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Chapter 25

Lactic Acid Bacteria as Source of Functional Ingredients

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http://dx.doi.org/10.5772/47766

1. Introduction

Lactic acid bacteria (LAB) are widespread microorganisms which can be found in any environment rich mainly in carbohydrates, such as plants, fermented foods and the mucosal surfaces of humans, terrestrial and marine animals. In the human and animal bodies, LAB are part of the normal microbiota or microflora, the ecosystem that naturally inhabits the gastrointestinal and genitourinary tracts, which is comprised by a large number of different bacterial species with a diverse amount of strains [1,2].

Phylogenetically the LAB belong to the *Clostridium* branch of Gram positive bacteria. They are non-sporenging, aero tolerant anaerobes that lack catalase and respiratory chain, with a DNA base composition of less than 53 mol% G+C [3,4]. According to their morphology LAB are divided to robs and cocci and according to the mode of glucose fermentation to homofermentative and heterofermentative. The homofermentative LAB convert carbohydrates to lactic acid as the only or major end-product, while the heterofermentative produce lactic acid and additional products such as ethanol, acetic acid and carbon dioxide [5,6]. Thus, the main metabolism of LAB is the degradation of different carbohydrates and related compounds by producing primarily lactic acid and energy. Although many genera of bacteria produce lactic acid as primary or secondary fermentation products, typical lactic acid bacteria are those of the Lactobacillales order, including the following genera: *Lactobacillus, Carnobacterium, Lactococcus, Streptococcus, Enterococcus, Vagococcus, Leuconostoc, Oenococcus, Pediococcus, Tetragonococcus, Aerococcus* and *Weissella* [7].

Many strains of LAB are among the most important groups of microorganisms used in the food and feed industries, although some of the genus Pediococcus cause deterioration of foods, which results in their spoilage [4]. LAB have been used in food preservation and for the modification of the organoleptic characteristics of foods, for example flavors and texture [2]. Various strains of LAB [8] can be found in dairy products (yoghurt, cheese), fermented
meats (salami), fermented vegetables (olives, sauerkraut), sourdough bread, etc [9]. The European Food Safety Authority (EFSA) has stated that several LAB strains can be considered to have “Qualified Presumption of Safety” QPS-status [9].

Moreover, nowadays, LAB play an important role in the industry for the synthesis of chemicals, pharmaceuticals, or other useful products (Figure 1). Also, the biotechnological production of lactic acid has recently reported that offers a solution to the environmental pollution by the petrochemical industry [10].

This chapter will discuss recent applications of LAB as source of probiotics, starter cultures, antimicrobial agents, vitamins, enzymes and exopolysaccharides, especially those that can satisfy the increasing consumer’s demands for natural products and functional foods in relation with human health.

2. Lactic acid bacteria as source of probiotics

Etymologically the term probiotics is derived from the Greek “probios” which means “for life”. In 1974 Parker [11] defined as probiotics “organisms and substances which contribute to intestinal microbial balance”. Fuller in 1989 [12] defined as probiotic “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance”. Later the Food and Agriculture Organization / World Health Organization defined probiotic bacteria as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” [13]. Since probiotics can colonize the gastrointestinal
tract and exert their beneficial effect long term, without requiring continuous medical intervention, they have been used for a century to treat a variety of mucosal surface infections (gut, vagina), but their use decreased after the appearance of antibiotics. However, today, probiotics are considered as an alternative solution to antibiotics due to the increasing spread of antibiotic resistance and the need for treatment cost reduction [14].

Microorganisms considered as commercial probiotics are mainly of the Lactobacillus genus with over one hundred species recognized, for example: L. acidophilus, L. rhamnosus, L. reuteri, L. casei, L. plantarum, L. bulgaricus, L. delbrueckii, L. helveticus [15-17]. Lactobacilli are Generally Recognized As Safe (GRAS) organisms [18,19].

Probiotic bacteria are very sensitive to many environmental stresses, such as acidity, oxygen and temperature [20,21] and they must fulfill some functional and physiological aspects such as [21,22]: a) Adherence to the intestinal epithelium and colonization of the lumen of the tract. b) Ability to stabilize the intestinal microbiota. c) Counteracting the action of harmful microorganisms. d) Production of antimicrobial substances. e) Stimulation of the immune response.


