# Lactic Acid Bacteria in Dairy Foods

Subjects: Biotechnology & Applied Microbiology

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Lactic acid bacteria (LAB) are regarded as 'Generally Recognized as Safe' (GRAS) and are commonly used in the dairy industry and also form part of the microbiota of the human intestine. LAB play a significant role in biopreservation because they produce a variety of antimicrobial metabolites during the development and fermentation processes. The use of antimicrobial-producing LAB in the production of dairy products, which can be incorporated into fermented or nonfermented dairy products, implies a processing advantage to improve the safety and quality of dairy products, providing an additional barrier against foodborne diseases. Among the most common antimicrobials are bacteriocins, which are ribosomally produced antimicrobial peptides. They can kill or inhibit undesirable bacterial strains, whether closely related or not, without harming themselves. This ability is especially relevant in the food industry. Notably, many LAB bacteriocins, including those derived from such bacteria, have shown efficacy against *Listeria monocytogenes*, a significant concern in traditional cheeses made from raw milk.

lactic acid bacteria        dairy foods        antimicrobial compounds

## 1. Bacteriocins in Dairy Foods

Bacteriocins are ribosomally synthesized short-length antimicrobial peptides produced by various groups of bacteria, especially LAB [1]. Bacteriocins produced by LAB are peptides mainly active against Gram-positive bacteria, including foodborne pathogens and food spoilage-related bacteria. Bacteriocins are categorized into various classes considering factors such as molecular size, physical properties, and the organisms that produce them. Class I comprises lantibiotics, due to the content of the unusual amino acid lanthionine, which are reputed for their heat stability and low molecular weight (around 5 kDa) [2]. Nisin A and its variants are the foremost examples of lantibiotics and have been the subject of extensive research [3]. Class II bacteriocins are distinguished by their simpler structures compared to lantibiotics. This class encompasses small, heat-stable peptides (around 10 kDa) that exhibit an amphiphilic helical conformation. They are further subdivided into three subclasses: II-A, II-B, and II-C [4]. Subclass II-A members stand out due to their potent antibacterial properties. These bacteriocins typically consist of 37–48 amino acid residues. Examples of this subclass include pediocins and enterocins [4]. Bacteriocins of subclass II-B, known as heterodimeric bacteriocins, are composed of two peptides. Lactococcin was the inaugural bacteriocin identified within this group. The mode of action involves dissipating the membrane potential and causing a reduction in the intracellular concentration of ATP [4][5]. Subclass II-C bacteriocins are characterized by their circular configuration, arising from the covalent bond between the C and N terminals. This leads to peptides adopting a cyclic tail conformation. Their action mechanism involves permeabilizing the cytoplasmic membrane of target cells, ultimately leading to cell lysis [4]. Bacteriocins in Class III are large, thermolabile proteins with molecular weights exceeding 30 kDa. A defining feature of this class is their ability to induce cell wall lysis in

target microbes [4]. Colicin, produced by *Escherichia coli*, serves as a representative example of Class III bacteriocins [4]. Furthermore, helveticin M is produced by *Lactobacillus crispatus*, helveticin J by *Lactobacillus helveticus*, and enterolysin A by *Enterococcus faecalis*, all falling under the category of Class III bacteriocins [6][7][8]. Finally, Class IV bacteriocins are characterized by their composition, which includes complex proteins conjugated with lipids or carbohydrates. Examples from this class include pediocin N5p and lactocin 27 [9].

The diverse classification of bacteriocins, ranging from heat-stable lantibiotics to complex protein conjugates, underscores their potential versatility in applications, particularly in dairy food preservation. These peptides, especially those produced by LAB, have received attention by the dairy industry for their capacity to combat foodborne pathogens and spoilage-related bacteria. A prime example is nisin, a bacteriocin produced by *Lactococcus* and some *Streptococcus* strains, renowned for its antimicrobial activities [10]. Additionally, LAB from *Lactobacillus* and *Leuconostoc* have been identified as prominent producers of class II bacteriocins, expanding the spectrum of potential antimicrobial agents [11]. Particularly noteworthy among these is the pediocin produced by *Pediococcus*, classified under class IIa bacteriocins, pediocin exhibits pronounced anti-listerial activity, finding substantial effectiveness in meat products [12]. In fact, there is an impressive array of approximately 30 class IIa bacteriocins that have been recognized, sourced from a variety of LAB genera including *Bifidobacterium*, *Lactobacillus*, *Lactococcus*, *Pediococcus*, *Leuconostoc*, *Streptococcus*, and *Enterococcus* [11].The *Enterococcus* species, for instance, produce enterocins that are particularly effective against strains like *Bacillus* and *Clostridium* species [13][14]. Studies also indicated that enterocin-producing *Enterococcus faecalis* strains exhibit inhibitory effects against *L. monocytogenes*, a pathogen of concern in fresh cheese [14].

