



A comprehensive overview of probiotic and antimicrobial attributes of lactic acid bacteria commonly employed in the dairy industry

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Abstract: It is commonly known that lactic acid bacteria (LAB) are employed as inoculants in the dairy sector. Various non-fermented and fermented foodstuffs for instance, alcoholic beverages, fermented vegetables, fermented cereals, fermented fruits, and dairy products, have been prepared by utilizing LAB as probiotics for a long time. Amongst many types of compounds formed by LAB such as H₂O₂, CO₂, lactic acid, and reuterin are promising sources of antimicrobials. These substances thwart the growth of harmful bacteria. Thus, probiotic strains of LAB serve as an alternative safe source of antibiotic and anti-inflammatory medicines. Here, the antimicrobial and probiotic potential of important newly reported genera of LAB and their diversity are compressively discussed in this review article along with the mechanisms of action of LAB antimicrobial compounds and their applications.

Keywords: Lactic Acid Bacteria; LAB; Probiotics; Antimicrobial Activity, industrial applications

1. Introduction

Lactic acid bacteria (LAB) are gram-positive, catalase-negative, coccobacilli or rods, non-flagellated. Their final output is lactic acid. Their presence is most common in various foodstuffs like fermented fruits, fermented vegetables, dairy products, and beverages (Enan et al., 2019). They inhabit numerous ecological niches ranging from the genital tract to the gastrointestinal tract and contribute to the normal microflora of humans and animals, but certain factors affect their distribution such as pH, substrate and oxygen availability, specific availability of substrates including their secretions and interactions (Soccol et al., 2010). The term LAB is not a phyletic class, instead, it represents the metabolic abilities of these bacteria. Their genome size ranges from 1.8 Mb to 3.3 Mb and has low GC content. LAB are being used in many ways including health improvement, the production of enzymes, metabolites, and macromolecules (Pfeiler & Klaenhammer, 2007). LAB produces a diverse range of compounds such as ethanol, H₂O₂, antifungal proteins, diacetyl, CO₂, acetaldehyde, and bacteriocins. They inhibit the growth of pathogenic bacteria through food fermentation using the compounds (Enan et al., 2019). The composition of commensal communal microflora utilized as probiotics belongs to the genera *Bifidobacteria* and *Lactobacillus*, which are also known as LAB. They are acid-tolerant, microaerophilic or facultatively anaerobic, and fermentative bacteria. The effectiveness of LAB as probiotics is due to their capability to survive bile salt and stomach acid. Similar to many other bacteria, LAB presents bacteriostatic or bactericidal attributes. The primary objective of probiotic therapy is to colonize the lower intestine of the host, facilitating the maintenance of gut microflora and eliminating enteric pathogenic bacteria (Jain et al., 2014). The main objective of this study is to determine LAB as probiotics and their antimicrobial abilities, contributing to robust human health and serving as substrates for various segments of the food industry to produce effective food products. In this review article, the antimicrobial and probiotic potential of important newly reported genera of LAB and their diversity are compressively discussed along with the mechanisms of action of LAB antimicrobial compounds and their applications.

2. Classification of LAB

The classification of LAB is generally based on various characteristics such as growth at different temperatures, morphological classification, range of sugar utilization, and the way LAB ferments glucose. LAB are not

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phylogenetically similar but they have several metabolic, physiological, and morphological similarities (Ayivi et al., 2020). This system of classification has consisted of several genera including *Leuconostoc*, *Lactobacillus*, *Streptococcus*, *Pediococcus*, *Lactococcus*, *Carnobacterium*, *Alloiococcus*, *Dolsigranulum*, *Tetragenococcus*, *Oenococcus*, *Weissella*, *Vagococcus* and *Enterococcus* (Mokoena, 2017). Furthermore, three diverse groups of *Lactobacilli* are facultatively hetero-fermentative bacilli, capable of fermenting hexose. Obligately homo-fermentative *lactobacilli*, catabolize the hexose but do not degrade pentose. Obligately hetero-fermentative *lactobacilli* can ferment both hexoses and pentoses (Enan et al., 2019). Moreover, a diverse range of new genera of LAB has been reported in Fig. 1. However, the most prominent probiotic genera among these are *Bifidobacteria* and *Lactobacilli* (Jain et al., 2014).

3. Niche of LAB

LABs are omnipresent and mostly found in various food sources (Enan et al., 2019). *Bifidobacteria* and *Lactobacilli* are beneficial and dominant microorganisms in the colon; they can thrive in the intestine without provoking any side effects. Yeasts and other LAB can also be utilized as probiotics in the form of various foods (Jain et al., 2014).

