Meat Science 183 (2022) 108661

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Review

Application of lactic acid bacteria for the biopreservation of meat products: A systematic review

Coral Barcenilla [a], Miroslav Ducic [b], Mercedes López [a,c], Miguel Prieto [a,c], Avelino Álvarez-Ordóñez [a,c,*]

[a] Department of Food Hygiene and Technology, Universidad de León, León, Spain
[b] Department of Veterinary Medicine, Faculty of Agriculture, University of Novi Sad, Novi Sad, Serbia
[c] Institute of Food Science and Technology, Universidad de León, León, Spain

ARTICLE INFO

Keywords:
Lactic acid bacteria
Biopreservation
Meat
Bacteriocins
Natural antimicrobials
Foodborne pathogens

ABSTRACT

The increasing concern of consumers about food quality and safety and their rejection of chemical additives has promoted the breakthrough of the biopreservation field and the development of studies on the use of beneficial bacteria and their metabolites as potential natural antimicrobials for shelf life extension and enhanced food safety. Control of foodborne pathogens in meat and meat products represents a serious challenge for the food industry which can be addressed through the intelligent use of bio-compounds or biopreservatives. This article aims to systematically review the available knowledge about biological strategies based on the use of lactic acid bacteria to control the proliferation of undesirable microorganisms in different meat products. The outcome of the literature search evidenced the potential of several strains of lactic acid bacteria and their purified or semi-purified antimicrobial metabolites as biopreservatives in meat products for achieving longer shelf life or inhibiting spoilage and pathogenic bacteria, especially when combined with other technologies to achieve a synergistic effect.

1. Introduction

In industrialized countries, consumers demand a continuous supply of a wide variety of foodstuffs with high quality and safety. For this purpose, products which maintain their quality attributes throughout a relatively long shelf life, from production until consumption, are in increasing demand. In addition, food quality and safety requirements must be strictly met, considering also the nutritional value and sensory properties of the end product (Saltmarsh & Insall, 2013).

In 2019, the zoonosis most commonly reported at European Union (EU) level was campylobacteriosis, with 220,682 confirmed cases, followed by salmonellosis, infections caused by Shiga toxin-producing *Escherichia coli* (STEC) and yersiniosis, with 87,923; 7,775 and 6,961 confirmed cases, respectively (EFSA, 2021). These diseases are caused by foodborne pathogenic bacteria which can contaminate meat and meat products during slaughtering or the manufacturing processes, causing debilitating or fatal effects in humans (Kim, Cho, & Han, 2013). *Listeria monocytogenes*, which in 2019 caused 2,621 confirmed cases in the EU, is the zoonotic agent with the highest case-fatality rate, with 300 reported deaths in 2019, which represents a 17.6% case-fatality rate and is showing a statistically significant increasing trend in the EU since 2009 (EFSA, 2021). Meat and meat products can be carriers of *L. monocytogenes,* with some recent foodborne outbreaks attracting much public attention. For instance, in August 2019, one of the largest listeriosis outbreaks ever reported was associated with a chilled roasted pork meat product. The outbreak caused a total of 227 confirmed cases, 226 in Spain, the majority reported in the region of Andalucía, and 1 case reported in France. Thirty-seven of these cases were pregnant women, with 5 reported miscarriages linked to the outbreak. Three deaths were reported among elderly people (Centro de Coordinación de Alertas y Emergencias Sanitarias, 2019; WHO, 2019).

The food industry has devoted intense research efforts to counteract the emergence of outbreaks caused by foodborne pathogenic bacteria. Thermal processing is commonly employed as a preservation method, but intense thermal treatments can entail unwanted organoleptic and nutritional effects onto food (Pisoschi et al., 2018). This problem is addressed by the food industry to a certain extent through the use of additives such as chemical preservatives, which allow responding to the

* Corresponding author at: Department of Food Hygiene and Technology, Universidad de León, León, Spain.
E-mail address: aalvo@unileon.es (A. Álvarez-Ordóñez).

https://doi.org/10.1016/j.meatsci.2021.108661
Received 17 April 2021; Received in revised form 19 August 2021; Accepted 21 August 2021
Available online 24 August 2021

requirements regarding variety, price, accessibility, convenience and quality of products, while reducing the intensity of technological treatments causing quality losses (Saltmarsh & Insall, 2013). Nevertheless, it is not uncommon to find rejection to certain food additives among consumers.

In the meat industry, the most relevant preservative substances that may be of safety concern from the consumer's perspective are nitrites and nitrates. On the one hand, they have been traditionally recognized for accomplishing several relevant functions in meat products, such as (i) their contribution to the development of the typical colour and flavour of cured meats, (ii) their bacteriostatic or bactericidal effect against some pathogens such as *Clostridium botulinum* (Majou & Christieans, 2018), (iii) or the inhibition of oxidation processes, which overall result in an extension of the shelf life of meat products. On the other hand, these additives can undergo nitrosation reactions to generate nitric oxide, a compound that in the presence of unprotonated secondary amines can form N-nitrosamines (Flores & Toldrá, 2020). The most frequently reported N-nitrosamines are N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosopiperidine (NPIP), N-nitrosopirrolidine (NPYR) and N- nitrosomorpholine (NMOR). Remarkably, NDMA and NDEA are considered the volatile N-nitroso compounds with the most powerful carcinogenic and genotoxic effects (Flores, Mora, Reig, & Toldrá, 2019; Flores & Toldrá, 2020). Nitrites and nitrates have been the focus of intense debate, although, at the levels used in meat products, they have been considered safe for consumers (EFSA, 2017a; EFSA, 2017b). However, they have been classified as probably carcinogenic to humans (group 2A) by the International Agency for Cancer Research, so new alternatives are being seeked to replace them or reduce their dose (IARC, 2010). Also, due to their possible health risk concerns, such as allergic reactions or carcinogenic effects, benzoates, sulfites, sorbates and phosphates are causing unfavorable opinions among consumers (Pisoschi et al., 2018). For example, phosphates are commonly used in processed meat products due to their various technological properties (pH stabilizers, increasing water holding capacity, improving texture, etc.), while the increased consumption of this type of products among infants, children and adolescents can be associated with health problems (Thangavelu, Kerry, Tiwari, & McDonnell, 2019). Therefore, there is an increasing interest in finding alternative additives from natural sources and developing novel food preservation methods, aiming to eliminate or reduce the use of unpopular preservatives in food (Kim et al., 2013; Pisoschi et al., 2018). In this field, the new spotlight is on the identification, development and evaluation of alternative additives from natural sources, and on the validation of novel nonthermal processing technologies such as high-pressure processing or non-thermal atmospheric plasma (López et al., 2019; Rendueles et al., 2011). Concurrently, there is also a need to evaluate the safety and mechanisms of action of these natural preservatives and novel technologies, and to elaborate proper safety regulations (Pisoschi et al., 2018).

Biopreservation strategies are those based on the use of natural substances derived from bacteria, fungi, plants or animals, with the aim of extending the shelf life of food products while guaranteeing their safety (Pisoschi et al., 2018). These substances can be primary and/or secondary metabolites obtained from microorganisms, vegetables, animal products, such as milk or eggs, or animal tissues, among others. Some of them are of great interest for the food industry because they can minimize lipid oxidation, reduce color losses and extend food shelf life. However, most authors circumscribe the concept 'biopreservation' strictly to the use of microorganisms and/or their metabolites to extend shelf life and enhance food safety. The most common approach is to use agents that have antimicrobial activity against bacteria responsible for food spoilage and, especially, agents which can combat foodborne pathogens (Ross, Morgan, & Hill, 2002; Settanni & Corsetti, 2008). An ideal biopreservation agent should only show specific antimicrobial activity against the targeted pathogenic or spoilage microorganism(s), and should not negatively influence the own intestinal microbiome of consumers (Pisoschi et al., 2018). Biopreservative agents can also be used as part of a hurdles technology approach, where they would be strategically combined with other barriers to fight food spoilage and ensure food safety. The rationale behind this combined approach is that synergism may occur by exposing the undesired microorganisms to a series of obstacles to their growth or survival. Moreover, if synergism occurs, lower doses of preservative agents and/or lower technological treatment intensities could be employed (Gálvez, Abriouel, López, & Omar, 2007).

Bacteriocin-producing bacteria and bacteriocins have been often suggested as promising natural preservatives. Nonetheless, some authors have indicated that the application of bacteriocins as additives may be limited by their elevated cost or by their limited effectiveness against certain pathogenic microorganisms in the end product. Consequently, research is still being conducted to improve their conditions of use. Bacteriocin characterization and purification have already allowed to develop databases for the automated identification of antimicrobial peptides and their genetic determinants from genomic data (Silva, Silva, & Ribeiro, 2018; van Heel, Jong, Montalbán-López, Kok, & Kuipers, 2013). Moreover, whole genome sequencing data also provide valuable information for confirming the safety (i.e., absence of virulence and antimicrobial resistance determinants) of bacteriocin-producing strains (Gálvez et al., 2007).

Bacteriocins are biologically active compounds with peptidic structure and antimicrobial activity that are ribosomically synthesized by some bacteria (Bastos, Coelho, & Santos, 2015; Silva et al., 2018). Their classification is complex, considering that they can differ in amino acid structure, biochemical properties, antimicrobial activity and mode of action (Zouhir, Hammami, Fliss, & Hamida, 2010). The first globally used classification was established by Klaenhammer (Klaenhammer, 1993), although it has undergone several modifications as new bacteriocins have been discovered. New classifications have been proposed which include four classes of bacteriocins. The most relevant ones for food biopreservation belong to Class I, which contains thermoresistant and ribosomically synthesized post-translationally modified peptides (RiPPs), and Class II, which predominantly includes unmodified peptides (Bolívar-Monsalve, Ramírez-Toro, Bolívar, & Ceballos-González, 2019; Cotter, Ross, & Hill, 2013).

Although some bacteriocins are active against both spoilage and pathogenic bacteria (Kumariya et al., 2019), most of the bacteriocins are active to a greater extent against Gram-positive bacteria (Pisoschi et al., 2018; Yildirim et al., 2018). Most species from bacteria and archaea can produce at least one bacteriocin (Caulier et al., 2019), although Gram-positive bacteria, and particularly lactic acid bacteria (LAB), have been the most widely studied as producers of bacteriocins of interest for biotechnological applications (Bastos et al., 2015).

LAB are part of the natural microbiota of fermented foods and also part of the intestinal microbiota of humans (Oppegård et al., 2007). Traditionally, they have been widely employed in fermentation processes, transforming carbohydrates to lactic acid and generating other biologically active compounds like organic acids, diacetyl, acetoin, polyols, hydrogen peroxide, antifungal and antibacterial peptides and flavor precursors (Egan et al., 2016). The vast majority of LAB have the status 'Generally Recognized As Safe' (GRAS) according to the U.S. Food and Drug Administration (FDA). The European Food Safety Authority (EFSA) has also granted the status of "Qualified Presumption of Safety" (QPS) to many LAB species (Table 1), included in the genera *Carnobacterium*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus* and the former *Lactobacillus* genus, recently reclassified into twenty-five new genera (EFSA, 2020; Zheng et al., 2020).

The increasing application of LAB as biopreservative agents in meat and meat products makes it necessary to systematically review the latest information available on the antimicrobial activity of LAB and their metabolites against meat spoilage microorganisms and foodborne pathogens. Hence, here a review of the state of the art of meat biopreservation was carried out considering the research articles published in the last 10 years and following the approach detailed in the supplementary file.

Table 1
LAB included in the 2019 updated list of QPS status recommended biological agents for safety risk assessments carried out by EFSA Scientific Panels and Units (EFSA, 2020).

