a genuine interest in the development of fruit-juice based functional beverages, fortified with the probiotic and prebiotic ingredients. The fruit juices have been suggested as an ideal medium for the functional health ingredients because they inherently contain beneficial nutrients, they have taste profiles that are pleasing to all the age groups, and because they are perceived as being healthy and refreshing [38]. The fruits and vegetables are rich in the functional food components such as minerals, vitamins, dietary fibers, antioxidants, and do not contain any dairy allergens that might prevent usage by certain segments of the population [26]. Current industrial probiotic foods are basically dairy products, which may represent inconveniences due to their lactose and cholesterol content [27]. Technological advances have made possible to alter some structural characteristics of fruit and vegetables matrices by modifying food components in a controlled way. This could make them ideal substrates for the culture of probiotics, since they already contain beneficial nutrients such as minerals, vitamins, dietary fibers, and antioxidants [39], while lacking the dairy allergens that might prevent consumption by certain segments of the population [26]. There is a genuine interest in the development of fruit juice based functional beverages with probiotics because they have taste profiles that are appealing to all age groups and because they are perceived as healthy and refreshing foods [39,40]. However, unsuitable contents of aromas (perfumery, dairy) and flavors (sour, savory) have been reported when *Lactobacillus plantarum* is added to juices [26].

According to Sheehan et al. [40], when adding *Lactobacillus* and *Bifidobacterium* to orange, pineapple and cranberry juice, extensive differences regarding their acid resistance were observed. All of the strains screened survived for longer in orange and pineapple juice compared to cranberry. Hardaliye is a lactic acid fermented beverage produced from the natural fermentation of the red grape, or grape juice with the addition of the crushed mustard seeds and benzoic acid. This beverage can be found in the Thrace region of Turkey. It is very well known and has been produced and consumed since ancient times. The mustard seed's eteric oils affect the yeasts and also give flavor to the final product. Benzoic acid inhibits, or decreases alcohol production by affecting the yeast. The LAB found in the beverage was *Lactobacillus paracasei* subsp. *paracasei*, *Lactobacillus casei* subsp. *pseudoplantarum*, *Lactobacillus brevis*, *Lactobacillus pontis*, *Lactobacillus acetotolerans*, *Lactobacillus sanfransisco* and *Lactobacillus vaccinostrercus* [41]. Yoon et al. [39] determined the suitability of the tomato juice as a raw material for the production of probiotic juice by *Lactobacillus acidophilus* LA3 *Lactobacillus plantarum* C *Lactobacillus casei* A4 and *Lactobacillus delbrueckii* D7. Yoon et al. [42] also evaluated the potential of red beets as the substrate for the production of probiotic beet juice by the above four species of LAB. All the lactic cultures were capable of rapidly utilizing the beet juice for the cell synthesis and lactic acid production. Yoon et al. [28] also developed a probiotic cabbage juice using LAB of *Lactobacillus plantarum* C *Lactobacillus casei* A4 and *Lactobacillus delbrueckii* D7. The fermented cabbage juice could serve as a healthy beverage for vegetarians and lactose-allergic consumers. Rakin et al. [43] enriched beetroot and carrot juices with the brewer's yeast autolysate before lactic acid fermentation with *Lactobacillus acidophilus*. The use of spent brewer's yeast from the brewery was important for the economic optimization of the fermentation. A mixture of beetroot and carrot juices has optimum proportions of pigments, vitamins and minerals [43].