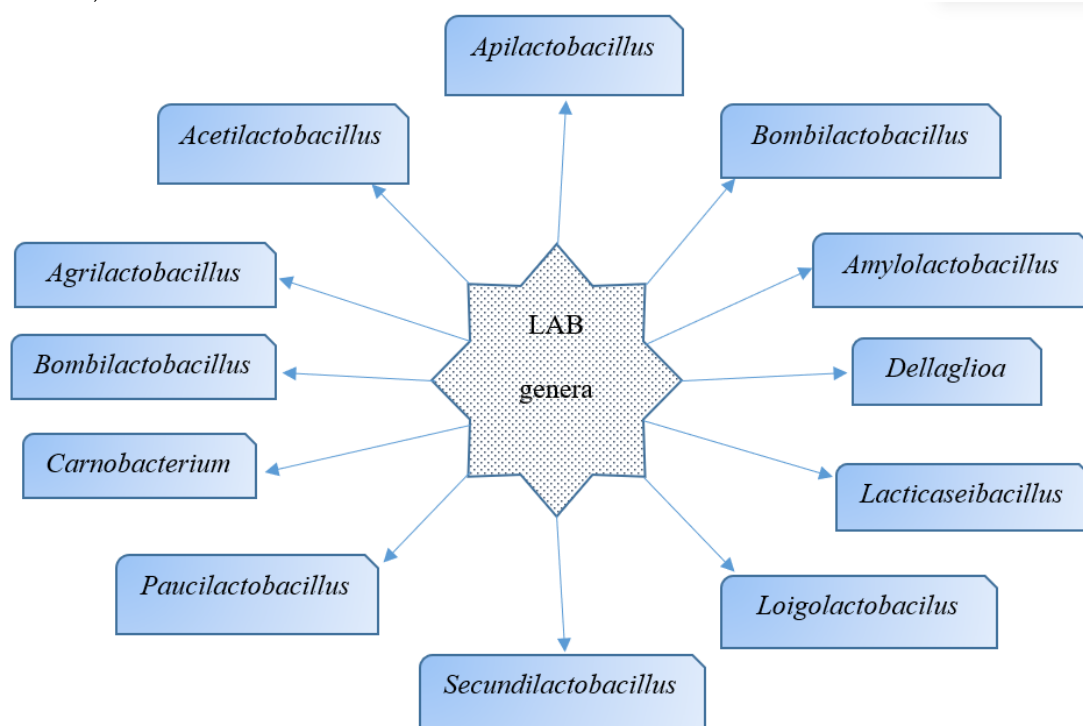


Fig.1. New reported genera of LAB (Ayivi et al., 2020).

4. LAB as probiotics

Probiotics are live microorganisms that contribute to human and animal health by competing with enteric pathogens. Moreover, they increase digestive capacity, reduce intestinal pH, and enhance mucus immunity. Due to these properties, probiotic bacteria create an unfavorable environment for the growth of enteric pathogenic bacteria, thus preventing them from colonizing the intestine (Ozen & Dinleyici, 2015). Lilly and Stillwell were the first to use the term probiotic in 1965, referring to material released by microorganisms that stimulate the growing conditions of other organisms (Lilly & Stillwell, 1965). They reduce the inflammatory responses of the host by strengthening the permeability barrier of the intestine, stabilizing the microbial environment of the gut, degrading antigens, and modifying their immunity and antigenicity. As a result, the gut microflora of the host becomes more resilient and stable to population disruptions (Ozen & Dinleyici, 2015). Probiotics can prevent or treat symptoms of constipation, irritable bowel syndrome, antibiotic-associated and acute diarrhea, diabetes, inflammatory bowel disease, and hypertension (Sanders et al., 2018). Functional foods, containing probiotics, offer various therapeutic advantages, such as anti-hypertension, anticancer, hypoglycemic characteristics, antioxidant effects, and immunomodulatory effects (Vargas-Bello-Pérez et al., 2019). Consequently, there have been significant commercial and medical interests in discovering novel probiotic strains that have positive health effects (Gao et al., 2021). For decades, LAB and *Bifidobacteria* species



have been the probiotics that received the most attention, as shown in Table 1. Moreover, factors such as tolerance to bile and acid conditions, the capacity to hydrolyze bile salt, cholesterol-lowering potential, the presence of antimicrobial properties, the absence of hemolysis, and the capacity to endure fermentation, have been employed to classify new LAB isolates as probiotics (Aspri et al., 2020).

Table 1. Some well-known lactic acid-producing bacteria serve as probiotics for human health (Moradi et al., 2021).

