

# Evaluation of Technological and Probiotic Abilities of Local Lactic Acid Bacteria

Lesly Samedi, Albert Linton Charles \*

Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology,  
1 Shuefu Road, Neipu, Pingtung 912 01, Taiwan

\*Corresponding author: [alcharles@mail.npust.edu.tw](mailto:alcharles@mail.npust.edu.tw)

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**Abstract** The definition of probiotics has been the topic of much discussion and the most recent definition from FAO/WHO stipulates that probiotics are “live microorganisms, administered in adequate amounts, confer a health benefit on the host”. Dairy products remain one of the most important sources of lactic acid bacteria. Today, these kinds of bacteria are of increasing interest as they are considered functional foods when combined with lactic acid bacteria. The identification and classification of isolates made difficult the research, since the benefits should only be pertinent to specific isolates. However, bacteria strains have a certain number of potential and well-established benefits. They may play a role in preventing and treating diarrhea and act on the immune system, improve lactose digestion, help the body to resist, and fight infection. Further researches need to be conducted to confirm the roles that lactic acid bacteria may play in antitumor effects, hyper cholesterol effects, preventing urogenital infections, alleviating constipation, and treating food allergies. In addition to food shelf-life and safety, consumers are showing interest in the relationship and bioactive roles of “functional foods” in preventing or managing non-transmissible chronic diseases. Since then, increased demand for non-dairy probiotic products has come from vegetarianism, milk cholesterol content, and lactose intolerance. Therefore, the establishment of the probiotic functional characteristics of isolated strains must be a key factor in the search for probiotic microorganisms and their inclusion in the food product design. This review presents a basic overview of the evaluation of technological and probiotic abilities of lactic acid bacteria strains and the determination of their probiotic properties.

**Keywords:** *lactic acid bacteria, microflora, non-dairy products, anti-oxidative properties, lactobacillus, probiotics*

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## 1. Introduction

The great availability of antibiotics in the 1950s brought about their widespread use as therapeutic agents and growth stimulators for farm animals. However, long use of probiotics applied in small doses to animal feed has resulted in increased bacterial resistance and the number of resistant bacterial populations thus making probiotic therapy difficult in both humans and animals [1,2]. Since 1969, the antibiotic supplementation also grows; although, promoters have a constant limit to those not involved in the treatment of diseases. Regardless of the purpose of their use and the way in which they are administered, antibiotics persist in the body after treatment, cause harmful side effects and diffuse into the muscles, milk and eggs [3,4]. For health reasons and technology, the presence of antibiotics in food is not allowed; therefore, we must look for some alternatives. Furthermore, probiotics have been applied by some breeders in preference to antibiotics and have received great research interest in animal health.

## 2. Definition and Composition of Probiotics

Among the many definitions formulated during the last decades, the following emerged: probiotics are preparations containing microorganisms and their metabolites, used as food additives, and which affect the host organism in a beneficial way [5,6,7]. In most cases, probiotics include microorganisms that will be able to grow and operate in the intestinal tract of the host animal. Abbassiliasi [8] reported several types of bacteria that are conducive to the production of probiotics. Most probiotics include strains of lactobacilli and (or) streptococci, and sporadically, Bifidobacterium. Some bacterial strains may be used as single or in synergy; the latter are more advantageous, since they have the possibility of a broad spectrum of antagonistic action against pathogens and also of a greater variety of preparations. In addition, a synergistic composition can act more efficiently than isolated strains. Understanding the mode of bacteria

probiotic action relies on the knowledge of microbial ecology in the intestinal tract [9,10].

The digestive system of the fetus is sterile, but at birth or soon after, it acquires the characteristic microflora of the species through contact with the mother and the environment. Later on, a very complex collection of about  $10^{14}$  cells including about 400 different types of microorganisms, establishes in a stable manner [11,12]. In this complex environment, there are several modes of interaction between different microorganisms as well as between microorganisms and host. As described in some previous works, the microflora of the intestinal tract helps the body to fight against infections. According to these authors [13,14], it takes 10 cells of *Salmonella enteritidis* to kill a mouse without germs while a conventional mouse can be killed with no less than  $10^7$  cells.

For a long time, dairy fermentation has had the reputation of possessing probiotic properties; but, for two decades of studies, lactic acid bacteria concerned the properties of the microorganisms in question. The lactic acid bacteria in human and animal guts are introduced via the fermented dairy products, food and nutritional supplements containing viable bacteria. These bacteria constitute an integral part of the gastrointestinal microecology of the organism in health and are involved in the metabolism from the host.