The mechanisms of health-improving properties of probiotics are still not completely understood, but are commonly suggested to relate to pathogen interference, exclusion or antagonism, immunomodulation, anticarcinogenic and antimutagenic activities, alleviation of lactose intolerance symptoms, reduction in serum cholesterol levels, reduction in blood pressure, prevention and decreasing incidence and duration of diarrhea, prevention of bacterial vaginosis and urinary tract infection, maintenance of mucosal integrity, and improved periodontal health [3, 44-48]. *Bifidobacteria* represent common inhabitants of the gastro intestinal track (GIT) of mammals, birds, and certain cold-blooded animals. Some bifidobacterial species (e.g. *Bifidobacterium bifidum* and *Bifidobacterium breve*) are frequently used as the probiotic ingredient in many functional foods. However, despite the generally accepted importance of *Bifidobacteria* as constituents of the human microbiota, there is only limited information available on their phylogeny, physiology, and genetics [49]. Besides, many LAB species are important for the food industry, since they are used as starters or adjunct cultures for the production of fermented foods. They play a recognized role in the preservation and microbial safety of fermented foods [50], thus promoting the microbial stability of the final products [51]. Protective effects are due to the production of organic acids, CO ethanol, hydrogen peroxide and diacetyl, antimicrobial compounds such as fatty acids, bacteriocins, and related proteinaceous compounds, and antibiotics [52-54]. A number of studies have addressed the development of probiotic cheeses including fresh cheeses such as Cottage, Crescenza and Minas cheeses, Cheddar cheese, Gouda cheese, semihard cheese, and white-brined cheese. These studies have demonstrated that cheeses have a great potential as a carrier to deliver probiotic bacteria to the consumer [55,56].

Many studies on probiotic cheeses have been addressed on maintaining a high probiotic population during product shelf-life. To this regard, several cheese varieties, such as Cheddar or Gouda, were shown to carry high numbers of different strains of probiotic bacteria for variable periods. For example, it was recently shown that probiotic lactobacilli in a semisoft cheese survived in the simulated human GIT and the cheese matrix did not affect the probiotic survival [57]. More generally, however, cheese does not always seem a favorable substrate for surviving of LAB probiotics because the cheese matrix itself can impose multiple stresses on bacterial survival [58]. Technological approaches, such as immobilization of probiotic or addition of protein hydrolysate, have then been suggested to improve strain viability in cheese [59]. One of the most applied methods for preservation of functionality and targeted delivery of bioactive food components is encapsulation. Both bioactive molecules and bioactive living cells may benefit from encapsulation. To this regard, microencapsulation of many types of probiotics may be mandatory for achieving the promised health benefits by promoting not only viability but, more importantly, functionality of the probiotics into the food matrix [60].

A special characteristic of cheeses is that, unlike other probiotic carriers, they are consumed after a ripening period that can vary from a few days to several months or even years. During ripening time, biochemical reactions take place and include the transformation of carbohydrates, lipids, and proteins into aroma compounds. *Lactobacilli* and *bifidobacteria* have a wide variety of proteolytic and peptidolytic enzymes, and therefore have the potential to influence proteolysis. If probiotic LAB showed positive impact on cheese quality, this would be an extra advantage besides the health benefits. This aspect is very important because it would positively influence the acceptability of the final product by the consumer. Unfortunately, the impact that the probiotic microbes have on textural and sensory properties of cheese is still poorly studied and, however, the few

available studies on this subject are giving conflicting results. The addition of cheese-isolated lactobacilli strains has been proposed to accelerate the ripening process or enhance sensory properties of the product [61,62]. A recent investigation on the impact of the proteolytic activity of three probiotic strains of *L. acidophilus*, *L. paracasei*, and *B. lactis* used as adjunct cultures in semihard (Pategr` as) cheese indicated that *L. acidophilus* played a major role in secondary proteolysis of probiotic cheeses; the probiotic strains produced acceptable functional foods, with similar acceptability scores to regular Pategr` as cheeses [59].

Established Benefits

LAB is important during lactose digestion. It is well-known that the presence of lactic acid bacteria, specifically *L. bulgaricus* and *S. thermophilus* in yogurt, improves lactose digestion [63]. It appears that the cell walls of the bacteria have to be intact (as is the case when the bacteria are alive) for the effect to occur [64]. Some possible mechanisms for the improved lactose digestion include: The lactase activity of the bacteria actually does the work of digesting lactose in the product once it reaches the intestine [65]. The slower transit time of yogurt may permit more time for the residual intestinal lactase and the yogurt bacteria to digest the lactose. Something in the yogurt may inhibit fermentation of lactose and thus reduce symptoms [66]. Sweet acidophilus milk (milk with *L. acidophilus* which has not been allowed to ferment) does not seem to alleviate the symptoms of lactose maldigestion. Although some work shows a small effect, most work shows no effect [67].