2.1. Mechanism of action of probiotics

Probiotics have multiple and diverse effects on the host. The main mechanisms of action of probiotic bacteria by which they improve mucosal defenses of the gastrointestinal tract include:

a. Antimicrobial activity: The probiotics block the colonization of pathogenic bacteria by decreasing luminal pH, inhibiting bacterial invasion and adhesion to epithelial cells and producing antimicrobial compounds such as bacteriocins and defensins, organic acids and hydrogen peroxide. The interaction of LAB with the mucosal epithelial cells of the gastrointestinal tract and the lymphoid cells in the gut enhance the gut immune response against ingested pathogens [26,27].

b. Enhancement of mucosal barrier function against ingested pathogens: It is achieved with the increasing mucus production through modulation of cytoskeletal and tight junctional protein phosphorylation. The probiotic bacteria compete with pathogenic bacteria for epithelial binding sites, inhibiting the colonization of strains like Salmonella and E. coli [28,29]. Probiotic bacteria interact with the epithelial cells of the gut, either directly (via cell compounds like DNA, lipoteichoic acids and cell-surface polysaccharides) or indirectly (through production of bioactive metabolites) [30]. The enhancement of mucosal barrier function may be an important mechanism by which probiotics benefit the host in various diseases such as Type 1 diabetes [31,32].
c. Immunomodulation: Specific strains of probiotics might influence the innate and the acquired immune system, thus playing an important role in human diseases. Probiotic bacteria may affect the epithelial cells, the dendritic cells, the monocytes / macrophages and the various types of lymphocytes (Natural killer cells, T-cells and T-cell redistribution) directly or secondarily [33,34]. This action of probiotics could be important for the elimination of neoplastic host cells [22]. Moreover, the effects of probiotics on B-lymphocytes and antibody production resulted in an increase in IgA secretion and the enhancement of response to vaccination [34]. Recently, it was also reported that probiotics can have positive effects on the respiratory system by preventing and reducing the severity of respiratory infections, because of an increase of IgA in the bronchial mucosa [35].

2.2. Probiotics and health

Functional properties of probiotics have been demonstrated for various therapeutic applications. Nevertheless, the health benefits provided by probiotics are strain-specific, therefore no probiotic strain will have all proposes benefits, not even strains of the same species [36]. Among the LAB probiotic strains *L. rhamnosus* GG and *L. casei* Shirota have the strongest human health efficacy in the management of lactose intolerance, rotaviral diarrhea and antibiotic associated diarrhea [17]. An optimal single oral dose, based on detection of the bacteria in human feces is $10^9$ bacterial colony forming units (CFU) [37], while in other reports $10^6$-$10^7$ CFU / g of food are considered adequate amounts [13,17,38].

Moreover, in animal nutrition dietary probiotics or direct fed microbials, term which is preferred in the USA, are able to help the maintenance of a healthy intestinal microflora. This microflora may serve to improve performance and health status of the animals, but also to suppress food born pathogens such as *Salmonella* and *Campylobacter*. These conditions are necessary for the production of safe meat and meat products [39]. For instance, the gastrointestinal tract of broilers can be colonized by ingested probiotic bacteria from the first days of their life, which results in shorter period for the achievement of microflora stability. Also, in another example the dietary inclusion of probiotics in young calves’ milk replacers may improve their growth performance [40].

Some of the beneficial effects of probiotics are well established as shown in Table 1.

<table>
<thead>
<tr>
<th>Lactic Acid Bacteria</th>
<th>Effects on human health</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus rhamnosus</em> GG</td>
<td>May shorten the course of rotavirus causing diarrhea.</td>
<td>41-45</td>
</tr>
<tr>
<td></td>
<td>Helps to alleviate the symptoms of ulcerative colitis and atopic dermatitis.</td>
<td></td>
</tr>
<tr>
<td><em>Lactobacillus casei</em></td>
<td>Reduces the severity and duration of diarrhea. It can stimulate the immune system of the gut and alleviates the symptoms of Crohn’s disease</td>
<td>17, 43, 46</td>
</tr>
</tbody>
</table>
Lactic Acid Bacteria as Source of Functional Ingredients

Lactic Acid Bacteria Effects on human health References

Lactobacillus acidophilus
Secretes lactic acid which reduces the pH of the gut and inhibits the development of pathogens (Salmonella spp, E. coli).
Reduces blood cholesterol.

Lactobacillus johnsonii
Effective in inhibition of H. pylori and against inflammation

Lactobacillus plantarum
Produces short-chain fatty acids that block the generation of carcinogenic agents by reducing enzyme activities

Lactobacillus fermentum
Effective in restoration of a normal microflora. Effective against bacterial vaginosis flora

Lactobacillus reuteri
Reduces the duration of diarrhea

Enterococcus faecium
Can reduce blood cholesterol leading to decreased blood pressure

Table 1. Lactic acid bacteria derived probiotics and human health

LAB derived probiotics have potential health benefits in the following situations:

1. Diarrheal diseases:
   a. Infective diarrhea. The most studied gastrointestinal condition treated by probiotics is acute infectious diarrhea in infants. Children represent a main target of studies due to the importance of limiting the spread of diseases and decreasing the need of antibiotics (Aureli et al. 2011). Clinical trials with LAB derived probiotics (L. rhamnosus GG; L. reuteri; L. casei; L. delbrueckii subsp. Bulgaricus) support the efficacy of these probiotics in preventing diarrhea [14,49,53], due to their direct or indirect interaction with the enterotoxins [54].
   b. Antibiotic associated diarrhea. A variety of probiotic bacteria, mainly lactobacilli have been used in the treatment and prevention of antibiotic associated diarrhea [55,56]. In a recent study L. acidophilus and L. casei seemed to be effective in reducing the risk of development of diarrhea [57]. Nevertheless, the results obtained were from pilot studies, so further investigation is needed to evaluate the efficacy of probiotics on such disorders.
   c. Clostridium difficile associated diarrhea. C. difficile is an opportunistic pathogen often responsible for diarrhea in vulnerable people. L. rhamnosus GG, has demonstrated positive effects on treated patients [14].
   d. Travelers diarrhea. Probiotics with Lactobacilli did not seem to be effective on such diarrhea, which is caused by bacteria, in particular enterotoxigenic E. coli [14,53].
   e. Radiation induced diarrhea. Although there is little research on this subject, probiotics seem to be promising in decreasing radiation diarrhea [53].
2. Inflammatory bowel disease

LAB may affect positively the intestinal mobility and relieve constipation, possibly through a reduction of the intestinal pH [58].

a. Pouchitis. It is a chronic inflammation of the ileal pouch. The treatment with probiotics such as *L. rhamnosus* GG and *L. acidophilus* reduced the risk of pouchitis due to decreased mucosal inflammation [59,60].

b. Crohn’s disease. This disease can involve the whole gastrointestinal tract and is characterized by inflammatory processes occurring deeper in the tissues. Among other typical treatments *L. rhamnosus* is used aiming at decreasing the rate and the severity of disease after surgery [61].

c. Ulcerative colitis. It is an acute or chronic disease only affecting the large bowel. LAB probiotics (*L. acidophilus*) provide some promising initial indications [53].

3. Irritable bowel syndrome

This term is used to describe a heterogenous group of gastrointestinal symptoms, like diarrhea, constipation, bloating and abdominal pain. *L. plantarum* strain 299V and *E. faecium* PR88 could be effective treatments against this syndrome [62,63].

4. Prevention of colon cancer

The anticarcinogenic effect of probiotics may be attributable to a combination of mechanisms like the induction of pro- or anti-inflammatory and secretary responses that could inhibit carcinogenesis [22]. In vitro studies with lactobacillus strains have shown anti-mutagenic activities. However, there is no evidence yet that probiotics can protect against the development of colon cancer in humans [64,65]. Although, it is hypothesized that the strains tested may have anti-carcinogenic effects by reducing the activity of the enzyme β-glucuronidase.

5. Helicobacter pylori

It is a common chronic bacterial infection in humans, which causes many problems, such as chronic gastritis, septic ulcers and gastric cancer. Probiotics like *L. salivarius*, *L. casei Shirota* and *L. acidophilus* appear to be promising in inhibiting the growth of *H. pylori* in vitro [66,67]. Moreover, *L. johnsonii* was also shown effective to inhibit *H. pylori* [68].

6. Lactose intolerance

It is the most common disorder of the intestinal carbohydrate digestion. In both adults and children it has been shown that probiotics can improve the lactose digestion by reducing the intolerance symptoms and slowing orocecal transit [69,70].

7. Blood cholesterol

Recently it has been suggested that some strains of probiotic bacteria, like *L. acidophilus*, *L. plantarum* and *Enterococcus faecium* could significantly reduce blood cholesterol and increase resistance of low density lipoprotein oxidation, leading to decrease of blood pressure [50-52].
8. Other disorders

The majority of probiotics use has focused on diseases related to the gut, but there are studies that evaluated probiotics, in allergic conditions, including atopic dermatitis, rhinitis, bacterial vaginosis and food allergies [53,71].

a. Atopic Dermatitis. It is the most common of the chronic skin disorders, known as eczema. Investigations have shown that probiotics like *L. rhamnosus* GG, can prevent or reduce the symptoms [42]. Even eczema can be prevented if mothers ingest probiotics during pregnancy and neonatal ingest them during the first 6 months of their life [53].

b. Bacterial Vaginosis. Probiotics (*L. rhamnosus* GR-1 and *L. fermentum* RC-14) are considered to have therapeutic benefits in vaginosis. Probably this is due to the large numbers of lactobacilli in the healthy vaginal microflora [72,73].

c. Other ailments. Probiotics such as *Lactococcus*, *Pediococcus* and *Leuconostoc* can prevent or limit mycotoxinogenic mould growth [74-78]. Moreover, LAB according to their bacterial strain could bind aflatoxin B1 both in vivo and in vitro [79]. It was reported in studies that *L. paracasei* ST11 reduced body and abdominal fat [80]. These probiotic bacteria seemed to have an anti-obese action. Probably intestinal bacteria may regulate body weight by affecting the host’s metabolic neuroendocrine and immune functions [80]. Additionally, probiotics may have anticariogenic effects, preventing and treating dental caries and generally be effective in the oral cavity and the treatment of periodontal disease [81,82].

