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*Review*

# Current Updates on *Limosilactobacillus reuteri*: Brief History, Health Benefits, Antimicrobial Properties, and Challenging Applications in Dairy Products

**Emília Maria França Lima [1,2], Maria Eduarda Marques Soutelino [3], Adriana Cristina de Oliveira Silva [3], Uelinton Manoel Pinto [1,2], Svetoslav Dimitrov Todorov [1,2] and Ramon da Silva Rocha [1,2,*]**

1 Laboratório de Microbiologia de Alimentos, Departamento de Alimentos e Nutrição Experimental, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo 05508-000, Brazil
2 Food Research Center (FoRC), Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo 05508-000, Brazil
3 Departamento de Tecnologia de Alimentos, Faculdade de Medicina Veterinária, Universidade Federal Fluminense, Niterói 24230-340, Brazil
* Correspondence: ramonrocha@usp.br

**Abstract:** This study aims to clarify the use of *Limosilactobaillus reuteri* (*Lmb. reuteri*) in dairy products, emphasizing its main characteristics and limitations through a comprehensive literature review. *Lmb. reuteri*, previously classified as *Lactobacillus reuteri*, is a lactic acid bacterium (LAB) generally present in the gastrointestinal tracts of humans and other animals, such as sheep, chickens, and rodents. *Lmb. reuteri* was reclassified as part of the genus *Limosilactobacillus* in April 2020, reflecting advancements in biomolecular research that identified distinct metabolic and biochemical characteristics among strains. This species is an important producer of reuterin, an antimicrobial compound facilitated through glycerol fermentation via specific enzymatic pathways. In addition, selected strains of *Lmb. reuteri* can be considered probiotic bacteria with numerous health benefits and that lead to well-being improvements. It is consistently related to improvements in gut health, immune function enhancement, and cholesterol reduction. Furthermore, its application in dairy products has gained prominence and is increasingly reported in the literature due to its technological and sensory benefits. Despite the challenges of its incorporation into the dairy matrix, largely due to the need to supplement these products, it has already demonstrated significant effects on several dairy products' technological, sensory, and quality characteristics. Future research should address challenges like strain-specific efficacy and regulatory hurdles for the application of *Lmb. reuteri* in foods.

**Keywords:** *Lactobacillus reuteri*/*Limosilactobaillus reuteri*; biopreservative; probiotic; human health; functional foods

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Academic Editor: Clara G. De los Reyes-Gavilsn

Received: 19 December 2024
Revised: 12 February 2025
Accepted: 13 February 2025
Published: 21 February 2025

**Citation:** Lima, E.M.F.; Soutelino, M.E.M.; Silva, A.C.d.O.; Pinto, U.M.; Todorov, S.D.; Rocha, R.d.S. Current Updates on *Limosilactobacillus reuteri*: Brief History, Health Benefits, Antimicrobial Properties, and Challenging Applications in Dairy Products. *Dairy* **2025**, *6*, 11. https://doi.org/10.3390/dairy6020011



## 1. Introduction

*Limosilactobacillus reuteri* (*Lmb. reuteri*, abbreviated according to recommendations from 2023 [1]), formerly known as *Lactobacillus reuteri*, belongs to the nonsystematic group of lactic acid bacteria (LAB), isolated from a variety of ecological niches, including the gastrointestinal tract of humans and other animals. Regarding its safety, there is no systematic evidence suggesting that the bacterium is pathogenic; moreover, several strains from the species have been reported to have health-promoting effects and have even been recommended to be applied as probiotics [2].

The discovery of *Lmb. reuteri* dates back to the mid-20th century, when it was originally classified as part of the *Lactobacillus fermentum* species (which was also reclassified in 2020 as *Limosilactobacillus fermentum* [3]). The similarity in biochemical and physiological behaviour, and heterofermentative profile were some of the arguments for this classification of early isolated strains. Moreover, in the 1960s, Gerhard Reuter suggested that some specificity in the physiology and biochemistry of some specific strains deserved additional attention and suggested that they be named *Lab. fermentum* biotype II [4]. However, with the development of biomolecular tools in bacteriology, sufficient differences were observed between biotype II and other biotypes of *Lmb. fermentum*, which, in 1980, were used as arguments for a distinct species, and a formal species identity, *Lactobacillus reuteri*, was proposed [5], named after Gerhard Reuter. Moreover, in April 2020, as part of the massive reclassification of former genus *Lactobacillus*, *Lactobacillus reuteri* was reassigned to the genus *Limosilactobacillus* and called *Limosilactobacillus reuteri* [3].

It was reported that *Lmb. reuteri* inhabits humans and other animals (including sheep, chicken [6,7], pig [8], and rodent gastrointestinal tracts [9,10]). Some authors have even suggested that *Lmb. reuteri* maybe the only species of the lactobacilli species present in the gut of every tested host animal [11], with the interesting finding that each host seems to harbor its own specific strain of *Lmb. reuteri* [9,12]. Further investigations in the last 30 years have shown that *Lmb. reuteri* contributes to the health of its host organism as beneficial microorganisms and, also, some authors have even suggested that several strains can be considered as probiotics [13]. Moreover, strains of *Lmb. reuteri* have been associated with different fermented food products, particularly dairy and meats [4,14–16], most probably related to cross-contaminations between the primary origin of the recoded strains in food products and the gut environment. *Lmb. reuteri* strains were reported to be one of the dominant fermenting organisms in sourdoughs, and were proposed to be associated with the production of specific exopolysaccharides and to play a role in the conversion of glutamine to glutamate, important features in improving bread quality [17].

