Probiotic lactic acid bacteria in biotechnology and the food industry: A review

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Abstract: This review explores the diverse applications and health benefits of probiotic lactic acid bacteria (LAB) through biotechnological applications in the food industry. While all LAB are indispensable for the production of fermented foods thanks to their ability to produce lactic acid and bacteriocins that act as natural preservatives, specific strains of probiotic LAB offer targeted health benefits. In addition to general benefits of LAB, probiotic strains significantly enhance gut microbiota, enhance human immunity, and exhibit antimicrobial properties. This review also delves into the mechanisms of action of probiotic LAB, focusing on adhesion, colonisation, and antioxidant production, emphasising their potential to advance nutritional innovations. Beyond food production, the broader category of LAB has transformative potential in industrial applications, particularly in the sugar industry, where their metabolic activity can improve sucrose extraction processes, promote microbial management, and reduce unwanted by-products. By understanding these aspects, the review underscores the importance of probiotic LAB in promoting health, efficiency, and sustainability across sectors.

Keywords: bacteriocins; food fermentation; dietary supplements; lactic acid; microbiota; sugar industry

Microorganisms that make up the normal flora are transmitted from mother to newborn during birth and are acquired from surrounding environments. This active colonisation provides a range of beneficial and potentially detrimental functions to the intestine and other systems of the body (Percival 1997). With hundreds of species in the gut, most of the microorganisms impact metabolic functions, immune responses, and health of the hosts (Nuriel-Ohayon et al. 2016). One group of live microbial species found in the population that provides this systemic balance is lactic acid bacteria (LAB). LAB are a natural group of cocci

or rod-shaped, catalase-negative, non-spore forming, and gram-positive bacteria. In general, they produce lactic acid as a major end-product of carbohydrate fermentation (Khalid 2011). This metabolic characteristic makes them highly valuable in the food industry, where they are widely used as starter cultures in the production of fermented foods. Additionally, LAB can produce bacteriocins, which are protein-based antimicrobial substances that serve as natural preservatives (Zacharof and Lovitt 2012). Due to their ability to withstand various stress factors in the gastrointestinal tract, they are valuable strains as probiotics.

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This review provides an overview of the mechanisms through which probiotic LAB exert beneficial effects, their applications in the biotechnology industry, and their potential therapeutic uses. By understanding these aspects, the contributions of probiotic LAB can be better evaluated, and innovative applications in functional foods can be explored. Furthermore, novel approaches can be considered to achieve a positive impact of controlled microbiota on the efficiency of processes in the sugar industry.

Lactic acid bacteria

LAB are a group of microorganisms characterised by unique metabolic, morphological, and physiological characteristics. They are naturally present on the healthy mucosal surfaces of animals and humans. Furthermore, they are commonly found in various fermented and other food products, including dairy products (e.g., yoghurt, cheese), beverages, cereal sourdoughs, meats, vegetables. These microorganisms are non-pathogenic and considered safe for human consumption. Certain LAB strains, classified as probiotics, offer specific health benefits by enhancing gut health, modulating the immune system, and exerting antimicrobial effects. These probiotic strains are often used in functional foods, which contain one or more bioactive components and offer salutary activities when consumed as a part of a balanced diet. Consequently, LAB have numerous biotechnological and other industrial applications, such as starter cultures that are used in the dairy industry as probiotics and bioconversion agents in nutritional supplements (Zhang and Cai 2014).

In recent years, LAB have been increasingly used as preservatives in food systems, offering a sustainable approach to prolong shelf life. Their metabolic conversion of sugars into lactic acid, a key microbicidal by-product, inhibits spoilage and can augment nutritional value (Rachwał and Gustaw 2024). LAB also produce bacteriocins, which have gained interest as natural food stabilisers. In addition, LAB generate hydrogen peroxide (H2O2) and exhibit an inhibitory effect by disrupting the membrane structure of pathogens in environments with detectable oxygen concentrations. The enzyme catalase neutralises H₂O₂ by breaking it down into water and oxygen, thereby inhibiting the growth of catalase-deficient pathogens (Zalán et al. 2005; Das and Bishayi 2009; Öncül and Yildirim 2020). To understand these protective and antimicrobial properties, a comprehensive examination of the general characteristics of lactic acid bacteria is essential.

General features, characterisation, and identification. LAB are a diverse group of non-motile, acid tolerant, and strictly fermentative bacteria that grow under microaerophilic conditions. While these microorganisms are not aerobic, they display aerotolerance. This property enables them to survive in low-oxygen environments (Khalid 2011; Wedajo 2015; Sionek et al. 2024). This tolerance can be attributed to their ability to detoxify reactive oxygen species through the action of antioxidant enzymes (such as peroxidases and superoxide dismutase) in their metabolism (Bryukhanov et al. 2022).

LAB are known for their lack of porphyrins and cytochrome. This deficiency makes them incapable of performing electron transport phosphorylation. Despite lacking a complete electron transport chain, LAB utilise substrate-level phosphorylation for energy production (Khalid 2011). Energy production occurs via fermentation pathways, and their metabolic activity and proliferation are supported by these sources. LAB thrive in environments enriched with acid derivatives, essential minerals, and vitamins. The optimal temperature range for their growth is from 30 to 45 °C (Anumudu et al. 2024). Their ability to adapt to changes in environmental factors (such as pH and nutrient levels) is supported by regulatory and metabolic mechanisms, contributing to their resilience.