Once the intestinal microflora is stabilized, it can be affected by both dietary factors and the environment, the most important of which is excessive hygiene, antibiotic therapy, and stress. In newborns, an excessive application of disinfectants may prevent the digestive tract from acquiring a microflora protective. In addition, oral antibiotic treatments remove this microflora even after its acquisition. The antibiotics, by destroying the protective microflora, permit the survival of drug-resistant pathogens [15,16,17]. The effects of such therapy, resulted by diarrhea, colitis and mucosa that can be reduced by the administration of preparations containing cells *Lactobacilli* and *Bifidobacteria*. However, antibiotics may also facilitate the colonization of the intestine by the probiotic bacteria in destroying the competitive microflora. Physical stress, emotional and dietetic factors usually lead to a reduction in the number of *Lactobacilli* and the growth of coliforms. The use of probiotics is valid whenever the balance of the intestinal microflora is broken, the organism itself being unable to return to normal. One of the most important motivating factors for the use of microorganisms as probiotics is that they allow to consider the replacement of antibiotics in animal nutrition. However, unlike the latter probiotics do not kill other bacteria but compete with them, inhibiting their growth, and ultimately leading to the restoration of biological balance [18,19,20].

The antagonistic activity of lactic acid bacteria is a fundamental probiotic property. It results in an inhibition of the growth of several kinds of harmful bacteria; and in some cases, by a bactericidal effect of recent reports it's revealed that lactic bacteria are able to inhibit growth or totally inactivate the following pathogens: *Salmonella ssp.*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Shigella spp.*, *Campylobacter spp.* and among the saprophytes, species of *Pseudomonas*, *Bacillus* and *Serratia* [21,22,23]. However, although the results of some

research have shown the broad spectrum of the bactericidal action of these bacteria, the proportion of highly active strains is very low and constitutes about 2%. It's found a protective action against salmonella in 48 different bacterial species isolated from faeces (among them species of *Lactobacillus* and *Streptococcus*) [24,25,26].

The bactericidal activity of lactic acid bacteria against other microorganisms living in the same environment is a complex and still poorly understood process. It is undoubtedly due to the competitive use of substrates and growth factors, a modification of the medium by changes in redox potential (Rh) and pH, the accumulation of primary and secondary metabolites, such as hydrogen peroxide, bacteriocins, and activation of the lacto peroxidase system. According to Lindgren and Dobrogosz [27], bacteriocin reuterin is an antimicrobial agent with a broad spectrum of effective action against gram-negative and gram-positive bacteria, molds, yeasts, and protozoa. Nisin and diplococcin are bacteriocins with a specific action (narrow spectrum); other bacteriocins are quoted in this article [27,28]. The lactic bacteria that colonize the digestive tract play an important role in the release of bile acids which, by their highly inhibitory action, lead to the regulation of the composition of bacterial populations in this digestive tract. Due to their antibacterial activity, specific cultures of lactic bacteria can protect against infections the food intended for animals and because of this, the same animals and human beings. One of the serious infections that can have serious consequences about people and animals is the salmonellosis. The number of human salmonella infections is high and remains at high levels. In 1991, salmonellosis was responsible for 2.2 million cases of food poisoning in Canada, costing taxpayers \$ 1.2 billion. Already in 1988, this cost reached 1.4 billion dollars in the USA. This cost is constantly increasing because of the increase in number of salmonellosis cases worldwide [29,30,31,32].

The ability of bacterial strains to colonize the tube digestive is derived from properties such as the adhesion, the action of bile and acids, their anaerobic character, and the possibility of competitive use of components nutritious. According to some authors, the competition for the adhesion on the intestinal wall is a significant mechanism of cell protection against infection; therefore, it plays an essential role for the reconstruction and regulation of the equilibrium between microbial populations in the intestinal tract. Several publications have revealed the elimination of negative effects of antibiotic therapy by simultaneous or subsequent administration of probiotic cultures of *Lactobacillus* and *Bifidobacterium* species. Beneficial effects of the colonization of the intestine and the prevention of infections with pathogenic serotypes of *E. coli* in experimental animals to newborns and piglets were obtained by applying *Bifidobacterium sp.* and *Lactobacillus salivarius* [33,34,35]. A strict relationship between lactic bacteria and intestinal walls was shown in poultry and piglets. A diversification of the adhesive properties have been shown according to the strains of the same species and the influence of the medium and the culture parameters on these properties. According to Fuller, membership is also a specific property to the host. Some organisms used as probiotics, capable to survive

and grow in the intestine can control undesirable microorganisms throughout host growth [36].