3. Lactic acid bacteria as source of starter cultures

3.1. Starter cultures and functional starter cultures in fermentation of foods

LAB for a long time have been applied as starter cultures in fermented foods and beverages, because they can improve nutritional, organoleptic, technological and shelf-life characteristics [83,84]. LAB initiate rapid and adequate acidification in the raw materials, through the production of various organic acids from carbohydrates. Lactic acid is the most abundant, followed by acetic acid, whilst LAB can also produce ethanol, bacteriocins, aroma compounds, exopolysaccharides and some enzymes [85]. Earlier the production of fermented foods and beverages was obtained on a spontaneous fermentation, due to the microflora naturally present on the raw materials. Later on, the direct addition of selected starter cultures to the food matrix was preferred by the food industry. The advantages were the high degree of control over the fermentation process and the standardization of the final product [84].

As starter culture can be defined a microbial preparation of a large number of one or more microorganisms which is introduced to a raw material aiming to produce a fermented food by accelerating and steering its fermentation process [86,87].

The industries of fermented foods mainly utilize commercial starter cultures for the direct inoculation to the food matrix, which are available as frozen and freeze dried concentrates or lyophilized preparations [88].
Recently the use of functional starter cultures in food and beverage fermentation is being explored. These cultures have at least one functional property, contributing in the improvement of the fermentation process, enhancing the quality and of the end safety product and conferring health benefits [84]. Nevertheless, the selection of starter cultures must also eliminate undesirable side effects like the formation of D-lactic acid or a racemate of lactic acid (DL) or the formation of biogenic amines [84,89].

### 3.2. Probiotics as functional starter cultures

A category of successful starter cultures are LAB produced probiotic cultures. Firstly, Metchnikoff [90] discovered the beneficial effects of LAB on human health, through the consumption of yoghurts and fermented milks. Currently probiotic cultures are used for a number of products such as yoghurt, yoghurt drinks, infant formulas, dietary supplements, etc [91]. Yoghurt is manufactured using *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* as starter cultures [17].

A manufacture in order to choose any probiotic microbial strain to be used as starter culture or better as a blend with a traditionally used starter culture (co-culture), must check the following aspects [92]: 1) The ability of the probiotics to grow in a medium to increase the cells counts. 2) The robustness of the organism to withstand the freezing and drying stages of preparation. 3) The tolerance to acidity of the gastric acid and the bile salts during their passage in the gastrointestinal tract. Thus, the probiotic strains must be stable in order to claim the health benefits [92].

LAB are used as starter cultures either in dairy or non-dairy products (Table 2).

<table>
<thead>
<tr>
<th>Genus</th>
<th>Application in dairy foods</th>
<th>Application in non-dairy foods</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus</em> spp</td>
<td>Cultured dairy products, cheese, yoghurt, kefir</td>
<td>Sausage, sourdough bread, fermented vegetables</td>
</tr>
<tr>
<td><em>Lactococcus</em> spp</td>
<td>Cheese, butter milk sour cream, cultured dairy products</td>
<td>-</td>
</tr>
<tr>
<td><em>Leuconostoc</em> spp</td>
<td>Cheese, cultured dairy products</td>
<td>Fermented vegetables</td>
</tr>
<tr>
<td><em>Streptococcus</em> thermophilus</td>
<td>Cheese, yoghurt</td>
<td>-</td>
</tr>
<tr>
<td><em>Pediococcus</em></td>
<td>-</td>
<td>Sausage, fermented vegetables</td>
</tr>
<tr>
<td><em>Tetragenococcus</em></td>
<td>-</td>
<td>Soy sauce</td>
</tr>
<tr>
<td><em>Oenococcus</em></td>
<td>-</td>
<td>Wine</td>
</tr>
</tbody>
</table>

**Table 2.** Lactic acid bacteria used as starter cultures in fermented foods
3.3. Functional starter cultures in fermented dairy products

Traditionally, LAB have been used in the fermentation of dairy products, as a simple and safe way of preserving such foods. The main species of LAB that can potentially be used as probiotic cultures in dairy products belong to the *Lactobacillus* spp (*L. acidophilus*, *L. lactis*, *L. casei*, *L. plantarum*, *L. rhamnosus*, *L. reuteri*, *L. delbrueckii subsp. bulgaricus*) or to the *Enterococcus* spp (*E. faecalis*, *E. faecium*) [17,92].

Dairy products are considered as ideal vehicles for delivering probiotics to the human gut. Yoghurt is considered the most important, followed by cultured buttermilk, kefir, cheeses, ice-cream [17,22,92] or frozen desserts like chocolate mousse [93]. Moreover proteolytic strains of LAB produced probiotics are used to release bioactive peptides called angiotensin I-converting enzyme inhibitors, which are examined for their hypotensive role [94]. Furthermore interaction between probiotics and starter cultures are possible, either as synergism (e.g. yoghurt) or antagonism (e.g. bacteriocins which exhibit antibiotic properties) [20].

A minimum viable LAB count of $10^6$ CFU/g in fermented dairy food is recommended for the claimed health benefits [95].