In this light, three strategies can be used to consider LAB and bacteriocins for natural preservation in the food sector. Each of these strategies offers distinct applications and implications within the industry. Firstly, incorporating LAB for dairy fermentation and protection: incorporating LAB directly into dairy products exploits the benefits of natural fermentation, enhancing flavor, texture, and nutritional value [15]. As these bacteria grow and proliferate within the dairy matrix, they consistently produce bacteriocins, ensuring a sustained defense against spoilage microorganisms and pathogens. Furthermore, the use of LAB resonates with modern consumers who are increasingly seeking cleaner labels and natural preservation methods. Nonetheless, the effectiveness of this biopreservation approach is intricately tied to the viability and activity of LAB. Factors such as pH, temperature, and the competitive dairy microbial environment can influence their performance [16]. Moreover, the use of LAB in food products can greatly influence flavor profiles. Specific strains of LAB, such as *Streptococcus thermophilus* and *L. lactis*, are approved and commonly used to enhance flavors in dairy products. These strains are known to introduce a tangy and pleasant acidic taste, often associated with fermented dairy products such as yogurt. On the other hand, certain strains, such as *Limosilactobacillus fermentum* and *Limosilactobacillus reuterin*, if not used judiciously, can produce flavors that may be perceived as off or less palatable. This can be attributed to the production of certain metabolites such as biogenic amines (e.g., histamine), diacetyl (which in excess can impart a strong buttery flavor), or acetic acid (which can give a sharp, vinegar-like taste [17]). Secondly, there is the direct application of pure bacteriocin to dairy products. Introducing purified bacteriocins into dairy products ensures a uniform and immediate antimicrobial defense. This method eliminates the waiting period associated with bacterial growth, providing prompt protection against potential spoilage agents and pathogens. Moreover, since it is the

isolated compound being added, there is minimal risk of introducing unintended flavors, maintaining the original taste profile of the dairy product. However, there are challenges to this approach. The extraction and purification processes for bacteriocins can drive up production costs. Additionally, while some bacteriocins possess a broad antimicrobial spectrum, others might be narrowly effective, requiring precise targeting of threats. There is also the concern of potential degradation due to the presence of proteolytic enzymes in certain dairy products, which might compromise bacteriocin structure and efficacy [18]. Lastly, using bacteriocin-producing fermented products in dairy: using fermented products derived from bacteriocin-producing strains offers a unique approach to dairy preservation. Incorporating such products can provide dual benefits, such as introducing rich flavors from fermentation and conferring the antimicrobial properties of the bacteriocins present. This approach differs from the first one because the initial method directly adds LAB to the dairy products, initiating immediate fermentation and bacteriocin production. In contrast, the latter incorporates already fermented products, capitalizing on existing bacteriocins without initiating a new fermentation process in the dairy product itself. This method also promotes sustainability, utilizing byproducts or excess from one dairy process to enhance another. However, this approach has challenges. The concentration and activity of bacteriocins in these fermented products can vary, potentially leading to inconsistent preservation outcomes. Depending on the fermented source, strong flavors might also be introduced that could overshadow the desired taste profile of the final dairy product [19].