Characterisation of LAB usually involves observation of small, round, opaque, grey-white colonies on selective agar plates [e.g. all purpose tween (APT) Agar, de Man Rogosa and Sharpe (MRS) Agar]. Traditional identification methods include biochemical tests such as catalase and nitrate negativity, carbohydrate fermentation, indole test, and tolerance assessments of varying concentrations of sugar and salt (Carr et al. 2002; Okcu et al. 2016; Khushboo et al. 2023). Apart from these techniques, advanced molecular methods have significantly enhanced LAB classification and identification. 16S rRNA gene sequencing, repetitive extragenic palindromic-PCR (Rep-PCR) fingerprinting, and whole-genomic analysis (WGS) provide detailed insight into their functional properties and genetic diversity. In particular, Rep-PCR offers a higher discriminatory power, and rapid results. This makes it a valuable tool for accurate and rapid identification of LAB species in various food samples, especially in combination with other methods (Meradji et al. 2023; Lahmamsi et al. 2024).

Taxonomic classification. LAB are primarily classified within the phylum Bacillota (formerly Firmicutes), class Bacilli, and order Lactobacillales. The LAB group comprises the families Aerococcaceae, Carnobacteriaceae, Enterococcaceae, Lactobacillaceae, Leuconostocaceae, and Streptococcaceae (Lahtinen et al. 2011; Zheng et al. 2020). These families, characterised by their fermentative metabolism, produce lactic acid as a primary end product of carbohydrate breakdown, and exhibit a range of traits from having beneficial probiotic features (e.g., Lactobacillaceae and some species of Carnobacteriaceae) to have harmful opportunistic pathogenicity (e.g., various strains of Enterococcaceae and Streptococcaceae) (Rossi 2023).

A 2020 taxonomic revision reclassified over 300 species previously distributed across seven genera and two families into a single family, Lactobacillaceae. This family now includes 31 genera, including Lactobacillus, Paralactobacillus, and Pediococcus. Members of Lactobacillaceae are generally known for their lactic acid production, playing essential roles in food fermentations and probiotics. In addition, the family Leuconostocaceae, which includes the genera Convivina, Fructobacillus, Leuconostoc, Oenococcus, and Weissella, represents the closest related group at the family level. These bacteria also produce lactic acid but differ in their ability to metabolise sugars via heterofermentative pathways, often contributing to the fermentation of food and beverages (Zheng et al. 2020; Qiao et al. 2022).

Nowadays, more precise classification and identification of LAB has been enabled in light of recent advances in biotechnological studies such as 16S rRNA gene sequencing and WGS. These methods have facilitated the separation of closely related strains within genera and the description of new species (e.g., *Lactiplantibacillus carotarum* and *Weissella fangxianensis*) (Xiang et al. 2023; Eilers et al. 2023). This expanding body of knowledge reveals the dynamic nature of LAB taxonomy and its potential impact on food and other applications.

Former Lactobacillus genus and related genera. Lactobacilli, formerly classified under the genus Lactobacillus and now distributed across multiple genera within the family Lactobacillaceae, are important taxa in the microbiology world (Zheng et al. 2020). They grow in carbohydrate-rich environments such as animal and human mucosal surfaces (oral cavity, intestines, and vagina). They can also be present in plants and plant-derived products and fermented or spoiled

foods. Species formerly classified under *Lactobacillus*, such as *Lacticaseibacillus casei* (*L. casei*) and *Lactiplantibacillus plantarum* (*L. plantarum*), play a crucial role as starter, adjunct, or protective cultures in the fermentation of vegetables, meat, and dairy products (Bernardeau et al. 2008; Gao et al. 2022). Their natural presence in these diverse environments makes them adaptable to various food substrates and highly resistant to acidic conditions prevalent in their ecological niches (Tripathi and Giri 2014).

These bacteria are Gram-positive, rod-shaped or cocci bacteria that do not form spores. They are catalase-negative, fermentative, aerotolerant or anaerobic, chemoorganotrophic, acidophilic, and require complex nutrients (e.g., carbohydrates, amino acids, peptides, fatty acid esters, salts, and vitamins) for growth. Most species are predominantly homofermentative, producing only lactic acid from sugars. However, some are heterofermentative, producing lactic acid along with other organic acids and carbon dioxide during fermentation (Stephen and Saleh 2023; Icer et al. 2023).

Species within the Lactobacillaceae family are classified according to their fermentation capabilities, more precisely the manner in which they break down sugars to produce energy. Homofermentative LAB mainly produce lactic acid, while heterofermentative ones form lactic acid, mannitol, carbon dioxide, and various organic acids. The first group, obligatory homofermentative species, ferments only hexoses (simple 6-carbon sugars) via Embden-Meyerhof-Parnas (EMP) pathway. This glycolytic process converts glucose into pyruvate, generating ATP and reduced nicotinamide adenine inucleotide (NADH), and producing lactic acid (Romano et al. 1979). Because these bacteria lack phosphoketolase activity, they cannot ferment pentoses (simple 5-carbon sugars) and gluconates (6-carbon sugar acids). The second group, facultative heterofermentative species, ferments hexoses by the EMP pathway and can also ferment pentoses and gluconates through aldolase and phosphoketolase activities. The final group, obligatory heterofermentative species, converts hexoses into lactic acid and ethanol, or acetic acid and carbon dioxide via the phosphogluconate pathway. They can also ferment pentoses through the same pathway (Zaunmüller et al. 2006; Abdel-Rahman et al. 2013; Delgado-Fernández et al. 2019). These different metabolic pathways reflect the diverse carbohydrate utilisation capabilities within the genus Lactobacillus, which is crucial for optimising industrial applications in food fermentation, probiotic development and other biotechnological processes.