Also Lactic acid bacteria may be useful in preventing and shortening the duration of several types of diarrhea [67]. A number of well-designed studies have noted that fermented milk products effectively prevent or treat infantile diarrhea [68]. Effects have been noted with *L. casei* and *B. bifidum*. A few small studies show that lactic acid bacteria can reduce the incidence of antibiotic-related diarrhea [69]. This suggests a role for lactic acid bacteria in immunosuppressed patients who routinely use antibiotics [70]. A few studies of traveller's diarrhea have demonstrated the effectiveness of lactic acid bacteria in decreasing the incidence of diarrhea [71,72]. Lactic acid bacteria can probably reduce diarrhea in several ways: Lactic acid bacteria compete with pathogens for nutrients and space in the intestines [69]. By-products of metabolism may have a direct effect against the pathogens. For example, *invitro* work shows that *L. casei*, *L. acidophilus* and *L. bulgaricus* can all produce antimicrobial agents such as acidophilin and bulgarican that can inhibit growth of pathogens [69]. Lactic acid bacteria may be effective against diarrhea due to effects on the immune system.

In addition, Lactic acid bacteria enhance immune system function at the intestinal and systemic levels. In humans, lactic acid bacteria have been shown to increase: B-lymphocytes or B cells, which recognize foreign matter [73], phagocytic activity, helping to destroy foreign matter [74], IgA-, IgG- and IgM-secreting cells and serum IgA levels, which would increase antibody activity [75], and γ -interferon levels, which help white blood cells fight disease [76]. Another way the body's defenses work is by the barrier provided by the mucus layers of the intestine. The mucosa provides a physical barrier, usually preventing foreign substances from passing through the gut. As well, a large variety of immune cells are found in the gut mucosa. This allows the gut to interact with the immune system. Lactic acid bacteria can stimulate immune activity in the intestinal mucosa [77]. In conditions such as allergy or auto-brewery syndrome (abnormal gut fermentation resulting in increased levels of blood ethanol), the permeability of the small intestine can increase, allowing undigested protein molecules to

pass through [78]. *Lactobacillus GG* has been shown to reverse gut permeability [79]. Probiotic bacteria may be able to play a role in treating food allergy. This was demonstrated in a recent experiment with infants known to have eczema due to a cow milk allergy [80]. Infants in the experimental group got hydrolyzed whey formula fortified with *LGG*, while those in the control group just got whey formula. The skin condition of the infants getting the *LGG* improved significantly compared to the control group. In addition, the experimental group had improved levels of factors associated with inflammation of the intestine.

Potential Benefits

Probiotics play an important role in the control of irritable bowel syndrome and inflammatory bowel diseases, suppression of endogenous/exogenous pathogens by normalization of the intestinal microbial composition, alleviation of food allergy symptoms in infants by immunomodulation, lowering serum cholesterol, improving lactose tolerance, and reducing risk factors for colon cancer by metabolic effects [44]. Probiotics play a therapeutic role by lowering cholesterol, improving lactose tolerance, nutritional enhancement and preventing some cancers and antibiotic associated diarrhoea [81]. Some LAB strains have hypocholesterolemic effects. For example, *L. acidophilus* can take up cholesterol in the presence of bile [82]. Other *invitro* research shows that cholesterol can precipitate with free bile salts in the presence of *L. acidophilus*, especially in an acid environment [83]. Thus, it has been hypothesized that one or both of these actions would take place *in vivo* and help lower serum cholesterol in humans. Various studies with fermented milk products have shown either no effect or a reduction in cholesterol levels. In conclusion, there is not yet good evidence to confirm a cholesterol-lowering effect of fermented milk products.

