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Review

The Application of Protective Cultures in Cheese: A Review

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Abstract: A number of non-thermal preservation strategies have been adopted from the dairy industry to improve cheese quality and safety. The application of lactic acid bacteria cultures that produce bacteriocins has been extensively studied as a means of bio-preservation. However, the application of purified bacteriocins as a bio-protective agent is limited in cheese. The application of protective cultures is another strategy, and the aim of the current review is to provide an overview of the application of commercial and autochthonous adjunct cultures on the bio-protection of cheese; both public health and spoilage aspects are considered.

Keywords: protective cultures; cheese; control of pathogens; control of spoilage

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1. Introduction

Cheese is a fermented dairy product which has been produced and consumed since the 7th millennium BC [1]. Cheesemaking practices have evolved throughout the years in different ways in different countries and more than 1400 traditional cheese varieties are produced worldwide, displaying a great diversity of organoleptic characteristics [2]. Since the first introduction of starter cultures at the end of the 19th century, a number of changes have been adoptedin the cheesemaking process and nowadays cheeses are produced in large quantities by fully controlled automated processes, and the use of commercial starter cultures is a prerequisite for a successful cheesemaking process [3]. Starter cultures are divided into defined- and mixed-strain cultures; defined-strain cultures are pure cultures with known physiological characteristics and technological properties [4]. Mixed-strain cultures contain different species or genera of lactic acid bacteria (LAB). The use of commercial starter cultures has been proved to be the best way to standardize cheese manufacture with small variations in the organoleptic characteristics. However, their extended use may reduce the microbiota diversity and its associated benefits [5].

LAB, including the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Enterococcus*, *Streptococcus* and *Pediococcus*, are the dominant population in raw milk [6,7]. As raw milk is the main source of the cheese microbiota, LAB from the starter and those not deliberately added as part of the starter or adjunct culture (that is, cultures that are added to cheese for purposes other than acidification [8–10]) constitute the main part of this microbiota [11,12]. This group of bacteria, either natural or selected, produce compounds that are essential for the acidification of the curd and the cheese flavour development during ripening; additionally, they produce antimicrobial peptides, like bacteriocins, which naturally inhibit undesirable microorganisms [11]. Other strains of non-LAB, yeasts and moulds are also present in the complex milk microflora [6].

The microbiota in cheese is formed from raw milk, the starter culture, the equipment used for the cheesemaking process and the environment; the microbiota ensures the diversity between cheeses throughout cheesemaking and maturation processes [5]. This microbiota is transformed according to the specific conditions of the ripening and maturation and includes LAB from the starter, and the autochthonous microbiota composed mainly of non-starter lactic acid bacteria (NSLAB) [12]. In parallel with the development of defined cultures, autochthonous or natural starter cultures have been developed for a number of cheeses [13–19]. The use of the latest DNA and RNA next generation sequencing technologies during recent years have greatly contributed to the understanding of the interactions between the autochthonous cheese microbial communities. These complex microbial communities with their enzymic system contribute to the complex biochemical activities occurring throughout cheese ripening and producing flavour compounds, which characterize the organoleptic properties of the final cheese and are much appreciated by consumers of all the world [5,20].

Bio-preservation is a natural way to protect food from spoilage and harmful contamination with either pathogenic or spoilage microorganisms [21]. Bio-preservation is an effective strategy to reduce food waste without using chemical preservatives. In addition, enhanced safety and extended shelf-life using indigenous or added adjunct cultures to control the growth of pathogenic and spoilage microbes can be achieved [22,23]. Chen et al. [22] reviewed the application of antifungal compounds and provided an overview of the mechanisms involved. Moreover, the preservation strategies of non-thermal and packaging technologies and the addition of natural antimicrobial agents and plant extracts and essential oils in cheese have been reviewed by Moula Ali et al. [23].

Protective cultures have been suggested as microorganisms which can reduce the risk of the growth and survival of both pathogenic and spoilage-causing microbes [21,24]. These are added in different cheeses to improve the safety and extend the shelf-life of the cheese.

LAB have been suggested to be used as protective cultures, as they have a long history in safe cheese production and their antagonistic interactions against pathogens and spoilage microbes are well known [25,26]. However, to finally apply LAB protective cultures in cheesemaking, a screening procedure is required and the selected strains should be phenotypically and genotypically characterized, to ensure their safety, and validated for their bio-protecting activities. In this context, Souza et al. reviewed the main strategies for the identification and characterization of the properties of bioprotective LAB [25].