Mechanisms of action of probiotic LAB in food and human health

Probiotic LAB demonstrate significant potential in nutritional and health applications, modulating biological systems and thus finding utility in functional foods and therapeutic interventions. They enhance gastrointestinal health, immune function, and metabolic processes by regulating intestinal barrier integrity, inflammatory responses, and nutrient absorption. Furthermore, certain probiotic LAB strains contribute to food preservation through bacteriocin production. These diverse mechanisms underscore their therapeutic and industrial relevance.

Adhesion and colonisation. The ability of probiotics to adhere to the gastrointestinal epithelium is crucial for colonisation, alteration of mucosal immune system, and inhibition of pathogens (Lau and Quek 2024). Probiotic adhesion mechanisms rely on aggregation capacity and the hydrophobic properties of cell surfaces. These aggregation processes include auto-aggregation, where microorganisms of the same species form colonies, and co-aggregation, where different genera cluster together. These interactions facilitate probiotic adherence to the intestinal tract, reducing pathogen colonisation (Bilginer and Çetin 2019; Karbowiak et al. 2022).

Alp and Kuleaşan (2020) examined the adhesion and competition abilities of Lacticaseibacillus casei DA4, Weissella cibaria DA28, Lactiplantibacillus plantarum DA100, Lactiplantibacillus plantarum DA140, and Loigolactobacillus coryniformis DA263 isolates against major gut pathogens using sheep intestines as a whole-tissue model. These probiotic LAB strains, isolated from fermented foods, demonstrate robust tolerance to acidic and bile-rich environments, a critical physiological attribute for probiotic efficacy within the gastrointestinal tract. They significantly reduced Clostridioides difficile (C. difficile) and Listeria monocytogenes (L. monocytogenes) populations by 3 log CFU⋅mL⁻¹ (CFU – colony forming unit). This reduction aligns with Hazard Analysis Critical Control Point (HACCP) safety standards for minimising foodborne risks (Todd 2004). Furthermore, these probiotic strains reduced adhesion rates by approximately 50%, demonstrating their probiotic potential in gastrointestinal health. Overall, this research highlights the value of whole-tissue models for providing insights into probiotic-mediated pathogen inhibition.

Antimicrobial activity. Thermal and non-thermal methods are commonly applied in food preservation to reduce microbial activity, but each have limitations.

The main challenge of thermal processes (e.g., pasteurisation or sterilisation) is preserving the nutritional value of foods at high temperatures. Non-thermal methods, such as bio-preservation, offer a safer and more efficient process. However, their effectiveness depends on specific conditions, such as presence and activity of beneficial microbial cultures. These cultures not only produce desired food products but also contribute to preservation by reducing the need for environmental stress and chemical preservatives (Andaluz-Mejía et al. 2022).

Numerous studies highlight the importance of antimicrobial compounds produced by LAB. These bacteria are well-regarded for their ability to synthesise various inhibitory metabolites, including lactic acid, ethanol, diacetyl, H₂O₂, and bacteriocins (De Vuyst and Leroy 2007). Bacteriocins, such as nisin and pediocin, are extensively utilised as natural food preservatives due to their antimicrobial efficacy. These compounds inhibit foodborne pathogens and spoilage microorganisms by disrupting cell wall biosynthesis and permeabilising bacterial cytoplasmic membranes (Sobrino-López and Martín-Belloso 2008). Additionally, they facilitate LAB dominance by inhibiting pathogen proliferation (De Vuyst and Leroy 2007; Orji et al. 2020; Gomez et al. 2021). Different inhibitory substances produced by LAB exhibit diverse antimicrobial properties; however, acidification of the environment through lactic acid production is often particularly effective in limiting competing bacterial growth. Their antagonistic effects against pathogens are well- documented and leveraged in various applications (Özoğlu et al. 2022).

In the study by Özoğlu et al. (2022), the lactic acid production and antimicrobial activity of putative probiotic and general LAB isolates from cheese, sucuk, and kefir were analysed. The study evaluated their inhibitory effects against various pathogens, including Staphylococcus aureus ATCC 43300 (methicillin- and oxacillin-resistant), Streptococcus mutans ATCC 25175, Bacillus subtilis ATCC 6633, Escherichia coli ATCC 25922, and Salmonella Enteritidis ATCC 13076, using an Agar Spot method. All isolates exhibited antimicrobial activity, with inhibition zones ranging from 1 to 24.5 mm. However, no antimicrobial activity was detected in the supernatants, suggesting that lactic acid was the primary inhibitory metabolite. High performance liquid chromatography (HPLC) analysis confirmed lactic acid production levels between 0.13 and 5.52 mmol·L⁻¹, which are moderately low compared to levels reported in other current studies. Although a general correlation existed between lactic acid produc-

tion and antimicrobial activity, the presence of inhibition in isolates with lactic acid levels below the detection limit of the HPLC method suggests the involvement of additional antimicrobial compounds. This study reinforces existing findings on antagonistic effects of LAB against pathogens via organic acid production.