### 3. Applications of Probiotics

The various products marketed as human or animal probiotics consist of either a single microorganism (so-called single-layer products) or a combination of several species (so-called multi-layer products). Today, probiotic products are marketed in three forms [37,38]:

- as a concentrate of cultures added to foods and beverages made from dairy products, fruits, and cereals;
- as an ingredient added to a food based milk or soy and to which it is possible to reach a high concentration by fermentation;
- as dried, concentrated, powdered, capsule or tablet cells.

Probiotics are usually associated with fermented dairy products. The probiotic products include cheeses, ice creams, and frozen yogurts as well as non-dairy foods and beverages. The survival of probiotics in products is affected by several factors during processing and storage processes [39,40]. New technologies, such as microencapsulation and immobilized cell technology, provide additional protection for probiotic organisms and new ways to include probiotics in food products. Moreover, manufacturers market new probiotic delivery vectors such as straws and bottle caps which, when pierced or broken, deliver doses therapeutics of probiotics in a food product [41,42].

#### 3.1. Stimulating the Growth of Farm Animals

The application of probiotics in animals meets the ultimate goal of increased livestock growth and performance. By their composition, probiotics act doubly in the attainment of this objective. By colonizing the gastrointestinal tract of the host, probiotic bacteria inhibit the growth of pathogens and, through nutrient supply, support the host organism. Indeed, certain strains of lactic bacteria used for the production of human and animal food have not only a beneficial effect on the hygienic quality of the product and the duration of storage, by inhibiting the development of harmful microflora; but, they also increase their nutritional values. They enrich this food with vitamin especially those of complex B, participate in the pre-digestion of proteins and lipids, hydrolyze and metabolize lactose, and degrade carcinogenic substances (nitrosamines) and anti-nutrients present in agricultural products. It has been shown that animal feed, fermented by well-selected lactic bacteria as well as yogurt containing living bacteria, stimulate growth and more efficient use of this food type [43]. Improving growth could be linked to the appearance of a protein fraction such as lysine, tryptophan, and methionine during the metabolism of lactic bacteria. It's argued that this yield is mainly due to the release by some strains of lactic acid bacteria. Other authors attribute this phenomenon to the biosynthesis of vitamins of group B, particular folic acid. Probiotics can be used as growth promoters in place of antibiotics and other dietary supplements. A significant statistical increase in chicken growth and egg production

was shown by Dilworth and Miles when adding probiotics to the diet [44,45]. Other researchers who have used lactobacilli in the feed of young pigs have achieved an increase in daily weight gain and better use of the mullet. The increase in weight gain was 2.5% for the use of Lactobacilli biomass and 8.6% for piglets receiving fermented milk with this type of bacteria. It's observed an increase in probiotic action on growth of piglets with supplementation with proteins hydrolyzed lacto-serum. A significant factor affecting the efficacy of the probiotic action is the number of live probiotic cells. A strong dietary influence on the population of lactobacillus species was demonstrated in intestinal contents [46]. The number of living cells was 1-2 orders of higher magnitude when examined individuals were fed a milk-vegetable diet compared to a diet exclusively of vegetables. The stimulation of animal growth is better when the preparation of probiotics contain both a high number of probiotic cells and nutrients that help the growth and action of bacteria in the intestine. In young animals, increased organ growth such as thymus, pancreas and thyroid glands is observed with the use of probiotics. A stimulation of the lymphatic system, blood system, and immune system is also observed resulting in acceleration of growth, higher weight gain, an acceleration of reproductive activity, and an intensification of resistance [47,48].

#### 3.2. Decreased Susceptibility to Stress

Stress is observed, particularly at weaning, when the animal is subjected to dietary changes, relocation, and transportation. The administration of probiotics has reduced the susceptibility to stress and improved the condition of animals; for instance, livestock can be transported without loss of weight. For example, Parkhurst found that viable cells of *Lactobacillus reuteri* reduce the effect of cold stress in young turkeys, which reduces their growth [49,50]. Moreover, viable cells in the presence of a specific substrate are effective to reduce the stress caused by protein deficiency in food. The use of probiotic preparations in the animal production industry reduces stress-related mortality of young animals, especially poultry, pigs, cows, and even fish. The possibility that *Lactobacillus casei* activates the immune system against the risk of rotavirus infection has been cited in reports on antibacterial activity and metabolic enhancement. The possibility that *Lactobacillus casei* activates the immune system against the risk of rotavirus infection. Food poisoning in the human body represents a serious concern because of their impact on public health as well as on the economy, particularly in the animal production sector. Although, chicken meat, and eggs are the main foods involved in salmonella contamination of humans, other types of food products from poultry or livestock are also involved. In addition, other microorganisms such as *Campylobacter*, *Listeria*, *Aeromonas*, *Toxoplasma*, and *Yersinia* species are also responsible pathogens for food poisoning and their proliferation must be better controlled. The site of contamination of poultry products by *Salmonella* is in the digestive tract. Probiotics that are *Salmonella* antagonists and applied by ingestion may eliminate the pathogen where they are found by acting on the first link in the