Carnobacterium divergens	*Lactobacillus delbrueckii*	*Lentilactobacillus hilgardii*	*Limosilactobacillus fermentum*
Companilactobacillus alimentarius	*Lactobacillus gallinarum*	*Lentilactobacillus kefiri*	*Limosilactobacillus mucosae*
Companilactobacillus farciminis	*Lactobacillus gasseri*	*Lentilactobacillus parafarraginis*	*Limosilactobacillus panis*
Fructilactobacillus sanfranciscensis	*Lactobacillus helveticus*	*Lentilactobacillus paraplantarum*	*Limosilactobacillus pontis*
Lacticaseibacillus casei	*Lactobacillus johnsonii*	*Leuconostoc citreum*	*Limosilactobacillus reuteri*
Lacticaseibacillus paracasei	*Lactobacillus kefiranofaciens*	*Leuconostoc lactis*	*Loigolactobacillus coryniformis*
Lacticaseibacillus rhamnosus	*Lactococcus lactis*	*Leuconostoc mesenteroides*	*Oenococcus oeni*
Lactiplantibacillus pentosus	*Lapidilactobacillus dextrinicus*	*Leuconostoc pseudomesenteroides*	*Pediococcus acidilactici*
Lactiplantibacillus plantarum	*Latilactobacillus curvatus*	*Levilactobacillus brevis*	*Pediococcus parvulus*
Lactobacillus acidophilus	*Latilactobacillus sakei*	*Ligilactobacillus animalis*	*Pediococcus pentosaceus*
Lactobacillus amylolyticus	*Lentilactobacillus buchneri*	*Ligilactobacillus aviaries*	*Secundilactobacillus collinoides*
Lactobacillus amylovorus	*Lentilactobacillus diolivorans*	*Ligilactobacillus salivarius*	*Streptococcus thermophilus*
Lactobacillus crispatus			

Most of the articles retrieved and thoroughly revised were based on the direct incorporation of LAB to meat products as a functional ingredient or as starter cultures during fermentation processes. There are different ways of directly applying LAB to a meat product, from a direct addition to the meat batter, to a surface spraying on fresh or ready-to-eat (RTE) products. In both cases, the agent can be added fresh or lyophilized. In other cases, the potential use of purified/semipurified biologically active compounds obtained from LAB was assessed or the effectiveness of these biopreservation agents when used within combined hurdles technology approaches or as part of an active packaging was tested (Fig. 1). The sections below critically discuss the most relevant information extracted from the retrieved articles in relation to each of these application strategies, which is also summarised in Tables 2–5.

2. LAB as a non-starter ingredient

L. monocytogenes is a major concern for meat producers due to its ubiquitous nature and its survival capacity under adverse conditions (Buchanan, Gorris, Hayman, Jackson, & Whiting, 2017; Letchumanan et al., 2018). As such, most of the investigations performed up to date on the biopreservation of meat products through the addition of functional LAB cultures focused mainly on designing strategies to eliminate this pathogen, but also *Salmonella* spp. or *Escherichia coli*. Sakaridis, Soultos, Batzios, Ambrosiadis, and Koidis (2014) assessed the potential of *Ligilactobacillus salivarius* (10^6 cfu/g) to inhibit *L. monocytogenes* and *Salmonella* spp. (both at 10^4 cfu/g) on chicken meat and skin (Table 3). All samples were stored at refrigeration temperature (7 °C) and were subjected to microbiological analysis after 0, 1, 2, 3, 4, 5, and 6 days. On the day 6 of analysis, they observed a statistically significant reduction of 0.7 and 0.5 log cfu/g for *L. monocytogenes* and *Salmonella* spp., respectively, on chicken skin. A slightly lower inhibition was observed in chicken meat. They also evaluated if the biopreservation agent caused changes in the sensorial properties of meat. LAB can produce a pH decrease which can lead to unpleasant flavours in food but also to inhibition of spoilage or pathogenic microorganisms. The sensorial evaluation at the end of the storage concluded that the addition of *L. salivarius* to chicken did not have any adverse effect on quality attributes of the food. It even inhibited slime production and improved overall appearance. This could be due to the fact that LAB populations remained practically stable throughout storage. Interestingly, although the authors did not detect LAB growth, the inhibition of the pathogens took place (Sakaridis et al., 2014).

The influence of temperature in the activity of biopreservative agents has been commonly assessed in the literature. A constant 2.5 log reduction of *L. monocytogenes*, which was reduced to levels below the limit of detection, was obtained in "chorizo" stored at 4 °C for 35 days when both *L. monocytogenes* and *Carnobacterium maltaromaticum* strains were incorporated at concentrations of 10^2 and 10^3 cfu/g, respectively (Table 4) (González, Yien, Castrillón, & Ortega, 2013). When the study was performed at 8 °C, during the first 7 days of storage the difference between the treated and control samples was of just 1 log unit, but from day 7 to day 35 *L. monocytogenes* counts remained under the limit of detection. These results confirm that the use of *C. maltaromaticum* as a biopreservative agent is a good choice due to its capacity to grow at low temperatures, as one of the prevailing LAB in fresh meat. In other study, García-Díez and Patarata (2017) obtained that higher storage temperatures (22 °C) result in safer biopreservative-treated products than lower storage temperatures (8 °C) because some LAB are not capable to grow at low temperatures, unlike psychrotrophic *L. monocytogenes*. In addition to storage temperature, García-Díez and Patarata (2017) also evaluated the effect of other components added in the meat batter, including salt (at 1.5 or 3%), *L. sakei* (6 log cfu/g) and 0.75% dextrose plus 0.75% lactose, in the growth of the pathogen (Table 4). They concluded that neither the addition of *L. sakei* nor the addition of dextrose and lactose achieved significant *L. monocytogenes* reductions in sliced "chouriço" sausage. However, high salt contents reduced *L. monocytogenes* counts. It is worth mentioning that after 7 days of storage both at 8 and 20 °C, *L. monocytogenes* was not detected in any of the samples (García-Díez & Patarata, 2017).

The use of *L. sakei* (10^5 cfu/g) for the control of *Salmonella* Choleraesuis (10^4–10^5 cfu/g) in a fresh pork sausage showed an average reduction of 2.4 log units in the pathogen, while in the absence of the

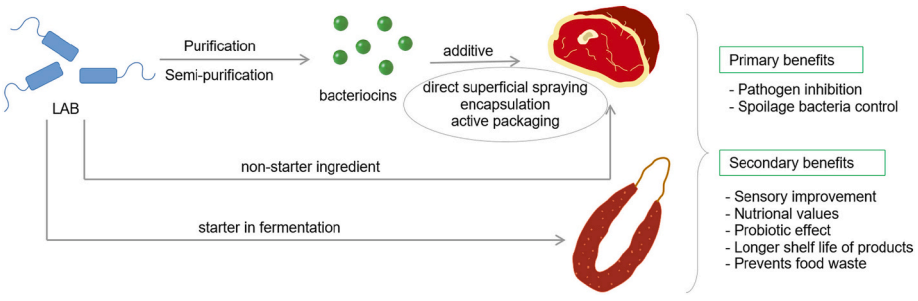

Fig. 1. Lactic acid bacteria, bacteriocins and their application in different meat products to achieve several benefits related to the inhibition of undesirable bacteria or towards improvements in the end product.

Table 2
Studies performed in beef products, testing diverse biopreservative agents against different target microorganisms.

Reference	Biopreservative agent	Product	Target microorganism(s)	Results observed
Khalili Sadaghiani et al. (2019)	*L. reuteri* or *L.plantarum* from beef in combination with garlic extract	Ground beef	*L. monocytogenes*	*L. reuteri* induced a 0.5 log reduction; *L. plantarum* induced a 0.7 log reduction. Combination of garlic extract (1%) with *L. reuteri* resulted in a 1.4 log reduction. Combination of garlic extract (1%) with *L. plantarum* resulted in a 1.5 log reduction.
Kamiloğlu et al. (2019)	*L. plantarum* strains – producers of bacteriocins/bacteriocin – like compounds	Sucuk sausages	*L. monocytogenes*	The difference between the initial and the final pathogen counts for the assays with *L. plantarum* S50, S51, S72, S74 and S85 were 2.7, 2.4, 1.0, 1.4 and 1.2 log, respectively.
Cosansu et al. (2010)	Bacteriocin-producing *P. acidilactici*	Dry fermented sausage during fermentation	*L. monocytogenes*	*L. monocytogenes* counts decreased by 3.3 log CFU/g during the 8-day ripening period. pH values for the samples with *P. acidilactici* decresased from 5.5 to 4.9.
Orihuel et al. (2018)	Bacteriocinogenic *Enterococcus mundtii* and curing agents (3% NaCl, 0.02% NaNO2, 0.0075% ascorbic acid, 0.75% sucrose and 0.75% glucose)	Beef sausage	*L. monocytogenes*	In competition with *E. mundtii*, *L. monocytogenes* shows a slight decrease at 96 h (0.7 cfu/g). When *E. mundtii* was in combination with curing additives, an enhanced antilisterial activity reached a >2 log cfu/g reduction.
Yildrim et al. (2016)	Lactococcin BZ	Fresh beef meat	Total aerobic psychrotrophic and mesophilic bacteria, lactic acid bacteria, total coliforms, faecal coliforms and *L. innocua*	Lactococcin BZ at 2500 AU/ml resulted in a 4.9 log decrease in mesophilic bacteria, 3.5 log reduction in psycotrophic bacteria and 3.9 log reduction in LAB. Also, in a $1.9{\cdot}10^4$ CFU/g reduction in coliforms and $1.04{\cdot}10^2$ CFU/g reduction in faecal coliforms. A concentration of 1600 AU/ml reduced *L. innocua* by 6 logs after 6 days.
Olaoye and Onilude (2010)	*P. pentosaceus* and *P. acidilactici*	Sliced fresh beef samples	*L. monocytogenes* and *S.* Typhimurium	Reduction in *Enterobacteriaceae*, *Staphylococcus* and coliforms. *L. monocytogenes* counts were below the detection limit on day 2. Counts of *S.* Typhimurium showed about a 2 log reduction.
Hu et al. (2019)	Partly purified bacteriocin from *C. maltaromaticum* combined with steam and chitosan	Sliced beef	*E. coli* and *S.* Typhimurium	No synergistic effects.
Arief et al. (2012)	Bacteriocin produced by *L. plantarum*	Beef meatballs	*S.* Typhimurium and *E. coli*	Addition of bacteriocin significantly reduced the total plate counts, which were about 1 log cfu/g lower than those for control and nitrite treated batches at day 0, 3 and 6. *E. coli* was not present at days 3 and 6 either with nitrite or with the bacteriocin.
Smaoui et al. (2016)	*Mentha piperita* essenthial oil with semi-purified bacteriocin BacTN635	Minced beef meat	Enterobacteriaceae	From 2.1 log to 2.6 log reduction depending on the concentration of essential oil and concentration of BACTN635. Extension of the shelf life for approximately 7 days.
Castellano et al. (2011)	Bacteriocin-producing *L. curvatus* and *L. lactis* in combination with Na_2EDTA	Frozen ground-beef patties	*E. coli*	The presence of the bioprotective cultures and the chelator resulted in a 1 log reduction for *E.coli* at day 0. Similar declines of indigenous coliforms were observed. Neither *E. coli* nor coliforms were inhibited in the absence of the chelator.
Castellano et al. (2010)	Lactocin producer strain of *L. curvatus*	Vacuum packed raw beef	*B. thermosphacta*	Not significant bioprotective effect was found for *Pseudomonas* sp., but *B. thermosphata* was effectively inhibited. It also controled the growth of spoilage lactic acid bacteria naturally present in meat.

biopreservative agent *S.* Choleraesuis was able to grow around 4.4 log units after 7 days of storage at 10 °C under vacuum atmosphere (Table 4) (Gelinski et al., 2019).

An increase in meat production and consumption is expected for the near future, especially in developing countries, where insufficient technological development jeopardizes food safety. A study performed in Nigeria proposed that biological agents can be exploited as an extra preservation technique. The results on local fresh beef inoculated with *Pediococcus pentosaceus* and *P. acidilactici*, added individually and in combination at a concentration of 10^6 cfu/g, and stored at room temperature (30 °C) exhibited a reduction of up to 4 log cycles in *Enterobacteriaceae*, and yeasts and moulds, as compared to the control samples at day 7 of storage, while *Staphylococcus* spp. counts were under the detection limit during the 7 days of storage (Table 2). Also, coliform counts were under the detection limit for batches inoculated with *P. pentosaceus*, the combination of both LAB, and *P. acidilactici* on days 1, 2 and 3, respectively. The impact on *L. monocytogenes* was promising for *P. pentosaceus*, both added individually and in combination, obtaining a bactericidal effect on day 2. However, *P. acidilactici* individually applied barely achieved 1 log reduction in the counts of the pathogen throughout the storage period. Similarly, *S.* Typhimurium counts were only reduced on day 3 for up to 1 log cfu/g with *P. acidilactici* and 2 log cfu/g with *P. pentosaceus* or the mixture of both LAB (Olaoye & Onilude, 2010). These findings are in agreement with those of García-Díez and Patarata (2017), who showed that high temperatures could help improve LAB functionality. In addition, Olaoye and Onilude (2010) also evaluated the impact of the LAB on pH and thiobarbituric acid (TBA) and free fatty acid (FFA) contents. The three LAB inoculated samples had pH values below 5 from day 2 of storage, which could explain, at least in part, the control of the undesired microorganisms. The lower contents of TBA and FFA in LAB inoculated samples showed that LAB cultures can also control the lipolytic and oxidative changes of fat and, consequently, maintain the quality attributes of fresh beef.