3.4. Functional starter cultures in fermented non-dairy products

3.4.1. Fermented meat and meat products

The preservation of meat and meat products by fermentation has been used from ancient times and it was based mainly on natural meat microorganisms. Recently, researchers begun to develop starter cultures for meat products, in order to ensure standard quality for the fermentation process [87]. In 1995 the first LAB meat starter culture used by Niven et al. [96] in the USA was a pure culture of *Pediococcus cerevisiae*. Essential requirements of meat LAB starter cultures are the immediate and rapid production of organic acids at the start of the fermentation, which will result in a pH below 5.1 [97]. Therefore, the original characteristics of the foods are changed, resulting in enhancement of the final products [98].

As commercial meat LAB starter cultures the species more used belong to the *Lactobacillus* and *Pediococcus* strains, which can be isolated from dry sausages [97], sauerkraut [20], or smoked salmon [99]. Strains of the above LAB were found to have the best survival activity under acidic conditions and high levels of bile salts [98]. The role of the starter culture as aforementioned is for the safety of foods by inactivating pathogens and spoilage microorganisms via the acid and bacteriocin production. Therefore, the production of biogenic amines is inhibited and microbial growth is suppressed, without the use of antibiotics [97].

Several studies have reported that LAB from meat and meat products can have antibiotic resistance [100]. Thus, before using novel starter cultures or probiotic cultures it is important to check that they do not contain transferable resistance genes [97]. In addition, the selection of LAB starter cultures for sausage production must not have amino decarboxylase activity.
Otherwise, biogenic amines will be produced in foods, such as histamine, tryptamine, tyramine, cadaverine, putrescine and phenylethylamine which have toxic effects [101].

3.4.2. Fermented vegetables

LAB fermentation of vegetables can be achieved due to the presence of carbohydrates. Usually fermented vegetable juices are produced from cabbage, red beet, carrot, celery and tomato [18,102]. Also, LAB play an important role in pickles and table olives fermentation, affecting the final flavour and shelf-life [103,104].

3.4.3. Starter cultures in silages

Ensiling is a traditional method of preserving forages and is widely used all over the world. It is based on natural fermentation, where LAB ferment water-soluble carbohydrates into organic acids, mainly lactic acid or acetic and formic acids, under anaerobic conditions. Inoculation of LAB is often used as silage additive to enhance lactic acid fermentation [18]. This results in decreasing pH, inhibiting detrimental anaerobes and preserving the nutritional value and palatability of the forage [105,106].

Among the LAB genera frequently used are *Lactobacillus plantarum*, *Enterococcus faecium*, *Pediococcus acidilactici*, *Pediococcus pentoseceus* and *Lactobacillus acidophilus*, with usual rates $10^5$-$10^6$ viable cells / g [107]. Feeding ruminants with silages that have been treated with LAB beyond improving their performance, it is believed to induce probiotic effects [108].

4. Lactic acid bacteria as source of antimicrobial agents

LAB derived probiotic bacteria display a wide range of antimicrobial activities. Some strains of LAB produce non specific antimicrobial substances (short chain fatty acids, hydrogen peroxide) while others produce toxins (bacteriosins, bacteriosin-like components) [109]. Short chain fatty acids (formic, acetic, propionic, butyric and lactic acids) which are produced during the anaerobic metabolism of carbohydrates, decrease the pH. It has been considered that these acids are responsible for the domination of mucosal ecosystems by LAB [110]. Also, hydrogen peroxide inhibits the growth of pathogens [111].

4.1. Bacteriocins

Most of bacteriocins originating by Gram positive bacteria are produced from LAB. They are proteins that have bacteriocidal activity against species closely related to the bacteriocin producing strains, which could be applied in food preservation and health care [112,113]. Traditionally bacteriocin production has been considered an important characteristic in the selection of probiotic strains, while nowadays it is considered that they may function within the gastrointestinal tract [114], perhaps as alternatives to antibiotics for medical and veterinary use [115]. Generally bacteriocins are cationic peptides which display hydrophobic or amphilitic properties and usually the bacterial membrane is the target for their action [116].
The majority of bacteriocin produced by LAB are distinguished from classical antibiotics because: a) They are ribosomally synthesized and have a relatively narrow killing spectrum. b) They can be divided into two main groups, produced by Gram-negative and Gram-positive bacteria [117,118].

Bacteriocins according to their structure and characteristics can be classified mainly in the following classes:

- Class I (lantibiotics), small peptides [119].
- Class II, small heat-stable proteins which are further divided into subclasses such as IIa (pediocin-like bacteriocins) and IIb (two peptide bacteriocins) [119].
- Class III (helveticin) [120].

Bacteriocins mainly produced by *Lactobacillus acidophilus* have strong antimicrobial capacity against various food pathogens [113]. Bacteriocins can act as bactericidal or bacteriostatic, a distinction which is strongly dependent on bacteriocin dose and degree of purification, physiological state of the indicator cells and experimental conditions such as incubation temperature, pH, presence of agents disrupting cell wall integrity, etc [121,122].