chain of contamination. This approach of implantation of a new flora presents a certain analogy with the beneficial effect of yogurt in the human being in the establishment of an intestinal flora destroyed by the previous action of antibiotics [51,52,53].

### 3.3. Decreased Level of Cholesterol in the Blood

The cholesterol is present in all animal cell membranes and plays a significant role in regular metabolism. Cholesterol is synthesized by the body according to its needs, and it is essential for the biosynthesis of certain steroid hormones such as adrenaline, sexual hormones, and bile acids. Cholesterol levels in the blood serum is affected considerably by the diet; for example, a high consumption of animal fat in the diet results in a high level of cholesterol in the blood. An excess of cholesterol leads to an atherosclerotic heart disease. Several researchers have shown a significant reduction in blood cholesterol levels after injecting yogurt, milk fermented with *Lactobacillus acidophilus*, and non-fermenting milk supplemented with live *Lactobacillus* spp [54,55,56]. In clinical trials, some reports have shown that the consumption of milk fermented by certain *Lactobacillus* strains leads to a decrease in the level of cholesterol in the blood because of the weakening of its synthesis process. According to Gilliland et al. [57], there is considerable diversity in the assimilation of cholesterol among *Lactobacillus acidophilus* strains. The ability of lactobacilli to degrade bile acids involved in cholesterol synthesis confirms their beneficial role in regulating human and animal metabolism [57,58]. The reduction of cholesterol in the blood can be attributed to (i) the assimilation of cholesterol by lactic bacteria and (or) (ii) the modulation of the ratio of high density lipoproteins and low density lipoproteins (HDL-LDL).

Reports have confirmed that lactic acid bacteria strains use substances localized in the cell walls to stimulate the immune system; however, the mechanisms involved remain unidentified. Recent studies have shown that *Bifidobacterium* sp. introduced into the digestive system stimulates the production of antibodies against food pathogens and allergens and protects the body against penetration into tissues. Animals with a complete intestinal microflora have a higher rate of immunoglobulins than those with a sterile gut [59,60]. *Lactobacillus* and *Enterococcus* cultures reduce the number of *Salmonella typhimurium* cells in the spleen. Some authors observed the ability of *Lactobacilli* to translocate from the intestine and survive for several days in the spleen, liver, and lungs; moreover, lactobacilli reportedly has a protective role against EMC virus. However, not all lactic acid bacteria have been characterized by their ability to stimulate the system immune [61,62]. Based on some studies, only 1 out of 23 strains of *Bifidobacteria* and none of 33 strains of *Streptococcus* showed abilities that newborns acquire *Lactobacillus* and *Bifidobacterium* species from their mothers; and thus, their colonized intestines show the importance of probiotics for the functioning of the immune system [56,63].

Some strains of lactic acid bacteria demonstrate the ability to degrade anti-nutritional factors present in animal

feeds such as phytates and glucosinates. On the other hand, the inhibition of the development of toxinogenic microorganisms prevents the synthesis of toxins. The anti-carcinogenic activity of *Lactobacillus bulgaricus* (*Lactobacillus delbrueckii* ssp. *bulgaricus*) was reported first by Ayebo et al. in 1981 [64]. Since then, publications on the protective role of *Lactobacilli* and *Bifidobacterium* have appeared against cancer. In their latest epidemiological studies, Ashby et al. [65] found that long-term yogurt consumption correlates with a reduced risk of development of colon and breast cancers [65]. These observations confer the anti-carcinogenic activities of the cultures present in the yogurt. Studies on the anti-carcinogenic mechanism of *Lactobacillus* spp. have made three observations: (i) inhibition of tumor cell growth by stimulation of the immune system; (ii) the suppression of enzyme-producing bacteria (such as glucosidase, glucuronidase, azo-reductase) responsible for the release of carcinogens from harmless complexes; and (iii) the destruction of carcinogens (nitrosamines) and the removal of nitro reductases involved in the intestinal tract in the synthesis of nitrosamines from nitrites present in the diet [66,67].