When a strain of *P. acidilactici*, in this case bacteriocinogenic, was applied (at 7 log cfu/g) on sliced turkey breast, *L. monocytogenes* counts remained lower than on the control batches, but a complete inhibition

Table 3
Studies performed in poultry products, testing diverse biopreservative agents against different target microorganisms.

Reference	Biopreservative agent	Product	Target microorganism(s)	Results observed
Balay et al. (2017)	Leucocin A from *Leuconostoc gelidum*	Wieners	*L. monocytogenes*	Around 1 log reduction after 16 days of storage at 7 °C
Cosansu et al. (2010)	Bacteriocin-producing Pediococcus acidilactici	Sliced turkey breast	*L. monocytogenes*	*L. monocytogenes* counts remained lower than in the control batch, although it was not completely inhibited during storage at 12 °C
Trinetta et al. (2010)	Sakacin A produced by *L. sakei*, delivered in some cases by a pullulan film	Ready-to-eat turkey deli meat	*L. monocytogenes*	Sakacin A directly applied to turkey reduced *L. monocytogenes* more than 2 log CFU/g after 3 weeks at 4 °C, while sakacin A-containing pullulan films reduced its populations 3 log CFU/g during the same time
Ruiz et al. (2010)	0.2, 0.3, 0.4, and 0.5% nisin treatment solutions	Ready-to-eat turkey ham	*L. monocytogenes* and LAB	Antimicrobial effectiveness of nisin increased as concentration increased. *L. monocytogenes* counts remained below 2 log cfu/g with the 0.5% treatment at 4 °C for 63 days. LAB counts were significantly lower for all nisin treatments when compared with the control
Sakaridis et al. (2014)	*L. salivarius*	Chicken meat and skin	*Salmonella* spp. and *L. monocytogenes*	After 6 days at 7 °C, reduction of *Salmonella* spp. on chicken skin was up to 0.5 log CFU/cm^2, while for *L. monocytogenes* up to 0.7 CFU/cm^2 log. Reduction of *Salmonella* spp. on chicken meat was up to 0.5 and for *L. monocytogenes* up to 0.7 log CFU/cm^2
Melero et al. (2012)	*L. pseudomesenteroides* combined with MAP (50% CO_2 and 50% O_2)	Fresh chicken meat burger	*L. monocytogenes* and *C. jejuni*	*L. pseudomesenteroides* reduced *L. monocytogenes* counts in 0.90 log CFU/g when packed under MAP. *C jejuni* was affected by freezing but only completely eliminated in combination with high O_2 MAP. The use of MAP extended the product's shelf-life up to 21 days
Chakchouk-Mtibaa et al. (2017)	Partially purified bacteriocin BacFL31 from *E. faecium*	Ground turkey meat	*L. monocytogenes, S.* Typhimurium,*S. aureus*	At 200 AU/g, the bacteriocin extended the shelf life up to 10 days, and at 400 AU/g up to 14 days. After 30 h, *L. monocytogenes* and *Salmonella spp.* counts were reduced by 3 logs. The BacFL31 treatment was less effective against *S. aureus*. After 30 h, 400 AU/g of bacteriocin reduced its numbers to 2.2 logs, from an initial level of 4 logs

during storage at 12 °C was not achieved (Table 3) (Cosansu, Geornaras, Ayhan, & Sofos, 2010). These authors obtained more satisfactory results when applying the same biopreservative agent as a starter culture in a fermented sausage (below described).

Two strains of *Lactobacillus* spp. and *Levilactobacillus brevis* were co-inoculated separately with *Yersinia enterocolitica* on pork meat samples and stored at 4 °C for 28 days (Table 4). Bacterial strains were inoculated by dipping the product in water containing concentrations of 5×10^7, 1×10^8 and 1×10^5 cfu/ml, respectively, for *Lactobacillus* spp., *L. brevis* and the pathogenic strain. The results demonstrated that as the storage period progressed the inhibition was stronger. *Yersinia* counts were on day 0 of 5.5 log cfu/ml and remained during the last day of storage (day 28) at 4.9 log cfu/ml with the addition of *Lactobacillus* spp. and 4.4 log cfu/ml with *L. brevis*, while in the control samples the counts of the pathogen increased to up to 7.9 log cfu/ml, achieving therefore a reduction of 3 and 3.5 log cfu/ml, respectively, and obtaining a bactericidal effect until the last day of storage (Angmo, Kumari, Savitri, & Chand Bhalla, 2016).

Some biopreservation studies have focused on the control of food spoilage microorganisms, such as *Brochrothrix thermosphacta*, associated with pork and lamb meat spoilage (Stanborough et al., 2017). Olaoye, Onilude, Ubbor, and Olaoye (2015) evaluated its survival capacity in pork meat when co-inoculated with two strains of *Lactococcus lactis* (Table 4). The *L. lactis* subsp. *lactis* strain was capable of diminishing *B. thermosphacta* counts to below the detection limit (<2 log cfu/g) after 48 h, while the *L. lactis* subsp. *hordinae* strain did not produce any significant reduction (the highest reduction was of 0.8 log cfu/g after 120 h of incubation). *L. lactis* subsp. *lactis* showed also favourable results against *Enterobacteriaceae* and *Staphylococcus* spp., reducing the counts of the former and inhibiting the latter ones to levels below the detection limit (<2 log cfu/g) (Olaoye et al., 2015).

Nutritional and sensory properties of meat products must be considered during the evaluation of novel biopreservatives. *Lactilactobacillus curvatus*, producer of lactocins 705 and AL705, was examined as a biocontrol agent at a concentration of 10^6 cfu/g in vacuum-packed fresh beef steaks stored during 60 days at 2 °C (Table 2). The counts of *B. thermosphacta* remained unchanged throughout the 60 days of storage while in the control batches a 2.5 log increase of this spoilage bacterium was observed. On the other hand, for *Pseudomonas* spp., no significant differences were observed between the control and treated batches. Along with microbiological analyses, microstructural characteristics of meat were evaluated and a 10-day delay for the appearance of tissue degradation signs was achieved when *L. curvatus* was used. Also, a trained panel evaluated the sensorial characteristics of the steaks and no significant differences between treated and control samples were appreciated during the first 30 days of storage. Moreover, samples treated with *L. curvatus* reached the end of the experiment (60 days) in better condition than the control samples according to odour, flavour and presence of off-odours and off-flavours. Only an “acid” off-flavour, typical of LAB, was developed in the treated batches, concurring with a slight but significant pH decrease (Castellano, González, Carduza, & Vignolo, 2010).

3. LAB as starter cultures in fermentation processes

LAB can be applied as starter cultures playing an important role in fermentation processes (Bartkiene et al., 2019). Five autochthonous *Lactobacillus plantarum* strains (S50, S51, S72, S74, and S85) selected due to their *in vitro* listericidal effect, were tested by Kamiloğlu, Kaban, and Kaya (2019) at a concentration of 10^7 cfu/g with *Staphylococcus xylosus* GM92 (10^6 cfu/g) in sucuk, a typical Turkish dry fermented sausage (Table 2). Strains were thought to produce bacteriocins and/or bacteriocin-like peptides, as treatment with proteases caused the loss of their inhibitory activity (Kamiloğlu et al., 2019). They observed reductions in *L. monocytogenes* counts (initially inoculated at 10^4 cfu/g) ranging from 1 to 2.7 log units for the different *L. plantarum* strains tested after 11 days of ripening. It was remarkable that the two most effective strains were also the ones with the highest acidification potential. They concluded that fast acidification, concurrently with the production of antimicrobial compounds during ripening, were fundamental for the control of *L. monocytogenes*. Similar conclusions regarding control of *L. monocytogenes* through pH modulation were achieved in the same beef product but using the bacteriocin-producing microorganism *P. acidilactici* (Table 2) (Cosansu et al., 2010). Although naturally occurring starter cultures controlled *L. monocytogenes* growth by approximately 1 log cfu/g, *P. acidilactici* produced a 3.3 log cfu/g reduction after 8 days of fermentation at temperatures of 22–24 °C. A pH decrease from 5.5 to 4.9 was seen in sucuk batches treated with *P. acidilactici*, but not in control samples. It is very likely that starter cultures formed by strains sourced from meat products and therefore better adapted to the meat matrix will

Table 4
Studies performed in pork products, testing diverse biopreservative agents against different target microorganisms.

Reference	Biopreservative agent	Product	Target microorganism (s)	Results observed
Ortiz et al. (2014)	*L. sakei* (sakacin K producer); Bactoferm F-LC	Batter for Iberian chorizo	*L. monocytogenes*	*L. sakei* induced a reduction of 4.4 and 5.4 log, respectively, at 7 °C and 20 °C. Bactoferm F-LC induced a reduction 2.5 and 2.3 log, respectively
Zanette et al. (2015)	*L. plantarum:* bacteriocin producer and non-bacteriocin producer strain	Pork Colonial sausages	*L. monocytogenes*	Both agents equally reduced *L. monocytogenes* by 1.7 log
Dussault et al. (2016)	Nisin and hop alpha acids	Ham	*L. monocytogenes*	Nisin applied in concentration of 20 ppm did not reduce *L. monocytogenes*
Castellano et al. (2018)	Bacteriocins from *L. curvatus* and *L. sakei*, in combination with nisin or organic acids	Frankfurters	*L. monocytogenes*	Bacteriostatic effect of bateriocins alone. Combination of bacteriocins and acids reduced *L. monocytogenes* under the detection limit up to day 6 of storage
Xie et al. (2018).	Film incorporating plantaricin BM-1	Pork meat	*L. monocytogenes*	Reduction of 1.4 $\log_{10}$ CFU/ g for *L. monocytogenes* compared with the control
Macieira et al. (2018)	*L. plantarum*	Chouriço	*L. monocytogenes*	No additional inhibition compared with the control
García-Díez and Patarata (2017)	*L. sakei*	Sliced chouriço sausages	*L. monocytogenes*	No influence on survival of the pathogen
Ghabraie et al. (2016)	Combination of essential oils, nisin, nitrite and organic acid salts, encapsulated.	Fresh pork sausage	*L. monocytogenes*	Antimicrobials (nitrite-100 ppm, nisin- 2.5 ppm, organic acid salts-1.5 % and essential oil-0.05 %) caused a reduction from initial 3 logs to1.8 log, after 7 days
Vaz-Velho et al. (2013)	*L. sakei* and *L. plantarum*, vacuum packed or packed under MAP (20% CO_2, 80% N_2)	Alheira paste	*L. monocytogenes*	2 log decrease with *L. sakei*. No differences between vacuum or MAP
González et al. (2013)	*C. maltaromaticum*	Chorizo	*L. monocytogenes*	Continuous reduction of 2.5 log at 4 °C. At 8 °C there was a initial reduction of 1 log, but from day 7 on there was a reduction to levels below the detection limit
Woraprayote et al. (2013)	Biocomposite film impregnated with pediocin PA-1/AcH	Raw sliced pork	*L. monocytogenes*	All treatments significantly reduced the *Listeria* population about 1.5–2 log cycles during storage at 4 °C
Castro et al. (2018)	Bacteriocin producing *P. acidilactici* or related supernatant alone or in combination with HPP	Non-smoked sterilized paste of Alheira sausages	*L. innocua*	After 60 days of storage (4 °C), *L. innocua* inactivation values reached 0.9 to > 1.5 log CFU/g. With high pressure, a reduction of up to roughly 4 logs was observed. Bacteriocin HA-6111-2 alone did not cause pathogen reduction
Casquete et al. (2018)	*L. sakei* or BLC35 culture	Sliced 'lombo'	*L. innocua*	Both cultures led to a reduction of 1–2 log CFU/g after 12 h. After 124 days of storage at 5 °C only *L. sakei* maintained this antilisterial effect, which was more evident at 40% CO_2/60% N_2
Nikodinoska et al. (2019)	*L. plantarum* non bacteriocin producing strain	Chorizo	*L. monocytogenes* and *Salmonella* spp.	Reduction of 2.6 and 3.8 logs with 75 mg/kg and 150 mg/kg added nitrite, respectively, after 7 days. No antimicrobial activity against *Salmonella* spp. growth
Gelinski et al. (2019)	*L. sakei*	Fresh pork sausage	*S. Choleraesuis*	Average reduction of 2.4 logs after 7 days stored at 10 °C
Wang et al. (2017)	Nisin, *Salmonella* bacteriophage and/ or potassium sorbate (PS)	Pork meat	*S.* Typhimurium	Nisin alone did not cause reduction. Phage treatments inhibited *Salmonella* to concentrations under the detection limit. Nisin-PS-phage treatment significantly lowered total viable counts
Vatanyoopaisarn et al. (2011)	*P. acidilactici* and *L. plantarum*	Thai fermented sausage	*S. aureus*	Total staphylococci remained unchanged. *S. aureus* was significantly reduced. During 2 days at room temperature (28–32 °C), *E. coli* counts did not increase and *Salmonella* sp. was reduced to an undetectable level
Olaoye et al. (2015)	*L. lactis* subsp. *lactis* I23 and *L. lactis* subsp. *lactis* E91	Pork meat	*B. thermosphacta*	Reduction of Enterobacteriaceae, no detection of *Staphylococcus* sp. and *B. thermosphacta* from 48 h in samples with strain I23. Mixed cultures were more effective than when used individually
Angmo et al. (2016)	*Lactobacillus* sp. and *L. brevis*	Refrigerated meat	*Y. enterocolitica*	*Y. enterocolitica* was reduced to 4.9 log CFU/ml (*Lactobacillus* sp.) and 4.4 log CFU/ml (*L. brevis*) on the 28th day of storage at 4 °C. The inhibition was higher as the incubation period was prolonged
de Azevedo et al. (2020)	Bacteriocin-like inhibitory susbstances (BLIS) from *P. pentosaceus* and nisin	Ready-to-eat pork ham	*L. seeligeri*	BLIS was effective in inhibiting the growth of *L. seeligeri* for 6 days at 4 °C (counts from 1.7 log CFU/g to below the detection limit)
Hongthong et al. (2020)	Sucrose (0.3% and 1.2%) and *L. plantarum*	Thai fermented sausage	Total microbial counts and LAB	The total microbial counts and LAB counts increased rapidly during fermentation at 30 °C and then decreased during storage at 4 °C