### 4.2. Traits of LAB derived bacteriocins

LAB derived bacteriocins are suitable to use as food preservatives due to their characteristics: a) protein nature – they are inactivated by proteases in the gastrointestinal tract. b) Non-toxic and generally non-immunogenic. c) Thermoresistant thus the antimicrobial activity remains after pasteurization and sterilization. d) Affect most of the Gram-positive bacteria. e) Genetic determinants generally located in plasmid facilitating genetic manipulation to increase the variety of natural peptides. f) Usually act on the bacterial cytoplasmic membrane having no cross resistance with antibiotics [122,123].

Some benefits of the use of bacteriocins as food preservatives are: a) extended shelf-life of foods. b) reduction of the risk of transmission of food born pathogenic bacteria. c) Amelioration of economic losses due to food spoilage. d) No addition of chemical preservatives. e) Decrease of the intensity of heat treatments resulting in better preservation of food nutrients and sensory properties of the food. f) Marketing of “novel” foods, less acidic, less salty and with higher water content [123].

Nicin (lantibiotic – class I) is the first bacteriosin produced by LAB (*Lactococcus lactis*) whilst today it is used in many countries as biopreservatives in foods [109]. Nicins have a dual mode of action: a) Binding to lipid II thus preventing correct cell wall synthesis and b) employing lipid II as a docking molecule to initiate a process of membrane insertion and pore formation which leads to rapid cell death [109]. Nisin-producing bacteria can be found in about 30% of human milk samples. This substance may protect mothers from mastitis and infants from toxification by pathogenic skin flora like *Staphylococcus aureus* [124]. Except from nicin, currently pediocin PA-1/AcH from several Pediococcus strains and enterocin AS-48 from *Enterococcus faecalis* are used as biopreservatives [119].
There are at least three ways in which bacteriocins can be incorporated into a food to ameliorate its safety: a) By using a purified or semi-purified bacteriocin preparation as food ingredient. b) By introducing an ingredient that has earlier been fermented with a bacteriocin producing strain. c) By using a bacteriocin-producing culture in fermented products to produce the bacteriocin in situ [125].

Additionally, bacteriocin production can contribute to the probiotic functionality of intestinal LAB, while in certain cases may be directly responsible for it, with respect to either beneficially modulating the gut microbiota or inhibiting some gastrointestinal pathogenic bacteria [30]. Consequently bacteriocins, derived from LAB can cover a broad field of applications, including the food industry and the medical sector, mainly in combination with other treatments to increase their effectiveness in humans and animals [126]. In the latter, bacteriocins can be used as growth promoters, instead of antibiotics, which have been banned in the European Union since 2006 [127].

5. Lactic acid bacteria as source of vitamins

Human life cannot exist without vitamins, because they are involved in essential functions e.g. cell metabolism and antioxidant activities. Humans cannot synthesize most of these vitamins, although it is well known that some intestinal bacteria like LAB can produce some vitamins (folate, vitamin B12 or cobalamin, vitamin K2 or menaguino, riboflavin and thiamine) [128,129]. The gut microbiota has been recognized as a source of some water-soluble vitamins, while such vitamins have also been reported as results of the LAB fermentation in yogurt, cheeses and other fermented foods.

5.1. Folate

Folate is the term used to describe the folic acid derivatives, such as the foly glutamates which are naturally present in foods and folic acid that is the chemically synthesized form of folate, commonly used for food fortification and nutritional supplements. Folate belongs to the B-group of vitamins and participates in many metabolic pathways like the biosynthesis of DNA and RNA and the inter-conversions of amino acids. Moreover, folate possesses antioxidant capacity that protects the genome by preventing free radical hack of DNA [130].

Dietary folate is essential for humans, since it cannot be synthesized by mammalian cells. Folate can be found in legumes, leafy greens, some fruits and vegetables, in liver and fermented dairy products [131], especially in yogurts, where it may be increased depending on the starter cultures used and the storage condition, to values above 200 μg / Lt [132]. Epidemiological studies indicated that folate deficiency is associated with a variety of disorders like Alzheimer’s disease, coronary heart diseases, osteoporosis and increased risk of breast and colorectal cancer [130,133].

LAB having the ability to produce folate belong to the Lactobacillus spp (L. lactis, L. plantarum, L. bulgaricus), Streptococcus spp. and Enterococcus spp. Nevertheless, some lactobacilli strains (L. gasseri, L. salivarius, L. acidophilus and L. johnsonii) used as both starter cultures and
probiotics, cannot synthesize folate due to their lacking in some genes involved in folate biosynthesis [130]. Furthermore, it has been reported that some starter cultures and probiotic lactobacillus strains in non-dairy foods utilize more folate than they produce [128,130]. For this reason nowadays the food industry focuses on the strategy to select and use folate producing probiotic strains, to produce fermented products with elevated amounts of “natural” folate concentrations, without increasing production cost, although increasing health benefits [130,133].