Probiotic LAB possess a notable capacity for fungal inhibition, complementing their antibacterial effects, thereby offering a food bio preservation strategy against spoilage and the production of toxigenic compounds caused by yeasts and moulds. Application of antifungal LAB strains as green preservatives in food matrices in situ has shown proven potential to outperform synthetic antimicrobials and provide a viable natural approach (Mokoena et al. 2021; Nasrollahzadeh et al. 2022). The antifungal efficacy of LAB, a common feature in fermented foods, is directly linked to the synthesis of a range of bioactive metabolites. These metabolites, encompassing organic acids that acidify the environment, as well as cyclic dipeptides, fatty acids, and reuterin, all play a role in suppressing fungal growth (Massinissa et al. 2019). Fitri et al. (2024) demonstrated that LAB isolated from yoghurt, especially Lactobacillaceae, exhibit significant antifungal activity, effectively inhibiting Aspergillus niger and Rhizopus stolonifer, key spoilage fungi in bakery products. The study further highlights that sourdough bread prepared with LAB formulations demonstrated an extended shelf life and improved organoleptic properties, reinforcing the potential of probiotic LAB as natural antifungal agents in food preservation. Future research may focus on biochemical and genetic characterisation to confirm probiotic LAB strains. Additionally, it is crucial to elucidate the intricate links between their antimicrobial activity, including both antibacterial and antifungal mechanisms, and the metabolism of key compounds like lactic acid. This comprehensive understanding is essential for ensuring food safety and optimising the application of probiotic LAB as natural bio-preservatives across diverse food applications.

Production of antioxidants. Oxidation is a physiological process that generates free radicals and reactive oxygen species (ROS), which can cause cellular damage and contribute to various chronic diseases. In food products, oxidation leads to rancidity, colour changes, and nutrient degradation, negatively impacting quality and shelf life. Synthetic chemical antioxidants such as butylated hydroxyanisole, butylhydroxytoluene, and propyl gallate have raised safety concerns. This has led to increased interest in natural alternatives, including lactic acid bacteria (Hu et al. 2023).

While LAB primarily ferment under anaerobic conditions, they can also grow in the presence of oxygen. They precisely synthesise a diverse array of antioxidants, including enzymatic antioxidants like superoxide dismutase (SOD), catalase, and glutathione peroxidase. Additionally, LAB produce non-enzymatic antioxidants, such as exopolysaccharides (EPS), glutathione, and certain bacteriocins. All of these contribute to potent free radical scavenging activities (Bryukhanov et al. 2022).

During fermentation, LAB enhance the antioxidant capacity of foods and transform phenolic compounds, further increasing their antioxidant potential. This dual role in fermentation and antioxidant production makes LAB valuable in developing functional foods designed to reduce oxidative stress and mitigate the risk of diseases like cancer. This antioxidant capacity, coupled with their ability to produce bioactive compounds such as peptides and vitamins, positions LAB as promising candidates for innovative probiotic supplements and nutraceuticals that promote overall well-being (Bryukhanov et al. 2022; Hu et al. 2023).

Gastrointestinal health. Approximately one thousand microorganisms colonise certain organs and tissues in the human body. This consortium primarily consists of bacteria, but also includes viruses, fungi, and various other prokaryotes and eukaryotes. Over 70% of these microorganisms are found in the nutrient-rich gastrointestinal system. The intestinal tract provides a diverse and dynamic ecosystem consisting of aerobic, anaerobic, and facultative bacteria. Following birth, the intestinal flora settles rapidly, and by the age of one, it begins to resemble a young digestive system. In adulthood, the intestinal microbiota is dominated by Bacteroides, Bifidobacterium, Streptococcus, Enterobacter, and several species within the Lactobacillaceae family. They are predominantly located on the mucosal layer.

Probiotics exert anti-inflammatory, antibacterial, and immunomodulatory effects by improving the intestinal barrier function. They achieve this by reinforcing tight junctions in the gut epithelium, reducing permeability, and preventing pathogen infiltration. Probiotic LAB species isolated from human and animal intestinal systems, such as *Lactobacillus acidophilus* and *Lacticaseibacillus paracasei*, are the most utilised bacteria. These species produce antimicrobial substances like bacteriocins and organic acids, which suppress the growth of harmful bacteria. They are gaining popularity in probiotic industrial processes to prevent various inflammations. Inflammatory bowel diseases

(IBDs), a group of non-infectious chronic illnesses, are characterised by intestinal ulceration. IBDs cause a variety of symptoms, such as abdominal pain, diarrhoea, weight loss, bleeding, and anaemia (Pithadia and Jain 2011). Saez-Lara et al. (2015) showed that probiotic LAB could alter the gut microbiota in IBD patients by promoting the growth of beneficial bacteria while suppressing pathogenic species, thereby contributing to improved gut homeostasis. Moreover, probiotic LAB modulates immune responses by potentiating anti-inflammatory pathways and reducing pro-inflammatory cytokines. Consistent with these findings, studies have shown that fermented milk containing Limosilactobacillus fermentum reduced IBD-induced inflammation after six weeks during a mouse model experiment. Furthermore, bioactive compounds in yoghurt containing Limosilactobacillus reuteri RC-14 and Lactiplantibacillus plantarum GR-1 increased T-cell levels in mice, offering a potential therapeutic avenue for regulating immune responses in the intestinal cells of IBD patients (Ağagündüz et al. 2021).

Probiotics also contribute to gut health through several mechanisms. They engage in competitive exclusion of pathogens by producing antimicrobial peptides. Additionally, they strengthen the intestinal mucosal barrier by upregulating tight junction proteins, including claudin 1 and occluding. Furthermore, probiotics regulate immune activity by modulating Toll-like receptor (TLR) signalling. They can also modulate levels of neurotransmitters, such as dopamine, gamma-aminobutyric acid (GABA), and serotonin, which influence gut mobility and other body systems (Latif et al. 2023).