better accomplish their antimicrobial activity *in situ*. On the other hand, on the basis that not all food matrices are similar, the promising results obtained in one given meat product will not necessarily be replicated in a different one, as it can be seen when comparing the effects of the addition of this *P. acidilactici* biocontrol agent on sucuk and on sliced turkey breasts (Cosansu et al., 2010).

Ortiz, López, Garriga, and Martínez-Suárez (2014) tested a *L. sakei* strain and the industrial meat starter culture *Bactoferm F-LC*, containing *P. acidilactici* (pediocin producer), *L. curvatus* (bavaricin producer) and *Staphylococcus xylosus*. Both bioprotective cultures were added independently on meat batter together with a *L. monocytogenes* cocktail (10^4 cfu/g) to evaluate their antilisterial effect (Table 4). Two different conditions were simulated, a slow fermentation at 7 °C and a fast fermentation at 20 °C. The pH significantly decreased on the first day only in batches fermented with bioprotective cultures at 20 °C, while in the control batches the decrease in pH was only observed on the third day of fermentation. At 7 °C, in all batches, the pH dropped after 3 days of fermentation, but values were lower for those with the bioprotective cultures. With regard to *L. monocytogenes* counts, whereas *Bactoferm F-LC* showed a bacteriostatic effect at 7 °C, *L. sakei*

Table 5
Studies performed in other meat-related products, testing diverse bio-preservative agents against different target microorganisms.

Reference	Biopreservative agent	Product	Target microorganism (s)	Results observed
Kumar et al. (2017)	Pediocin from *P. pentosaceus* and *Murraya koenigii* berries extract	Goat meat emulsion	*L. innocua*	Around 1.4, 3.0 and 4.1 log reductions in *L. innocua* counts was noted at day 3, 6 and 9, respectively
Rivas et al. (2018)	Cell free supernatant with sakacin G from *L. curvatus*	Natural and artificial casings	*L. innocua*	Growth reduction was up to 0.5 log/cm^2, 0.6 log/cm^2 and 1.8 log/cm^2 in the natural, cellulose and collagen casing, respectively. Collagen casings were a better carrier of the sakacin G, while in the rest of casings only a bacteriostatic effect was observed
Hammou et al. (2010)	Nisin solutions at 0, 100, 150 and 200 μg/g. Each combined with salt at 0, 4, 7 and 12% (w/v)	Sheep natural casings	*L. monocytogenes*	Nisin alone had no antilisterial effect before 20 days. At days 20–90 all nisin treatments reduced the *L. monocytogenes* population. A bactericidal effect was evident in all samples in combination with salts, with reductions of approximately 1 log cfu/g after 90 days
Hammou et al. (2011)	Oregano essential oil (EO) and nisin	Sheep natural casings	*E. coli*	Nisin at 800 or 1600 IU/g did not show any antibacterial activity against *E. coli* strains, while the EO did. Synergy between EO and nisin was observed
Wijnker et al. (2011)	Different nisin solutions	Natural sausage casings	*Clostridium sporogenes* spores	Nisin caused a reduction of approximately 1 log of *Clostridium* viable spores during outgrowth

exerted a bactericidal effect at 7 °C and 20 °C, achieving nearly a 2 log reduction in the initial *L. monocytogenes* counts. The fastest inhibit ion was observed in the batch with *L. sakei* at 20 °C, where *L. monocytogenes* counts started to decrease on the second day of fermentation (Ortiz et al., 2014). These results show that LAB not only help to reduce the pH more rapidly, but also compete with pathogenic bacteria for the available nutrients and produce antagonistic primary and secondary metabolites, ensuring the safety of the product, effect that is more rapidly achieved at higher temperatures, which agree with the conclusions drawn by García-Díez and Patarata (2017) and Olaoye and Onilude (2010).

Both bacteriocinogenic and non-bacteriocin producing LAB can be added to meat products as starter cultures. Two *L. plantarum* strains, one producing a bacteriocin and other which did not produce any bacteriocin, were added at 10^6 cfu/g and assessed during the fermentation and drying for 19 days of colonial sausages (Table 4). No significant differences in effectiveness were observed between both of them, which achieved a 1.7 log cfu/g reduction in the concentration of *L. monocytogenes* (initially inoculated at 10^4 cfu/g) (Zanette, Dalla, & dos Santos, 2015).

The reduction of *S. aureus*, *E. coli* and *Salmonella* was studied in Thai fermented sausages by Vatanyoopaisarn, Prapatsornwattana, Kuhakongkeat, and Phalakornkule (2011), who added three LAB, *P. acidilactici*, *L. plantarum* CP1-15 and *L. plantarum* CP2-11, to control them (Table 4). When the *Lactobacillus* strains were individually applied at 10^6 cfu/g, *S. aureus* and *E. coli* were not significantly reduced. However, when dual starters were used (*P. acidilactici* with *L. plantarum* CP1-15 and *P. acidilactici* with *L. plantarum* CP2-11), counts of *S. aureus* were significantly reduced as compared with the control, and *Salmonella* counts were reduced to levels below the detection limit. Sausages with the dual starter *P. acidilactici* and *L. plantarum* CP1-15 obtained a higher score in appearance, taste, texture and overall acceptability (Vatanyoopaisarn et al., 2011). In other Thai fermented sausage, *L. plantarum* was suggested as an attractive alternative to improve the quality of the product (Table 4). With the starter, pH values decreased faster than in non-inoculated batches and the fermented sausages had significantly higher counts of LAB. The authors also evaluated the addition of sucrose and recommended an inoculation of 10^7 cfu/g of *L. plantarum* and a sucrose level of 0.3% (Hongthong, Chumngoen, & Tan, 2020).

Macieira et al. (2018) tested in a traditional Portuguese fermented dry meat sausage bacteriocinogenic *L. plantarum* cultures, both fresh and lyophilized (10^6 cfu/g), but obtained no further inhibition of *L. monocytogenes* (initially inoculated at 10^5 cfu/g), as compared with the control batches (Table 4). The authors attributed this either to the fact that the high hydrophobic matrix of the product can protect the pathogen from bacteriocins or to the low concentration of the bio-preservative agents. Regarding the sensory evaluation, lower hardness was obtained in the inoculated samples, which was attributed to the impact on the protein hydrolysis, and lower brightness and a strange odour were detected at the end of storage in comparison with the control batches (Macieira et al., 2018).

4. Purified or semipurified bacteriocins

Purified or semi-purified bacteriocins or other metabolites of bacterial origin can be also used to control undesired bacteria in meat products. Lactococcin BZ, produced by *L. lactis* spp. *lactis* BZ, was added to fresh beef at concentrations ranging from 200 to 2,500 AU/ml (Table 2). The results obtained showed that the higher the amount of bacteriocin added, the higher the inhibitory activity observed, with 4.9, 3.9 and 3.5 log reductions for total mesophilic bacteria, LAB and total psychrotropic bacteria being obtained, respectively, at the end of storage (12 days) (Yildrim, Yerlikaya, Öncül, & Sakin, 2016). *Listeria innocua was also* inoculated at a concentration of 10^6 cfu/g, together with the two lactococcin BZ preparations, at 800 and 1,600 AU/ml. Reductions of 4.5 and 6 log units, respectively, were found for this microorganism on day 6 (Yildrim et al., 2016).

Bacteriocins produced by indigenous LAB have shown potential to be used as an alternative to nitrites. Plantaricin, produced by *L. plantarum* 2C12, isolated from Indonesian local beef, was purified and added at a 0.3% (v/v) during grinding in beef meatballs, which were later stored for 6 days at 4 °C (Table 2). The treatment showed the same effectiveness against *E. coli* as a treatment with 0.3 % nitrites, with counts below the detection limit at day 3 and 6, while around 0.6 log cfu/g was detected for the control samples and the samples containing nitrites. Furthermore, the samples with the addition of plantaricin showed the lowest total plate counts. In addition, the application of the bacteriocin did not lead to significant physical or nutritional changes (Arief, Jenie, Suryati, Ayuningtyas, & Fuziawan, 2012). When bacteriocins are used to inhibit Gram-negative bacteria, they first need to cross the cell wall. The combination of bacteriocins with chemical or physical treatments that disrupt the cell wall is frequently proposed as a successful synergistic approach. However, some bacteriocins are also active against Gram-negative bacteria, which may be attributed to the fact that there is a

membrane protein that can act as a bacteriocin-specific receptor (Acuña, Picariello, Sesma, Morero, & Bellomio, 2012).

Another study undertaken with ground turkey meat evaluated the activity of BacFL31, a partially purified bacteriocin from *Enterococcus faecium,* against *L. monocytogenes*, *S.* Typhimurium and *S. aureus* (Table 3). The bacteriocin was added at two different concentrations in turkey meat: 200 and 400 AU/g, and the target bacteria was inoculated at 10^4 cfu/g. At the lowest concentration tested, the shelf life of the product was of 10 days, while at the highest concentration it was of 14 days. *L. monocytogenes* and *S.* Typhimurium were inhibited, with their counts being reduced by 3 log units after 30 h of incubation at 4 °C, while the highest concentration of bacteriocin diminished *S. aureus* counts around 1.8 log units (Chakchouk-Mtibaa et al., 2017).

Leucocin A, a class II bacteriocin, has been suggested as a good candidate to be used in meat products due to its proved antilisterial activity and its stability under extreme conditions. In order to improve the bactericidal activity, or any other functional aspect, of a bacteriocin, variants with minor changes in the amino acid sequence can be produced. Balay, Dangeti, Kaur, and Mcmullen (2017) synthesized an analogue of leucocin A by replacing the amino acid asparagine in the residue 17 by leucine, obtaining the leucocin N17L variant. They determined if the activity against *L. monocytogenes* could be enhanced in the presence of natural spoilage microbiota on RTE poultry meat (Table 3). Unfortunately, the synthesized compound did not show as good antilisterial activity as the original bacteriocin. Nevertheless, *in situ* experiments with leucocin A on the surface of wieners reduced the counts of *L. monocytogenes*. However, the presence of some spoilage bacteria could reduce the antimicrobial activity of the bacteriocin.