## 4. Criteria for the Selection of Lactic Ferments

### 4.1. Definition of Lactic Ferments

A ferment is a microbial preparation of a large number of cells, a single microorganism or more, added to raw material to produce a fermented food product accelerating and orienting its fermentation process. The lactic acid bacteria group plays an important role in these processes and has a long history of industrial application. Currently, starters or lactic ferments are defined as pure cultures or mixtures of lactic acid bacteria screened and used for the production of fermented products such as yogurts, kefir, and cheeses [68,69].

In the fermentation industry, lactic acid bacteria are very often associated, either with each other or with other microorganisms (non-lactic bacteria, yeasts, or molds) forming mixed cultures where different types of interactions can occur. All of these interactions govern the structure of microbial communities and their activities. They are classified into two categories: (i) positive interactions that are characterized by the stimulation of one or more microorganisms and (ii) the negative interactions that correspond to an inhibition of growth and metabolic activity [70,71].

#### 4.1.1. Positive Interactions

The review differentiates commensalism where one of the partners is stimulated by the production of an essential substance or by the destruction of an inhibitory factor, and mutualism where, in this case, the interaction is beneficial to both partners [72,73].

#### 4.1.2. Negative interactions

There are various mechanisms of inhibition of microorganisms. If the inhibition occurs by the production of inhibiting substances and if only one of the two

microorganisms is inhibited by the other, it is necessary to speak of amensalism. On the other hand, if the mechanisms of inhibition are reciprocal, it is then a phenomenon of competition. This competition may be exercised vis-à-vis the space available (contact inhibition) and / or the availability of substrates. Antagonism refers to a reciprocal struggle between the two populations by the production of generally specific inhibitory molecules [72,74].

The selection of lactic ferments relies on numerous criteria to meet both the specifications requested by the user and the constraints imposed by the producer. These criteria may be related to the technological features of the strains, their performance, and their safety. They differ according to the type of product desired, the characteristics of the raw materials to be processed and the technology applied [75].

#### 4.1.3. Security Criteria

The bacteria that can be produced and used as lactic ferments must not be pathogenic and unable to generate toxic substances. This is the case for most species of lactic acid bacteria, which have GRAS status (Generally Recognized As Safe) with the exception of certain enterococci [76,77].

#### 4.1.4. Technological Skills

##### 4.1.4.1. Acidifying Ability

The acidifying function is the most sought after metabolic property of lactic acid bacteria used in the food industry. It manifests itself in the production of lactic acid from the fermentation of carbohydrates during bacterial growth [78,79]. The physico-chemical and microbiological consequences can be summarized as follows [80]:

- Accumulation of lactic acid participating in the flavor of fermented foods;
- Gradual lowering of the pH of culture media and food matrices;
- Limitation of the risk of development of pathogenic flora and alteration in the final products;
- Destabilization of casein micelles, coagulation of milks and participation in syneresis. For a given ferment, it is necessary to allow a high rate of acidification and / or to reach a predefined final level of acidity. The level of acidity depends on the specifications of the product, which will determine the choice of strains [71].

##### 4.1.4.2. Proteolytic Ability

Growth to cell densities that allow lactic acid bacteria to perform fermentation functions relies on a proteolytic system capable of satisfying all amino acid requirements by hydrolyzing proteins. Lactic acid bacteria demonstrate different potentialities, related to their enzymatic equipment, for the use of the nitrogen fraction. Lactobacilli are generally more proteolytic than Lactococci [71,81,82].

##### 4.1.4.3. Lipolytic Ability

Lipolytic properties are generally low in lactic acid bacteria. Lactococci are considered more lipolytic than

*Streptococcus thermophilus* and lactobacilli. However, they may be of interest for certain cheese applications [75]. In general, the esterase preferentially hydrolyzes the esters formed with short-chain (C2-C8) fatty acids and lipases, which are active on emulsified substrates containing long-chain (> C8) fatty acids. These enzymes are involved in the hydrolysis of mono, di and triglycerides [75,83,84].

##### 4.1.4.4. Flavoring Ability

Lactic acid bacteria are capable of producing numerous aromatic compounds (such as:  $\alpha$ -aceto-lactate, acetaldehyde, diacetyl, acetoin and 2, 3-butanediol, ethanol, and acetate mainly from lactose, citrate, amino acids and fats. This feature is particularly important when making fermented milks, fresh cheeses, creams and butter, whose main aroma is related to this activity microbial [85,86,87].