The success of any bio-preservation strategy, applied in cheese, depends on the in situ antimicrobial efficacy of the LAB metabolites [21]. Several reviews have studied the application of bacteriocin-producing LAB in cheese, mainly focusing on nisin and/or other lactococcal bacteriocins [20,27–38]. Gonzalez-Gonzalez et al. [31] reviewed the functional properties of bacterial cultures used for different dairy applications and Rangel-Ortega et al. reviewed the food safety issues related to the artisanal cheeses and two strategies for the control of the main pathogenic bacteria, that is, applying LAB and natural compounds [32]. In addition, Ahansaz et al. recently reviewed antimicrobial agents, that is, bacteriocins, organic acids and other metabolites produced by LAB in dairy products; the authors highlighted the limitations in applying bacteriocins in cheese due to the degradation caused by certain peptidases [33].

The aim of the current review is to provide an overview of the application of commercial and autochthonous adjunct cultures on the bio-protection of cheese, both as a control measure for pathogenic bacteria and for spoilage microorganisms. Cheeses were categorized into thirteen groups according to their moisture content and the special cheesemaking practices [2].

2. Antimicrobial Mechanisms

Protective cultures in cheese will exert their antimicrobial activity depending on several factors, i.e., the initial level of contamination, the type of microbial contaminant and technological parameters (i.e., maturation time, storage temperature). It should be mentioned that protective cultures primarily have the potential to stop or delay the onset of further contamination, rather than act on initial high concentrations of microbial contaminants [37]. The three main antimicrobial mechanisms of protective cultures in cheese are briefly described below.

2.1. Metabolites

The protective cultures usually exhibit an amensalism relationship, i.e., interactions in which one type of microbe negatively affects another type, without being affected itself. In dairy fermentations, strains of LAB produce metabolites such as acetic, lactic, propionic, phenyllactic and hydroxyphenyl lactic acids, which have been shown to inhibit the growth of certain bacteria and fungi. The inhibitory effect is either attributed to the reduction in the pH, or to their undissociated forms by diffusing through the cell membranes and releasing H+ ions that acidify the cell cytoplasm. Moreover, the antimicrobial activity is related to the denaturation of membrane proteins, blocking transmembrane transport, proton gradient interference, enzyme inhibition, and reactive oxygen species production, which disturbs the cell metabolism, resulting in growth inhibition [25]. Other LAB antimicrobials include H_2O_2, fatty acids (decanoic, coriolic), diacetyl and acetoin and bacteriocins [37]. In the case of acetoin or diacetyl, these compounds can interact with arginine, compromising the structure of some proteins; another possible mechanism is that diacetyl can link to DNA molecules, promoting its unfolding [25]. Finally, reuterin produced by *Limosilactobacillus reuteri* is reported as a potent compound with broad-range antimicrobial activity that inhibits fungi but also Gram-negative bacteria. *Limosilactobacillus reuteri* uses a CoA-dependent pathway, in which 3-hydroxypropionaldehyde (3-HPA, the active antimicrobial system) is obtained from glycerol in a reaction catalysed by the coenzyme B12-dependent glycerol/diol dehydratase [19].

2.2. Bacteriocins

Bacteriocins are peptides or proteins produced in the ribosome and secreted, mainly, by Gram-positive bacteria such as LAB. They have distinct mechanisms of action and can be divided according to their promotion of a bactericidal effect or bacteriostatic, inhibiting further cell growth [38]. Bacteriocins are divided into three major classes, i.e., Class I, small post-translationally modified peptides; Class II, unmodified bacteriocins; and Class III, larger peptides (>10 kDa, thermo-labile), with each one subdivided into subclasses. Class I bacteriocins comprise the well-known lantibiotics (nisin, lacticin 3147), Class II examples include cyclized peptides (enterocin AS-48), pediocins produced by *Pediococcus* spp., while a novel Class III bacteriocin is Helveticin-M, produced by *Lactobacillus crispatus* [39]. The mode of action of bacteriocins has been extensively studied [25,32–40] and they generally exert their antibacterial effect by targeting the cell-envelope-associated mechanisms and by pore formation, resulting in a variation in the cytoplasm membrane potential, disrupting the proton motive forceand ultimately causing cell death. Nisin is capable of both mechanisms, i.e., pore formation and the inhibition of cell wall biosynthesis, which are combined within the same molecule for potent antimicrobial activity. Other bacteriocins can kill their target cells through the inhibition of gene expression and protein production [38,41]. Nisin, lacticin 3147 and 481, pediocin AcH, thermophilin, macedocin, reuterin and enterocin AS-38 and KP are some of the bacteriocins that have been effectively applied in different cheeses [29,35,38].However, the low yields, the complexity and the cost of purification and the inactivation through proteolytic enzymes during ripening are the main limiting factors for the addition of bacteriocins in cheese production [19,26,29,35,38].