Among all purified bacteriocins tested up to now, nisin (E-234) is the only one allowed as food additive in the European Union (European Commission, 2011). An assay performed testing different nisin concentrations against *L. monocytogenes* in RTE turkey ham demonstrated that at day 0 all nisin treatments achieved up to 4 log reductions in *L. monocytogenes* when compared with the control batch (Table 3). It was reported that the higher the concentration of nisin the higher the inhibitory effect observed. Remarkably, 2 log reductions in *L. monocytogenes* counts were still obtained after 63 days of storage at 4 °C for samples treated with the highest nisin concentration (0.5%) (Ruiz, Williams, Djeri, Hinton, & Rodrick, 2010).

Although not recognized as a pathogen, *Listeria seeligeri* can also cause sporadic human infections and, therefore, it has also been employed in experimental studies with artificially contaminated foods. De Azevedo et al. (2020) compared the antimicrobial activity of the industrial biopreservative Nisaplin with that of a bacteriocin-like inhibitory substance (BLIS) produced by *P. pentosaceus* in vacuum packed RTE pork ham artificially contaminated with *L. seeligeri* (Table 4). Between day 2 and 6 of storage at 4 °C, *L. seeligeri* counts in the BLIS-inoculated samples were <1 log cfu/g. However, in samples treated with Nisaplin, *L. seeligeri* counts were similar to those obtained for the control batch until the second day of storage, when a progressive decay in counts started until concentrations <1 log cfu/g were reached at day 6. Nonetheless, BLIS-inoculated samples showed a slight recovery of *L. seeligeri* from day 6 to the end of the experiment (day 10), while Nisaplin treated samples showed *L. seeligeri* concentrations <1 log cfu/g until the end of the experiment.

Although most of the research studies retrieved were carried out in beef, pork and poultry products, a few articles dealt with casings or goat meat. In the case of casings, preservation with brines or with dry salt appears to be the ideal environment for the control of pathogenic non-spore-forming bacteria. However, if the environmental conditions become favourable, the remaining spores could germinate, thus being necessary the implementation of additional barriers. When nisin was employed to evaluate the reduction of *Clostridium sporogenes* spores outgrowth on casings, the model showed that nisin can cause a reduction of approximately 1 log of viable *C. sporogenes* (Table 5) (Wijnker, Weerts, Breukink, Houben, & Lipman, 2011).

The effectiveness of cell free supernatants (CFS) containing sakacin G, produced by *L. curvatus*, added individually to porcine, ovine, bovine, cellulosic and collagen casings, showed variable results depending on the type of casing used. Porcine casings containing sakacin G showed statistically significant reductions in *L. innocua* counts from the day 2 of storage at 5 °C (Table 5). Collagen casings also exhibited significant lower *L. innocua* counts when treated with CFS containing sakacin G from the fifth day of storage and a 2 log reduction was achieved at the end of the trial (35 days). For ovine, bovine and cellulosic casings, a bacteriostatic effect until the end of the experiment was observed (Rivas, Cayré, Campos, & Castro, 2018).

5. LAB and/or their metabolites as part of a hurdle technology strategy

Several studies have tested the effectiveness of different LAB combined with other biopreservatives. In a study carried out in ground raw beef, two LAB strains, *Limosilactobacillus reuteri* and *L. plantarum*, were tested individually and in combination with a garlic extract (1%) at a concentration of 10^7 cfu/g, resulting in higher reductions of *L. monocytogenes* (initially inoculated at 10^5 cfu/g) and also of aerobic mesophilic bacteria when LAB and garlic extract were combined (Table 2) (Khalili Sadaghiani, Aliakbarlu, Tajik, & Mahmoudian, 2019). After 12 days of storage at 4 °C, the *L. reuteri* strain caused a 0.5 log cfu/g reduction of *L. monocytogenes* counts while its combination with the garlic extract resulted in a 1.4 log cfu/g reduction. The *L. plantarum* strain alone reduced 0.7 log cfu/g but, when combined with the garlic extract, caused a 1.5 log cfu/g reduction of the pathogenic microorganism. For aerobic mesophilic bacteria, significant statistical differences were also found when combining the LAB strains with garlic extract, reaching values 1.4–1.6 log cfu/g lower than in control batches. Interestingly, the sensory analysis revealed that regarding colour, odour and overall acceptability, the samples with combined treatments received higher scores than the control samples and also than the samples with only LAB at the end of the storage period.

When various antimicrobial agents are used together, it is crucial to choose the most appropriate combination, so that favourable outcomes or even synergism can take effect. The addition of chelating agents can make the outer membranes of Gram-negative bacterial cells more permeable to hydrophobic peptides such as bacteriocins (Belfiore, Castellano, & Vignolo, 2007). The combination of Na_2EDTA (ca. 48 mM) with bacteriocin-producing strains of *L. curvatus* and *L. lactis* (at 10^7 cfu/g) in frozen ground-beef patties, stored during 9 days at 5 °C, showed better ability than the use of the chelator alone to control *E. coli* O157:H7 and indigenous coliforms (Table 2). Moreover, in absence of Na_2EDTA, neither *E. coli* nor coliforms were inhibited by the bioprotective cultures (Castellano, Belfiore, & Vignolo, 2011).

Castellano et al. (2018) tested in frankfurters the antilisterial activity of various dipping solutions containing bacteriocins produced by *L. curvatus* CRL705 (533 UA/ml) or *L. sakei* CRL1862 (266 UA/ml), nisin (2500 UI/ml) or organic acids (2.5% lactic acid and acetic acid) (Table 4). The most effective treatment was the combination of the bacteriocin from *L. sakei* CRL1862 with organic acids, which reduced the pathogen counts (initially at 3–4 log cfu/ml) to below the detection limit from day 6 until the end of the 36-day storage at 10 °C.

The combination of the semi-purified bacteriocin BacTN635, from *L. plantarum*, at 500–1,000 AU/g, and the essential oil of *Mentha piperita*, at 0.25–0.5% v/w, resulted in an extension of the shelf life of minced beef meat by approximately 7 days at 4 °C, taking into account that the shelf life of minced beef meat could be limited by lipid oxidation, metmyoglobin accumulation, aerobic bacteria and sensory attributes (colour, appearance, odour and overall acceptability) (Table 2). The strongest biopreservation effect and sensory acceptability was achieved with the highest concentrations of essential oil (0.5%) and bacteriocin (1,000 AU/g) in combination (Smaoui et al., 2016). Samples containing the highest concentration of both biopreservatives obtained the following values on

day 7: 1.05 mg of malonaldehyde/kg of sample (a 2 TBARS value is the limit value of acceptability), 21.8% metmyoglobin (being 40% the limit value of acceptability) and 3.2 log cfu/g of aerobic plate counts (a value of 6.7 cfu/g indicates the end of shelf-life of raw minced meat).

Hu, Balay, Hu, McMullen, and Gänzle (2019) observed that the addition of partially purified bacteriocins from *C. maltaromaticum* increased the activity of chitosan against *E. coli* and *Salmonella* in an *in vitro* experiment. However, when this was tested on beef, no synergistic effects were observed (Table 2). These differences can be attributed to the fact that the food matrix is a complex ecosystem in which several populations interact affecting the total community structure, unlike *in vitro* assays which are much simpler. Also, the efficacy of antimicrobial compounds depends on their concentration and the microbial load. If the antimicrobial substance is added at low concentrations, it could be difficult to control a target microorganism added at high concentrations (Fangio & Fritz, 2014). Thus, it may be convenient to design the experiments as close as possible to real industrial conditions, where contamination is not commonly at very high levels. Hence, an important aspect to consider when combining biopreservation agents in a meat product is the quantity/concentration to apply. Dussault, Vu, and Lacroix (2016) observed an absence of influence of a mixture of nisin and hop alpha acids on the growth of *L. monocytogenes* in ham (Table 4). Only this one, among the seven preservation strategies evaluated (sodium nitrite, pH modulation, sodium chloride, sodium acetate, sodium lactate syrup, calcium propionate, and nisin and hop alpha acids), failed to provide positive results and this was attributed to the selection of inadequate concentrations of nisin and hop alpha acids to perform the assay (only 20 ppm).

As a part of the hurdles technology approach, modified atmosphere packaging (MAP) and freezing temperatures can also be employed in combination with biopreservative agents. In a trial by Melero, Diez, Rajkovic, Jaime, and Rovira (2012), a culture of *Leuconostoc pseudomesenteroides* was applied at 6 log cfu/g in chicken meat burgers in combination with a 50% CO_2/50% O_2 MAP to control the growth of *L. monocytogenes* and *Campylobacter jejuni* (Table 3). The target pathogens were additionally subjected to −18 °C for 48 h to stress their cells. When the bioprotective culture was combined with MAP *L. monocytogenes* counts were reduced in 0.9 log cfu/g. Freezing temperatures were not effective against *L. monocytogenes* but they were against *C. jejuni.* In fact, the combination of high-O_2 MAP with freezing eliminated this microorganism (Melero et al., 2012).

On the other hand, no significant additional reductions were observed with a Portuguese salami-like product, "Alheira", when *L. sakei* was combined with packing under vacuum or MAP (20% CO_2–80% N_2), with a constant 2 log reduction of *L. monocytogenes* being observed during the first 7 days of storage at 4 °C regardless of the experimental condition (Table 4) (Vaz-Velho et al., 2013), meaning that the gas atmosphere did not influence the growth of the LAB or the pathogen, and only the presence of *L. sakei* was sufficient to control *L. monocytogenes*.

Casquete et al. (2018) evaluated the antimicrobial effect of *L. sakei* and a commercial LAB starter culture (with *L. curvatus*, *S. xylosus* and *P. acidilactici*) against *L. innocua* in a cured-smoked pork loin (Table 4). The assay was performed in combination with two modified atmosphere packaging conditions (20% CO_2/80% N_2 and 40% CO_2/60% N_2), which showed no influence in the growth or survival of the biopreservative agents used. *L. innocua* numbers decreased between 1 and 2 log cfu/g at the early stages of storage in the presence of both LAB cultures. Despite this, the commercial culture did not show any significant effect, as compared to the control batch, at the end of the study (124 days), while *L. sakei* combined with a 40% CO_2/60% N_2 atmosphere reduced *L. innocua* counts in 5 log cfu/g, being much more effective.

Other promising approach to control *L. innocua* was reported by Castro et al. (2018), who showed the synergistic effect between pediocin bacHA-6111-2 and 300 MPa (10 °C, 5 min) high hydrostatic pressure treatments in a traditional Portuguese fermented product (Table 4). Several *L. innocua* concentrations, ranging from 4 to 8 log cfu/g, were used. While a bacteriostatic effect was observed with the highest concentration, for lower concentrations of *L. innocua*, closer to natural contamination levels, pediocin decreased *L. innocua* numbers for up to 2 log cfu/g.

Cocktails of *L. monocytogenes* and *Salmonella* spp. were used as target microorganisms by Nikodinoska et al. (2019) in a "chorizo" sausage model to test the efficacy of *L. plantarum* as a biopreservative (Table 4). The biopreservative agent was added to the product in the presence of 150 mg/kg $NaNO_2$, 75 mg/kg $NaNO_2$ or with no nitrite added. Counts of *L. monocytogenes* were significantly lower with the addition of the biopreservative, achieving a 3.8 log cfu/g reduction with the maximum nitrite concentration and a 2.6 log cfu/g reduction with the reduced nitrite concentration. In samples without nitrite, *L. plantarum* was capable of significantly reducing *L. monocytogenes* counts but not until the end of the experiment. The approach suffered the limitation of a lack of antimicrobial activity against *Salmonella* spp. (Nikodinoska et al., 2019). Although the nitrite free treatment was not as effective as the halved-nitrite treatment, the combination of LAB with 75 mg/kg $NaNO_2$ was a good hurdle approach to ensure food safety. In agreement with this study, Orihuel et al. (2018) observed that the antilisterial activity of bacteriocinogenic *Enterococcus mundtii* was enhanced when curing additives (3% NaCl, 0.02% NaNO2, 0.0075% ascorbic acid, 0.75% sucrose and 0.75% glucose) were also used in a beef sausage model, reaching up to a 2 log cfu/g reductions (Table 2).