Milk products fermented with some strains of *L. acidophilus* and bifidobacteria shorten intestinal transit time. This effect may be useful for those with constipation, such as the elderly [84]. A well-controlled human study is needed to confirm this. Several lactic acid bacteria may help prevent initiation of colon cancer. It has also been demonstrated that lactic acid bacteria slow the growth of experimental cancers, although the results are not long-term. It appears that lactic acid bacteria can reduce the levels of colon enzymes that convert procarcinogens to carcinogens. Specifically, lactic acid bacteria can reduce levels of the enzymes β -glucuronidase, nitroreductase, and azoreductase. Lactic acid bacteria may also be involved in the direct reduction of procarcinogens, for example, by taking up nitrites and by reducing the levels of secondary bile salts [85]. In most reports, these effects only occur during the period of time that the bacteria are consumed. Changes in enzyme activity in humans have been observed with *L. acidophilus* and *B. bifidum* [86], and *LGG* [87]. Animal studies show fewer tumors in those exposed to a carcinogen, in the presence of *LGG*, compared to the animals exposed to the carcinogen without the benefit of *LGG* [88]. In humans, epidemiological reports show that populations eating fermented dairy products have a decreased risk of colon cancer [89]. However, there is not yet a clear relationship between lactic acid bacteria intake and cancer prevention.

Lactic acid bacteria may reduce candidal vaginal infections. This is still speculative, however it would be research worth pursuing. One small study showed that women with recurrent vaginal candidiasis who ate 8 oz. daily of a yogurt containing *L. acidophilus* had fewer occurrences of vaginal candidiasis than during the control period in which they ate no yogurt [90]. This was a cross-over study which started with 21 women. Eight of those who started in the treatment

group refused to cross over to the control phase since they experienced so many fewer infections. Thus only 13 women completed the study.

Lactic acid bacteria show some promise against stomach ulcers. Work with a specific strain of *L. acidophilus* demonstrated that *L. acidophilus* competes effectively (*invitro*) against *Helicobacter pylori* for attachment sites, limiting the number of *H. pylori* that can attach to the cell wall [91]. Infection with *H. pylori* is a risk factor for stomach ulcers. A small study of patients with ulcers showed that *Bifidobacteria bifidum* promoted healing of gastric ulcers in 50% of the patients and eradication of *H. pylori* from the mucous membranes in 30% of the patients.

The Mechanism of Action of Probiotic Bacteria

It is accepted that LAB exert beneficial effects through two mechanisms: direct effects of the live microbial cells, known as the “probiotic effect” or indirect effects during fermentation where these microbes act as cell factories for the generation of secondary metabolites with health-promoting properties [92]. The effects of probiotics can be classified in three modes of action. The first is related with the modulation of the host’s defenses which is most likely important for the prevention and treatment of infectious disease and also for treatment of intestinal inflammation. Probiotics may influence the immune system by means of products such as metabolites, cell wall components or DNA. In fact, these products can be recognized by the host cells sensitive for these because of the presence of a specific receptor [93]. The main target cells are generally the gut epithelial and the gut-associated immune cells. Finally, the interaction between probiotics and the host’s immune cells by adhesion might be the triggering signaling cascade leading to immune modulation [94].

The second mechanism of action can be described by a direct effect on other microorganisms which can be commensal and/or pathogenic. In this case, the therapy and the treatment of infections are concerned but restoration of the microbial balance in the gut is an important factor too [95]. Probiotics have the ability to be competitive with pathogens and therefore allow for preventing their adhesion to the intestine [96]. Eventually, probiotics have the ability to affect some microbial products such as toxins and host products like bile salts and food ingredients [97]. However, it is important to know that these three mechanisms of action are strain-dependent, and to date the modes of action of probiotic bacteria are not yet fully known [98].