*Lmb. reuteri* is a well-studied species as a producer of reuterin; however, reports have shown evidence that other LAB can also be considered to be reuterin producers. An example is *Loigolactobacillus coryniformis* WBB05, shown to be a reuterin producer when cultured in aerobic conditions [18]. An important point is that the production of reuterin by *Lmb. reuteri* (and maybe even *Lob. coryniformis*) involves a specific biochemical pathway. For the metabolic process of producing reuterin, glycerol fermentation is essential [19]. The metabolic processes include the enzyme glycerol dehydratase, where this coenzyme B12-dependent enzyme converts glycerol into 3-hydroxy propionaldehyde (3-HPA), also known as reuterin [20,21].

An interesting characteristic is that representatives from *Lmb. reuteri* are few of the LAB that can produce reuterin, an interesting broad-spectrum antimicrobial metabolite, with specific its inhibitory properties against pathogens and spoilage bacterial species [20,21]. In the last few decades, with the development of the concept of probiotics, the representatives strains of *Lmb. reuteri* have been suggested to be probiotic, in which their health benefits are not only associated with production of reuterin, but also with advantages associated with effective contributions to the improvement of gut health, enhancing immune function, and reducing cholesterol levels [10]. In the literature and as commercial products, strains of *Lmb. reuteri* have been suggested as probiotics for improving gut and oral health, and even linked to benefits related to potential positive effects on the gut-brain axis [10,22].

With the development of bioinformatic research tools, extensive comparative genomic analyses have revealed distinct genetic profiles of *Lmb. reuteri* that contribute to their unique metabolic capabilities and health benefits [10]. These differences highlight each bacterium's unique roles in health and their specific applications as probiotics [10,22].

It was reported that some strains of *Lmb. reuteri* can be beneficial by improving the balance of the gut microbiota, where, in addition to the previously reported improvement and maintenance of a healthy balance between the main phyla of gut populations [22], some strains can even actively contribute to the digestion of food components and processes of absorption of nutrients [23]. Some strains of *Lmb. reuteri* can actively contribute to processes of improvement of some gastrointestinal disorders, such as irritable bowel syndrome (IBS) and diarrhea [24], or the reinforcement of immune defenses, by enhancing the body's natural defenses against pathogens [25], or can even be involved in the modulation of immune responses via reducing the likelihood of autoimmune diseases [26]. It was suggested that strains of *Lmb. reuteri* can bring benefits to the host related to their positive effect of reducing dental plaque and the risk of oral inflammations [27]. Moreover, as a consequence of actively reducing some oral-associated pathogens, beneficial strains of *Lmb. reuteri* can play a role in combatting bad breath [28]. Furthermore, for specific strains of *Lmb. reuteri* anti-inflammatory properties have been reported that can benefit overall health [24], including beneficial contributions to improving skin conditions like eczema [29].

In the food and health industry, strains of *Lmb. reuteri* have been applied as probiotic supplements and as part of fermented foods or as pharmaceutical supplements with probiotic properties [30]. When consumed and they reach the gut, *Lmb. reuteri* beneficial strains may play a positive role in improving digestive health. Several randomized controlled trials pointed out that *Lmb. reuteri* DSM 17938 can reduce diarrhea incidence and duration in children and adults [31]. Scientific evidence has shown the anti-inflammatory properties of *Lmb. reuteri* strains. A study by Zhang et al. [32] evaluated three strains of *Lmb. reuteri* regarding their anti-inflammatory properties and beneficial assets in protecting against alcohol-induced intestinal barrier damage and suggested their modulation of the immune responses in a mouse model [32].

Probiotic strains of *Lmb. reuteri* can be beneficial in wound healing recovery due to increased collagen deposition, as shown successfully in an animal model [33]. Moreover, they may be associated with the production of reuterin but not excluding the presence of several additional antimicrobial metabolites that can be produced by LAB [34]. *Lmb. reuteri* LMG P-27481 was found to significantly reduce the growth of *Clostridium difficile* and decrease colitis in a mouse animal model [35]. Several studies have explored the probiotic role of *Lmb. reuteri* in various human body sites, including the gastrointestinal tract, urinary tract, and skin, and also in breast milk, highlighting its broad potential benefits [21,36].

This review aims to present relevant studies on the use of *Lmb. reuteri* in dairy products, emphasizing its history, technological applications, main health benefits, and pathogen control, in addition to a critical analysis of its main limitations and challenges.

## 2. Technological Applications of *Limosilactobacillus reuteri* in Dairy Products

*Lmb. reuteri* has been widely used as an important probiotic and producer of antimicrobial metabolites in the formulation of several dairy products, especially fermented milk [37], yogurts [38–40], and cheeses [41–44], demonstrating a high survival capacity in dairy matrices and secondary effects on parameters such as pH, water activity, aromatic profile, texture, and viscosity. Table 1 presents the main technological effects of applying *Lmb. reuteri* in dairy products. In this scenario, the processing of fermented products without refrigeration and with low post-acidification is of great interest to the industry, as it reduces transportation and storage costs and expands the market economically [45]. *Lmb. reuteri* is a suitable alternative, as it can present high stability and the controlled production of organic acids during product storage. Chen et al. [38] found that *Lmb. reuteri* WHH1689 presented a high survival rate (4.89 $log_{10}$ CFU/mL) in drinkable yogurts after 145 days of storage at 28 °C. Furthermore, when fermented and stored at 37 °C for 95 days, the yogurt-like product fermented by this microorganism presented very weak post-acidification, with a pH maintained at 4.03. On the other hand, Fayyaz et al. [39] reported high post-acidification of *Lmb. reuteri* IBRC-M10755 (5%) in fermented milk at 42 °C for 5 h, with a reduction in the pH from 4.2 to 3.4, an increase in the water holding capacity (%) from 19.54 to 25.62, and an increase in the hardness (g) from 22 to 25 ($p < 0.05$) after 30 days of refrigerated storage. Compared to samples fermented by *Lactobacillus helveticus* and *Lactobacillus acidophilus*, the fermented milk with *Lmb. reuteri* added exclusively also showed a higher apparent viscosity on the 10th day of storage (0.250) ($p < 0.05$), in addition to higher scores for color (between 3.9 and 4.1) and texture (between 3.7 and 3.9) in a sensory analysis.