The limitation of some biopreservative agents for the control of *Salmonella* in pork products has been also reported by Wang et al. (2017). Nisin and a *Salmonella* bacteriophage were applied to fresh pork meat previously inoculated with *S.* Typhimurium (at 3 log cfu/g) (Table 4). The bacteriophage, alone and in combination with nisin, produced a decrease in the counts to levels under the detection limit (<1 log cfu/g). However, nisin treatment alone did not reduce *Salmonella* counts in fresh chilled pork (Wang et al., 2017).

In relation to goat meat, pediocin from *P. pentosaceus* together with an extract of *Murraya koenigii* berries were evaluated for their antimicrobial and antilisterial effect in a goat meat emulsion (Table 5) (Kumar, Kaur, Shahi, Kairam, & Tyagi, 2017). Analysis of aerobic plate counts, psychrotrophic counts, and counts of *Enterobacteriaceae* and *L. innocua* were conducted at day 0, 3, 6 and 9 of storage at 4 °C. Aerobic plate counts and psychrotrophic counts showed a 2.2 and 1.7 log reduction, respectively, at day 9 of storage in comparison with the control batch. In addition, similar results regarding aerobic plate counts were obtained from pediocin supplemented samples and a nitrite treated sample. These authors thus stated that pediocin and nitrite could have similar antimicrobial activities in goat meat. *Enterobacteriaceae* counts also suffered a 1 log reduction during the storage period, as compared with the control batch, but the greatest effect was observed for *L. innocua*. Samples containing pediocin and berries extract showed a 1.4, 3, and 4.1 log reduction of *L. innocua*, respectively, at day 3, 6 and 9 of storage. When that treatment was compared with a nitrite treatment, significantly lower counts were observed when the bacteriocin was used, revealing the promising antilisterial effect of pediocin (Kumar et al., 2017).

As previously mentioned, salt or brines are common techniques to preserve casings, hence Hammou, Skali, Idaomar, and Abrini (2010) evaluated the combination of nisin (0, 100, 150, and 200 μg/g) and salt (0, 4, 7 and 12% w/v) at different concentrations to prevent the growth of *L. monocytogenes* in casings (Table 5). Nisin alone did not show any antilisterial effect before 20 days of storage at 6 °C, but from day 20 to day 90 all nisin treatments were effective against *L. monocytogenes*. When nisin was applied with 4% salt, there was an evident antilisterial effect regardless of the nisin concentration. In all samples, the addition of nisin in combination with salt produced a greater inhibitory effect than the use of salt alone. The same authors reported similar synergistic effects in casings when nisin was combined with *Origanum compactum* essential oil to inhibit *E. coli*, although the bacteriocin used alone had no impact on the pathogen's populations, when compared with the control samples (Table 5) (Hammou, Skali, Idaomar, & Abrini, 2011). The samples treated with oregano essential oil plus nisin presented higher

inhibitory effects on *E. coli* than both treatments applied individually, probably due to the fact that carvacrol and thymol, the most abundant individual components of the oregano essential oil, are able to destabilize the bacterial outer membrane thus facilitating the antimicrobial activity of nisin.

6. LAB metabolites in active packaging

Consumers are not only concerned about their health, demanding new ways of preserving food, but also demand ecologically friendly and biodegradable packaging materials. This has led to the development of new biopolymers. Pullulan is an extracellular polysaccharide produced by *Aureobasidium pullulans* whose edible and biodegradable films are colourless, tasteless and odourless, and allow a controlled release of antimicrobial molecules to the food matrix. This polysaccharide was tested with the bacteriocin sakacin A in RTE turkey breasts (Table 3) (Trinetta, Floros, & Cutter, 2010). When sakacin A was added directly to the meat, populations of *L. monocytogenes* were reduced in more than 2 log cfu/g after 3 weeks of storage at 4 °C, while sakacin A incorporated in a pullulan film reduced them by 3 log cfu/g after the same time period. This result demonstrates that these types of films permit the persistent migration of the antimicrobial compound to the food matrix over time. In addition, the antimicrobials are protected from being degraded by cross-reactions with other food components, thus maintaining their activity along the product shelf life.

Xie et al. (2018) rehearsed a plantaricin solution absorbed in an active polyvinylidene chloride film and determined the antimicrobial effect on fresh pork (Table 4). After 7 days of cold storage, the counts of *L. monocytogenes* were 1.4 log cfu/g lower than those observed for the control sample. Other assay by Woraprayote et al. (2013) evaluated a novel biocomposite film made of poly lactic acid and sawdust particles that enhanced the adsorption of the biopreservative pediocin PA-1/AcH using a diffusion coating technique. All treatments applied by these authors with the biocomposite film with pediocin PA-1/AcH on raw sliced pork significantly reduced the populations of *L. monocytogenes* about 1.5–2 log units after 14 days of storage at 4 °C (Table 4).

Some studies have demonstrated the advantages of microencapsulation techniques due to the slow release of biopreservative agents, which are also protected from being degraded by the food matrix. Ghabraie, Vu, Huq, Khan, and Lacroix (2016) tested several antimicrobial agents encapsulated into alginate-cellulose nanocrystal microbeads, and found it to be an effective delivery methodology (Table 4). These formulations were based on the utilization of the following four agents: essential oils (0.025–0.05%), 100–200 ppm of nitrite, organic acid salts (1.55–3.1%) and nisin (12.5–25 ppm). They obtained more than 2.6 and 1.5 log cfu/g reductions in the counts of *L. monocytogenes* in sausages at day 7 of storage at 4 °C, as compared to those obtained for the control batches, for formulation A (0.05%, v/w mixed essential oils, 1.55% w/w mixed organic acid salts, 12.5 ppm nisin and 100 ppm nitrite) and formulation B (0.025% v/w mixed essential oils, 1.55% w/w mixed organic acid salts, 12.5 ppm nisin and 100 ppm nitrite), respectively.

7. Conclusions and future perspectives

LAB have a long history of safe application in fermentation processes. However, as biopreservation agents they are still underutilized despite the aforementioned promising results on meat products. Indeed, with regard to bacteriocins, up to now, the only purified bacteriocin that has been officially approved for a direct use as additive in the European and United States food legislation is nisin (Codex Alimentarius, 1995; FDA, 2019) for certain types of foods. Pediocin PA-1 is also commercialized as a crude extract based on a fermentation of a bacteriocin-producing strain (Back et al., 2016).

LAB and their antimicrobial metabolites show potential to be used as an alternative to widely employed additives as they meet the consumers´ demands and ensure the safety of the product. In addition, they help accomplish longer shelf life, control the growth of spoilage and pathogenic microorganisms, and provide better sensorial characteristics to meat products. Some authors have even highlighted that they could be a viable alternative to tackle resistant and challenging microorganisms (Camargo, Todorov, Chihib, Drider, & Nero, 2018; Pisoschi et al., 2018). Moreover, biopreservation seems to be a good alternative to solve the large economic losses that industries suffer when products become contaminated.

Most of the findings emerging from the literature suggest that both LAB and purified bacteriocins (or bacteriocin-like substances) show a consistent antimicrobial activity when they are applied during *in vitro* assays. Although their use in real meat products at lab scale generally lead to a lower antibacterial activity due to the complexity of these foods, promising results have also been obtained. Further difficulties may arise when these lab-based trials are upscaled at industrial level under real processing conditions. The main challenges could be related to the regulatory framework, as the approval of novel bacteriocins as food additives could be tangled, and to their formulation in the meat product, as the dose of the biopreservative agent needs to be fine-tuned so that the required antimicrobial activity is achieved without the development of undesirable effects on the end product.

On the other hand, it must not be assumed that LAB and their metabolites will represent a silver bullet which will provide the unique and final solution to quality and safety issues in the meat industry. Some of the limitation to their use here discussed are related to the fact that not all natural antimicrobials demonstrate the same activity in different food matrices or against different target microorganisms. In addition, their performance varies depending on the time and temperature of storage, pH range of the product, and the interactions with other food components or members of the food microbiota. Therefore, improvements in their use, alone or within combined hurdles approaches, ought to be investigated. An important issue for further research would be the strategy followed for incorporating the active compound into the food. In this field, microencapsulation or active packaging may be a valuable alternative for gradually dosing the antimicrobial and avoiding its degradation during the shelf life. Also, the combination of the biopreservative agents with other methods of food preservation could provide better results. For instance, their combination with modified atmosphere packaging or high-pressure processing could result in a powerful synergistic effect. The combined use of cocktails of LAB or their bacteriocins has been barely investigated, although this could also be a promising approach and may show complementary antimicrobial activities against several pathogenic and/or spoilage microorganisms.

Other very important topic where research is being focused in recent years is the use of genomic and metagenomic techniques for the identification and characterization of environmental microorganisms in food processing chains and of the microbiome of food products. These techniques will facilitate the design of novel biopreservation strategies and the assessment of their efficacy and possible impact on the microbiological quality of the product. Nevertheless, although food safety is a great concern, and seems possible to positively manage it through biopreservation, other sensory, toxicological and healthy attributes ought to be also evaluated.

Declarations of interest

None.

Acknowledgments

This study was supported by the project MASTER (Microbiome Applications for Sustainable food systems through Technologies and Enterprise), which received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 818368.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meatsci.2021.108661.

References

Acuña, L., Picariello, G., Sesma, F., Morero, R. D., & Bellomio, A. (2012). A new hybrid bacteriocin, Ent35–MccV, displays antimicrobial activity against pathogenic Gram-positive and Gram-negative bacteria. *FEBS Open Bio, 2*, 12–19. https://doi.org/10.1016/j.fob.2012.01.002.

Angmo, K., Kumari, A., Savitri, M., & Chand Bhalla, T. (2016). Antagonistic activities of lactic acid bacteria from fermented foods and beverage of Ladakh against Yersinia enterocolitica in refrigerated meat. *Food Bioscience, 13*, 26–31. https://doi.org/10.1016/j.fbio.2015.12.004.

Arief, I. I., Jenie, B. S. L., Suryati, T., Ayuningtyas, G., & Fuziawan, A. (2012). Antimicrobial activity of bacteriocin from indigenous Lactobacillus plantarum 2C12 and its application on beef meatball as biopreservative. *Journal of the Indonesian Tropical Animal Agriculture, 37*(2), 90–96. https://doi.org/10.14710/jitaa.37.2.90-96.

de Azevedo, P. O. S., Mendonça, C. M. N., Seibert, L., Domínguez, J. M., Converti, A., Gierus, M., & Oliveira, R. P. S. (2020). Bacteriocin-like inhibitory substance of *Pediococcus pentosaceus* as a biopreservative for *Listeria* sp. control in ready-to-eat pork ham. *Brazilian Journal of Microbiology, 51*, 949–956. https://doi.org/10.1007/s42770-020-00245-w.

Back, A., Borges, F., Mangavel, C., Paris, C., Rondags, E., Kapel, R., … Cailliez-Grimal, C. (2016). Recombinant pediocin in *Lactococcus lactis*: increased production by propeptide fusion and improved potency by co-production with PedC. *Microbial Biotechnology, 9*(4), 466–477. https://doi.org/10.1111/1751-7915.12285.

Balay, D. R., Dangeti, R. V., Kaur, K., & Mcmullen, L. M. (2017). Purification of leucocin A for use on wieners to inhibit Listeria monocytogenes in the presence of spoilage organisms. *International Journal of Food Microbiology, 255*, 25–31. https://doi.org/10.1016/j.ijfoodmicro.2017.05.016.

Bartkiene, E., Bartkevics, V., Mozuriene, E., Lele, V., Zadeike, D., & Juodeikiene, G. (2019). The safety, technological, nutritional, and sensory challenges associated with lacto-fermentation of meat and meat products by using pure lactic acid bacteria strains and plant-lactic acid bacteria bioproducts. *Frontiers in Microbiology, 10*, 1–4. https://doi.org/10.3389/fmicb.2019.01036.

Bastos, M. C. F., Coelho, M. L. V., & Santos, O. C. S. (2015). Resistance to bacteriocins produced by gram-positive bacteria. *Microbiology, 161*, 683–700. https://doi.org/10.1099/mic.0.082289-0.

Belfiore, C., Castellano, P., & Vignolo, G.Ã. (2007). Reduction of *Escherichia* coli population following treatment with bacteriocins from lactic acid bacteria and chelators. *Food Microbiology, 24*, 223–229. https://doi.org/10.1016/j.fm.2006.05.006.