Immune enhancement. Probiotic LAB are essential for modulating and enhancing the human immune system (Doo et al. 2024). They achieve this by interacting with immune cells in the gut-associated lymphoid tissue, stimulating the production of immunoglobulins, and influencing cytokine secretion. Regular consumption of probiotics and lactic acid-fermented foods can bolster immunity against illnesses and infections. Maintaining immunological activity requires frequent interactions between beneficial bacteria and immune cells in a stable gut environment. Probiotic bacteria stimulate and enhance serum antibodies (Immunoglobulin A (IgA), Immunoglobulin G (IgG), and Immunoglobulin M (IgM)), contributing to immune defence. This immunomodulatory effect also involves balancing anti-inflammatory and pro-inflammatory cytokines levels, thereby regulating immune function and maintaining equilibrium (Ayivi et al. 2020; Mazziotta et al. 2023).

Beyond their metabolic effects, the cell walls of probiotic LAB contain molecules like peptidoglycans and lipoteichoic acids (LTAs), which serve as key immunostimulatory agents. Studies show that various strains, such as *Lactiplantibacillus plantarum* CJW55-10, *Lactiplantibacillus pentosus* CJW18-6, *Lact. pentosus* CJW56-11, and *Pediococcus acidilactici* CJN2696, significantly enhance IgA secretion by stimulating lamina propria cells (LPCs) from Peyer's patches (specialised gut-associated lymphoid tissues) (Hattori-Muroi et al. 2023; Choi et al. 2023). Additionally, LTAs from the cell wall of well-known probiotic *Lacticaseibacillus rhamnosus* GG activate pattern recognition receptors, promoting IgA production and enhancing immune surveillance (Matsuzaki et al. 2022).

A study by Costabile et al. (2017) explored how a combination of the probiotic *Lacticaseibacillus rhamnosus* GG and soluble corn fibre (SCF) affected immune function in senior individuals. Results showed that this symbiotic interaction enhanced natural killer (NK) cell activity and reduced the levels of the pro-inflammatory cytokine IL-6, suggesting a beneficial effect on both the microbial and immune systems.

Bacteriocins are ribosomally synthesised antimicrobial peptides produced by lactic acid bacteria. They have gained attention for their bio-preservative properties, pathogen-targeting abilities, and immuno-modulatory effects (Mihaylova-Garnizova et al. 2024). Bacteriocins exhibit diverse mechanisms of bacterial inhibition and are being explored as potential antibiotic alternatives due to their unique modes of action. They can bind to or disrupt bacterial cell membranes, interfere with DNA, RNA, and protein synthesis, and ultimately cause cell death (Gu 2023).

Beyond their antimicrobial activity, bacteriocins modulate the immune system by regulating the levels of anti- and pro-inflammatory cytokines, and stimulating IgA and IgG production, enhancing host immune defence (Guryanova 2023). Additionally, acetic acid and bacteriocins disrupt bacterial membranes, leading to cell leakage and death. Bacteriocins from lactic acid bacteria such as lactococcin A (LcnA) and pediocin PA-1 (PA-1) induce membrane permeabilisation by targeting the mannose phosphotransferase system (man-PTS) receptor, causing ion leakage and cytoplasmic collapse (Li et al. 2023). Acetic acid enhances bacteriocin efficacy by increasing outer membrane permeability, allowing peptides like PA-1 to penetrate and disrupt membrane potential and ATP gradients, leading to cell lysis (Wang et al. 2020). These diverse

functionalities of LAB, from immune modulation to antimicrobial activity, position them as promising candidates for future health interventions.

Anticancer activity. Malignant tumours remain a leading cause of death worldwide (Mohemed et al. 2022). While existing medical treatments (chemotherapy and radiation) effectively suppress cancer cell growth and progression, they often cause significant adverse side effects. Consequently, the search for innovative bacterial-based anticancer substitutes is a central concern in oncological research (Mughal and Kwok 2022). As alternatives to conventional therapies, probiotic LAB and their bioactive metabolites show promise in enhancing treatment strategies by modulating immune responses, inhibiting pathogenic microorganisms, and regulating metabolic pathways (Wu et al. 2021; Latif et al. 2023).

Probiotic LAB and their metabolites (e.g., bacteriocins, organic acids, and peptides) interact with key cellular pathways associated with cancer. These interactions influence inflammation, proliferation, angiogenesis, apoptosis, and metastasis, primarily mediated by increased production of short-chain fatty acids (SC-FAs). SCFAs, particularly butyrate, induce apoptosis, suppress cancer cell growth, and boost antioxidant defences. Probiotic LAB strains, such as Limosilactobacillus reuteri, contribute to cancer suppression through multiple mechanisms, including downregulation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-KB) signalling for cell proliferation, reduction of carcinogenic bile salts, and modulation of other related pathways. These findings highlight the significant potential of LAB probiotics in cancer prevention and treatment (Garbacz 2022; Latif et al. 2023). While the health-promoting properties of probiotic LAB, particularly their role in SCFAs production and anticancer activity, have attracted considerable attention, their applications extend beyond the medical field to biotechnological areas.