Expanding on this concept, there are clear examples of bacteriocins effectively used in dairy products to improve their quality and safety. For instance, lactacin F from *Lactoplantibacillus plantarum* has been employed in yogurt to improve its safety [20]. In a model fresh cheese, lacticin 481 produced by *L. lactis* L3A21M1 effectively reduced *L. monocytogenes* presence [21]. Skim milk utilized nisin Z from *L. lactis* W8 to extend its shelf life [22]. Additionally, nisin A was added to cottage cheese to inhibit the growth of *L. monocytogenes* [23]. Nisin, produced by *L. lactis* subsp. *lactis*, is a noteworthy bacteriocin among those derived from LAB. Recognized for its broad-spectrum activity against Gram-positive bacteria and its proven safety for human consumption, it has been approved as a natural food preservative in numerous countries. Dairy products, especially milk, are among its main applications [24]. Nisin is structured with unique amino acids, which give rise to its distinctive rings formed by thioether bonds [24]. Incorporating nisin into dairy products is an effective strategy to inhibit the proliferation of notable pathogens, including *L. monocytogenes* and *Staphylococcus aureus* [25]. Nisin exerts its antibacterial properties through a dual-action mechanism (**Figure 1**). Firstly, it binds to the cell wall precursor, lipid II, inhibiting cell wall synthesis. Simultaneously, this binding facilitates the formation of pores in the bacterial cell membrane, disrupting the integrity and leading to cell death [16][26].
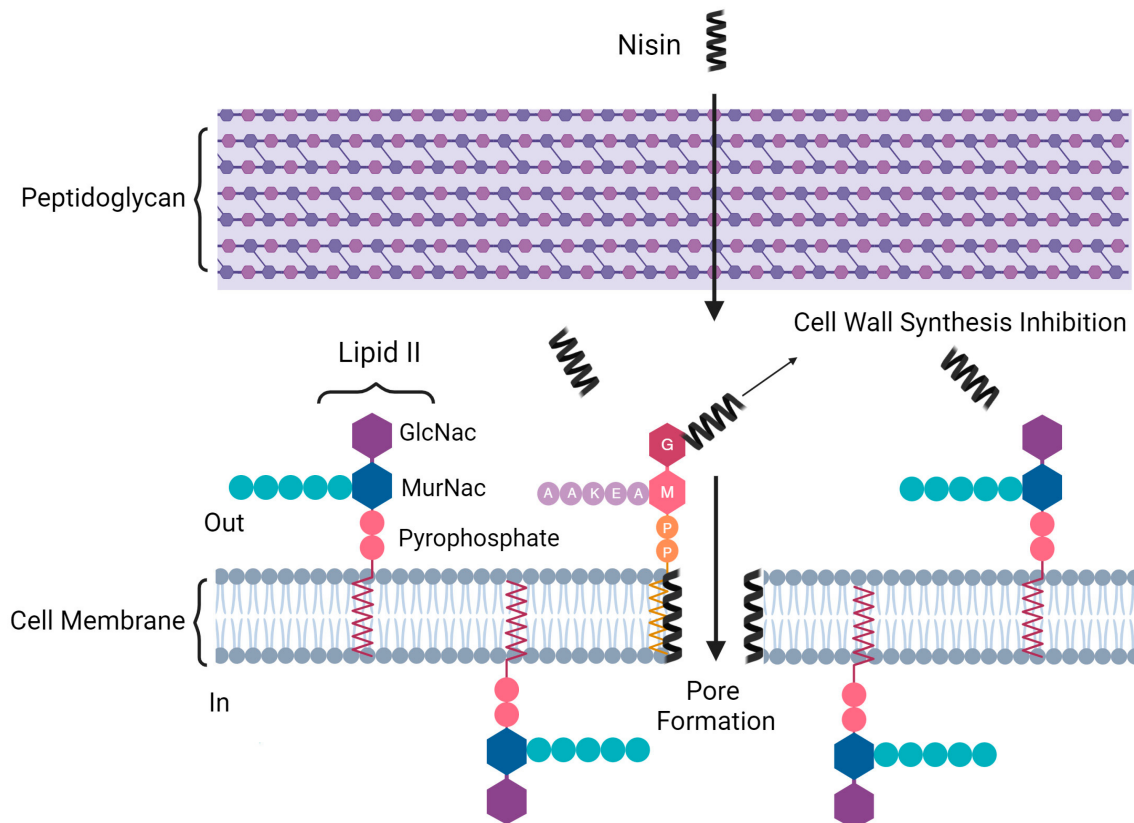
**Figure 1.** Mechanism of action of nisin [16][26].