Probiotic microbes	LAB	
Lactobacillus	<i>Lactobacillus acidophilus</i>	
	<i>Lactobacillus paracasei</i>	
	<i>Lactobacillus casei</i>	
	<i>Lactobacillus delbrueckii</i>	
	<i>Lactobacillus johnsonii</i>	
	<i>Lactobacillus rhamnosus</i>	
	<i>Lactobacillus plantarum</i>	
	<i>Lactobacillus sakei</i>	
	<i>Lactobacillus gasseri</i>	
	<i>Lactobacillus bulgaricus</i>	
	<i>Lactobacillus lactis</i>	
	<i>Lactobacillus brevis</i>	
	Bifidobacteria	<i>Bifidobacteria lactis</i>
<i>Bifidobacteria breve</i>		
<i>Bifidobacteria infantis</i>		
<i>Bifidobacteria longum</i>		
<i>Bifidobacteria bifidum</i>		
<i>Bifidobacteria animalis</i>		
<i>Bifidobacteria adolescentis</i>		
Others	<i>Leuconostoc mesenteroides</i>	
	<i>Lactococcus lactis</i>	
	<i>Streptococcus thermophilus</i>	

Exploring the prospective probiotic attributes of LAB obtained from the milk of various animal species has garnered more attention in recent years. Particularly, indigenous camel species have drawn interest as a rare source of LAB with possible health advantages. Indigenous camels have co-evolved with their habitat to establish unique microbial communities that are suited to dry and semi-arid environments. These microbial communities, which include LAB, are essential for the fermentation and storage of camel milk. Furthermore, LAB can be ingested as probiotics to benefit the health of humans and animals. Generally, the probiotic attributes of LAB are the survival in host tract, adherence to the epithelium of the host intestine, and the hindrance of the invasion or growth of injurious microbes, such as *E. coli* and *Salmonella sp.*, in animal intestines. For LAB cells that have survived in bile and gastric conditions, the ability to adhere to host intestinal epithelium can give a competitive advantage and is crucial for bacterial persistence in the GI tract. The competitive exclusion of harmful microorganisms may be influenced by such a characteristic. Additionally, it has been hypothesized that some LAB strains may have additional positive health effects, such as enhancing human immunity (M. Kumar & Ghosh, 2012). The idea that some probiotic bacteria can augment the immune response is supported, for example, by studies using *L. casei* given to mice that revealed, an increase in the activity of natural killer cells from the mesenteric node cells and not from Peyer's patches cells from spleen cells. In mice administered with different probiotic bacteria, spleen cells produce much more gamma interferon and had significantly greater proliferative responses to the mitogens such as concanavalin A which is a T cell mitogen, and lipopolysaccharide which is a B cell mitogen. Some LAB strains of vegetable origin, sourced from plant material and different fermented meals, have been found to play a role in immune boosting, anticancer, and antibacterial properties. (M. Kumar & Ghosh, 2012).

5. Delivery of probiotic strains

To deliver probiotics into the animal and human body, food matrices are employed as carriers. These matrices serve as vehicles to transport probiotics through the gastrointestinal tract, enhancing their colonization. Yogurt and fermented milk stand out as optimal food carriers for delivering probiotic bacteria to both human and animal bodies. To derive maximum benefits from probiotics, it is essential to incorporate them into the daily diet consistently. Occasional consumption may not yield the full spectrum of advantages. Furthermore, the physicochemical characteristics of yogurt and fermented milk position them as the optimal food carrier matrices for delivering probiotic strains into the body, surpassing alternatives like ice cream and cheese carriers (Srinu et al., 2013).

6. Probiotic functional food products

Humans consume probiotics in three different forms: 1) through food items, whether fermented or non-fermented; 2) as culture concentrates introduced to food, available in dry or deep-frozen forms for both industrial and household use; and 3) in the form of nutritional supplements, presented as powdered pill, or tablet products. The most widely accepted method of introducing probiotic flora is through food products (Granato et al., 2010). Foods categorized as functional contain chemical and/or microbiological components that, beyond their nutritional value, can positively influence one or more target bodily processes related to health and well-being or contribute to lowering the risk of disease. Probiotic-containing foods belong to the functional foods group, constituting a significant portion of approximately 60-70% of the entire functional food market. Over the past thirty years, there has been considerable attention on dairy products featuring probiotic bacteria. These products encompass a range of items such as sour cream, ice cream, fermented milk, frozen dairy desserts, cheese, various types of baby food, buttermilk, regular and flavored liquid milk, milk powder, concentrated milk, and whey-based beverages (Granato et al., 2010). Various non-dairy items have also been recently introduced, encompassing morning cereals, baby meals, fruit juice, cereal-based products, vegetarian options, oat-based desserts, confectionery products, and baby foods. A few important LABs that inhabit various dairy and non-dairy fermented foods are listed in Table 2.