5.2. Vitamin B12
Vitamin B12 or cobalamin is required for the metabolism of fatty acids, amino acids, nucleic acids and carbohydrates [134]. Vitamin B12 cannot be synthesized by mammals and must be obtained from exogenous sources like foods or the intestinal microbiota [128]. It has been reported that among the microorganisms some members of the \textit{Lactobacillus} spp have the ability to produce this vitamin. In particular a probiotic strain of \textit{L. reuteri} which exhibits hypocholesterolaemic activity in animals can produce B12 [135].

Vitamin B12 deficiency can cause various pathological disorders that affect the haematopoietic (pernicious anaemia), nervous and cardiovascular system. Furthermore, this deficiency in male animal models influenced the number of offspring which showed growth retardation and decrease in some blood parameters [136].

5.3. Vitamin K
Vitamin K is involved in blood clotting, tissue calcification, atherosclerotic plaque and bones and kidneys function [137]. Vitamin K is present as phylloquinone (Vitamin K\textsubscript{1}) in green plants and as menaquinone (K\textsubscript{2}) produced by some intestinal bacteria, like LAB and especially strains of the genera \textit{Lactococcus}, \textit{Lactobacillus}, \textit{Enterococcus}, \textit{Leuconostoc} and \textit{Streptococcus} [128]. Vitamin K deficiency has been involved in some clinical disorders like intracranial hemorrhage in newborn infants and possible bone fracture resulting from osteoporosis [129]. LAB producing menoquinone could be useful to supplement vitamin K requirements in humans [138].

5.4. Riboflavin
Riboflavin or vitamin B\textsubscript{2} is necessary in cellular metabolism, being the precursor of coenzymes acting as hydrogen carriers in biological redox reactions [129]. Although, riboflavin is present in many foods such as dairy products, meat, eggs, green vegetables, its deficiency occurs with damages in the liver, skin and changes in the brain glucose metabolism [128,129], with symptoms like hyperaemia, sore throat, edema of oral and mucous membranes, cheilosis and glossitis [139].

Currently, riboflavin-producing LAB strains were isolated and used as a convenient biotechnological application for the preparation of bread (fermented sourdough) and pasta to enrich them with vitamin B\textsubscript{2} [140].
6. Lactic acid bacteria as source of enzymes

LAB possess an extensive collection of enzymes many of which have the potential to influence the composition and the processing, organoleptic properties and quality of foods and feeds. LAB release various enzymes into the gastrointestinal tract and exert potential synergistic effects on digestion and alleviate symptoms of intestinal malabsorption [141]. In other cases these organisms may serve as a source for the preparation of enzyme extracts that are able to function under the environmental conditions of fermentation [142]. The enzymatic activity has been studied mainly in LAB isolated from wine or other fermented foods like cheeses and yoghurt [143,144]. Species of Lactococcus and Pediococcus are the LAB most commonly associated with fermented foods [143]. The LAB produced enzymes and in particular amylases which are the most stable can be used in sourdough technology for the natural improvement of bread texture [145]. Moreover, LAB contribute to the aroma and flavor of fermented foods. Certain peptidases produced by Lactococcus lactis subsp. cremoris improved the sensory quality of cheese [146]. In addition, proteolysis and lipolysis may enhance the flavour of most varieties of cheese [147]. LAB strains isolated from a traditional Spanish Genestoso cheese were evaluated for the enzymatic activity and it was reported that dipeptidase activity of high level was found for Lactococcus spp, enterolytic activity was detected for Enterococcus spp., while carboxypeptidase activity was very low or undetectable [147].

Also, enzymes play an important role in winemaking. Wine flavor and aroma apart from aromas originating in grapes and alcoholic fermentation, is derived mainly from the activity of the LAB, through the action of their enzymes. These bacteria grow in wine during malolactic fermentation, following alcoholic fermentation, while a broad range of secondary modifications improve the taste and flavor of wine [144].

7. Lactic acid bacteria as source of exopolysaccharides

7.1. Definition and classification of exopolysaccharides

A number of LAB can produce a variety of long chain sugar polymers, called exopolysaccharides (EPS) which are mainly employed for the production of fermented dairy products. They are synthesized either extracellularly from sucrose by glycanascrases or intracellularly by glycosyltransferases from sugar nucleotide precursors [148]. These EPS can be classified according to their chemical composition and biosynthesis mechanism as homopolysaccharides, consisting of a single type of monosaccharide and heteropolysaccharides consisting of repeating units of two or more types of monosaccharides, substituted monosaccharides and other units like phosphate, acetyl and glycerol [149-151].