Studies have shown that bacteriocins, such as leucocin A and sakacin A, are frequently utilized to counteract *L. monocytogenes* in dairy items [27][28]. Meanwhile, nisin, produced by *L. lactis*, has gained approval as a food preservative in several nations to suppress harmful bacteria in foods like ricotta cheese and other processed cheeses [29]. A study introduced a novel class III bacteriocin, NX371 from *L. acidophilus* NX2-6. This bacteriocin presents promising for its use in preserving milk and Mozzarella cheese, as it notably curtails pathogenic proliferation in dairy items. Furthermore, its resistance to heat and varying pH levels surpasses that of nisin [30]

Beyond specific applications of bacteriocins in dairy, these compounds have broader implications for food safety. The overall advantages of bacteriocins in food preservation underscore their significance. They can be degraded by proteases in the digestive tract, reducing consumer concerns about accumulation [31]. Moreover, they demonstrate effectiveness against a range of spoilage and pathogenic bacteria. In a study by Ageni et al. [32], bacteriocins displayed robust antagonistic effects against several pathogens, with *Pseudomonas aeruginosa*, *E. coli*, and *Clostridium* spp. being particularly sensitive. Thermal stability is another important characteristic of bacteriocins, aligning well with food processing requirements. Many food processing methods involve heat, making the heat stability of bacteriocins a valuable trait for preservation. Confirming this, both Moigani and Amirinia, and Ageni et al. noted the remarkable ability of bacteriocins to maintain their antimicrobial or preservative function for 15 minutes at 121 °C, an attribute that proves invaluable in food safety procedures based on heat treatments [32][33]. The comprehensive study by Ibarra-Sánchez et al. [25] sheds light on both the challenges and emerging practices in utilizing nisin for dairy product conservation. The study confirms the efficacy of nisin in dairy products

through various strategies, including antimicrobial packaging, bioengineering, encapsulation, and combined antimicrobials. Specifically for cheese, active antimicrobial packaging with nisin offers a robust defense against contamination either during or after processing. However, it must be noted that its protective action is confined to the cheese's surface. Addressing this limitation, incorporating encapsulated nisin into antimicrobial packaging emerges as a promising technique. This approach could elevate both stability and microbiological safety, ensuring the preservation of food's nutritional and sensory quality [25].

# 2. Organic Acids and Their Antimicrobial Properties

In fermented milk, lactic acid stands out as the primary organic acid, as result of the metabolic activity of lactose break down by LAB. However, depending on the specific LAB strains and their fermentation pathways, other organic acids, including formic and acetic acid, may also be present, especially from the processes of hetero-fermentative LAB [15]. LAB can operate as either homo-fermenters or hetero-fermenters. Homo-fermenters predominantly metabolize hexoses to produce lactic acid as the sole or primary fermentation end-product. Their metabolic pathway of choice is the Embden–Meyerhof–Parnas (EMP), often referred to as glycolysis. The EMP pathway is a linear process converting glucose to pyruvate, which is then reduced to lactic acid. This results in an efficient lactic acid production, creating an acidic environment that not only helps in food preservation but also gives the characteristic tangy taste to fermented foods [25]. Prominent examples of homo-fermenting LAB species include *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Ligilactobacillus salivarius*, *Lactobacillus helveticus*, *L. lactis*, and *S. thermophilus* [26]. In contrast, hetero-fermenting LAB have a more diverse metabolic output. They can produce lactic acid, acetic acid, alcohol, and carbon dioxide from their metabolic activities. These bacteria metabolize sugars through the phosphoketolase pathway. When breaking down pentose sugars, they typically produce lactic acid, acetic acid, and $CO_2$. For hexoses, the end products can be lactic acid, ethanol, and $CO_2$, with possible minor production of acetic acid. The carbon dioxide produced by hetero-fermenters is crucial in some food applications, such as leavening in sourdough bread. Examples of hetero-fermenting LAB include *Lentilactobacillus buchneri*, *Levilactobacillus brevis*, and *L. fermentum* [34][35].On the other side, facultative hetero-fermenters such as *L. plantarum*, exhibit remarkable metabolic versatility. When metabolizing hexose sugars such as glucose, they can utilize the EMP pathway for homo-fermentation, producing primarily lactic acid, or switch to the phosphoketolase pathway for hetero-fermentation, leading to lactic acid, ethanol, and $CO_2$. The choice between these pathways is influenced by various factors: environmental conditions such as pH and oxygen levels; substrate concentrations, which can shift the balance of end-products; and the strain's genetic makeup that predisposes it to prefer one pathway over another. In slightly acidic conditions, they might favor homo-fermentation, as it produces lactic acid, further inhibiting competitors. In more neutral conditions, hetero-fermentation can be advantageous, producing varied metabolites such as ethanol and $CO_2$, which offer different ecological benefits. Oxygen presence can also affect the choice. Limited oxygen might push LAB towards homo-fermentation for efficient energy production, while trace oxygen levels might tilt the balance towards hetero-fermentation to maintain cellular redox balance through ethanol production. Moreover, high glucose concentrations often steer LAB towards homo-fermentation, maximizing ATP production. However, a mix of sugar types, such as hexoses and pentoses, can push them towards hetero-fermentation, exploiting the diverse sugar array. Furthermore, the inherent genetic predisposition of