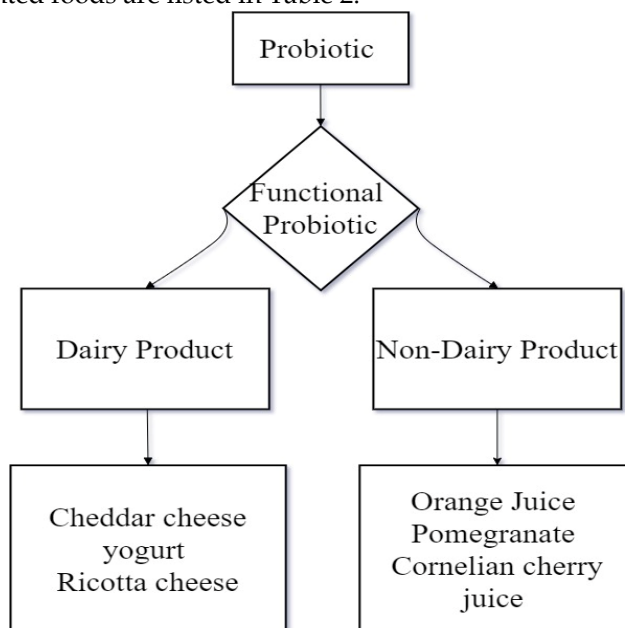


Fig.2. Few important functional probiotic food products (Latif et al., 2023).

Table 2. Fermented food containing LAB.

Fermented products	LAB	References
	Dairy products	
Hard cheeses without eyes	<i>L. lactis subsp. lactis</i> <i>L. lactis subsp. cremoris</i>	(Mathur et al., 2020)
Butter and buttermilk	<i>L. lactis subsp. lactis</i>	



	<i>L. lactis</i> subsp. <i>lactic</i> var. <i>diacetylactis</i>	(Ayivi et al., 2020)
	<i>L. lactis</i> subsp. <i>cremoris</i>	
kefir	<i>Leucmenesteroides</i> subsp. <i>Cremoris</i>	
	<i>Lb. kefir</i>	(Mathur et al., 2020)
	<i>Lb. kefiranofacies</i>	
	<i>Lb. brevis</i>	
Cheeses with small eyes	<i>L. lactis</i> subsp. <i>lactis</i>	(Ayivi et al., 2020)
	<i>L. lactis</i> subsp. <i>lactic</i> var. <i>diacetylactis</i>	
	<i>Leucmenesteroides</i> subsp. <i>cremoris</i>	
Fermented probiotic milk	<i>Lb. casei</i>	(Mathur et al., 2020)
	<i>Lb. acidophilus</i>	
	<i>Lb. rhamnosus</i>	
	<i>Lb. johnson</i>	
	<i>B. lactis</i>	
	<i>B. bifidum</i>	
	<i>B. breve</i>	
Yogurt	<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i>	(Ayivi et al., 2020)
	<i>S. thermophilus</i>	
	Fermented meats	
Fermented sausage (Europe)	<i>Lb. saki</i>	(Parlindungan et al., 2021)
	<i>Lb. curvatus</i>	
	Fermented vegetables	
Sauerkraut	<i>Leuc. mesenteroides</i>	(Bautista et al., 2020)
	<i>Lb. plantarum</i>	
	<i>P. acidilactici</i>	
	<i>P. cerevisiae</i>	
	<i>Leuc. mesenteroides</i>	
	<i>Lb. brevis</i>	
Fermented olives	<i>P. acidilactici</i> ,	(Ashaolu & Reale, 2020)
	<i>P. pentosaceus</i>	
	<i>Lb. Plantarum</i>	
Pickles	<i>Lb. plantarum</i>	(Bautista et al., 2020)
	<i>Leuc. mesenteroides</i>	
	<i>Lb. pentosus</i>	
	<i>Lb. Plantarum</i>	
Fermented vegetables	<i>Lb. fermentum</i>	(Ashaolu & Reale, 2020)
Eggplant, mustard, beets,	<i>Lb. fermentum</i>	(Bautista et al., 2020)
peppers,	<i>Lb. pentosus</i>	
tomatoes, carrots, capers,	<i>Lb. Plantarum</i>	
cabbage	<i>P. pentosaceus</i>	
	<i>Lb. paracasei</i>	
	<i>Lb. pantheris</i>	
	<i>P. acidilactici</i>	
	<i>Lb. curvatus</i>	
	<i>W. consult</i>	
	<i>W. soli</i>	
	<i>Enterococcus faecium</i>	
Leeks	<i>Leuc. mesenteroides</i>	(Ashaolu & Reale, 2020)
	<i>Lb. Plantarum</i>	
	<i>Lb. saki</i>	
Soyabean	<i>Leuconostocs</i>	(Bautista et al., 2020)
	<i>L. lactis</i>	