##### 4.1.4.5. Texturing Ability

The ability of lactic acid bacteria to synthesize exopolysaccharides (EPS) plays an important role in the consistency and rheology of processed products. The *Lb. delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus* producing EPS are used in the manufacture of yogurts, in order to improve the texture, avoid syneresis, and increase viscosity of the finished products. The use of EPS produced by Lc strains. *Lactis ssp. cremoris* is very promising for the structure and viscosity of fermented milk products [69,88].

##### 4.1.4.6. Antimicrobial Activity

Lactic acid bacteria produce a variety of antimicrobial compounds that are used in the fermentation and bio-conservation of food [89]. Organic acids, such as lactic acid, acetic acid or propionic acid, made during the fermentation of carbohydrates, can inhibit yeasts, molds and bacteria. Hydrogen peroxide produced by lactic acid bacteria accumulates in the environment and can inhibit certain microorganisms. Lactobacillus bacteria synthesize carbon dioxide as a secondary metabolite. Its accumulation in the external environment creates anaerobiosis that is toxic to certain aerobic microorganisms in the food. Diacetyl can inhibit the growth of Gram-negative bacteria, yeasts and molds [90,91]. The bacteriocins produced by lactic acid bacteria are antimicrobial substances variable molecular weight. They have an inhibitory activity directed against the bacteria close to the producing strain and their spectrum of action is generally narrow. The best known are: nisin, diplococcin, acidophilin and bulgarican [92,93]. Most bacteriocins produced by lactic acid bacteria share the same mode of action, based on the formation of pores in the membrane of the target bacteria [94,95].

##### 4.1.4.7. Performance

The selection of a lactic ferment must take into account the performance criteria of the bacteria. The bacteria must meet some of the following specificities [96]:

- Resistance to bacteriophage and mechanical treatments;
- Tolerance to growth inhibitors (antibiotics, sodium chloride, sucrose, acidity, ethanol and elevated temperature);
- Ability to freeze or lyophilize and preserve;
- Behavior in the presence of oxygen;

- Growth at suboptimal temperatures;
- Compatibility with other strains;
- Ease of use.

Lactic acid bacteria have quite diverse metabolic activities and ability to adapt to different environments. This diversity is responsible for their wide range of applications on an industrial scale [97]. In the food industry, these microorganisms allow the conversion of a wide variety of raw materials, leading to many products: sausages, fermented milks, cheeses, fermented olives, and some wines. Of these applications, the dairy industry is arguably the largest user of commercial lactic ferments [98,99]. Lactic acid bacteria are also used in the chemical industry (lactic acid production), in the medical field (in particular for the treatment of intestinal dysfunctions), and in the food additives industry (production of exopolysaccharides). They are also used in the production of bacteriocins and therapeutic proteins [69].

## 5. Main Species of Lactic Acid Bacteria with Probiotic Potential

The most frequent and most reported species in the literature are of the genus *Bifidobacterium* and *Lactobacillus*, but we must also mention strains of the genus *Enterococcus* and *Streptococcus* [100,101]. The main species of lactic acid bacteria with probiotic activity are listed in Table 1.

Table 1. The main species of lactic acid bacteria with probiotic activity [81]

<i>Lactobacillus</i> species		
<i>Lb. acidophilus</i>	<i>Lb. gasseri</i>	<i>Lb. paracasei</i>
<i>Lb. amylovorus</i>	<i>Lb. johnsonni</i>	<i>Lb. rhamnosus</i>
<i>Lb. crispatus</i>	<i>Lb. casei</i>	<i>Lb. plantarum</i>
<i>Bifidobacterium</i> species		
<i>Bf. Lactis</i>	<i>Bf. Animalis</i>	<i>Bf. infantis</i>
<i>Bf. longum</i>	<i>Bf. bifidum</i>	<i>Bf. Breve</i>
<i>Bf. adolescentis</i>		
Other lactic acid bacteria		
<i>Lc. Lactis</i>	<i>St. diacetylactis</i>	<i>En. Faecalis</i>
<i>Ln. mesenteroides</i>	<i>St. intermedius</i>	<i>En. Faecium</i>
<i>P. acididilactici</i>	<i>St. thermophilus</i>	

## 6. Properties and Selection Criteria for Probiotic Strains

Probiotics have properties that vary depending on the species or microbial strain. It is necessary to know the genus and species of the strain used because the probiotic effects are specific to the microbial strain. The non-pathogenicity (safety) of strains is a very important criterion, and strains recognized as GRAS (Generally Regarded As Safe) are usually favored. However, the criterion of viability or survival remains essential in the selection of probiotics that must arrive alive at the target site, namely the intestine, and therefore resist the various defense mechanisms of the host. Bacteria are administered orally and face major obstacles to digestive transit including acid pH, bile salts, and pancreatic enzymes

[102,103,104]. Table 2 summarizes the criteria used in different laboratories for the screening of probiotics.