the strain plays a definitive role. Some LAB strains, due to specific genes or regulatory elements, might inherently lean more towards one pathway, regardless of environmental conditions. Finally, in the presence of pentoses such as xylose, these LAB typically employ the phosphoketolase pathway for hetero-fermentation, producing lactic acid, acetic acid, and $CO_2$. This metabolic flexibility provides an adaptive advantage, allowing them to efficiently process substrates in varied fermentation contexts, influencing both preservation and flavor nuances of the resulting products [34][35].

While the intricacies of the metabolic pathways employed by LAB highlight the vast spectrum of organic acids produced, it is essential to emphasize the direct implications these acids have on food aroma, safety, and preservation. Among these organic acids, acetic acid is a potent compound with notable antimicrobial properties. Not only does it play a role in imparting a distinct tangy flavor to fermented foods, but its efficacy against a range of spoilage and pathogens, especially fungi, is commendable. The antifungal activity of acetic acid is vital in the context of dairy fermentations, where mold and yeast contaminations can compromise product quality and safety. Acetic acid exhibits antifungal properties in dairy products primarily by penetrating the fungal cell membrane in its undissociated form. Once inside the cell, it dissociates into acetate ions and protons, leading to a decrease in intracellular pH. This acidic environment disrupts essential enzymatic reactions, hindering the fungal cell metabolic processes. Additionally, the osmotic imbalance caused by the accumulation of acetate ions compromises the integrity of the cell membrane, resulting in leakage of cellular contents and inhibiting fungal growth [36]. Beyond acetic acid, there's Phenyllactic Acid (PLA), which is synthesized from the amino acid phenylalanine and demonstrates broad antifungal activity by disrupting fungal cell membranes and energy metabolism. Furthermore, organic acids such as lactic acid, formic acid, and propionic acid, each contribute uniquely to the antifungal effect. For instance, formic and propionic acids penetrate fungal cells and disrupt their internal pH balance and metabolic functions. Recognizing the diverse antifungal arsenal of LAB, encompassing acetic acid, PLA, and other organic acids, is crucial. Their collective and potentially synergistic effects can significantly enhance the preservation and safety of dairy products [36]. Delving deeper into the antimicrobial mechanisms of acetic acid and other organic acids can provide insight into their potential applications in enhancing the shelf life and safety of dairy products. Fungal contamination restricts the shelf-life of fermented dairy products, leading to both food wastage and economic challenges [36]. While conventional methods such as heat treatment and air filtration are employed to decrease contamination during the production [37], there is an increasing consumer preference for natural preservation methods, such as biopreservation [38]. In this context, LAB strains exhibiting antifungal properties present a promising solution. Research indicates that certain LAB strains, including *L. plantarum*, *Lacticaseibacillus rhamnosus*, and *Lacticaseibacillus casei*, possess inherent antifungal activities [38][39][40][41]. The primary antifungal mechanisms attributed to LAB in dairy settings are the synthesis of specific antifungal compounds and a reduction in pH [36]. Of these compounds, acetic acid, a metabolite produced by LAB, stands out for its significant antifungal effects in dairy products [41]. Intriguingly, the inhibitory concentration of acetic acid is enhanced when in the presence of lactic acid, suggesting a synergistic action between the two [42].

Lactic acid, a primary byproduct of LAB metabolism, is instrumental in lowering the pH of fermented dairy products. This reduction in pH plays a critical role, resulting in a higher proportion of undissociated acetic acid and enhancing its antimicrobial potency [43]. Furthermore, Garnier et al. [44] conducted an in vitro research on three fermented

dairy products produced (a reconstituted 10% low heat milk supplemented with 45% anhydrous milk fat and an ultrafiltration permeate supplemented with 1% yeast extract) by *Acidipropionibacterium jensenii* CIRM-BIA1774, *L. rhamnosus* CIRM-BIA1952, and *Mucor lanceolatus* UBOCC-A-109193. All three products demonstrated strong antifungal properties. Their investigation revealed propionic and acetic acid as the primary antifungal constituents, enhanced by lactic and butyric acids. Garrote, Abraham, and De Antoni [45] reinforced this observation, pinpointing acetic and lactic acids as the primary agents behind the antimicrobial strength of kefir. Further research by Lind, Jonsson, and Schnürer identified acetic and propionic acids as chief antagonists against fungi such as *Aspergillus fumigatus* and *Aspergillus nidulans*, whereas lactic acid exhibited a lesser effect. It is also important to note that the inhibitory potential of these acids on fungal growth tends to diminish with increasing pH [46].