2.3. Ecological Competition

Protective cultures engage in competitive exclusion where they outcompete the spoilage agent for nutrients and oxygen. Competitive exclusion as a major bioprotective mechanism of lactobacilli against fungal spoilage in fermented milk products is discussed by Siedler et al., where it was concluded that the mechanism of nutrient depletion constitutes an alternative strategy to chemical preservation [42]. Furthermore, protective cultures can take part in quorum sensing—where the microbial cultures ‘feel’ their environment and adjust their performance to deal with these new challenges [39]. The strong antibacterial (antilisterial) effect of ecological competition as expressed in smear-ripened cheeses by some undefined consortia has been reported by Mayo et al., where the cheese ecosystem, its interactions and their effect on safety and quality are extensively discussed [40].

3. Control of Pathogens

Most studies for the application of protective LAB cultures to control pathogens in cheese have been carried out in model cheeses; however, there are also a number of studies on industrial and artisanal cheeses (Table 1, [43–81]).

Table 1. Applications of protective cultures to control pathogenic bacteria in different cheeses.

Cheese Category	Cheese	Protective Culture	Target Microorganism(s)	Main Findings	Reference
Model	Fresh cheese	Three nisin producing *Lc. lactis* strains	*L. monocytogenes*	Numbers of *L. monocytogenes* were reduced in model cheese by 2 Log units during 7 days of storage	[43]
Model	Miniature fresh cheese	*Lc. lactis* 16FS16-9/20234-11FS16 and *Lpb. plantarum* 1/14537-4A/20045)	*L. monocytogenes*	Reduction in the growth of *L. monocytogenes* by 3–4 Log units	[44]
Model	Model cheese	*Staph. equorum* SE3	*L. monocytogenes*	*Staph. equorum* inhibited the growth of *L. monocytogenes* (<1 Log unit)	[45]
Model	Model cheese	Commercial protective cultures and bacterial fermentates	*L. monocytogenes*	The growth of *L. monocytogenes* was delayed by the protective cultures	[46]
Model	Laboratory-scale cheese	*Lc. lactis* CSK2775 and *Lpb. plantarum* LMG P-26358	*L. innocua*	Combination of the two cultures was suggested for industrial use	[47]
Model	Cheddar-like	*Lcb. paracasei*	*B. cereus*, *L. monocytogenes*	*Lcb. paracasei* inhibited both pathogens	[48]
Hard	Cheddar	Autochthonous LAB	*L. monocytogenes*	*C. crustorum*, *Lpb. plantarum* and *Lmb. fermentum* decreased the levels of *L. monocytogenes* in cheese	[49]
Hard	Graviera	Enterocin-producing *E. faecium*	*L. monocytogenes*	*E. faecium* KE82 is suggested as a protective culture, but the indigenous bacteriocin-producing LAB might contribute to the inhibition of *L. monocytogenes* in Graviera	[50]
Hard	Pecorino Sardo PDO	*Lpb. plantarum* (commercial) and an autochthonous LAB (*Lb. delbruekii* ssp. *sunkii*).	Protection against *L. monocytogenes*	*Lb. delbruekii* ssp. *sunkii* was as effective as the commercial culture for the protection against *L. monocytogenes*	[51]
Semi-hard	Uncooked pressed cheese	Single or combined cultures of 18 selected bacterial strains	*E. coli* O26:H11 and O157:H7	*H. alvei*, *Lpb. plantarum* and *Lc. lactis* reduced the growth of STEC by 3 Log units	[52]
Semi-hard	Semi-hard cheese	*Lc.lactis* nisin Z producers (44SGLL3, 29FL1 and 41FL1)	*L. monocytogenes* and *Stap. aureus*	*Lc. lactis* 41FL1 reduced *Staph.aureus* counts by 1.7–3.5 Log units; no effect on *L.monocytogenes* was observed	[53]