Table 2. Selection criteria used in laboratories for the screening of probiotics [105]

Criteria	Purpose
<ul style="list-style-type: none"> <li>• Resistance to gastric acidity</li> <li>• Resistance to bile salts</li> </ul>	<ul style="list-style-type: none"> <li>• Survival during passage through the stomach and duodenum</li> <li>• Survival during passage through the small intestine</li> <li>• Effective "acid barrier" production in the intestine</li> </ul>
<ul style="list-style-type: none"> <li>• Acid production (from glucose and lactose)</li> <li>• Adhesion to mucus and / or human epithelial cells</li> <li>• Production of Antimicrobial substances</li> <li>• Heat resistance</li> <li>• Good technological properties.</li> </ul>	<ul style="list-style-type: none"> <li>• Effective colonization, reduction of the sites of adhesion of pathogens on the surface</li> <li>• Inhibition of the development of pathogenic germs</li> <li>• Survival during the transformation process</li> <li>• Stability, growth on a large scale, survival in the product, bacteriophage resistance</li> </ul>

### 6.1. Resistance to Gastric Acidity

The survival of bacteria in gastric juice depends on their ability to tolerate low pH conditions. The passage time can be from one hour to four hours depending on the individual and diet. Therefore, some authors suggest that suitable probiotic strains should survive a pH of 2.5 in a culture medium for four hours [106].

### 6.2. Resistance to Bile Salts

In the small intestine, bile salt tolerance is an important factor contributing to the survival of probiotics. Bacteria that survive the acidic conditions of the stomach must then deal with the detergent action of bile salts released in the duodenum after ingestion of fatty meals. Bacteria can reduce the emulsifying effect of bile salts by hydrolyzing them with hydrolases, thus decreasing their solubility [106,107].

### 6.3. Adhesion to Epithelial Cells

The ability to adhere to the intestinal layer is a recommended selection criterion for the choice of probiotics, since it is a condition for colonization of the bowels. Adhesion is the first defense mechanism against the invasion of pathogenic bacteria. It is based on performing a set of *in vitro* and *in vivo* tests using cells of animal and / or human origin [108,109]. In addition to the adhesion to the epithelial cells of the intestine, probiotics can attach to the mucus that covers the enterocytes or to the various microorganisms found in the gastrointestinal tract [110].

### 6.4. Production of Antimicrobial Substances

Lactic acid bacteria synthesize bactericidal/bacteriostatic molecules such as organic acids, hydrogen peroxide, carbon dioxide, diacetyl and bacteriocins. These antimicrobial mechanisms have been exploited to improve the preservation of food [89,111].

## 6.5. Antibiotic Resistance

Lactic acid bacteria are naturally resistant to many antibiotics due to their structure and physiology. Temmerman et al. [112] showed that 68.4% of isolated probiotics are resistant to one or more antibiotics. Strains of lactobacillus were found to be resistant to kanamycin (81%), tetracycline (29.5%), erythromycin (12%) and chloramphenicol (8.5%); whereas, 38% of *Enterococcus faecium* isolates were found to be resistant to Vanomycin [112]. In most cases the resistance is not transmissible; however, it is possible that the plasmid coding for antibiotic resistance is transferred to other species and genus. This is a significant reason for choosing missing strains of potential resistance transfer [113]. European authorities have recently concluded that some bacteria used for food production could pose a risk to human and animal health because of host strains with transmissible resistance genes. Therefore, before initiating a probiotic culture, it is important to verify that the bacterial strains involved do not contain transmissible genes for antibiotic resistance [106].

## 6.6. Technological Criteria

In addition to safety and functional properties, technological criteria are also taken into consideration in the selection of probiotic strains. According to Benmechernene et al., [114], these criteria are:

- good sensory properties;
- phage resistance;
- viability during the technological treatment;
- stability in the product and during storage.

Several beneficial effects on health have been associated with the consumption of probiotics. Table 3 illustrates the diversity of beneficial health effects documented and reported in the literature.