Sourdough	Fermented cereals	
	<i>Lb. sanfransicensis</i>	(Xu et al., 2020)
	<i>Lb. farciminis</i>	
	<i>Lb. fermentum</i>	
	<i>Lb. brevis</i>	
	<i>Lb. plantarum</i>	
	, <i>Lb. amylovorus</i>	
	<i>Lb. Reuters</i>	
	<i>Lb. points</i>	
	<i>Lb. panis</i>	
	<i>Lb. Alimentarius</i>	
Fermented maize products	<i>W. cibaria</i>	
	<i>Lb. fermentum</i>	(Ashaolu & Reale, 2020)
	<i>Lb. plantarum</i>	
	<i>Lb. delbrueckii subsp. bulgaricus,</i>	
	<i>Lb. helveticus</i>	
Fish product	<i>lactis subsp. cremoris</i>	
	Fermented fish	
	<i>Lb. alimentarius</i>	(Gupta et al., 2021)
Kiwis	<i>Carnobacterium piscicola</i>	
	Fermented fruits	
Cherries	<i>Lb. plantarum</i>	(Li et al., 2021)
	<i>Lb. plantarum</i>	(Szutowska, 2020)
Pineapple	<i>Leuc. mesenteroides subsp.</i>	
	<i>mesenteroides</i>	
	<i>P. pentosaceus</i>	
Papayas	<i>Lb. plantarum</i>	(Li et al., 2021)
	<i>Lb. Russian</i>	
Rice wine	<i>Lb. plantarum</i>	(Szutowska, 2020)
	<i>Lb. pentoses</i>	
	<i>W. cibaria</i>	
	<i>W. confusa</i>	
Wine	Alcohol beverages	
	<i>Lb. sakei</i>	(Song et al., 2022)
	<i>P. acidilactici</i>	
	<i>Oenococcus oeni</i>	(Song et al., 2022)

**B. Bifidobacterium, L. Lactococcus, Lb. Lactobacillus, Leuc. Leuconostoc, P. Pediococcus, S. Streptococcus, W. Weissella*

7. LAB and their antimicrobial activity

LAB produces various antimicrobial substances categorized into two groups: compounds having high molecular weight like bacteriocins, and compounds having low molecular weight such as diacetyl, CO₂, and H₂O₂ (Şanlıbaba & Güçer, 2015). LAB's antimicrobial properties arise from the production of these substances. The acid produced in the environment creates an acidic condition that hinders the growth of pathogens, ensuring the safety and quality of dairy products.

7.1. Lactic acid (LA)

The key organic acid generated as a by-product of the fermentation process carried out by these bacteria is lactic acid, which is the main antibacterial agent. LA is released by the bacteria in both L- and D-isomeric forms. The stereoisomers of lactic acid exhibit diverse potential antimicrobial properties against various microbial agents. The L-lactic acid isomer demonstrates greater inhibitory activity compared to the D-lactic acid (Reis et al., 2012). It has been demonstrated that LAB can produce antimicrobial compounds, which are essential in preventing the growth of harmful bacteria. This antimicrobial activity is one of the many positive impacts that LAB can have on human health (de Souza et al., 2023). It has been demonstrated that LAB strains from camel milk have antibacterial activity against a variety of diseases. Various substances including organic acids particularly acetic acid and lactic acid, as well as hydrogen peroxide (H₂O₂) and bacteriocins such as nisin and related substances, possess this activity. These antimicrobial substances play a vital function in inhibiting bacterial growth and survival, contributing to the maintenance of gut health (Darbandi et al., 2022). Abd Allah et al., (2023), reported the different bacterial genera with enhanced antimicrobial properties, about 50.8% of the isolated bacteria



showed antibacterial activity against the tested pathogens, and genome shuffling of *Lactobacillus plantarum* among potent strains proved the highest antimicrobial activity and the removal of organic acid function also showed the reduced effect of antibacterial activity. In another study, it was reported that these organic acids produced during fermentation, establish an environment with an acidic nature which prevents the growth of infectious bacterial strains (Leroy & De Vuyst, 2004).

7.2. Mechanism of action of lactic acid

Lactic acid, the most abundant, disrupts the internal pH balance and essential metabolic processes of pathogens. Acetic and propionic acids disrupt cell membranes and inhibit ATP synthesis in pathogenic bacteria (Leroy & De Vuyst, 2004). These organic acids play a crucial role in maintaining microbial balance and inhibiting pathogenic growth during fermentation. Lactic acid inhibits various metabolic processes and interferes with active transport, as a result, disturbs cell membrane potential, and ultimately reduces intracellular pH (Reis et al., 2012). Lan & Chen (2023) reported the role of LAB in maintaining the homeostatic vaginal environment to increase the conception rate in infertile women with vaginal microecological disorder, they treated the patients with metronidazole synergistic with LAB capsules and just gave metronidazole to control group. After treatment significant change in pH value and hydrogen peroxide concentration was noticed in the experimental group than the control and the pregnancy rate of about 26% and 96% of the total effect rate was observed in the experimental group than the control. Thus, LAB can lower the vaginal pH, regulate the amount of H₂O₂ and immunological factors, and rectify the imbalance of the vaginal microecological environment, all of which can increase the conception rate of patients. However, the reduction of pH and lactic acid production relies on numerous factors, for instance, the species of bacteria, the nature of the culture, and other growth-stimulating factors. Lactic acid exhibits a broad mode of action, inhibiting the activity of molds, bacteria, and yeasts. Many microorganisms, including bacteria, yeast, and fungi, produce LA in its undissociated form under low pH conditions. Bacteria exhibit varying levels of sensitivity to different types of bacteria. At a pH of 5, lactic acid demonstrates inhibitory activity against spore-forming bacteria but does not affect molds and yeasts. It interacts with arginine-binding proteins to affect arginine utilization, which inhibits the development of many Gram-negative bacteria (Reis et al., 2012).