Understanding antimicrobial implications of organic acids in dairy products is crucial, but so is the appreciation of their impact on the sensory and textural qualities of these products. Organic acid concentrations, particularly lactic acid, have far-reaching effects beyond inhibitory action against spoilage organisms. For instance, over-acidification in cheese is considered a defect, leading to an undesired crumbly texture and overly tangy flavor. Ensuring the right level of acid production is crucial not just for achieving the desired taste and texture but also for maintaining the structural integrity of the cheese [47][48][49].

Alongside understanding the critical balance of acid production in dairy, advancements in production techniques are equally important. Cubas-Cano et al. [50] further emphasized the possibilities brought forth by innovative process configurations. Techniques such as fed-batch and simultaneous saccharification and fermentation (SSF) not only streamline production but also enhance resource utilization efficiency, thereby potentially reducing the production costs. Fed-batch processing, a method where substrates are added incrementally to the fermentation vessel instead of a singular addition at the start, has become a key approach in modern fermentation processes. This strategy effectively manages microbial growth rates by adjusting substrate availability, avoiding problems such as overpopulation or running out of substrate. Additionally, adding feed over time can lengthen the production phase, possibly increasing yield. Another important method is SSF, where the breaking down of complex sugars and fermentation happen at the same time. This combined process removes the need for separate steps in breaking down sugars, making it more direct to turn complex sugars into end-products. Plus, as fermentation and the breakdown of sugars occur together, any potential harmful compounds produced are quickly used up or changed by the fermenting microbes [50]. The continuous evolution of these methods opens the door for more sustainable and efficient production pipelines in the food and biotech sectors. Beyond yield improvement, these advancements reflect the industry's broader goal: achieving high-quality, consistent, and scalable production that can cater to global demands while minimizing environmental footprints.

# 3. Antimicrobial Compounds Diversity in Dairy Foods

Dairy fermented foods are common in many diets around the world. They are valued for their taste and texture and the many health-boosting compounds they contain. LAB particularly add these compounds during fermentation, making the food both healthy and safe. LAB are effective inhibitors of pathogens and significantly hinder the activities of food-spoiling organisms. When introduced to food, they can suppress harmful gut pathogens and

reduce toxic elements in the intestines [51]. Additionally, LAB enhance the nutritional value and texture of dairy items, including yogurt and cheese. They achieve this while also fostering gut health through the production of antibacterial agents [52][53]. Commercially, LAB are popularly utilized as starter cultures due to their range of metabolic abilities. Besides the well-known bacteriocins and organic acids, LAB produce many other bioactive molecules that deserve attention in the context of dairy fermentation (**Figure 2**) [54][55]. Specifically, milk fermentation involves various LAB species such as *Streptococcus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, and *Lactobacillus* [53]. For instance, *L. rhamnosus* is effectively employed as a probiotic in fermented dairy products and its incorporation in cheese production could mitigate the risk of pathogen proliferation, speed up cheese maturation, and enhance cheese flavor [56].
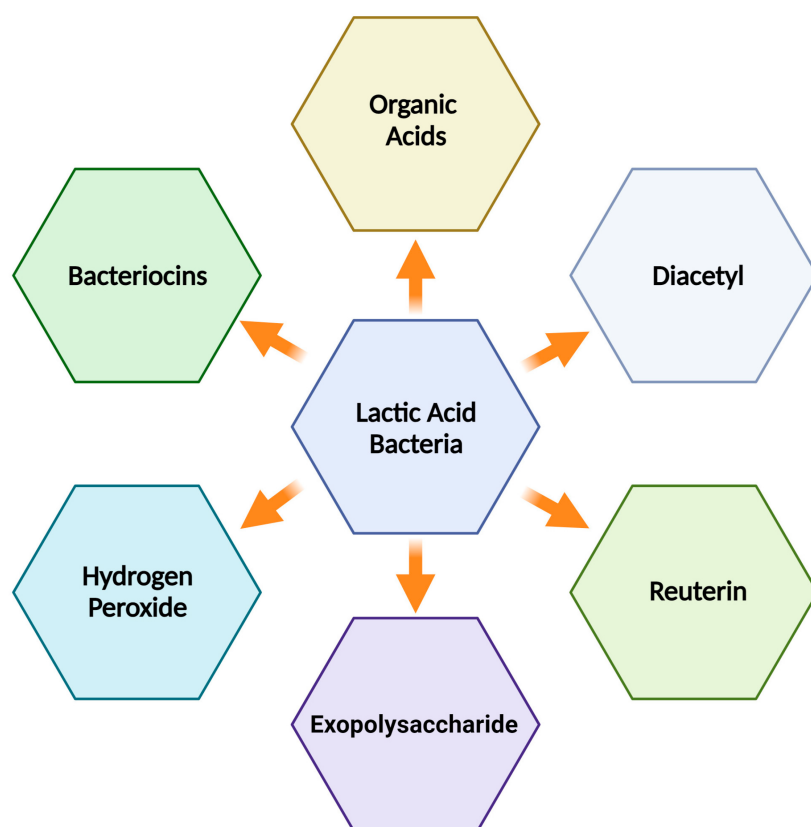