Another study performed by Al-Nabuls [44] with white cheeses brined at temperatures of 10 and 25 °C and NaCl concentrations of 10% and 15% (*w*/*w*), revealed a high survival rate (5.78 to 5.83 log10 CFU/mL) of *Lmb. reuteri* strains (SS730, S3608, MM-2, CF2, and RC14) after 28 days of storage. Furthermore, adding *Lmb. reuteri* did not significantly impact the water activity of the samples ($p > 0.05$). It reduced the pH (6.48 to 4.69) only in cheeses subjected to brine at 10% NaCl and a temperature of 25 °C ($p < 0.05$). The fermentation conditions of the studies indicate that time, temperature, and the salinity of the matrix can modify the metabolic dynamics of *Lmb. reuteri*, the amount of organic acids produced and their consequent microstructural, physicochemical, and sensory alteration in the dairy matrix. For example, salt concentrations above 10% appear to partially inhibit the activity of *Lmb. reuteri* through osmotic stress, while temperatures below 30 °C for a more extended period can prolong its latency and growth phase, reducing its enzymatic activity.

**Table 1.** Some examples of applications of *Limosilactobacillus reuteri* strains in dairy products.

| Dairy Product | *Lmb. reuteri* Strain | Fermentation Conditions | Supplementation | Main Results | Reference |
|---|---|---|---|---|---|
| Yogurt * | ATCC53608 | 40 °C for 4 h (pH between 5 and 5.5) | 200 mM glycerol | *Lmb. reuteri* associated or not with 200 mM glycerol did not result in significant changes in pH, acidity, soluble solids, color, or rheological aspects of a yogurt during 28 days of storage at 4 °C ($p > 0.05$). The synthesis of reuterin by *Lmb. reuteri* in the product reached a maximum value of 33.97 ± 4.90 mM after supplementation with glycerol ($p < 0.05$). | [37] |
| Drinkable yogurt | WHH1689 | 28 or 37 °C | - | High survival rate of 4.89 and 4.65 log10 CFU/mL after 145 days of storage (28 °C and 37 °C respectively). Very weak post-acidification after 95 days of storage at 37 °C with pH maintained at 4.03. | [38] |
| Yogurt | IBRCM10755 | 42 °C for 5 h | - | Reduced pH and increased water activity and hardness after 30 days of storage. Increased viscosity and higher scores for color and texture after 10 days of storage. | [39] |

**Table 1.** *Cont.*

| Dairy Product | *Lmb. reuteri* Strain | Fermentation Conditions | Supplementation | Main Results | Reference |
|---|---|---|---|---|---|
| Semi-hard sheep's cheese | INIA P572 | - | 30 mM glycerol | *Lmb. reuteri*–glycerol association increased cell-free aminopeptidase activity, overall proteolysis and free amino acids ($p < 0.05$). *Lmb. reuteri*–glycerol reduced hardness and elasticity, increased pH and gave the cheese a pink appearance after 90 days of storage ($p < 0.05$). In the sensory evaluation, *Lmb. reuteri*-glycerol resulted in increased flavor scores ($p < 0.05$) and the scores for texture and color quality were not affected ($p > 0.05$). Reuterin synthesis was identified only in cheese with added *Lmb. reuteri*–glycerol. | [41] |
| Semi-hard sheep's cheese | INIA P572 | 32 °C to 25 min | 30 mM glycerol | *Lmb. reuteri*-glycerol increased the formation of twelve volatile compounds but decreased the formation of five ($p > 0.05$). *Lmb. reuteri*-glycerol did not impact the odor and aroma scores of sheep cheese ($p > 0.05$), but significantly decreased odor intensity scores ($p < 0.05$) after 90 days of storage. Samples with added *Lmb. reuteri*-glycerol received higher scores for "cheesy" aroma, and lower scores for "milky", "caramel" and "yogurt-like" aroma ($p < 0.05$). | [42] |
| Fresh cream cheese | DSM20016 | - | 5 g of FOS in 1 L of pasteurized milk | The addition of *Lmb. reuteri* resulted in lower sensory scores for color, texture, and overall acceptance, but higher scores for odor ($p < 0.05$). The addition of FOS did not impact the sensory parameters of the samples added with *Lmb. reuteri* ($p > 0.05$); and resulted in a significant increase in *Lmb. reuteri* counts after 28 days of storage at 4 °C ($p < 0.05$). | [43] |
| Cheese | SS730, S3608, MM-2, CF2 e RC14 | 37 °C for 30 to 40 min | - | High survival rate (5.78 to 5.83 log10 CFU/mL) after 28 h of storage. No significant change in the water activity of the samples ($p > 0.05$). Reduction in pH in cheeses subjected to brine at 10% NaCl and a temperature of 25 °C ($p > 0.05$). | [44] |

-: Information not provided in the referenced work; * term yogurt used according to the cited reference.