Application of lactic acid bacteria as probiotics in food biotechnology

In recent years, consumers have increasingly sought natural food alternatives. Products including probiotic bacteria, especially members of the *Lactobacillaceae* family, are gaining popularity (Liang et al. 2024a). The global market for functional foods that aid digestion has expanded rapidly. Statistical analysis from 2023 indicates that global financial revenue from probiotic supplements reached USD 87.7 billion. This trend is expected to continue in the forthcoming years (Lei et al. 2024). To meet this demand, a variety of prod-

ucts enriched with probiotics, including cereal products, dairy- and soy-based goods, fruits, and beverages, have been offered to consumers. These probiotics with mixed starter cultures are available as dietary supplements in capsule, tablet, medicinal, or powdered form (Ranadheera et al. 2017). Food biotechnology has also played a significant role in harnessing the power of these microorganisms, leading to the development of innovative food products such as yoghurt, cheese, and other fermented foods (Sundarraj et al. 2018).

Building on market expansion and growing biotechnological applications, the following sections delve into the fundamental points of LAB as probiotics. Key topics include their contributions to starter culture development, applications in food and beverages, advancements in microencapsulation technologies, and considerations for designing effective probiotic products.

Probiotic cultures. Lactic acid bacteria were first defined by Fuller (1989) as live microbial supplements that positively impact the hosts via improving the balance of microorganisms in their intestines. Salminen et al. (1999) later broadened this definition to include microbial cell preparations or cell components that enhance health and overall well-being. Probiotic LAB strains are widely available as commercial products in dietary supplements and food, providing manufacturers with various options for functional food development (Cappello et al. 2023). Advances in biotechnology have enabled probiotic LAB to produce bioactive compounds (exopolysaccharides, conjugated linoleic acids, etc.) that enhance the health benefits of edible products. Furthermore, modern tools like CRISPR-Cas9 allow for the metabolic engineering of LAB strains to improve their probiotic properties and optimise functional food formulations (Abedin et al. 2024). This can be accomplished by using an enzyme known as Cas9 endonuclease to create precise cuts in the genome. Thus, precise gene knockouts and base rearrangements can be facilitated. For instance, CRISPR-Cas9 systems have been used with customised single guide RNA (sgRNA) to repair templates, delete target genes, or insert biosynthetic pathways (Goh and Barrangou 2021). Such processes can increase the production of bioactive metabolites, such as exopolysaccharides and conjugated linoleic acids, unlocking new possibilities for innovative probiotic products.

Within food biotechnology, the targeted use of naturally occurring or metabolically engineered probiotic cultures is widely utilised to achieve desired product characteristics. Specifically, recent research highlights their ability to enhance bioactive properties in cheese,

with strains like *Lactiplantibacillus plantarum* A3 and *Limosilactobacillus reuteri* WQY-1 improving cheese ripening and antioxidant activity (Liang et al. 2024b). Furthermore, emerging strains such as *Weissella confusa* strain GCC_19R1, isolated from rice-based fermented food, show probiotic potential as a starter culture for the production of novel functional foods and dietary supplements (Nath et al. 2021).

Applications in fermented foods. Fermented foods have historically been considered safe and have been widely adopted worldwide. These products undergo microbial or enzymatic transformations to achieve desired biochemical changes. Early examples include alcoholic beverages from grains and fruits, as well as dairy products (e.g. yoghurt), which have roots in the Middle East and India. Additionally, East Asia has a rich tradition of fermented vegetables such as kimchi. In these processes, a diverse range of lactic acid bacteria (LAB), including both general starter cultures and probiotic strains from various genera within *Lactobacillaceae*, are essential. In ancient times, environmental conditions naturally selected these microorganisms, enabling them to modify and preserve food (Caplice and Fitzgerald 1999). Today, biotechnology provides precise control over fermentation, even in regions where mass-produced food is abundant. This fundamental role of probiotic LAB strains, deeply rooted in traditional practices, is particularly evident in the production of cheese.

In traditional raw milk cheeses, certain LAB strains act as starter cultures and may also possess probiotic properties, whether added or naturally present. During cheese maturation, probiotic LAB significantly influence texture, flavour, and aroma by contributing to the development of the secondary microbiota. Their preservative properties stem from the production of metabolites such as lactic acid, acetic acid, hydrogen peroxide, and bacteriocins. Probiotic LAB strains, especially indigenous ones, offer promising alternatives for food preservation due to their ability to inhibit undesirable microorganisms. Furthermore, some LAB strains in raw milk cheeses demonstrate probiotic properties, such as tolerance to gastrointestinal conditions and production of bioactive compounds with health-promoting effects. Advances in high-throughput DNA sequencing (HTS) methodologies have deepened understanding of traditional cheese microbiota, potentially leading to the development of improved LAB starter cultures and more refined cheese-making techniques to enhance consistency and quality (Coelho et al. 2022).

Beyond cheese, probiotic LAB also play a key role in yoghurt production. Generally, it is produced through the symbiotic action of two bacterial strains: Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus (Uzunsoy et al. 2023). While the chemical composition of yoghurt is highly similar to its raw material, milk, it differs due to substances added during manufacturing and changes resulting from bacterial fermentation. During fermentation by LAB, lactose in the milk is converted to lactic acid, proteins are broken down into peptides and amino acids, and fats are transformed into fatty acids (Çakıroğlu 2003; Yang et al. 2025). According to developments in food technology, fermentation approaches can now be optimised through metabolic engineering. This enables the introduction of modified LAB strains that can enhance the nutritional value and quality of yoghurt and other products in the dairy industry (Yilmaz et al. 2024).