Table 1. *Cont.*

Cheese Category	Cheese	Protective Culture	Target Microorganism(s)	Main Findings	Reference
Semi-hard	Coalho	*Lcb. rhamnosus* EM1107	*Staph. aureus*, *Salmonella enteritidis*, *L. monocytogenes* and *E. coli*	*Lcb. rhamnosus* exhibited different inhibition rates against *Staph. aureus*, *Salmonella enteritidis*, *L. monocytogenes* and *E. coli*	[54]
Semi-hard	Coalho	*Lcb. paracasei*	*Staph. aureus* and *L. monocytogenes*	*Lcb. paracasei* delayed the growth of *Staph. aureus* and *L. monocytogenes* in Coalho cheese	[55]
Semi-hard	Pressed uncooked cheese	*Lb. reuteri* INIA P57	*L. monocytogenes* and *E. coli* O157:H7	Reuterin production was enhanced with glycerol and resulted in the control of the pathogenic bacteria	[56]
Semi-hard	Artisanal cheese	*E. faecium* CRL1879	*L. monocytogenes*	*E. faecium* CRL1879 ensured an efficient control of *L. monocytogenes* for up to 30 days without altering the organoleptic properties of the artisanal cheese	[57]
Semi-hard	St. Nectaire	Complex cheese microbial consortium	*L. monocytogenes*	The species composition of the microbial consortium is the most important factor for the antimicrobial activity	[58]
Semi-hard	Minas (semi-hard)	*Lvb. brevis* 2-392, *Lpb. plantarum* 1-399 and *E. faecalis* (1-37, 2-49, 2-388 and 1-400)	*L. monocytogenes*	*L. monocytogenes* was inactivated (reduction by 4–5.8 Log units) during the ripening of semi-hard cheeses by the mix of LAB added	[59]
Soft	Minas (soft)	*Lvb. brevis* 2-392, *Lpb. plantarum* 1-399 and *E. faecalis* (1-37, 2-49, 2-388 and 1-400)	*L. monocytogenes*	Selected LAB strains presented a bacteriostatic anti-listerial effect (reduction by 0.6–1.75 Log units) in Minas soft cheese	[59]
Soft	Minas Frescal	*Lpb. plantarum* 49 and *Lcb. paracasei* 108	*L. monocytogenes*	*Lpb. plantarum* 49 and *Lcb. paracasei* 108 reduced the counts of *L. monocytogenes* by 2.8 Log units	[60]
Soft	Soft cheese	*Lb. sakei* and *Lpb. plantarum*	*L. monocytogenes*	Strains of LAB reduced the growth of *L. monocytogenes* by 1 Log unit in the soft cheese	[61]
Soft	Fresh cheese	*Lc. lactis* (autochthonous)	*L. monocytogenes*	The application of *Lc. lactis* reduced the growth of *L. monocytogenes* by 1 Log unit in fresh cheese	[62]
Soft	Fresh cheese	Autochthonous LAB	*L. monocytogenes*	Autochthonous LAB inhibited the growth of *L. monocytogenes* in the soft cheese	[63]
Soft	Kareish	*Lpb. plantarum*	*B. cereus*	*Lpb. plantarum* decreased the counts of *B. cereus* in Kareish cheese	[64]
Soft	Queso fresco	*Lb. curvatus*, *Lb. sakei*, *P. acidilactici*, and *Leuc. carnosum* (commercial)	*L. monocytogenes*	The LAB cultures did not show any inhibitory effect on *L. monocytogenes*	[65]

Table 1. *Cont.*

Cheese Category	Cheese	Protective Culture	Target Microorganism(s)	Main Findings	Reference
Soft	Soft sheep milk cheese	*Lpb. plantarum* (commercial)	*L. monocytogenes*	*Lpb. plantarum* can control *L. monocytogenes* growth without affecting the characteristics of the cheese	[66]
Soft	Torta del Casar	*Lcpb. casei* 116 and *Lc. garvieae* 151	*L. monocytogenes*	*Lcpb. casei* 116 and *Lc. garvieae* 151 inhibited the growth of *L. monocytogenes* during the ripening of the cheese	[67]
Soft	Soft cheese	*Bif. breve* and *Bif. animalis*	*L. monocytogenes*	Probiotic cultures resulted in the decrease in *L. monocytogenes* counts in soft cheese	[68]
Soft	UF cheese	*Lc. lactis* ssp. *lactis* and *E. durans*	*L. monocytogenes*	*E. durans* and *L. lactis* were suggested for the control of *L. monocytogenes* in UF cheese	[69]
Dutch-type	Gouda	*Lpb. plantarum* LMG P-26358	*L. innocua*	The addition of *Lpb. plantarum* LMG P-26358 with a nisin producer was found to eliminate *L. innocua in Gouda cheese*	[70]
White-brined	Beyaz	*Lc. lactis* L54	*L. monocytogenes*	*Lc. lactis* L54 inhibited the growth of *L. monocytogenes* in Beyaz cheese	[71]
White-brined	Domiati-type	Autochthonous LAB	*Staph. aureus*	*Lcb. rahmnosus* has antimicrobial activity against *Staph. aureus* and could be used as protective culture in soft cheese	[72]
White-brined	Domiati-type	*Lpb. plantarum*	*Staph. aureus*	The mixed culture of *Lpb. plantarum* strains showed improvement of the safety and quality of Domiati-type cheese	[73]
Pasta filata	Nite	Fresco DVS 1010, culture A, *Lb. acidophilus* LA145, *Lcb. rhamnosus* VT1 and *Lcb. rhamnosus* GG	Coagulase-positive staphylococci and *E. coli*	The best inhibitory effect for Nite cheese was observed with Fresco DVS 1010 and *Lcb. rhamnosus* GG	[74]
Bacterial surface-ripened	Smear-ripened cheese	*Lc. lactis* DPC4275	*L. monocytogenes*	The lacticin 3147 producer reduced the counts of *L. monocytogenes* by 3 Log units; regrowth was observed during the ripening	[75]
Blue-veined	Gorgonzola	Autochthonous LAB	*L. monocytogenes*	*Lc. lactis* showed inhibition on the growth of *L. monocytogenes* at 4 °C	[76]
Acid-coagulated	Cottage	*Lc. lactis* (nisin A, Z and lacticin 481 producers)	*L. monocytogenes*	Only weak abilities to reduce *L. monocytogenes* were reported from the bacteriocin-producers in Cottage cheese	[77]
Acid-coagulated	Cottage	*Lcb. rhamnosus* (non-bacterio-cinogenic)	*L. monocytogenes*	Inhibition of *L. monocytogenes* was found to be caused through competitive exclusion, by depletion of manganese	[78]