**Table 3. The main beneficial effects attributed to probiotics [117]**

Intestinal effects	Effects on the immune system	Other effects
Control of the following disorders: <ul style="list-style-type: none"> <li>• Bad digestion of lactose</li> <li>• Diarrhea due to rotavirus and diarrhea-associated antibiotics</li> <li>• Irritable bowel syndrome</li> <li>• Constipation</li> <li>• Helicobacter pylori infection</li> <li>• Bacterial overgrowth in the small intestine</li> <li>• Inflammatory chronic diseases of the intestine</li> </ul> Prevention of necrotizing enterocolitis of the newborn.	<ul style="list-style-type: none"> <li>• Immune modulation</li> <li>• Suppression of allergic reactions by reduction of inflammation</li> <li>• Reduced risk of infection with common pathogens (Salmonella, Shigella)</li> </ul>	Reduced risk of: <ul style="list-style-type: none"> <li>• Certain cancers (colorectal, bladder, cervix, breast)</li> <li>• Coronary artery disease:</li> <li>Urinary tract disease</li> <li>• Upper respiratory infection and related infections</li> <li>Reduction of serum cholesterol and blood pressure</li> </ul>

Probiotics can be considered as a mean of transporting the active ingredients they contain (enzymes, wall components, antimicrobial substances) to their target of

action in the digestive tract. The mechanisms of action of probiotics on the host are complex, often multiple and depend on the considered bacterial strain; they act in particular by inhibiting the undesirable bacteria, by neutralizing the toxic products, by improving the alimentary digestibility and by stimulating the immunity; this suggests that it is necessary a direct contact of these probiotics with the various constituents of the intestinal barrier, such as endogenous microflora, intestinal mucus, epithelial cells. They are also a source of vitamins (mainly of group B), and assimilable mineral salts [115,116].

## 7. Perspectives in the Study of Probiotics

Current research is oriented towards possible selection of the most effective strains of probiotic bacteria. That selection can be based on the verification of different strains found in nature; this approach is expensive and requires a lot of effort. In addition, the number of strains that can be tested are limited. It would be more advantageous to use selection systems that retain only those strains with the unwanted characteristics whose definition is essential [118,119]. However, although many studies show the positive or negative effects of probiotics, so far little is known about the selection criteria of the strains. In the field of antagonistic properties of strains against other bacteria, the data is still rare. The growth potential of probiotic strains in human intestines and their ability to colonization of the intestine. Among the strains of the same species, there are large variations in antibacterial effects against pathogens. Adhesion to the mucosa intestinal tract is a specific property of the host and the strains suitable for the preparation of probiotics [20,120]; for example, piglets may be ineffective in the case of other animals. Adhesive abilities also vary between strains of the same species and, while a given strain of *Lactobacillus acidophilus* is an effective probiotic, another may be totally ineffective. Also, if a culture must survive and multiply in the intestinal tract, it must have the ability to grow in the presence of bile and this capacity varies by strains [121,122,123]. Finally, the presence of viable and active organisms at the time of consumption requires a degree of stability in microorganisms during preparation and storage prerequisite for the consumption of probiotics. Therefore, further efforts will be needed to define the mechanisms by which the expected benefits of probiotics are manifested as well as appropriate selection tests. It is unlikely that a single strain will deliver all the benefits; hence, methods must be developed to obtain cultures of many suitable bacteria strains to function in the target host [124,125]. Undoubtedly more information concerning the origin of the activity of the probiotics is necessary, which will sustain research work on the genetic improvement of the strains. It may be possible to combine the survival skill in the intestine with the ability to produce metabolites responsible for probiotic effects. Radical development of genetic engineering could promote such possibilities. Thus McCarthy showed that *Lactobacillus acidophilus* isolated from pigs can be transformed genetically to enable their colonizing the gastric epithelium of mice [124,126]. Teuber using molecular techniques made an attempt to

define the adhesion and antagonistic probiotic properties of bacteria in the intestine [127].

The probiotic LAB uses and their applications have shown a significant increase over the past two decades. Furthermore, the probiotics industry also faces challenges when it claims health benefits. In addition to viability and sensory acceptance, it must be noted that the selection, processing, and inoculation of strains must be taken into account. Indeed, it has been shown that the viability of probiotics throughout the storage period in addition to recovery levels in the gastrointestinal tract are important factors. For this reason, the safe criteria and biochemical properties of the probiotics based on their sources are key of acceptability as GRAS so that the food industry might develop more dairy and non-dairy food model for lactose-intolerant and vegetarian consumers.

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## Conflict of Interest

The authors declare no conflict of interest.

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