Bolívar-Monsalve, J., Ramírez-Toro, C., Bolívar, G., & Ceballos-González, C. (2019). Mechanisms of action of novel ingredients used in edible films to preserve microbial quality and oxidative stability in sausages - A review. *Trends in Food Science & Technology, 89*, 100–109. https://doi.org/10.1016/j.tifs.2019.05.011.

Buchanan, R. L., Gorris, L. G. M., Hayman, M. M., Jackson, T. C., & Whiting, R. C. (2017). A review of *Listeria monocytogenes*: An update on outbreaks, virulence, dose-response, ecology, and risk assessments. *Food Control, 75*, 1–13. https://doi.org/10.1016/j.foodcont.2016.12.016.

Camargo, A. C., Todorov, S. D., Chihib, N. E., Drider, D., & Nero, L. A. (2018). Lactic acid bacteria (LAB) and their bacteriocins as alternative biotechnological tools to control *Listeria monocytogenes* biofilms in food processing facilities. *Molecular Biotechnology, 60*, 712–726. https://doi.org/10.1007/s12033-018-0108-1.

Casquete, R., Fonseca, S. C., Pinto, R., Castro, S. M., Todorov, S., Teixeira, P., & Vaz-Velho, M. (2018). Evaluation of the microbiological safety and sensory quality of a sliced cured-smoked pork product with protective cultures addition and modified atmosphere packaging. *Food Science and Technology International*, 1–10. https://doi.org/10.1177/1082013219825771.

Castellano, P., Belfiore, C., & Vignolo, G. (2011). Combination of bioprotective cultures with EDTA to reduce *Escherichia coli* O157:H7 in frozen ground-beef patties. *Food Control, 22*(8), 1461–1465. https://doi.org/10.1016/j.foodcont.2011.02.018.

Castellano, P., González, C., Carduza, F., & Vignolo, G. (2010). Protective action of *Lactobacillus curvatus* CRL705 on vacuum-packaged raw beef. Effect on sensory and structural characteristics. *Meat Science, 85*(3), 394–401. https://doi.org/10.1016/j.meatsci.2010.02.007.

Castellano, P., Peña, N., Ibarreche, M. P., Carduza, F., Soteras, T., & Vignolo, G. (2018). Antilisterial efficacy of *Lactobacillus* bacteriocins and organic acids on frankfurters. Impact on sensory characteristics. *Journal of Food Science and Technology, 55*(2), 689–697. https://doi.org/10.1007/s13197-017-2979-8.

Castro, S. M., Silva, J., Casquete, R., Queirós, R., Saraiva, J. A., & Teixeira, P. (2018). Combined effect of pediocin bacHA-6111-2 and high hydrostatic pressure to control *Listeria innocua* in fermented meat sausage. *International Food Research Journal, 25*(2), 553–560. http://www.ifrj.upm.edu.my/25%20(02)%202018/(14).pdf.

Caulier, S., Nannan, C., Gillis, A., Licciardi, F., Bragard, C., & Mahillon, J. (2019). Overview of the antimicrobial compounds produced by members of the *Bacillus subtilis* group. *Frontiers in Microbiology, 10*(302), 1–19. https://doi.org/10.3389/fmicb.2019.00302.

Centro de Coordinación de Alertas y Emergencias Sanitarias. (2019). *Informe de fin de seguimiento del brote de listeriosis*. Consumo y Bienestar Social: In *Ministerio de Sanidad*. Retrieved from https://www.mscbs.gob.es/profesionales/saludPublica/ccayes/alertasActual/listeriosis/docs/Informe_cierre_Listeriosis_20190927.pdf Accessed April 18, 2020.

Chakchouk-Mtibaa, A., Smaoui, S., Ktari, N., Sellem, I., Najah, S., Karray-Rebai, I., & Mellouli, L. (2017). Biopreservative efficacy of bacteriocin BacFL31 in raw ground Turkey meat in terms of microbiological, physicochemical, and sensory qualities. *Biocontrol Science, 22*(2), 67–77. https://doi.org/10.4265/bio.22.67.

Codex Alimentarius. (1995). Norma general para los aditivos alimentarios. *Codex Stan*, 192–1995.

Cosansu, S., Georgnaras, I., Ayhan, K., & Sofos, J. N. (2010). Control of *Listeria monocytogenes* by bacteriocin-producing *Pediococcus acidilactici* 13 and its antimicrobial substance in a dry fermented sausage sucuk and in turkey breast. *Journal of Food and Nutrition Research, 49*(4), 206–214. Retrieved from: https://www.cabdirect.org/cabdirect/abstract/20103371930 Accessed April 4, 2020.

Cotter, P. D., Ross, R. P., & Hill, C. (2013). Bacteriocins – A viable alternative to antibiotics? *Natural Reviews Microbiology, 11*, 95–105. https://doi.org/10.1038/nrmicro2937.

Dussault, D., Vu, K. D., & Lacroix, M. (2016). Development of a model describing the inhibitory effect of selected preservatives on the growth of *Listeria monocytogenes* in a meat model system. *Food Microbiology, 53*, 115–121. https://doi.org/10.1016/j.fm.2015.09.011.

EFSA. (2017a). Re-evaluation of potassium nitrite (E 249) and sodium nitrite (E 250) as food additives. *EFSA Journal, 15*. https://doi.org/10.2903/j.efsa.2017.4786. Article 04786.

EFSA. (2017b). Re-evaluation of sodium nitrate (E 251) and potassium nitrate (E 252) as food additives. *EFSA Journal, 15*. https://doi.org/10.2903/j.efsa.2017.4787. Article 04787.

EFSA. (2020). The 2019 updated list of QPS status recommended biological agents in support of EFSA risk assessments. *EFSA Journal, 17*, 1–5. https://doi.org/10.5281/zenodo.3648682.

EFSA. (2021). The European union one health 2019 zoonoses report. *EFSA Journal, 19*(2). https://doi.org/10.2903/j.efsa.2021.6406.

Egan, K., Field, D., Rea, M. C., Ross, R. P., Hill, C., & Cotter, P. D. (2016). Bacteriocins: Novel solutions to age old spore-related problems? *Frontiers in Microbiology, 7*, 1–21. https://doi.org/10.3389/fmicb.2016.00461.

European Commission. (2011). Commission regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. Retrieved from: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R1129&from=EN Accessed July 19, 2020.

Fangio, M. F., & Fritz, R. (2014). Potencial use of a bacteriocin-like substance in meat and vegetable food biopreservation. *International Food Research Journal, 21*(2), 677–683. Retrieved from: http://www.ifrj.upm.edu.my/21%20(02)%202014/35%20IFRJ%2021%20(02)%202014%20Maria%20253.pdf Accessed July 17, 2020.

Flores, M., Mora, L., Reig, M., & Toldrá, F. (2019). Risk assessment of chemical substances of safety concern generated in processed meats. *Food Science and Human Wellness, 8*, 244–251. https://doi.org/10.1016/j.fshw.2019.07.003.

Flores, M., & Toldrá, F. (2020). Chemistry, safety, and regulatory considerations in the use of nitrite and nitrate from natural origin in meat products - Invited review. *Meat Science, 171*, 1–12. https://doi.org/10.1016/j.meatsci.2020.108272.

Food and Drug Administration. (2019). Food additive status list. Retrieved from https://www.fda.gov/food/food-additives-petitions/food-additive-status-list Accessed February 5, 2020.

Gálvez, A., Abriouel, H., López, R. L., & Omar, N. B. (2007). Bacteriocin-based strategies for food biopreservation. *International Journal of Food Microbiology, 120*, 51–70. https://doi.org/10.1016/j.ijfoodmicro.2007.06.001.

García-Díez, J., & Patarata, L. (2017). Influence of salt level, starter culture, fermentable carbohydrates, and temperature on the behaviour of *L. monocytogenes* in sliced chouriço during storage. *Acta Alimentaria, 46*(2), 206–213. https://doi.org/10.1556/066.2017.46.2.10.

Gelinski, J., Baratto, C., Casagrande, M., Oliveira, T., Megiolaro, F., Soares, F. A., … Fonseca, G. (2019). Control of pathogens in fresh pork sausage by inclusion of *Lactobacillus sakei BAS0117*. *Canadian Journal of Microbiology, 841*, 831–841. Retrieved from: https://tspace.library.utoronto.ca/bitstream/1807/96531/1/cjm-2019-0136.pdf Accessed January 21, 2021.

Ghabraie, M., Vu, K. D., Huq, T., Khan, A., & Lacroix, M. (2016). Antilisterial effects of antibacterial formulations containing essential oils, nisin, nitrite and organic acid salts in a sausage model. *Journal of Food Science and Technology, 53*(6), 2625–2633. https://doi.org/10.1007/s13197-016-2232-x.

González, H. M. I., Yien, W., Castrillón, V. J. A., & Ortega, P. A. (2013). Addition of *Carnobacterium maltaromaticum* CB1 in vacuum packaged chorizo and morcilla, to inhibit the growth of *Listeria monocytogenes*. *Vitae, 20*(1), 23–29. Retrieved from: http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0121-40042013000100003 Accessed July 16, 2020.

Hammou, B. F., Skali, N., Idaomar, M., & Abrini, J. (2011). The antimicrobial effect of *Origanum compactum* essential oil, nisin and their combination against *Escherichia coli* in tryptic soy broth (TSB) and in sheep natural sausage casings during storage at 25 and 7°C. *African Journal of Biotechnology, 10*(71), 15998–16005. https://doi.org/10.5897/AJB11.1046.

Hammou, F. B., Skali, S. N., Idaomar, M., & Abrini, J. (2010). Combinations of nisin with salt (NaCl) to control *Listeria monocytogenes* on sheep natural sausage casings stored at 6°C. *African Journal of Biotechnology, 9*(8), 1191–1195. Retrieved from: https://www.ajol.info/index.php/ajb/article/view/78254 Accessed May 26, 2020.

van Heel, A. J., Jong, A., Montalbán-López, M., Kok, J., & Kuipers, O. P. (2013). BAGEL3: automated identification of genes encoding bacteriocins and (non-)bactericidal posttranslationally modified peptides. *Nucleic Acids Research, 41*, 448–453. https://doi.org/10.1093/nar/gkt391.

Hongthong, N., Chumngoen, W., & Tan, F. J. (2020). Influence of sucrose level and inoculation of *Lactobacillus plantarum* on the physicochemical, textural, microbiological, and sensory characteristics of Isan sausage (Thai fermented pork sausage). *Animal Science Journal, 91*(1), 1–8. https://doi.org/10.1111/asj.13312.

Hu, Z. Y., Balay, D., Hu, Y., McMullen, L. M., & Gänzle, M. G. (2019). Effect of chitosan, and bacteriocin – producing *Carnobacterium maltaromaticum* on survival of *Escherichia coli* and *Salmonella Typhimurium* on beef. *International Journal of Food Microbiology, 290*, 68–75. https://doi.org/10.1016/j.ijfoodmicro.2018.10.003.

IARC. (2010). *IARC monographs on the evaluation of carcinogenic risks to humans. 94* pp. 1–464). Retrieved from: https://monographs.iarc.who.int/wp-content/uploads/2018/06/mono94.pdf Accessed July 16, 2020.

Kamiloğlu, A., Kaban, G., & Kaya, M. (2019). Effects of autochthonous *Lactobacillus plantarum* strains on Listeria monocytogenes in sucuk during ripening. *Journal of Food Safety, 39*(3), 1–6. https://doi.org/10.1111/jfs.12618.

Khalili Sadaghiani, S., Aliakbarlu, J., Tajik, H., & Mahmoudian, A. (2019). Anti-listeria activity and shelf life extension effects of *Lactobacillus* along with garlic extract in ground beef. *Journal of Food Safety, 39*(6), 1–8. https://doi.org/10.1111/jfs.12709.

Kim, S. J., Cho, A. R., & Han, J. (2013). Antioxidant and antimicrobial activities of leafy green vegetable extracts and their applications to meat product preservation. *Food Control, 29*, 112–120. https://doi.org/10.1016/j.foodcont.2012.05.060.

Klaenhammer, T. R. (1993). Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiology Reviews, 12*, 39–86. https://doi.org/10.1111/j.1574-6976.1993.tb00012.x.