Starter Design and Functionality

Although many probiotic LAB strains are already known and applied in commercial probiotic fermented milks throughout the world [99], the market of biofunctional dairy products, including probiotics, is continuously asking for implementing and diversifying the range of available products. To this regard, there is a growing need to identify new biofunctional strains, new strategies to assure survival of these cultures, and different sources from which to isolate strains. During the fermentation process, LAB also influences the sensory properties of a product, including the flavor development. Flavor compounds are formed by various processes, e.g. the conversions of lactose and citrate (glycolysis and pyruvate metabolism), fat (lipolysis), and proteins (proteolysis, peptidolysis, and amino acids catabolism) [100]. For these reasons, many LAB species find wide industrial applications, mainly as starter or complementary cultures, in several food fermentations. The most commonly food-associated genera belong to *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Oenococcus*, and *Streptococcus* [101]. The possibility to include strains isolated from nondairy sources in probiotic preparations could

extend the range of available strains to be proposed as candidate probiotics. An important factor limiting the availability of new probiotic cultures is linked to the industrial costs of detection, characterization, and clinical validation of new candidate LAB strains of probiotic interest. This led to the development of different sets of simple *invitro* screening tests.

Selection Criteria of Probiotics

Many *invitro* tests are performed when screening for potential probiotic strains. The first step in the selection of a probiotic LAB strain is the determination of its taxonomic classification, which may give an indication of the origin, habitat and physiology of the strain. LAB is associated with habitats that are rich in nutrients, such as various food products and plant materials. They can be found in soil, water, manure, sewage, and silage and can ferment or spoil food. Particular LAB is inhabitants of the human oral cavity, the intestinal tract, and the vagina, and may have a beneficial influence on these human ecosystems. All these characteristics have important consequences on the selection of the novel strains [102].

The initial screening and selection of probiotics includes testing of the following important criteria: phenotype and genotype stability, including plasmid stability; carbohydrate and protein utilization patterns; acid and bile tolerance and survival and growth; intestinal epithelial adhesion properties; production of antimicrobial substances; antibiotic resistance patterns; ability to inhibit known pathogens, spoilage organisms, or both; and immunogenicity. The ability to adhere to the intestinal mucosa is one of the more important selection criteria for probiotics because adhesion to the intestinal mucosa is considered to be a prerequisite for colonization.

So, the host must be immuno-tolerant to the probiotic. On the other hand, the probiotic strain can act as an adjuvant and stimulate the immune system against pathogenic microorganisms. It goes without saying that a probiotic has to be harmless to the host: there must be no local or general pathogenic, allergic or mutagenic/carcinogenic reactions provoked by the microorganism itself, its fermentation products or its cell components after decrease of the bacteria [103].

| General | Property | |
|------------------------|--|------------------|
| Safety criteria | Origin | |
| | Pathogenicity and infectivity | |
| | Virulence factors—toxicity, metabolic activity and intrinsic properties, i.e., antibiotic resistance | |
| Technological criteria | Genetically stable strains | |
| | Desired viability during processing and storage | |
| | Good sensory properties | |
| | Phage resistance | |
| | Large-scale production | |
| Functional criteria | Tolerance to gastric acid and juices | |
| | Bile tolerance | |
| | Adhesion to mucosal surface | |
| | Validated and documented health effects | |
| Desirable criteria | physiological | Immunomodulation |

| |
|--|
| Antagonistic activity towards gastrointestinal pathogens, i.e., <i>Helicobacter pylori</i> , <i>Candida albicans</i> |
| Cholesterol metabolism |
| Lactose metabolism |
| Antimutagenic and anticarcinogenic properties |

Table 1: Key and desirable criteria for the selection of probiotics in commercial applications [104].