Beverages. The application of probiotic LAB in beverage production extends beyond traditional roles. These microorganisms enhance both sensory attributes and functional qualities of probiotic beverages. Products containing strains such as *Lactiplantibacillus plantarum*, *Limosilactobacillus fermentum*, and other genera within the *Lactobacillaceae* family can improve digestion and immune function while potentially reducing the risk of various diseases (Gizachew et al. 2024). These LAB strains can be added to dairy-based drinks like yoghurt and kefir, as well as non-dairy alternatives such as fruit and vegetable juices, and alcoholic and non-alcoholic drinks (Shah et al. 2024).

Fermentation not only increases probiotic content but also enhances aroma, consistency, and storage life of the beverages (Anumudu et al. 2024). To ensure consistent probiotic benefits, it is essential that these products maintain a minimum of 6.0–7.0 log CFU·mL⁻¹ of viable bacteria throughout their shelf life, aligning with international standards (Özcan et al. 2023). By selecting specific strains and optimising substrates, manufacturers can create tailored probiotic beverages to meet diverse consumer preferences and dietary requirements, thus expanding the market for functional and health-promoting drinks (Mattila-Sandholm et al. 2002).

To develop innovative probiotic beverages, scientists have analysed the fermentation of single and mixed cereal substrates using LAB. Rathore et al. (2012) studied the fermentation of malt, barley, and a mixture of barley and malt using *Lactobacillus acidophilus* NCIMB 8821 (National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland, UK) and *L. plantarum* NCIMB 8826 at 30 °C for 28 h. Results showed a rapid

LAB growth in malt-containing media with lactic acid production ranging from 0.5 to 3.5 g·L⁻¹. Cell concentrations had reached between 7.9–8.5 log CFU⋅mL⁻¹ within 6 h. The pH level had dropped below 4.0. This indicates an increase in acidity of the medium, which is a typical indicator of successful fermentation. In addition, mixed cereal fermentations yielded cell populations comparable to single cereal flours, but with notable differences in lactic acid production. More recently, Gizachew et al. (2024) demonstrated the potential of six LAB isolates, mainly Weissella confusa and Limosilactobacillus fermentum, to ferment Ethiopian cereal-based beverages Naaqe and Cheka. Their study found pH levels below 4.6 and cell counts ranging from 5.75-9.02 log CFU·mL⁻¹, ensuring food safety. Remarkably, the LAB strains obtained from Naage exhibited higher growth rates and acidification ability compared to spontaneous fermentation. It also showed that specific LAB starter cultures are promising for improved fermentation efficiency and consistency. These findings are similar to previous studies, emphasising the importance of strain selection to produce high-quality probiotic beverages.

Designing the final probiotic product. To maximise the efficacy of new probiotic products in technological designs, it is essential to select appropriate strains. These microorganisms must then not only enhance the well-being of the host but also exhibit resilience to survive processing, storage, and gastrointestinal conditions. Probiotics are rarely found in their pure form in food products, so they are typically incorporated into food matrices that act as carrier systems. There are two primary ways for incorporating probiotics into food products: growing them directly in the final product or adding them through encapsulation (Arratia-Quijada et al. 2024).

The first method involves fermenting and growing probiotic bacteria directly within the food matrix. This approach produces food-based probiotic products that can be categorised as dairy products (e.g., yoghurt, cheese, milk, fermented milk, and ice cream) or non-dairy products (e.g., fruit and vegetable juices, cereals, chocolate, bread, and meat). This method naturally integrates probiotics into the foods and allows fermentation to impart unique characteristics to the designed products (Flach et al. 2018).

The second approach involves safely adding probiotics to the post-production product via encapsulation. Microencapsulation offers an effective strategy to protect probiotic cells from environmental stress, thereby enhancing their stability and viability during storage and digestion. This technique minimises the degradation of probiotics under adverse conditions and can also prevent undesirable sensory changes in fermented foods. For instance, studies indicate that the addition of microencapsulated probiotics, specifically *Lacticaseibacillus casei*, *Bifidobacterium bifidum*, *Lactobacillus acidophilus*, and *Bifidobacterium lactis*, did not noticeably change the sensory qualities of various food products, including ice cream, sausages, mayonnaise, cheese, and yoghurt (Feucht and Kwak 2013; Liao et al. 2017; Sharma et al. 2022; Arratia-Quijada et al. 2024).

Beyond strain selection and integration methods, certain essential steps are necessary to consider for the stability, efficacy, and safety of final probiotic products through optimised delivery forms. Approaches such as spray-dried or freeze-dried formulations play a critical role in maintaining viability and structural integrity of the cells, particularly by mitigating the adverse effects of dehydration on cell membranes and proteins. Advances in microencapsulation techniques, coupled with the use of protective encapsulants like a combination of skim milk, trehalose, and sucrose, have proven effective in enhancing cell survival during drying and storage (Dianawati et al. 2016). Among these, spray drying is widely used technique due to its cost-effectiveness, high stability, and scalability, providing optimal protection against heat, oxygen, and pH fluctuations (Bagdat et al. 2024). Alginate, a commonly used encapsulating agent, has demonstrated effectiveness in probiotic protection. A study by Tan et al. (2022) investigated calcium-alginate-sucrose formulations for encapsulating probiotic Lacticaseibacillus rhamnosus GG (Figure 1), achieving high bacterial viability post spray drying and exposure to simulated gastric fluid.