Kumar, Y., Kaur, K., Shahi, A. K., Kairam, N., & Tyagi, S. K. (2017). Antilisterial, antimicrobial and antioxidant effects of pediocin and *Murraya koenigii* berry extract in refrigerated goat meat emulsion. *LWT - Food Science and Technology, 79*, 135–144. https://doi.org/10.1016/j.lwt.2017.01.028.

Kumariya, R., Garsa, A. K., Rajput, Y. S., Sood, S. K., Akhtar, N., & Patel, S. (2019). Bacteriocins: classification, synthesis, mechanism of action and resistance development in food spoilage causing bacteria. *Microbial Pathogenesis, 128*, 171–177. https://doi.org/10.1016/j.micpath.2019.01.002.

Letchumanan, V., Wong, P., Goh, B., Chiau Ming, L., Pus-parajah, P., Hei Wong, S., … Lee, L. (2018). A review on the characteristics, taxanomy and prevalence of *Listeria monocytogenes*. *Progress in Microbes and Molecular Biology, 1*. https://doi.org/10.36877/pmmb.a0000007.

López, M., Calvo, T., Prieto, M., Múgica-Vidal, R., Muro-Fraguas, I., Alba-Elías, F., & Álvarez-Ordóñez, A. (2019). A review on non-thermal atmospheric plasma for food preservation: mode of action, determinants of effectiveness, and applications. *Frontiers in Microbiology, 10*. https://doi.org/10.3389/fmicb.2019.00622. Article 622.

Macieira, A., Barros, D., Pinheiro, R., Fonseca, S., Albano, H., & Teixeira, P. (2018). Effects of *Lactobacillus plantarum* bacteriocinogenic culture on physicochemical, microbiological, and sensorial characteristics of "chouriço vinha d'alhos", a traditional Portuguese sausage. *Journal of Food Quality and Hazards Control, 5*, 118–127. https://doi.org/10.29252/jfqhc.5.4.2.

Majou, D., & Christieans, S. (2018). Mechanisms of the bactericidal effects of nitrate and nitrite in cured meats. *Meat Science, 145*, 273–284. https://doi.org/10.1016/j.meatsci.2018.06.013.

Melero, B., Diez, A. M., Rajkovic, A., Jaime, I., & Rovira, J. (2012). Behaviour of non-stressed and stressed *Listeria monocytogenes* and *Campylobacter jejuni* cells on fresh chicken burger meat packaged under modified atmosphere and inoculated with protective culture. *International Journal of Food Microbiology, 158*(2), 107–112. https://doi.org/10.1016/j.ijfoodmicro.2012.07.003.

Nikodinoska, I., Baffoni, L., Di Gioia, D., Manso, B., García-Sánchez, L., Melero, B., & Rovira, J. (2019). Protective cultures against foodborne pathogens in a nitrite reduced fermented meat product. *LWT - Food Science and Technology, 101*, 293–299. https://doi.org/10.1016/j.lwt.2018.11.022.

Olaoye, O. A., & Onilude, A. A. (2010). Investigation on the potential application of biological agents in the extension of shelf life of fresh beef in Nigeria. *World Journal of Microbiology and Biotechnology, 26*(8), 1445–1454. https://doi.org/10.1007/s11274-010-0319-5.

Olaoye, O. A., Onilude, A. A., Ubbor, S. C., & Olaoye, O. A. (2015). Control of *Brochothrix thermosphacta* in pork meat using *Lactococcus lactis* subsp. *lactis* I23 isolated from beef. *Applied Food Biotechnology, 2*(3), 49–55. Retrieved from www.apiweb.biomerieux.com Accessed June 6, 2020.

Oppegård, C., Rogne, P., Emanuelsen, L., Kristiansen, P. E., Fimland, G., & Nissen-Meyer, J. (2007). The two-peptide class II bacteriocins: structure, production, and mode of action. *Journal of Molecular Microbiology and Biotechnology, 13*(4), 210–219. https://doi.org/10.1159/000104750.

Orihuel, A., Bonacina, J., Vildoza, M. J., Bru, E., Vignolo, G., Saavedra, L., & Fadda, S. (2018). Biocontrol of *Listeria monocytogenes* in a meat model using a combination of a bacteriocinogenic strain with curing additives. *Food Research International, 107*, 289–296. https://doi.org/10.1016/j.foodres.2018.02.043.

Ortiz, S., López, V., Garriga, M., & Martínez-Suárez, J. V. (2014). Antilisterial effect of two bioprotective cultures in a model system of Iberian chorizo fermentation. *International Journal of Food Science and Technology, 49*(3), 753–758. https://doi.org/10.1111/ijfs.12362.

Pisoschi, A. M., Pop, A., Georgescu, C., Turcuş, V., Olah, N. K., & Mathe, E. (2018). An overview of natural antimicrobials role in food. *European Journal of Medicinal Chemistry, 143*, 922–935. https://doi.org/10.1016/j.ejmech.2017.11.095.

Rendueles, E., Omer, M. K., Alvseike, O., Alonso-Calleja, C., Capita, R., & Prieto, M. (2011). Microbiological food safety assessment of high hydrostatic pressure processing: A review. *LWT Food Science and Technology, 44*, 1251–1260. https://doi.org/10.1016/j.lwt.2010.11.001.

Rivas, F. P., Cayré, M. E., Campos, C. A., & Castro, M. P. (2018). Natural and artificial casings as bacteriocin carriers for the biopreservation of meats products. *Journal of Food Safety, 38*(1). https://doi.org/10.1111/jfs.12419. Article 12419.

Ross, R. P., Morgan, S., & Hill, C. (2002). Preservation and fermentation: past, present and future. *International Journal of Food Microbiology, 79*, 3–16. https://doi.org/10.1016/S0168-1605(02)00174-5.

Ruiz, A., Williams, S. K., Djeri, N., Hinton, J. A., & Rodrick, G. E. (2010). Nisin affects the growth of *Listeria monocytogenes* on ready-to-eat turkey ham stored at four degrees Celsius for sixty-three days. *Poultry Science, 89*(2), 353–358. https://doi.org/10.3382/ps.2008-00503.

Sakaridis, I., Soultos, N., Batzios, C., Ambrosiadis, I., & Koidis, P. (2014). Lactic acid bacteria isolated from chicken carcasses with inhibitory activity against *Salmonella* spp. and *Listeria monocytogenes*. *Czech Journal of Food Sciences, 32*(1), 61–68. https://doi.org/10.17221/414/2012-cjfs.

Saltmarsh, M., & Insall, L. (2013). Food additives and why they are used. In M. Saltmarsh (Ed.), *Essential guide to food additives* (pp. 1–13). https://doi.org/10.1039/9781849734981-00001.

Settanni, L., & Corsetti, A. (2008). Application of bacteriocins in vegetable food biopreservation. *International Journal of Food Microbiology, 121*, 123–138. https://doi.org/10.1016/j.ijfoodmicro.2007.09.001.

Silva, C. C. G., Silva, S. P. M., & Ribeiro, S. C. (2018). Application of bacteriocins and protective cultures in dairy food preservation. *Frontiers in Microbiology, 8*. https://doi.org/10.3389/fmicb.2018.00594. Article 594.

Smaoui, S., Ben, A., Lahmar, A., Ennouri, K., Mtibaa-chakchouk, A., Sellem, I., … Bouaziz, M. (2016). Bio-preservative effect of the essential oil of the endemic *Mentha piperita* used alone and in combination with BacTN635 in stored minced beef meat. *Meat Science, 117*, 196–204. https://doi.org/10.1016/j.meatsci.2016.03.006.

Stanborough, T., Fegan, N., Powell, S. M., Tamplin, M., & Chandry, P. S. (2017). Insight into the genome of Brochothrix thermosphacta,a problematic meat spoilage bacterium. *Applied Environmental Microbioloy, 83*(5), 1–20. https://doi.org/10.1128/AEM.02786-16.

Thangavelu, K. P., Kerry, J. P., Tiwari, B. K., & McDonnell, C. K. (2019). Novel processing technologies and ingredient strategies for the reduction of phosphate additives in processed meat. *Trends in Food Science & Technology, 94*, 43–53. https://doi.org/10.1016/j.tifs.2019.10.001.

Trinetta, V., Floros, J. D., & Cutter, C. N. (2010). Sakacin a-containing pullulan film: an active packaging system to control epidemic clones of *Listeria Monocytogenes* in ready-to-eat foods. *Journal of Food Safety, 30*(2), 366–381. https://doi.org/10.1111/j.1745-4565.2010.00213.x.

Vatanyoopaisarn, S., Prapatsornwattana, K., Kuhakongkeat, T., & Phalakornkule, C. (2011). Potential use of lactic acid bacteria with bacteriocin-like activity against *Staphylococcus aureus* as dual starter cultures in Thai fermented sausage "Sai Krok Prew". *International Food Research Journal, 18*(2), 697–704. Retrieved from: http://www.ifrj.upm.edu.my/18%20(02)%202011/(32)%20IFRJ-2010-238.pdf Accessed May 5, 2020.

Vaz-Velho, M., Jácome, S., Noronha, L., Todorov, S., Fonseca, S., Pinheiro, R., … Teixeira, P. (2013). Comparison of antilisterial effects of two strains of lactic acid bacteria during processing and storage of a portuguese salami-like product alheira. *Chemical Engineering Transactions, 32*, 1807–1812. https://doi.org/10.3303/CET1332302.

Wang, C., Yang, J., Zhu, X., Lu, Y., Xue, Y., & Lu, Z. (2017). Effects of *Salmonella* bacteriophage, nisin and potassium sorbate and their combination on safety and shelf life of fresh chilled pork. *Food Control, 73*, 869–877. https://doi.org/10.1016/j.foodcont.2016.09.034.

WHO. (2019). Listeriosis – Spain. Retrieved from: https://www.who.int/csr/don/16-september-2019-listeriosis-spain/en/ Accessed May 5, 2020.

Wijnker, J. J., Weerts, E. A. W. S., Breukink, E. J., Houben, J. H., & Lipman, L. J. A. (2011). Reduction of *Clostridium sporogenes* spore outgrowth in natural sausage casings using nisin. *Food Microbiology, 28*(5), 974–979. https://doi.org/10.1016/j.fm.2011.01.009.

Woraprayote, W., Kingcha, Y., Amonphanpokin, P., Kruenate, J., Zendo, T., Sonomoto, K., … Visessanguan, W. (2013). Anti-listeria activity of poly(lactic acid)/sawdust particle biocomposite film impregnated with pediocin PA-1/AcH and its use in raw sliced pork. *International Journal of Food Microbiology, 167*(2), 229–235. https://doi.org/10.1016/j.ijfoodmicro.2013.09.009.

Xie, Y., Zhang, M., Gao, X., Shao, Y., Liu, H., Jin, J., … Zhang, H. (2018). Development and antimicrobial application of plantaricin BM-1 incorporating a PVDC film on fresh pork meat during cold storage. *Journal of Applied Microbiology, 125*(4), 1108–1116. https://doi.org/10.1111/jam.13912.

Yildirim, S., Röcker, B., Pettersen, M. K., Nilsen-Nygaard, J., Ayhan, Z., Rutkaite, R., … Coma, V. (2018). Active packaging applications for food. *Comprehensive Reviews in Food Science and Food Safety, 17*, 165–199. https://doi.org/10.1111/1541-4337.12322.

Yildrim, Z., Yerlikaya, S., Öncül, N., & Sakin, T. (2016). Inhibitory effect of Lactococcin BZ against *Listeria innocua* and indigenous microbiota of fresh beef. *Food Technology and Biotechnology, 54*(3), 317–323. https://doi.org/10.17113/ft b.54.03.16.4373.

Zanette, C., Dalla, H. S., & dos Santos, L. B. (2015). Effect of *Lactobacillus plantarum* starter cultures on the behavior of *Listeria monocytogenes* during sausage maturation. *International Food Research Journal, 22*(22), 844–848. Retrieved from http://www.ifrj.upm.edu.my Accessed May 26, 2020.

Zheng, J., Wittouck, S., Salvetti, E., Franz, C. M. A. P., Harris, H. M. B., Mattarelli, P., … Felis, G. E. (2020). A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *International Journal of Systematic and Evolutionary Microbiology, 70*(4), 2782–2858. https://doi.org/10.1099/ijsem.0.004107.

Zouhir, A., Hammami, R., Fliss, I., & Hamida, J. B. (2010). A new structure-based classification of gram-positive bacteriocins. *The Protein Journal, 29*(6), 432–439. https://doi.org/10.1007/s10930-010-9270-4.