Different strains of *Lmb. reuteri* also influence the quality of dairy products differently. *Lmb. reuteri* IBRC-M10755 [39] demonstrates high β-galactosidase activity and more efficient lactic acid production, resulting in a faster reduction in the dairy product matrix pH. These microorganisms may also be more tolerant to low pH and continue to produce organic acids during product storage. In contrast, *Lmb. reuteri* WHH1689 [38] may reduce its metabolic activity as the pH decreases.

A relevant observation is that most studies on applying *Lmb. reuteri* in dairy products address matrix supplementation to increase *Lmb. reuteri* viability and reuterin production in situ. Biopreservation systems for yogurt-like product and cheese from *Lmb. reuteri* associated with glycerol were first described by Langa et al. [46]. This compound is the most commonly used in conjunction with *Lmb. reuteri*, as it can promote cryoprotective, osmoprotective, protein stabilization, and anti-oxidative damage effects [47], in addition to acting as a source of carbon and energy for the synthesis of reuterin during its anaerobic metabolism [41]. Preliminary studies have shown that the association of this compound with *Lmb. reuteri* does not impair milk fermentation by starter bacteria [37].

Ortiz-Rivera et al. [37] reported that *Lmb. reuteri* ATCC 53608, associated or not with 200 mM glycerol and under fermentation conditions of 40 °C for 4 h, did not result in significant changes in the pH, acidity, soluble solids, color, and rheological aspects of yogurt-like product during 28 days of storage at 4 °C ($p > 0.05$). Furthermore, the synthesis of reuterin by *Lmb. reuteri* in the product reached a maximum value of 33.97 ± 4.90 mM after supplementation with glycerol (g/kg) ($p < 0.05$). This alternative has also been efficient in cheese processing [41,42]. Garde et al. [41] applied a combination of *Lmb. reuteri* INIA P572 and 30 mM glycerol to semi-hard sheep cheese. In the subsequent experiments, they found greater cell-free aminopeptidase activity (Lys-p-NA of 2.94 and Leu-p-NA of 2.35), general proteolysis (2.01 absorbance at 340 nm), and free amino acids (FAAs) (17.53 g/kg) compared to the control cheese or with the addition of only *Lmb. reuteri*. The *Lmb. reuteri*–glycerol association also reduced the hardness from 0.24 to 0.20 (Jules) and elasticity from 1.65 to 0.70 (Newtons/mm$^2$), increased the pH from 4.92 to 5.25, and gave the cheese a pink appearance after 90 days ($p < 0.05$). The sensory evaluation (10-point scale) significantly increased the taste quality scores from 5.15 to 6.33, and the texture and color quality scores were not affected ($p > 0.05$). The authors also reported that in situ reuterin production by *Lmb. reuteri* was detected only in the cheese supplemented with glycerol, representing a promising alternative to boosting the antimicrobial activity in this product.

Gómez-Torres et al. [42] found that supplementation with *Lmb. reuteri* INIA P572 in combination with 30 mM glycerol could also increase the formation of twelve volatile compounds but decrease the formation of five in sheep cheese. The authors reported that *Lmb. reuteri*–glycerol treatment did not impact the odor and aroma scores of cheese containing *Lmb. reuteri* ($p > 0.05$). However, it resulted in a significant decrease in odor intensity scores (5.88 to 4.52) ($p < 0.05$) at the end of 90 days of storage. Furthermore, this sample received significantly higher scores for "cheesy" aroma (4.32) and lower scores for the "milky" (2.38), "caramel" (1.87), and "yogurt-like" (1.92) aroma attributes ($p < 0.05$). Gómez-Torres et al. [42] concluded that *Lmb. reuteri* combined with glycerol can create an adequate biopreservation system in cheeses and positively influence the biochemical processes associated with ripening and the development of flavor, aroma, and texture. From another perspective, Speranza et al. [43] studied supplementation with prebiotics to optimize the application of *Lmb. reuteri* DSM 20016 in fresh cream cheese. The authors found that adding fructooligosaccharide (FOS) to the cheese increased the *Lmb. reuteri* count from 8.26 to 9.74 Log CFU/g after 28 days of storage at 4 °C. However, compared to the addition of *Bifidobacterium animalis*, the addition of *Lmb. reuteri* to the cheese resulted in lower scores for color (3.89), texture (3.21), and general acceptance (4) but a higher score for odor (3.91) ($p < 0.05$), with no significant influence of FOS ($p > 0.05$). Compared to glycerol, FOS appeared to have less impact on the sensory profile of yogurt fermented with *Lmb. reuteri*, an alternative to maintaining quality parameters.

Natural ingredients as a source of prebiotics can also favor the aromatic profile and increase the functional and sensory properties of yogurts produced with *Lmb. reuteri*. Mohan et al. [40] produced a yogurt with *Lmb. reuteri* DPC16 under fermentation conditions of 35 °C (until the pH reached 4.5) and reported the greater production of lactic and propionic acids in samples supplemented with Manuka honey than samples supplemented with inverted syrup or without sweetener. Other researchers have also reported that the production of organic acids and aromatic compounds by *Lactobacillus* can significantly increase when associated with prebiotics during storage [48–50].