7.3 Bacteriocins

Bacteriocins are substances produced during the initial stages of bacterial growth that exhibit antimicrobial activity. They are proteins or polypeptides characterized by being cationic, amphiphilic, and heat-stable (Widyastuti et al., 2014). These antimicrobial peptides, generated by (LAB), selectively inhibit closely related bacteria, offering a competitive advantage (Cotter et al., 2013). Nisin, derived from *Lactococcus lactis*, disrupts bacterial cell walls and impacts pathogens such as *S. aureus* and *L. monocytogenes* (Drider et al., 2006). Sivarooban et al., (2008), combined nisin with either a grape seed extract (GSE) or a green tea extract (GTE), this combination harmed specific cells of a strain of *L. monocytogenes*. Lactacin, produced by *Lactococcus lactis* and *Lactobacillus* species, creates pores in bacterial cell membranes, targeting pathogens like *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* sp. (Cotter et al., 2013). Additional bacteriocins, like pediocin, plantaricin, and sakacin, demonstrate efficacy to a variety of bacteria, including *L. monocytogenes*. The varied bacteriocins produced by LAB present potential applications in food preservation and therapeutics. Low-molecular-weight compounds have been observed to inhibit pathogen replication in vitro. Short-chain fatty acids, for example, lactic acid, are at the top of this list (Soomro et al., 2002). *Lactobacilli* also produce high molecular weight bacteriocins (class III) and low molecular weight bacteriocins (LMWB). The LMWB are antimicrobial peptides. Mice exhibit resistance to infection by the hazardous food-borne pathogen *L. monocytogenes*, a phenomenon attributed to the broad activity spectrum of the class II bacteriocin Abp118 formed by *L. salivarius* strain UCC118 (Qiao et al., 2020). Since Abp118-negative mutants were unsuccessful in defending animals, Abp118 is unequivocally identified as the primary mediator of protection. Probiotics, in addition to low molecular weight bacteriocins (LMWB), also produce certain antibiotics. Fernández et al., (2008), applied a 6ug/ml of nisin solution to the memory areola and nipple of eight lactating women who had a symptom of disease (staphylococcal mastitis) caused by *S. aureus*, and they observed that in comparison to the control group, the nisin group's bacterial burden was statistically lower. On day 14 of the experiment, the nisin group did not exhibit any clinical indications of staphylococcal mastitis. This diverse array of bacteriocins offers various health benefits by effectively eliminating hazardous microbes.

Table 3. Bacteriocins are formed by the LAB and their properties.

Bacteriocins	Bacterial strains	Properties	References
Pediocin AcH	<i>Pediococcus acidilactici</i>	Broad spectrum, plasmid-mediated	(Jin et al., 2020)
Helveticin J	<i>Lactobacillus helveticus</i> 482	Narrow spectrum, chromosomally mediated, bactericidal	(Antonio et al., 2021)
Nisin	<i>Lactococcus lactis</i> subsp. <i>lactis</i> ATCC 11454	Bactericidal, Lantibiotic, broad-spectrum, chromosome/plasmid-mediated, formed lately in the growth cycle	(Şanlıbaba & Güçer, 2015)
Carnobacteriocin	<i>Carnobacterium piscicola</i> LV17	Narrow spectrum, produced early in the growth cycle, plasmid-mediated	(Kim et al., 2016)
Leucocin	<i>Leuconostoc gelidum</i> UAL 187	Bacteriostatic, broad-spectrum, produced early in the growth cycle plasmid-mediated	(Pujato et al., 2022)

7.4. Hydrogen peroxide

Hydrogen peroxide is produced by LAB under aerobic conditions, and it has antimicrobial activity. The antimicrobial activity of hydrogen peroxide is attributed to the oxidation of sulfhydryl groups which denature various enzymes and increases membrane permeability. In addition to its direct effects on enzymes and membrane permeability, hydrogen peroxide also serves as a precursor to free radicals, such as OH⁻ and O₂⁻ that harm the DNA of the bacteria (Oelschlaeger, 2010). Hydrogen peroxide damages the DNA in *E. coli* with a lower concentration and higher concentration also reported to cause death in *E. coli* due to damaging effects on not-target sites (Linley et al., 2012). Hydrogen peroxide also serves as a precursor to free radicals that are bactericidal, including hydroxyl, and superoxide radicals that damage the DNA of the bacteria. Hydrogen peroxide has a strong oxidizing effect on the cellular proteins and the membrane lipids, and it is produced by using enzymes such as NADH peroxidase, flavoprotein oxidoreductases, α-glycerophosphate oxidase, and NADH oxidase. This synthesized hydrogen peroxide stops the growth of pathogenic and psychotropic bacteria (Oelschlaeger, 2010). Nakajima et al., (2003), isolated *Lactobacillus lactis* subsp. *lactis* AI 62 and observed the effect of H₂O₂ formed by the aforesaid strain. They determined that the amount of 300-800 ppm of H₂O₂ prevents the growth of psychotropic bacteria present in milk, particularly *Listeria*, *Aeromonas*, and *Yersinia* sp.