Table 1. *Cont.*

Cheese Category	Cheese	Protective Culture	Target Microorganism(s)	Main Findings	Reference
Acid-coagulated	Symbiotic cheese spread	*Lb. sakei* 2a and inulin	*L. monocytogenes*	*Lb. sakei* 2a has been suggested to control *L. monocytogenes* in the cheese spread	[79]
Whey cheeses	Anthotyros	Crude enterocin ABP extract	*L. monocytogenes*	Enterocin ABP extract showed a decreasein *L. monocytogenes* counts, probably associated with the acidification of the cheese	[80]
Whey cheeses	Anari	*E. faecium* DM 224, DM 270 and DM 33	*L. monocytogenes*	*E. faecium* DM 33 was found to decrease *L. monocytogenes* counts by more than 4 Log units	[81]

LAB: Lactic acid bacteria, A.: Aeromonas, B.: Bacillus, Bif.: Bifidobacterium, C.: Companilactobacillus, E.: Enterococcus, H.: Hafnia, L.: Listeria, Lc.: Lactococcus, Lb.: Lactobacillus, Lcb.: Lacticaseibacillus, Lpb.: Lactiplantibacillus, Ltb.: Latilactobacillus, Lvb.: Levilactobacillus, Lmb.: Limosilactobacillus, Leuc.: Leuconostoc, P.: Pediococcus, Staph.: Staphylococcus, Str.: Streptococcus.

Lactococcus lactis and *Lactiplantibacillus plantarum* strains were studied in model cheeses against *Listeria monocytogenes* with variable results (Table 1). Nisin producing *Lc. lactis* strains have been applied in a variety of model cheeses and Cheddar cheese slurries to prevent the growth of *L. monocytogenes* [43,82]. In some cases, *Lc. lactis* was applied in combination with other LAB and the combined action was found to be more efficient in reducing the growth of *L. monocytogenes* [44]. The combination of *Lpb. plantarum* strain with a strain of *Lc. lactis* that produced nisin reduced the growth of *L. monocytogenes* in a model ripened cheese; the combination was more effective than the single action [47]. The application of *Staphylococcus equorum* was studied by Bockelmann et al. and the authors reported strong anti-listerial activity [45].

The application of autochthonous LAB has been studied in several hard cheeses, such as Cheddar, Graviera and Pecorino Sardo [49–51]. Graviera cheese is a hard, ripened cheese and the addition of *Enterococcus faecium* on *L. monocytogenes* was studied [50]. Meloni et al. evaluated thermophilic LAB to control *L. monocytogenes* growth in Pecorino Sardo PDO cheese [51].

A number of research papers have been published on the application of protective cultures in semi-hard cheeses. The application of LAB and *Hafnia alvei* was successfully studied as bio-protective cultures in raw milk cheese against *Salmonella* spp. and Shigatoxin producing *Escherichia coli* (STEC) [52]. Callon et al. reported that a combination of *H. alvei*, *Lpb. plantarum* and *Lc. lactis* was the most inhibitory, reducing STEC O26:H11 and O157:H7 by up to 3 Log CFU/g [52]. LAB consortia, isolated from the cheese rind of Saint-Nectaire and then added to the cheese surface, demonstrated a higher anti-listerial activity as compared to the cheese made with a defined starter culture [58]. *Lactobacillus brevis*, *Lpb. plantarum* and *E. faecalis*, where applied in semi-hard cheese, reduced *L. monocytogenes* counts by up to 4 Log CFU/g [59].