**Figure 2.** Diversity of antimicrobial compounds produced by LAB in dairy fermentation.

Reuterin (3-hydroxypropionaldehyde) is one of the prominent examples, which is produced by some strains of *L. reuteri* during the fermentation of dairy products such as cheese and yogurt, and it exhibits broad activity against foodborne pathogens [57][58]. Beyond reuterin, compounds such as diacetyl also emerge during fermentation, adding to the diverse antimicrobial arsenal of LAB [57]. Reuterin works by inducing oxidative stress inside microbial cells. This compound has a multi-targeted mechanism, interacting with thiol groups in enzymes and other cellular proteins, leading to impaired function. Additionally, reuterin disrupts microbial cell membranes, alters intracellular pH, and causes DNA damage [59]. On the other hand, diacetyl, a volatile compound naturally found in fermented dairy products, primarily exhibits its antimicrobial activity by lowering the internal pH of microbes. The acidic environment created by diacetyl destabilizes the microbial cell membrane, leading to a loss of essential molecules

and eventual cell death [60]. The synergistic action of reuterin and diacetyl has been emphasized, with these natural antibacterials enhancing the safety of acidified dairy products [57]. Recent studies have also expanded our understanding of these compounds. Sun et al. [61] reported that the reuterin system regulates intestinal flora and has anti-infection, anti-inflammatory, and anti-cancer properties. Additionally, Ortiz-Rivera et al. [62] evaluated the antimicrobial potential of reuterin, both in vitro and as part of a fermented milk product. Their findings indicated a stronger susceptibility of Gram-negative bacteria compared to Gram-positive bacteria to reuterin's action. Significantly, the presence of reuterin did not compromise the quality aspects of fermented milk, such as pH and acidity, indicating its efficacy as a preservative agent. In a separate study, Assari et al. [63] investigated LAB isolates for their antibacterial effects against pathogens such as *L. monocytogenes* and *S. aureus*. Their results indicate significant antimicrobial activity from diacetyl production. Besides its antimicrobial properties, diacetyl also plays a role in hydrolyzing milk proteins, enhancing food digestibility, and contributing to flavor [64].

Furthermore, the lactoperoxidase system (LPOS) has been highlighted as a promising preservative agent to combat foodborne pathogens [65]. The lactoperoxidase system is a naturally occurring antimicrobial in milk. At its core is the enzyme lactoperoxidase, which catalyzes the oxidation of thiocyanate (SCN-) in the presence of hydrogen peroxide ($H_2O_2$), yielding hypothiocyanite ions (OSCN-). These ions are particularly reactive and play a crucial role in the antimicrobial action. The hypothiocyanite ions disrupt bacterial cell membranes by oxidizing the sulfur-containing amino acid residues in proteins and enzymes. This oxidation damages bacterial membrane integrity, causing increased permeability, loss of vital molecules, and eventually cell lysis [66]. Al-Baarri et al. [65] examined the efficacy of the LPOS against *E. coli* in fresh cow milk and its related products to assess its antibacterial potency. They used enzymatic reactions, such as that with hydrogen peroxide, to derive the antimicrobial agents from LPOS. Their findings indicate that LPOS serves as a potent antibacterial agent.