7.5 Reuterin

A broad-spectrum antibiotic, effective for yeast, fungi, protozoa, viruses, and bacteria. Lastly, it is crucial to highlight that many probiotics also produce microcins, which are peptides with a limited spectrum of activity. Proteins within the bacteriocin family, with a molecular weight of 420 kDa, exhibit unique antibacterial activity (Widyastuti et al., 2021). For instance, it has been documented that the *Lactobacillus reuteri* strain ATCC55730 produces the antibiotic reuterin (3-hydroxypropionaldehyde) (Meade et al., 2020). Gram-positive bacteria, produce Group III bacteriocins which is another term for these proteins. However, it's noteworthy that Gram-negative bacteria are also capable of producing similar proteins, referred to as bacteriocins. It is possible to demonstrate the production of various bacteriocins with antibacterial effects, showing activity against both Gram-positive and Gram-negative bacteria (Widyastuti et al., 2021). Arqués et al., (2004), reported that reuterin along with different bacteriocins formed by the LAB showed the antibacterial ability to kill food-borne pathogens like *Listeria monocytogenes* in milk. They evaluated the synergistic combination of reuterin with nisin, and enterocin AS-48, and observed the enhanced effect of reuterin in combination with nisin on *S. aureus*.

7.6 De-conjugated bile acids

Bile salts can be converted into de-conjugated bile acids by probiotic bacteria. Comparatively, the antibacterial activity of deconjugated bile acids is higher than that of the host's production. The mechanisms by which probiotic bacteria defend themselves against these "self-made" metabolites, or whether they exhibit resistance to de-conjugated bile acids, remain unclear (Oelschlaeger, 2010).



7.7 Health benefits associated with LAB

Certain strains of *Lactobacillus* probiotics have demonstrated efficacy in promoting digestive health. They contribute to improved digestion and absorption, enhance gut transit time, and alleviate symptoms associated with gastrointestinal disorders. These strains of *Lactobacillus* play a key role in optimizing nutrient absorption and digestion, thereby supporting overall digestive health. Certain strains of *Lactobacillus* can improve the gastrointestinal tract's capacity for nutrition absorption and digestion. These probiotics generate enzymes, including amylases, proteases, and lipases, facilitating the breakdown of complex carbohydrates, proteins, and fats into simpler forms readily absorbable by the body (Hill et al., 2014). LAB has been shown to improve gut transit time, which refers to the time it takes for food to pass through the digestive system. A decrease in gut transit time not only helps prevent constipation but also supports regular bowel movements. Strains of *Lactobacillus*, including *Lactobacillus acidophilus* and *Lactobacillus plantarum*, have been observed to expedite colonic transit time and enhance stool consistency. Extensive research has been conducted on *Lactobacillus* strains, examining their effectiveness in diminishing the duration and severity of diarrhea, especially in cases of acute infectious diarrhea. *L. rhamnosus* GG has demonstrated effectiveness in lessening the period of acute infectious diarrhea in both adults and children (Guandalini et al., 2000; Szajewska et al., 2001). It works by competing with pathogenic bacteria for adhesion sites, producing antimicrobial substances, and modulating the gut immune response to help restore gut health.

Abdominal discomfort, bloating, and irregular bowel movements are the symptoms of IBS (irritable bowel syndrome), a persistent gastrointestinal ailment for which LAB has shown promise in treating this disease. *Lactobacillus acidophilus* NCFM has been specifically studied and shown to reduce abdominal pain and improve overall well-being in individuals with IBS (O'Mahony et al., 2005).