Coelho et al. isolated eight bacteriocin producer strains, identified as *Lc. lactis* and *Enterococcus faecalis* from Pico cheese; the authors reported that the adjunct cultures in situ controlled the growth of *L. monocytogenes*, anda blend of two bacteriocin producing *Enterococcus* ssp. optimized the reduction in *L. monocytogenes* counts in fresh cheese [63]. Autochthonous LAB strains have shown bio-protective activities against pathogens [11]; *Carnobacterium maltaromaticum* has showed antibacterial activity in different French soft cheeses, inhibiting the growth of *Psychrobacter* spp. and *L. monocytogenes* [82]. The inhibition of *L. monocytogenes* was reported by strains of *Lactobacillus sakei* and *Lpb. plantarum* in soft cheese [61]. However, Lawton et al. [65] reported that the bacterial cultures did not significantly inhibit the growth of *L. monocytogenes* in Queso fresco. Martin et al. evaluated the effect of selected protective cultures of *Lactocaseibacillus casei* and *Lactococcus garvieae* on the organoleptic characteristics of Torta del Casar cheese and reported no significant effects [67]. In addition, selected strains of *Lc. lactis* and *Enterococcus durans* have also been reported to inhibit the growth of *L. monocytogenes* in ultrafiltered cheese [69]. The application of protective cultures has been studied in Beyaz and Domiati cheeses; Meral Aktas et al. evaluated a nisin-producing *Lc. lactis* strain for its efficacy to control the growth of *L. monocytogenes* in Beyaz cheese [71]. O'Sallivan et al. demonstrated the use of bacteriocin produced by LAB for the bio-preservation of bacterial surface-ripened cheeses [75]. *Lb. sakei*, *Lc. lactis* and *Carnobacterium* strains selected from Gorgonzola cheese have been showed to inhibit *L. monocytogenes*, up to 2 Log CFU/g, in Gorgonzola [76]. Interestingly, the combination of selected LAB with antimicrobial compounds, that is, acid/sodium lactate and L-sodium lactate, has been suggested [83]. The growth of *L. monocytogenes* was inhibited by strains of *Lb. sakei* in synbiotic cheese spread and the expression of the genes *sak* P and *sak* Q encoding for bacteriocins production was reported [79]. Gensler et al. studied the impact of ten commercial protective cultures on both the antimicrobial activity against *L. monocytogenes*, STEC and *Salmonella* spp. and the growth starter cultures, that is, mesophilic, thermophilic and adjunct cultures (*Arthrobacter nicotianae* and *Brevibacterium linens*); the authors discussed the importance of identifying protective cultures with limited impact on the starter and adjunct cultures for specific cheeses [84]. van Gijtenbeek et al. studied the competitive exclusion as a bio-protective mechanism against the growth of *Listeria* spp. in cottage cheese [78]. The growth of the starter culture, that is, *Streptococcus thermophilus* and *Lactococcus lactis*, was not influenced by reduced manganese levels [78]. Finally, the incorporation of bacteriocin in active packaging has been suggested by Contessa et al. [85]; the authors reported the reduction in *L. monocytogenes* by 3 Log units [85].

An important aspect of cheese safety is the reduction in biogenic amines, as many Gram-negative bacteria have been reported to produce cadaverine, histamine or putrescine [11]. Renes et al. showed that the use of an adjunct culture composed of *Lc. lactis*, *Lc. lactis* ssp. *cremoris* and *Lpb. plantarum* effectively reduced the amount of biogenic amines in cheese [86].

4. Control of Spoilage Microorganisms

A presentation of the most important applications of protective cultures to control spoilage bacteria in cheese is shown in Table 2 [87–113]. Most studies have been conducted to control fungi and spoilage bacteria such as clostridia, pseudomonads, Enterobacteriaceae and coliforms.

Table 2. Applications of protective cultures to control spoilage microorganisms in different cheeses.

Cheese Category	Cheese	Protective Culture	Target Microorganism(s)	Main Findings	Reference
Model	Cheese slurries	*Lc. lactis* ssp. *lactis* 32 and encapsulated nisin-A	*Cl. tyrobutyricum*	Application of *Lc. lactis* was able to control the growth of *Cl. tyrobutyricum*	[87]
Model	Model cheese	*Lc. lactis* ssp. *lactis* INIA 415 (nisin- and lacticin 481 producer)	*Cl. beijerinckii* INIA 63	Bacteriocin producer *Lc. lactis* resulted in the prevention of late blowing in model cheese	[88]
Model	Cheese-mimicking matrix	LAB	Fungi	The antifungal activity was found to be strain-dependent and the fermentation substrate had a strong effect	[89]
Model	Miniature Caciotta	*Lpb. plantarum*, *Lcb. paracasei*, *Lvb. brevis* and *Lb. sakei*	*Pen. chrysogenum* ATCC 9179 and *Asp. flavus* ATCC 46283	Single and combined adjunct cultures reduced the mould growth by more than 2 Log units after 15 and 30 days of ripening	[90]