The production of EPS by LAB has also gained attention in the food sector, specifically in dairy. These EPS influence the texture and consistency of fermented foods and play a vital role in providing antimicrobial benefits in such products [67]. Moreover, EPS represent a protective coating that can shield LAB by forming a physical barrier, blocking harmful bacteria, and trapping essential nutrients, giving EPS-producing bacteria an advantage over others [68]. Research by Angelin and Kavitha [69] and others has shown that EPS produced by LAB can exhibit protective effects both in vitro against a spectrum of pathogens, including those residing in the digestive system. However, the challenges of integrating EPS at industrial level include their modest yields and variability across different EPS-producing bacterial strains. Thus, there is a pressing need to explore new strategies for enhancing EPS production for prospective industrial uses [67]. While the potential of LPOS and EPS produced by LAB in dairy products has been increasingly recognized, several aspects require deeper exploration. The scalability of the use of LPOS as an antimicrobial agent in industrial dairy production is yet to be thoroughly evaluated. Given the global demand for dairy products and the constant need for safer preservation techniques, integrating natural antimicrobial agents such as LPOS could revolutionize dairy processing. Similarly, while the antimicrobial and textural benefits of EPS are evident, the exact mechanisms by which these compounds exert their effects are still being unraveled. This knowledge is crucial for manipulating LAB strains to consistently produce optimal EPS amounts and quality for specific dairy applications. The economic aspects of integrating these compounds on a

large scale, such as cost–benefit analyses comparing traditional preservatives versus LPOS or EPS, are also essential to consider.

Beyond the direct production of antimicrobial agents such as EPS, LAB also employ sophisticated communication systems to coordinate their activities. This communication, known as quorum sensing (QS), represents another dimension of how these beneficial microbes influence the fermentation process and, in turn, the safety and quality of dairy products. Traits regulated by QS, including bacteriocin production and acid stress tolerance, influence not just foodborne pathogens but also the shelf-life of food products [70][71]. Central to QS systems is the synthesis of autoinducers (AIs), low molecular weight signaling molecules, which are recognized and responded to by nearby bacterial cells [72][73]. Notably, several bacteriocins, potent antimicrobial peptides produced predominantly by Gram-positive bacteria, are synthesized in a QS-regulated manner [74]. QS is well-recognized in food microbiology due to its association with foodborne pathogenicity, spoilage, and biofilm formation [75]. Numerous studies indicate that various QS mechanisms are present in fermented foods. This suggests that modifying the involved QS systems can positively influence the quality of fermented foods [70]. One study evaluated the AI-2 activity in lactic-fermented foods and found varying AI-2 signaling intensities. Based on these findings, it is understood that interactions that take place both within and between species in kimchi likely involve AI-2 signaling activities, potentially influencing product variety [75]. Additionally, AI-2 signaling in LAB enhances the beneficial properties of fermented foods. QS molecules identified in spoiled products influence microbial diversity and metabolic actions. These molecules could serve as essential markers for monitoring dairy product quality during storage and for preventing spoilage [76].

This suggests potential avenues to positively influence fermented food quality by modulating inherent QS systems [70]. A comprehensive understanding of QS in LAB reveals a tri-component system: an autoinducing peptide (AIP), a membrane-bound histidine kinase (HK) sensor, and an intracellular response regulator (RR) [77][78].

In summary, dairy-fermented foods harbor a rich assortment of antimicrobial compounds, significantly enhancing the nutritional, safety, and sensory attributes of the product. LAB play an instrumental role in producing these compounds, with reuterin, $H_2O_2$, diacetyl, LPOS, and EPS as just a few noteworthy examples. Their combined effects safeguard the products against harmful pathogens and impart unique textures and flavors that consumers appreciate. However, challenges remain, particularly in scaling up the use of these compounds for industrial applications and ensuring consistent production. Additionally, the complex QS mechanisms employed by LAB underscore the complexity and sophistication of microbial interactions during fermentation. Applying these interactions and antimicrobial agents offers promising opportunities for the future of dairy processing. Moreover, the exploration of microbial compounds should not be limited to well-known metabolites.

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