Safety of Probiotic LAB

Lactic acid bacteria traditionally used in fermented dairy products have a long history of safe use. However, as interest grows in using new strains, safety testing will become important. *Lactobacillus GG*, one of the newer strains, has undergone extensive testing for the safety and efficacy of its use. It was approved in 1992 by the United Kingdom Advisory Committee on Novel Foods and in 1996 by the Japanese functional food authorities [105]. Probiotic strains must be safe for human consumption. Probiotic strains such as *Lactobacillus* species, *Bifidobacterium* species, and *Streptococcus* species have long history of safe use and are GRAS. However, there have been some reports that have associated LAB with clinical pathological conditions such as bacteraemia and endocarditis [106]. These reports raised concerns about the safety of probiotic bacteria. Because there was no international consensus on methodology to assess efficiency and safety of probiotics, the FAO and WHO undertook work to compile and evaluate the scientific evidence on functional and safety aspects of probiotics [13]. In the selection process of probiotic strains, safety aspects include, among others, specifications such as origin, lack of harmful activities, and absence of acquired antibiotic resistance. It is well recognized that probiotic effects are strain, condition, and dose specific. Therefore, one of the first prerequisites documenting the microbiological safety of bacterial cultures intended for probiotic or nutritional use is the accuracy of their taxonomic identity. Potential probiotic strains must be identified by internationally accepted methods and named according to the International Code of Nomenclature and strains must be deposited in an internationally recognized culture collection [13]. Strain identification, which is done by phenotype and genotype methods, is important to link a strain to a specific health effect as well as to enable accurate surveillance and epidemiological studies. In LAB, recent studies highlighted that reliable identification and strain characterization can be obtained by molecular methods [107].

Another key aspect for safety is the specification of the strain origin. Potential probiotic cultures have been isolated from a variety of sources including animal, human, and food sources. However, there is now growing evidence that strains are host specific and for that reason it is generally accepted that strains to be used for human applications should be human isolates [108]. However, many food-associated LAB, such as *L. (para)casei* and *L. plantarum*, which constitute the majority of the LAB species found in most cheese varieties, have been selected as candidate probiotics and some of them are currently used in commercial probiotic products [109]. Of course, whatever the origin or the taxonomic identity, the candidate probiotic strains need a series of *in vitro* tests and animal trials to verify the absence of β -hemolytic activity and other harmful enzymatic activities such as β -glucosidase, N-acetyl- β -glucosaminidase, and β -glucuronidase activities, which all have been associated with health disorders or intestinal diseases [110,111]. For example, β -glucuronidases liberate toxins and mutagens that have been glucuronated in the liver and excreted into the gut with

the bile. This can lead to high local concentrations of carcinogenic compounds within the gut, thus increasing the risk of carcinogenesis [112]. Increased efforts have been devoted in recent years to gain more insight into the diffusion of antibiotic-resistance phenotypes within food-associated LAB, with particular emphasis on those applied as starter cultures or probiotics. Presently available literature data support the view that, in antibiotic challenged habitats, LAB (especially enterococci) like other bacteria are involved in the transfer of resistance traits over species and genus border, with important safety implications. The prevalence of such bacteria with acquired, genetically exchangeable, resistances is high in animals and humans that are regularly treated with antibiotics [113]. This underlines the importance to include the antibiotic susceptibility profiles within the selection criteria of candidate probiotics.

Conclusion

The uses of probiotic LAB and their applications have shown tremendous increase in the last two decades. Probiotics can turn many health benefits to the human, animals, and plants. Applications of probiotics hold many challenges. In addition to the viability and sensory acceptance, it must be kept in mind that strain selection, processing, and inoculation of starter cultures must be considered. Probiotics industry also faces challenges when claiming the health benefits. It cannot be assumed that imply adding a given number of probiotic bacteria to a food product will transfer health to the subject. Indeed, it has been shown that viability of probiotics throughout the storage period in addition to the recovery levels in the gastrointestinal tract are important factors [114]. For this purpose, new studies must be carried out to: test ingredients, explore more options of media that have not yet been industrially utilized, reengineer products and processes, and show that lactose-intolerant and vegetarian consumers demand new nourishing and palatable probiotic products.

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