Table 2. *Cont.*

Cheese Category	Cheese	Protective Culture	Target Microorganism(s)	Main Findings	Reference
Model	Cheese matrix	*W. confusa* W5 and W8, *W. paramesenteroides* W9, *W. cibaria* W25 and *Lpb. plantarum* Q4C3	*Asp. niger* IOC 207 and *Pen. chrysogenum* IOC 132	The single LAB strains showed antifungal activities in the model cheese against both fungi targets; however, these activities were reduced when combined with a commercial culture	[91]
Hard	Cheddar (shredded)	Autochthonous LAB	Fungi	All strains of *Lpb. plantarum* prolonged the shelf life of Cheddar	[92]
Hard	Cheddar	*Lb. amylovorus* DSM 19280	*Pen. expansum*	The inoculation of *Lb. amylovorus* adjunct delayed the growth of the mould on the surface	[93]
Hard	Pecorino Siciliano	LAB	*Pseudomonas* spp. and *Enterobacteriaceae*	The levels of enterobacteria and pseudomonads were not detectable after five months of ripening	[94]
Semi-hard	Cheddar (semi-hard)	*Lvb. brevis* SJC120 in whey gelatin film	Fungi	The active packaging showed antifungal activity in Cheddar	[95]
Semi-hard	Experimental	23 strains of *Lactobacillus*, *Leuconostoc* and *Propionibacterium* spp.	*Pen. commune*, *M. racemosus*, *G. geotrichum*, *Y. lipolytica*	The combination of different LAB and propionibacteria allowed the development of two antifungal combinations	[96]
Semi-hard	Experimental	Fermentates from *Lcb. rhamnosus* CIRM-BIA1952, *Pr. jensenii* CIRM-BIA1774 and *M. lanceolatus* UBOCC-A-10919	Fungi	The fermentate from *Pr. jensenii* CIRM-BIA1774 showed the greatest antifungal activity and most selected fermentates delayed the growth of spoilage moulds	[97]
Soft	Fresh cheese	Autochthonous LAB	Gram-negative bacteria	*C. maltaromaticum* and *Lcb. rhamnosus* lowered psychotropic bacteria by almost 3 Log CFU/g in the soft cheese	[98]
Soft	Fresh cheese	Autochthonous LAB	*Asp. flavus*, *Asp. parasiticus*	*Lpb. plantarum* PIN showed remarkable antifungal activity	[99]
Soft	Queso fresco (soft cheese)	*Lcb. rhamnosus* species (commercial)	Fungi	Commercial protective cultures vary in performance against yeasts and moulds	[100]
Soft	Soft cheese (low salt)	*Lcb. rhamnosus*	Aerobic spore-forming bacteria	Combination with nisin and lysozyme	[101]
Dutch-type	Dutch-type	*Lb. paracasei* LPC37, *Lb. acidophilus* NCFM and *Lcb. rhamnosus* HN001	Coliform bacteria, *Enterococcus* ssp., yeasts and moulds	The application of LAB protective cultures was suggested	[102]
Dutch-type	Gouda	*D. hansenii* and/or *P. acidilactici* *combined with* cysteine-rich antifungal protein PgAFP	*Asp. parasiticus*	The combination of *D. hansenii* and the cysteine-rich antifungal protein PgAFP resulted in the inhibition of *Asp. parasiticus*	[103]

Table 2. *Cont.*

Cheese Category	Cheese	Protective Culture	Target Microorganism(s)	Main Findings	Reference
White-brined	White-brined cheese	*Lcb. rhamnosus* and *Lpb. plantarum* (commercial)	*Enterobacteriaceae* and coliform bacteria	The use of *Lcb. rhamnosus* was recommended for white-brined cheese	[104]
Pasta-filata	Burrata	*Lcb. rhamnosus* and *Lpb. plantarum* (commercial)	Spoilage bacteria	The combination of MAP and protective culture extended the shelf-life of Burrata cheese	[105]
Pasta-filata	Burrata	*Lpb. plantarum* LPAL and *Lcb. rhamnosus* LRB	Staphylococci, coliforms and *Pseudomonas* spp.	The use of *Lpb. plantarum* LPAL and *Lcb. rhamnosus* LRB extended the shelf-life of Burrata cheese	[106]
Pasta filata	Grottone	*Lcpb. casei* LC4P1 (commercial)	*Cl. sporogenes*	The protective culture resulted in an inhibition of the PAB starter development	[107]
Pasta-filata	Kashar	*Lpb. plantarum* and *Lc. lactis* ssp. *lactis*	*Clostridium* spp.	The co-inoculum resulted in 1 Log unit reduction in *Cl. sporogenes counts*	[108]
Acid-coagulated	Cottage	*Lcb. rhamnosus*, *Bifid. animalis* ssp. *lactis*	Fungi	*Lcb. rhamnosus* alone or in combination with *Bif. animalis* ssp. *lactis* inhibited mould growth	[109]
Acid-coagulated	Cottage	Mix of *Lacticaseibacillus* spp. and *Lactiplantibacillus* spp., *Lcb. rhamnosus*	Fungi	The protective cultures were not very effective against yeast, whereas they delayed the spoilage of at least one mould strain	[110]
Acid-coagulated	Cottage	*Lpb. plantarum*	*Pen. commune*	All *Lpb. plantarum* isolates were found to prevent the visible growth of *Pen. commune* on Cottage cheese	[111]
Whey cheese	Ricotta fresca	*Carnobacterium* spp. (commercial)	*Pseudomonas* spp	*Carnobacterium* spp. inhibited the growth of *Pseudomonas* spp.	[112]
Whey cheese	Ricotta fresca	*E. faecium*, *Lpb. plantarum*, *Lcb. rhamnosus* or *Carnobacterium* spp. or the fermentate MicroGARD 430 (commercial)	*Pseudomonas* spp. and Enterobacteriaceae	Different reduction rates were observed in the concentrations of *Pseudomonas* spp. and Enterobacteriaceae	[113]