The manufacturing process must follow strict quality control measures to ensure consistency, safety, and regulatory compliance. From the selection of raw materials to final packaging, rigorous controls are essential to maintain product reliability and consumer confidence. As stated previously, advanced analytical tools such as HTS can be used to monitor microbial composition, thereby further improving safety and quality standards (Syromyatnikov et al. 2022). By taking these measures and leveraging biotechnological advancements, probiotic products can achieve better efficacy, stability, and consumer appeal (Ljungh and Wadström 2006). Furthermore, the adoption of sustainable practices in production ensures long-term viability and is compatible with environmental goals.

The new proactive strategy for lactic acid bacteria in the sugar industry. The sugar industry has long

been concerned with the study and monitoring of microorganisms during the production process. The key concern is the prevention of sugar losses due to the presence of microorganisms and the formation of other products such as organic acids, gases (CO₂ or H₂) or mucilaginous substances. The activity of microorganisms during production adversely affects the setting of process parameters such as high temperature, low pH values and limited oxygen availability, as well as the development and use of antimicrobial agents to control microbial growth (Birke et al. 2024).

The requirements of technical microbiological sterility and the requirements of technological quality of the extraction process should always be compared. When the temperature is increased above 75 °C, the metabolism of microorganisms is suppressed (which would meet the microbiological requirement), but at the same time, pectin substances are transferred from the pulp to the juice in an increased manner, deteriorating the quality of the raw juice (Bretschneider 1980; Both et al. 2013). If the juice is to be extracted as sterile as possible in the operation, this can be achieved at temperatures around 75 °C and lower only with the simultaneous use of disinfectants. As already mentioned, the main sugar losses occur when extracting juice from sweet pulps by the extraction process. The obtained raw juice represents an ideal environment for the activity of microorganisms, both in terms of juice composition (content of sucrose, pectin, organic acids, proteins, amino acids, etc.), pH value (a pH value in the range of 5.5-6.0 is suitable for bacterial growth) and temperature (Henke et al. 2024). The most common metabolite of sucrose degradation is lactic acid (lactic fermentation, Lactobacillus, about 80% of decomposed sugar) and acetic acid (acetic fermentation, Acetobacter,

Dry particle collector

about 20% of decomposed sugar) (Berlowska et al. 2018; Abedi and Hashemi 2020). Lactic acid releases from the cossettes during extraction and reduces the pH of the juice below 5.8 at 20 °C (Henke et al. 2024).

A new proactive approach (strategy) addresses microbial diversity in beet sugar factories and calls for better use of the beneficial properties of microorganisms to decompose undesirable substances and promote desirable ones. The main goal is permanently improving the sucrose extraction process. Some positive effects have been observed through bacterial activity, such as that the acidic by-products (lactic acid) of bacterial metabolism lowering the pH, leading to higher stability of sugar beet pulp during pressing (Prati and Maniscalco 2013). In addition, the beneficial metabolic processes have been recognised, such as the preferential utilisation of monosaccharides instead of sucrose or the selective degradation of undesirable substances (e.g. reduction/conversion of nitrites to elemental nitrogen) (Zhang et al. 2022). Modern sequencing techniques continue to identify unknown bacteria with the potential to improve the process (Moser et al. 2021; Bill et al. 2024). Investigation of these bacteria could offer valuable insights for better microbial management in beet sugar production. However, extensive research is necessary to identify and characterise suitable bacterial species and to best utilise them. Still unexplored but desirable properties include the ability to hydrolyse raffinose and to exhibit dextranase activity (Eggleston and Monge 2005; Jiménez 2009; Jaśkiewicz et al. 2024). The effort to develop a technologically beneficial microbiota applicable in the extraction process would mean a major transformation in sugar production, instead of fighting against the existing microbiota, to exploit

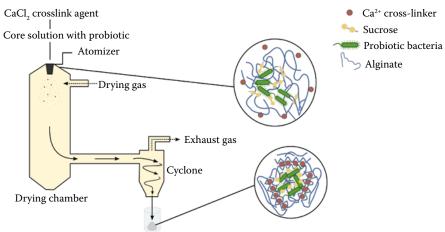


Figure 1. Schematic diagram depicting the calcium-alginatesucrose in situ alginate crosslinking during spray drying (Tan et al. 2022)

its potential (Gaoyu et al. 2024). In this case, procedures based on appropriate stimulation of optimised microbiota, the use of immobilised enzymes and bacteria, or the use of some of the methods of genetic engineering, seem advantageous. By a more comprehensive understanding of the microbiota and the use of its activities, the sugar industry could benefit from improved processes and a more sustainable approach to beet sugar extraction (Birke et al. 2024).

CONCLUSION

LAB are valued for their consistent and efficient metabolic processes, producing organic acids and other bioactive metabolites that improve food safety, extend shelf life, and enhance sensory qualities. These properties make probiotic LAB an integral part of fermentation and preservation of beverages and food. Beyond their traditional applications, various LAB strains are gaining prominence as probiotics and therapeutic agents. Emerging research underscores their multifaceted role in health promotion, exploring their potential to combat gastrointestinal disorders, modulate immune responses, and address antibiotic resistance. The sugar industry, which has traditionally focused on eradicating microorganisms to mitigate sugar loss, is undergoing a paradigm shift. Instead of simply controlling microbial activity, the industry is exploring the strategic use of beneficial microbial communities, including LAB, to optimise sucrose extraction processes. This innovative approach exemplifies a transition from microbial suppression to harnessing microbial functionality, mirroring the advances in food biotechnology. Such synergistic strategies demonstrate the potential to improve both production efficiency and sustainability, establishing a framework for continued innovation in food production and industrial applications.

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