## 3. Health Benefits of *Limosilactobacillus reuteri*

### *3.1. Oral Health*

It has been clearly shown that some strains of *Lmb. reuteri* can be beneficial and effective probiotic bacteria improving oral health, and their role in the reduction in gingivitis and periodontal disease has been scientifically explored. Studies reported on the probiotic properties of *Lmb. reuteri* in managing gingivitis and periodontal disease found a reduction in inflammation processes and inhibition of associated diseases by pathogenic bacterial species in the mouth [27,51]. Moreover, *Lmb. reuteri* strains were effective in reducing dental plaque, considered one of the main contributors to cavities and gum disease [52]. Strains of *Lmb.* reuteri have been reported for their contribution to combating bad breath by reducing the levels of odor-causing bacteria through the production of various antimicrobials, including reuterin [30], or decrease oral inflammation, where overall oral health was also improved after the application of *Lmb. reuteri* beneficial strains [27]. The fact that *Lmb. reuteri* strains can produce different antimicrobials, and that some of them even have effective inhibitory properties against *Candida* spp. pointed to another possible beneficial application [53]. These benefits make *Lmb. reuteri* a valuable addition to oral health care routines, and could be in the form of probiotic supplements and certain dental products.

### *3.2. Role of Limosilactobacillus reuteri in Mental Health Through the Gut–Brain Axis*

*Lmb. reuteri* has been reported to have beneficial properties in improving humans mood and anxiety; specifically, probiotic strains can play a key role in the production of serotonin. This neurotransmitter plays a crucial role in mood regulation and it has been suggested that some strains may help reduce stress and anxiety by modulating the gut–brain axis [54]. The probiotic properties of some strains of *Lmb. reuteri* were reported regarding improvements in individuals with autism spectrum disorder (ASD), with potential improvements in social behavior. Research performed in mouse models with induced ASD showed positive results, including improved social behaviors, potentially linked to increased oxytocin levels [55]. Moreover, it has been suggested that strains of *Lmb. reuteri* may be beneficial in reducing neuroinflammation, linked to cognitive decline and neurodegenerative diseases [21], or via strengthening the intestinal barrier; *Lmb. reuteri* may improve and even prevent inflammation affecting brain health [21].

### *3.3. Safety of Limosilactobacillus reuteri*

Most strains of *Lmb. reuteri* are generally considered safe for application in human and other animals. However, some important safety issues should be considered. As it has clearly been suggested in recent decades, the safety of microbial cultures needs to be a priority in evaluation and suggestions for any microbial culture to be deemed beneficial in foods. The fact that some species have a long history of safe use or have received a Generally

Recognized As Safe (GRAS) status by the FDA of the USA or a Qualified Presumption of Safety (QPS) by the European Food Safety Authorities (EFSA) does not exclude the need to evaluate the safety profile of specific strains.

It has been reported that most individuals can tolerate *Lmb. reuteri* well. However, some potential side effects may include mild gastrointestinal symptoms like diarrhea or constipation [56]. Moreover, in some specific cases, side effects can be associated with supplement components of pharmaceutical preparations, where, in addition, to the bioactive component (the probiotic itself), the commercial preparations contain additives, stabilizers, flavors, or sweeteners.

A concern raised by some research groups is related to the long-term application of probiotics and the possibility of overloading the gut with specific strains and even changes in the balance between different taxa in the gastrointestinal tract [57]. Even though it has been reported that, in some cases, the application of *Lmb. reuteri* strains for up to 6 months does not show any indications for concern, this is a factor that must be specifically evaluated for each strain intended for commercialization as a probiotic [58].

As was suggested for *Lmb. reuteri*, and for most probiotics, in some specific cases, such as a compromised immune system (e.g., due to chemotherapy or HIV), some applications of probiotics may have negative consequences for consumers [59]. The same rules must always be applied to pregnant women, where applications of any probiotic need to be in accordance with a physician's prescription and monitoring [60].

Potential interactions with medications can occur with probiotics, including *Lmb. reuteri*. A simple example is the fact that antibiotics can exert negative effects on probiotics when consumed in combination. The timinge of the application of probiotics and antibiotics needs to be different to avoid reducing their effectiveness. Moreover, some authors suggest that moderate antibiotic resistance in probiotics can be considered beneficial, so some antibiotics and probiotics could be applied in combination [61]. However, when addressing this issue, it is important to confirm that any observed antibiotic resistance in probiotic cultures is not associated with mobile genetic elements in order to rule out possible scenarios of horizontal genes transfer [62].

The use of LAB and their metabolites for biopreservation has gained attention as a strategy to enhance food safety, particularly through the use of reuterin. Despite the good antimicrobial activity, the application of reuterin in foods and food contact surfaces should be evaluated in light of its potential toxicity effect, particularly considering that some molecules of the reuterin system, such as acrolein may be toxic. As mentioned by Ponzio et al. [63], one aspect that still raises questions concerns acrolein (one of the molecules that are part of the reuterin system, as seen in Section 4) and the potential risk of certain diseases arising from its interaction with the human body. To date, no studies in the existing literature deal with this risk in the long term. For instance, the World Health Organization classifies acrolein as a Class 2A carcinogen [64]. Even though there are concerns about the safety of reuterin, studies show that *Lmb. reuteri* consumed as probiotic can positively affect the gut microbiome and function, with important implications to reducing colon tumor growth [65,66]. For food consumption with reuterin, further research is needed to establish safe daily intakes.

## 4. Pathogen Control and Antimicrobial Properties of *Limosilactobacillus reuteri*

The antimicrobial properties of *Lmb. reuteri* are due to its capacity to produce a range of inhibitory compounds including lactic acid, acetic acid and ethanol due to its heterofermentative metabolism, bacteriocin (reutericin 6), reutericyclin (an antimicrobial compound related to tetramic acids) and reuterin [21,67]. Despite the possibility of producing multiple antimicrobial compounds, several strains of *Lmb. reuteri* differ in their capacity to produce these different molecules.