LAB: Lactic acid bacteria, A.: Aeromonas, Asp.: Aspergillus, B.: Bacillus, Bif.: Bifidobacterium, Cl.: *Clostridium*, C.: Companilactobacillus, D.: *Debaryomyces*, E.: Enterococcus, G.: Galactomyces, Lc.: Lactococcus, Lb.: Lactobacillus, Lcb.: Lacticaseibacillus, Lpb.: Lactiplantibacillus, Ltb.: Latilactobacillus, Lvb.: Levilactobacillus, Lmb.: Limosilactobacillus, Leuc.: Leuconostoc, M.: Mucor, P.: Pediococcus, Pen.: Penicillium, Pr.: Propionibacterium, Staph.: Staphylococcus, Str.: Streptococcus, Y.: Yarrowia, W.: *Weissella*.

Nisin- and lacticin 481-producing *Lc. lactis* as a starter was shown to delay late blowing defects in the manufacture of model cheeses; the cheese made with clostridial spores and *Lc. lactis* INIA 415-2 showed late blowing defect after 120 days of ripening, without altering its sensory characteristics [88]. Souza et al. investigated the antagonist activity of *Weissella* spp. and *Lpb. plantarum* isolated from Brazilian artisanal cheese and dairy environments against *Aspergillus* and *Penicillium* spp. [91]. Acid-coagulated cheeses such as cottage cheese also benefit from the addition of protective cultures to control the spoilage fungi, as shown by numerous studies [108–111]. The application of commercial and autochthonous protective cultures to control *Pseudomonas* spp. and Enterobacteriaceae has been studied for Ricotta fresca [112,113]. Makki et al. demonstrated that commercial LAB cultures vary in their antifungal activities [110].

Shi and Maktabdar reviewed the antifungal activities of LAB against different spoilage moulds [114] and Li et al. reviewed the antifungal activities of *Lpb. plantarum* and its potential in bio-preservation and the mechanisms for the elimination of mycotoxins [115]. Moreover, Erfani et al. performed a systematic review on probiotic bacteria as bio-preservative cultures and reported that *Lpb. plantarum* showed significant antifungal activities [116]. Further research is needed to empower the optimal cheesemaking parameters to enable protective culture to produce antifungal metabolites in cheese, and this knowledge could help in the selection of LAB strains for specific cheeses. Additional studies are recommended to characterize the interaction potential with starter and other NSLAB. The selected cultures should be tested in industrial scale cheesemaking process.

5. Conclusions

Nisin and other bacteriocins such as enterocins, lactacins and pediocins have been effectively studied as antimicrobial agents to control *L. monocytogenes* in a variety of cheeses; however, their commercial application is limited. Protective cultures composed of specific strains of the LAB species such as *Lc. lactis*, *Lpb. plantarum*, *Lcb. paracasei*, *Lcb. rhamnosus*, *Lvb. brevis*, *Lb. sakei*, *E. faecium* and *Carnobacterium* spp., *Bifidobacterium* spp. and *Propionibacterium* spp. have been used to control the growth of pathogenic and spoilage bacteria in different cheeses. The application of protective cultures has been demonstrated to bean important, clean-label strategy for the control of pathogens, mainly *L. monocytogenes* and spoilage bacteria in cheese, as an additional measure to the application of good hygiene and manufacture practices throughout the whole cheesemaking process. Single strains or combinations of strains have been suggested, but further research is needed to evaluate the effects on the cheese microbial ecology, physico-chemical, organoleptic and nutritional characteristics of each type, before being applied to manage the microbiological risks. The combined use of protective cultures with other natural bio-preservatives and other treatments has showed promising results.

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