Homopolysaccharides are further divided into fructans including levan and inuline-type and glucans including dextran, mutan, alteran and b-1, 3 glucan [152]. On the other hand, heteropolysaccharides demonstrate little structural similarity to one another. Their production is influenced by the bacterial growth, phase, medium composition (carbon and
nitrogen source), pH and temperature [153]. They can be produced by Lactococcus spp. and Lactobacillus spp. and they play a crucial role in the food industry [151]. Homopolysaccharides can be introduced in sourdough products, influencing the structural quality and backing ability in bakery products, while heteropolysaccharides are used as food additives in dairy products [154]. EPS contribute to the organoleptic quality of the fermented foods, in texture, taste perception, mouth-feel and stability [153,155]. The above researchers reported that there is no information about the effects of bacterial EPS in non-dairy foods, such as meat products, sauerkraut and vinegar. Although EPS are tasteless, they prolong the time that the milk product spends in the mouth, enhancing its delicacy through an improved volatilization of the intrinsic flavors [153,155].

7.2. Applications of exopolysaccharides in the industry

In the last years, EPS derived from LAB have received increasing interest because of their GRAS status and their properties. EPS can improve the rheology of fermented foods (viscosity and elasticity) as natural biothickeners, emulsifiers, gelling agents and physical stabilizers to bind water and limit syneresis [153,156]. In particular commercial products like LAB dextran could be utilized apart from foods in gel filtration products, in the pharmaceutical industry, as blood volume expander and flow improver, in chemistry as paper and metal plating processes, in enhanced oil recovery and in chromographic media. Furthermore, levan can find use in the food industry as biothickener, while alteran as low-viscosity factor, extender, etc [128,151,157]. Additionally, EPS may produce oligosaccharides having prebiotic properties that could find important applications in functional foods [158]. The successful application of EPS in the manufacture of fermented milks is determined by the ability to bind water, interact with proteins and increase the viscosity of the milk serum phase [159]. Although, many LAB strains are able to produce EPS, their yield is low [149] and their industrial applications for the improvement of the properties of food products are limited [155].

7.3. Potential health benefits of exopolysaccharides

Apart from the technological benefits some EPS derived from LAB are claimed to have beneficial physiological effects on consumer’s health. These benefits are detectable at very low concentrations [153]. The EPS due to their increased viscosity in foods may remain for longer time in the gastrointestinal tract and therefore be beneficial to the transient colonization by probiotic bacteria [153,160]. Another health benefit is the generation of short chain fatty acids by colonic microflora degradation in the gut. Several of these fatty acids are possibly involved in the prevention of colon cancer [153,159]. In addition, LAB synthesized EPS appear to have anti-tumor, anti-ulcer, immuno-modulating and cholesterol-lowering activity [155].

7.4. Factors limiting the use of exopolysaccharides

The EPS used in the industry represent only a small fraction of the biopolymers used. The reasons are their economical production which needs a global knowledge of their
biosynthesis and an adapted bioprocess technology [151]. Moreover, large scale production of LAB derived EPS is low, since LAB being anaerobes, are relatively inefficient in converting energy from carbohydrates, compared to aerobes [149,161]. So this technological barrier must be overcome for cost effective production of EPS. Furthermore, the genetic instability of EPS production is a problem to industrial applications, resulting in loss or reduction of production or change in the composition of EPS [162].

Increasing the knowledge on EPS structure may lead to the production of the “designer” EPS, including the modification primary in structure by altering their physical properties, their function and their production levels. However, for such production of EPS legal approval and the acceptance by the consumers and the food industries are required [151,163]. However, if these biomolecules are to be developed commercially, they must be cost effective.

7.5. Negative effects of exopolysaccharides

In some circumstances EPS cause food spoilage. For instance during the fermentation of wine or cider the final products receive undesirable properties. The EPS synthesis is responsible for dental plaque that results in dental caries. Moreover, the accumulation of EPS cause many technical and hygienic problems in the cheese and milk industries [156,164].

8. Lactic acid bacteria as source of low-calorie sweeteners

Recently, low calorie sugars produced from LAB have attracted the interest of researchers, industries and consumers, since they can find application as vital food ingredients mainly in foods marketed as “diabetic foods”, like sugar-free candies, cookies and chewing gums [165,166]. Manitol, sorbitol, xylitol, tagatose and thehalose are sweeteners produced by LAB. These substances are polyols, i.e. sugar alcohols and can be produced in food fermentation processes. They can be incorporated directly to foods or be produced in the food by LAB, leading to the production of foods containing such sweeteners [165]. Leuconostoc and Lactobacillus spp. seem to be the most promising producers of these sweeteners [167,168].

A number of health benefits have been attributed to these LAB produced low calories sweeteners, like low glycemic index, osmotic diuretics, weight control, antiplaque, prebiotic. These GRAS substances could be used especially by children, diabetic patients and weight watchers [165,166].

9. Conclusion

Lactic acid bacteria are very promising sources for novel products and applications, especially those that can satisfy the increasing consumer’s demands for natural products and functional foods. They can be used in the diet of humans and animals, with particular role in their health status. Despite recent advances, the study of LAB and their functional ingredients is still an emerging field of research that has yet to realize its full potential.
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10. References