Reuterin is the general term given to a mixture of different forms of 3-hydroxypropionaldehyde (3-HPA), including its monomer (3-HPA), 3-HPA dimer, 3-HPA hydrate, acrolein, and 3-hydroxypropionic acid [64,68]. It is considered the most important antimicrobial system produced by *Lmb. reuteri*. The term "reuterin" generally refers to 3-HPA since this is the main antimicrobial compound in the reuterin system [64]. It is synthesized during glycerol fermentation under anaerobic conditions and exhibits broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi, yeasts, and even protozoa [67,68].

The antimicrobial activity of reuterin is related to its ability to induce oxidative stress in target cells. Reuterin causes the depletion of free thiol groups in glutathione (GSH), affecting proteins, including important microbial enzymes, thereby disrupting the cellular redox balance and leading to bacterial cell death. Consequently, reuterin inhibits bacterial growth through thiol group modification, demonstrating its broad negative impact on numerous cellular targets [68,69]. Regarding its antifungal activity, a more recent in silico study concluded that its mechanism operates at the enzyme level by inhibiting catalase, catalase-peroxidase, and spore-specific catalase in *Aspergillus flavus*. Reuterin induces cell ROS accumulation by competing for binding to enzyme active sites [70]. The broad spectrum of activity may affect metabolic processes of pathogens or spoilage microorganisms (and even beneficial organisms), resulting in inhibition of their growth [19].

Reuterin (3-HPA) is a neutral, low-molecular-weight, water-soluble compound that remains active across a wide pH range and is resistant to proteolytic and lipolytic enzymes. These characteristics make it suitable for various applications, including food preservation [71,72]. Since reuterin is stable under various processing conditions, the system helps maintain the quality and safety of the food products. Additionally, its application does not negatively affect the sensory properties of the food [73].

Most strains of *Lmb. reuteri* from human origin can produce reuterin [21]. *Lmb. reuteri* converts glycerol into reuterin through a process catalyzed by the enzyme glycerol dehydratase, where the production of reuterin is influenced by factors, including glycerol concentration, pH, temperature, and the growth stage of the bacterial culture [21,68,74]. Sun et al. [74] reported that for the strain *Lmb. reuteri* DPC16, the highest yield of reuterin was achieved with specific conditions such as 21 g/L of biomass of a 24-h-old culture converting a 300 mmol/L glycerol solution at 30 °C and pH 6.2 [74]. However, Mishra et al. [75] reported the optimal production of reuterin from *Lmb. reuteri* BPL-36 was when the strain was cultured for 2.5 h in water-glycerol medium (267 mM concentration of glycerol), with pH 6.0, incubation temperature 37 °C, where the yield of reuterin was 58.69 mM. Interestingly, some other species can also produce reuterin, including members of the genus *Bacillus*, *Klebsiella*, *Citrobacter*, *Enterobacter*, and *Clostridium* and lactobacilli [71]. Strains of *Lmb. reuteri*, as well as reuterin itself, are effective against a variety of foodborne pathogens and spoilage microorganisms, as described in Table 2. The antimicrobial property of reuterin is particularly effective against enteropathogens related to the gut environment of humans and other animals [53].

In the study of Lin et al. [76], *Lactobacillus gasseri*, *Ligilactobacillus salivarius* and *Lmb. reuteri* were isolated from intestinal microbiota in fecal samples from dairy cows. The cell-free supernatants of *Lmb. reuteri* exhibited outstanding inhibitory activity against some *Salmonella* serovars (*S.* Enteritidis, *S.* Typhimurium, and *S.* Dublin) and also displayed potent inhibitory activity against *Mycobacterium avium* subsp. *paratuberculosis*, which is also an important pathogen.

The antimicrobial activity of *Lmb. reuteri* against *Escherichia coli* strains has also been evaluated in many studies. Recently, Ali et al. [77] observed that *Lmb. reuteri* PSC102, isolated from pig fecal samples, significantly inhibited the growth of enterotoxigenic *E. coli.* Bertin et al. [78] observed that *Lmb. reuteri* LB1-7, a strain derived from raw cow milk, possesses antimicrobial activity against an enterohaemorrhagic *E. coli* O157:H7 strain via hydroxypropionaldehyde (HPA) under strict anaerobiosis. The growth inhibition of *E. coli* O157:H7 was also observed by the presence of *Lmb. reuteri* strains in white-brined cheeses under different storage conditions [44].

Interestingly, Asare et al. [68] evaluated the co-culture of *Lmb. reuteri* and *Campylobacter* strains with or without glycerol, demonstrating the sensitivity of *Campylobacter jejuni* and *Campylobacter coli* isolates to reuterin. *C. jejuni* was killed only during co-cultures in the presence of glycerol, suggesting the production of reuterin by *Lmb. reuteri*.

The use of LAB and their metabolites for biopreservation has gained growing attention in recent decades as a strategy to enhance food safety and prolong the shelf life of food products, in addition to having a natural appeal. The reuterin system holds promise as a food preservative due to its potent antimicrobial activity against different microorganisms and its high stability under different food processing conditions [44,64]. Reuterin can be used in food products through in situ production by adding *Lmb. reuteri* and food-grade glycerol directly to the food. This method does not require regulatory approval or label declarations [64]. Moreover, purified reuterin can also be added, but this approach may require regulatory approval depending on the region, considering regulatory agency exigences.