The exact mode of action by which *Lactobacillus* strains employ their effects in IBS is still under investigation but may involve modulation of the microbial flora in the gut and interaction with the gut-brain axis. It has been reported that different *Lactobacillus* strains stimulate the generation of immune-regulatory cytokines, such as interferon-gamma and interleukin-10. IL-10 is an anti-inflammatory cytokine that is essential for controlling and inhibiting excessive immune responses, and inflammation, fostering immune balance, and mitigating inflammation. IFN- γ is a pro-inflammatory cytokine that plays a critical role in activating immune responses against pathogens (Plaza-Díaz et al., 2019). *Lactobacillus* strains contribute to immune regulation and the induction of appropriate immune responses by fostering the production of these cytokines. These strains have the potential to boost the activity of various cells, mainly Natural killer cells and macrophages. One subset of cytotoxic lymphocytes, known as NK cells, is essential to the innate immune response against malignant and infectious cells. LAB strains have been found to enhance macrophage activity which are phagocytic cells that engulf and eliminate pathogens, promoting efficient pathogen clearance and immune defense (Rocha-Ramírez et al., 2017). These immune-modulating properties of *Lactobacillus* strains are attributed to their interactions with the gut-associated lymphoid tissue (GALT) and the mucosal immune system, which are crucial components of the immune system located in the gut. Specific surface structures and components in *Lactobacillus* strains enable interactions with immune cells, triggering immune responses. Studies have suggested that supplementing with specific *Lactobacillus* strains during early life may decrease the risk of infants developing atopic dermatitis and food allergies. These findings imply that certain *Lactobacillus* strains, such as *L. rhamnosus* GG, have the potential to modulate immune responses and reduce the risk of allergic disorders.

7.8. Lactic acid in the food industry

The significance of LA and its by-products in the food sector appears to be connected to the bacterial activity of organisms, specifically LAB. A unique metabolic process known as "fermentation" is the way that LAB bacteria including, *Lactobacillus*, *Enterococcus*, *Streptococcus*, and *Lactococcus* spp. produce LA from hexoses. Because of these factors, the food industry frequently uses fermentative bacteria as starting cultures for the industrial processing of various fermented products (Ameen & Caruso, 2017). In a wide range of prepared foods, lactic acid is utilized as an acidulant, flavoring, pH buffering agent, or inhibitor of bacterial spoiling. It tastes rather acidic when compared to other dietary acids. It works excellently as a pickling and preservation. The storage period of chicken and fish can be prolonged by adding an aqueous solution of lactic acid to their packaging (Das & Goyal, 2012). LA is suggested for use as an acidity controller for various food groups, including UHT and sterilized creams, whey protein cheese, frozen veggies, seaweeds, nuts, seeds, salt alternatives, and

newborn formulas designed for specific medical needs. In any case, GMPs (good manufacturing practices) always set maximum amounts, except for one category 13.2 called "complementary foods for infants and young children," where the limit is 2000 g per kg (Ameen & Caruso, 2017).

8. Applications of probiotics as functional foods

The pharmaceutical and food sectors are likely to be intrigued by the possible treatments, as the modification of the gut microbiota has demonstrated promise in the prevention and treatment of several illnesses. For example, the businesses Seres Health and Rebiotix are at present developing a standardized commercially manufactured fecal microbiota transplant (FMT) and a specified microbial cocktail, respectively. These therapeutic modalities can be utilized as alternatives to FMT, as they are specifically designed for the treatment of *Clostridium difficile* infection (CDI). It is expected that synthetic microbial communities designed for transplants would adhere to regulations related to manufacturing, mode of action, and safety (El Hage et al., 2017). Moreover, a variety of fermented dairy products naturally contain probiotics such as fermented dairy beverages, sour cream, kefir, yogurt, cheese, cultured buttermilk, and koumiss. However, LAB in dairy products is not always proven to be probiotic along with no harmful aspects. Therefore, LAB are commonly used as starter cultures for fermenting dairy products (Gao et al., 2021). *L. bulgaricus* and *S. thermophilus*, are two bacterial strains that ferment and acidify milk resulting in yogurt and fermented milk. The product that results from this procedure is thicker and has a longer shelf life. Yogurt has high quantities of bioavailable calcium, which makes it a great source of nutrients. Additionally, it is a great source of potassium, phosphorus, vitamin A, vitamin B12, and vitamin B2. It also provides important biologically essential proteins and fatty acids. Therefore, yogurt stands as a highly nutritious meal and an excellent method for absorbing probiotics (Hadjimbei et al., 2022). The production of yogurt using multiple strains has demonstrated superior properties. Despite this, traditionally produced yogurt exhibits less efficacy compared to probiotic strains. Potential probiotic strains, such as *L. acidophilus* and *L. plantarum*, are utilized in yogurt production and play an essential role in minimizing the presence of bisphenol A, an estrogenic substance in yogurt (Gao et al., 2021). A variety of fermented milk products is found globally, with cheese being one of them. Its characteristics are based on the specific bacterial strains used in the fermentation of milk. The synergistic combination of all LAB results in the development of more distinct flavors of cheese products (Widyastuti et al., 2014).

9. Conclusion

Lactic acid bacteria (LAB) have been gaining a huge importance in recent days due their application in food, health, and agriculture. Their antimicrobial compounds have been employed in various food industries. LAB has huge potential and still need to explore their potential at strain level from various sources. Many strains of LAB have been declared as probiotics, however, their use in developing countries is still limited specially to treat various ailments due to their higher price.

10. References

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