Some examples of applications of reuterin include dairy products, where reuterin was used to extend the shelf life of dairy products by inhibiting foodborne pathogens and spoilage bacterial species [64]. Pilote-Fortin et al. [79] demonstrated the antifungal and anti-yeast activity of reuterin in milk and yogurt. In stirred yogurt (4 °C), the addition of reuterin at 10 mM allowed total inhibition of *Mucor racemosus* and *Rhodotorula mucilaginosa* within 1 week and reduced *Aspergillus niger* counts by 3 log cycles over 4 weeks. Vimont et al. [80] also demonstrated the antifungal activity of purified reuterin against many fungal and yeasts in vitro, besides in yogurts added with *M. racemosus* and *Penicillium chrysogenum*. Similarly, Ortiz-Rivera et al. [37] observed that *Penicillium expansum* was the most sensitive microorganism to reuterin in a fermented milk product. Since fungi are highly problematic spoilage microorganisms in dairy products, these findings suggest that reuterin can be applied as a preservative in dairy products.

In meat products, such as sausages, cold cuts, and processed subproducts, reuterin plays a leading role in the preservation of the meat products by reducing the growth of foodborne pathogens and spoilage, including *Listeria monocytogenes* and *Salmonella* spp. [64]. Some authors suggested the use of reuterin to preserve vegetables. In fact, some studies confirmed that reuterin can preserve minimally processed fresh lettuce, enhancing its safety and shelf life [68]. Reuterin was also used to preserve fruit juices, preventing microbial spoilage without affecting the food products' sensory characteristics or nutritional value [64].

Montiel et al. [81] also tested the use of reuterin to control foodborne pathogens in fishery products. Purified reuterin (10 AU/g) demonstrated a significant inhibitory effect on the growth of *L. monocytogenes* in cold-smoked salmon under moderate or severe temperature abuse. These findings suggest that reuterin could serve as a hurdle technology to enhance safety and extend the shelf life of seafood products.

Another application of reuterin that has been studied is in edible packaging. Recently, Rodrigues et al. [82] observed the inhibitory effect of *Lmb. reuteri* and reuterin incorporated into edible films against foodborne pathogens, including *Bacillus cereus*, *Clostridium perfringens*, *Pseudomonas aeruginosa*, and some fungi. The authors concluded that edible films containing reuterin could be a promising alternative for food preservation due to the reuterin antimicrobial action. In another approach, Hernández-Carrillo et al. [72] demonstrated that pectin-based edible coatings enriched with reuterin, and lemon essential oil have the potential to preserve strawberries, as they reduced spoilage by *Penicillium* sp., extending the shelf life of the fruit beyond the study period (31 days). Furthermore, Xu et al. [83] reported that sub-inhibitory concentrations of reuterin effectively reduced biofilm formation and the motility of *C. perfringens*, presumably through *quorum sensing* (QS) inhibition.

**Table 2.** Recent studies on antimicrobial activity of *Limosilactobacillus reuteri* and reuterin against spoilage and pathogen microorganisms.

| ***Lmb.* reuterin Strains** | **Concentration/ Methodology** | **Target Microorganisms** | **In Vitro/In Situ Assay** | **Reference** |
|---|---|---|---|---|
| *Lmb. reuteri* strains | Viability of *E. coli* and *Lmb. reuteri* | *E. coli* O157:H7 | In situ: white-brined cheese | [44] |
| Cell-free supernatants (CFS) of *Lmb. reuteri* | 100 µL or 200 µL, tested in solid medium | *S.* Enteritidis, *S.* Typhimurium, and *S.* Dublin | In vitro | [76] |
| *Lmb. reuteri* PSC102 | Strains: time-kill assay CFS: disk diffusion method | Enterotoxigenic *E. coli* | In vitro | [77] |
| *Lmb. reuteri* LB1-7 and HPA | Agar spot test | *E. coli* O157:H7 | In vitro | [78] |
| *Lmb. reuteri* cells | Inhibitory effect of *Lmb. reuteri* strains was calculated from the log response ratio. | *B. cereus*, *C. perfringens*, *P. aeruginosa*, *Fusarium oxysporum*, *Colletotrichum gloeosporioides*, *Alternaria alternata*, and *Penicillium digitatum* | In vitro and in situ: edible alginate-konjac gum film containing *Lmb. reuteri* cells. | [82] |
| Reuterin synthesis by *Lmb. reuteri* ATCC53608 in fermented milk | Survival of spoilage and pathogenic microorganisms with reuterin | *P. expansum*, *E. coli*, *L. monocytogenes*, *Staphylococcus aureus*, and *Salmonella* spp. | In situ: fermented milk product | [37] |
| Reuterin produced by *Lmb. reuteri* PTA5_F13 | 24 h co-cultures with or without glycerol | *C. jejuni* and *C. coli* | In vitro | [68] |
| Reuterin produced by *Lmb. reuteri* ATCC55730 | *Penicillium* sp. growth in solid medium and visual observation of the fruits (27 days) | *Penicillium* sp. conidia | In situ: reuterin in pectin edible coatings incorporated into strawberries | [72] |
| Purified reuterin produced by *Lmb. reuteri* ATCC53608 | Purified reuterin (1 to 10 mM); and agar diffusion assay | *R. mucilaginosa*, *A. niger*, *P. chrysogenum*, and *M. racemosus* | In situ: milk and yogurt | [79] |

**Table 2.** *Cont.*

| *Lmb.* reuterin Strains | Concentration/ Methodology | Target Microorganisms | In Vitro/In Situ Assay | Reference |
|---|---|---|---|---|
| Purified reuterin produced by *Lmb. reuteri* ATCC53608 | Purified reuterin: fungicidal effect at 15.6 mM or less | In situ: antifungal activity against *P. chrysogenum* and *M. racemosus* | In vitro and in situ: yogurts. | [80] |
| Reuterin produced by *Lmb. reuteri* | Inhibition measured by growth, cell morphology, biofilm formation, and motility | *C. perfringens* ATCC 13124 | In vitro | [83] |
| Purified reuterin produced by *Lmb. reuteri* INIA P579 | Purified reuterin (10 AU/g); inhibition measured by optical density (growth curves). | *L. monocytogenes* | In situ: cold-smoked salmon | [81] |

In some referenced works, strain identifications were not provided.

## 5. Challenges and Limitations in the Use of *Limosilactobacillus reuteri* in Dairy Products

Despite the numerous probiotic, technological and antimicrobial benefits of *Lmb. reuteri*, its application in dairy products may present limitations, mainly related to its stability, impact on product quality, and the need for food matrix supplementation.

In some cases, *Lmb. reuteri* may present low survival and reduced metabolism when exposed to acid, osmotic, and thermal stresses [84], which limits its application in various formulations. On the other hand, studies by Fayyaz et al. [39] and Al-Nabuls et al. [44] concluded that some strains of *Lmb. reuteri* exhibit high resistance to acidic environments but can also cause intense post-acidification, resulting in undesirable changes in flavor, increased viscosity or excessively firm texture in yogurt and other fermented milk products. Furthermore, viable counts higher than $1.5 \times 10^8$ CFU/mL can cause textural defects in yogurt [85]. Therefore, the careful selection of the strain and adjustment of fermentation conditions can represent obstacles to developing new products, mainly due to potential physicochemical and sensory alterations in the dairy matrix.

Another factor that demands attention is that *Lmb. reuteri* can produce biogenic amines, especially histamine and tyramine, under certain environmental conditions [86]. These substances are often responsible for food poisoning and allergies in sensitive consumers [87]. Body et al. [86] found that NaCl concentrations in sheep's milk cheese of $\geq$3% ($w/v$) can significantly inhibit the growth of different strains of *Lmb. reuteri* (CCM 3642, 3643, and 3644), but also their tyramine and histamine production ($p < 0.05$). Determining manufacturing processes and salt concentrations suitable for satisfactory growth of *Lmb. reuteri*, but with controlled production of allergenic substances, can also be challenging to manufacture high-quality and safe products.

As observed in Section 2, matrix supplementation can significantly impact the growth of *Lmb. reuteri* and its metabolite production. However, this alternative can also present challenges related to the appropriate choice of the supplement and its concentration. For example, Champagne et al. [85] found that enriching milk with sugars, minerals, or peptone-based ingredients did not improve the growth of *Lmb. reuteri* in drinkable yogurt fermented at 42 °C for 4 h. Furthermore, Martín-Cabrejas et al. [88] reported that the development of a slightly pink color was related to some compounds produced when higher levels of glycerol were present in cheese produced with *Lmb. reuteri*, but not due to reuterin production.

Overall, the findings in the literature reveal the complexity of the interactions of *Lmb. reuteri* with environmental factors and reinforce the need for further research on the effects of its application in different dairy matrices. In addition, the scarcity of studies on new supplementation strategies for this microorganism limits the launch of new formulations, especially with functional appeal.

## 6. Conclusions

This review highlights the diverse applications of *Lmb. reuteri* in dairy products, emphasizing its technological versatility, health benefits, and potential in pathogen control. Current evidence demonstrates the species' promise as a probiotic, contributing to oral and gut–brain health, while also offering antimicrobial properties against undesirable microorganisms through reuterin production.

Despite the numerous benefits of *Lmb. reuteri*, its application in food products still presents limitations, mainly related to its stability and impact on product quality. Advances in encapsulation and delivery systems can potentially enhance the efficacy and stability of *Lmb. reuteri*, ensuring its viability throughout processing and storage. Studying strains with a higher tolerance to low pH, high osmolarity, and adverse conditions could also guide research to address some of the existing limitations.

Other challenges, such as strain-specific efficacy, regulatory hurdles, and variability in product performance need to be addressed. Future research should focus on optimizing these aspects, enabling the integration of *Lmb. reuteri* in different food matrices and exploring its potential within the food industry, combining health benefits with technological innovation. Looking ahead, innovations in plant-based dairy alternatives present an opportunity to broaden the applications of *Lmb. reuteri*, catering to consumer demand for inclusive food options.

**Author Contributions:** Conceptualization, U.M.P., S.D.T. and R.d.S.R.; methodology, E.M.F.L., M.E.M.S., A.C.d.O.S., U.M.P., S.D.T. and R.d.S.R.; investigation, E.M.F.L., M.E.M.S., A.C.d.O.S., U.M.P., S.D.T. and R.d.S.R.; writing—original draft preparation, E.M.F.L., M.E.M.S., A.C.d.O.S., U.M.P., S.D.T. and R.d.S.R.; writing—review and editing, A.C.d.O.S., U.M.P., S.D.T. and R.d.S.R.; visualization, A.C.d.O.S., U.M.P., S.D.T. and R.d.S.R.; supervision and project administration, R.d.S.R. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

**Acknowledgments:** E.M.F.L. acknowledges the São Paulo Research Foundation (FAPESP, Brazil) for the financial support (grant #2024/13311-9); M.E.M.S. acknowledges the Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (Process #88887.903769/2023-00); U.M.P. acknowledges a scholarship from the National Council for Scientific and Technological Development (CNPq) Process #306685/2022-1; S.D.T. acknowledges the São Paulo Research Foundation (FAPESP, Brazil) for the financial support (grant #2023/05394-9 and #2024/01721-8), and E.M.F.L., U.M.P., S.D.T. and R.S.R. acknowledges the São Paulo Research Foundation (FAPESP, Brazil) for the financial support to the Food Research Center (grant #2013/07914-8).

**Conflicts of Interest:** The authors declare no conflicts of interest.

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