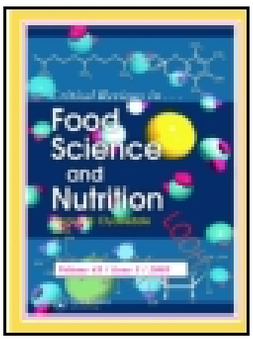

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REVIEW

Microbial biopreservatives for controlling the spoilage of beef and lamb meat: their application and effects on meat quality

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ABSTRACT

Biopreservation is a recognized natural method for controlling the growth of undesirable bacteria on fresh meat. It offers the potential to inhibit spoilage bacteria and extend meat shelf-life, but this aspect has been much less studied compared to using the approach to target pathogenic bacteria. This review provides comprehensive information on the application of biopreservatives of microbial origin, mainly bacteriocins and protective cultures, in relation to bacterial spoilage of beef and lamb meat. The sensory effect of these biopreservatives, an aspect that often receives less attention in microbiological studies, is also reviewed. Microbial biopreservatives were found to be able to retard the growth of the major meat spoilage bacteria, *Brochothrix thermosphacta*, *Pseudomonas* spp., and *Enterobacteriaceae*. Their addition did not have any discernible negative impact on the sensory properties of meat, whether assessed by human sensory panels or instrumental and chemical analyses. Although results are promising, the concept of biopreservation for controlling spoilage bacteria on fresh meat is still in its infancy. Studies in this area are still lacking, especially for lamb. Biopreservatives need more testing under conditions representative of commercial meat production, along with studies of any possible sensory effects, in order to validate their potential for large-scale industrial applications.

KEYWORDS

Antimicrobials; bacteriocins; protective cultures; sensory effects; meat shelf-life; spoilage bacteria

Introduction

Meat is a perishable product. At slaughter, the inherent protective barriers (e.g., skin) and natural defense mechanisms (e.g., antimicrobial peptides) of live animals break down. This allows microorganisms to grow rapidly and decompose muscle tissues. Changes caused by microorganisms lead to the formation of discoloration, off-odors, and slime, making meat become unacceptable to consumers and limiting the product shelf-life (Comi 2017; Iulietto et al. 2015). Although endogenous muscle enzymatic and non-enzymatic chemical reactions and physical changes contribute to the spoilage of muscle tissues, the effect is minor compared to the action of microorganisms (Sofos et al. 2013).

Shelf-life affects everyone in the food supply chain. For consumers, inadequate shelf-life often leads to dissatisfaction, which will then affect the acceptance and sale of the product. As a result, supermarkets generally do not accept products with less than 75% of shelf-life remaining (Robertson 2013). Product distribution, both to international markets and local retailers, can also be limited by shelf-life, which is particularly disappointing for distributors in the current era of globalization of food trade. The perishable nature of meat not only causes substantial economic losses but also contributes to other global issues, such as food insecurity and food waste (Odeyemi et al. 2020). Therefore, manufacturers generally attempt to maximize product shelf-life, taking into consideration the costs and requirements of distributors, retailers, and consumers (Robertson 2013).

At present, many food products in the market are preserved using multiple methods (hurdle technology). Refrigeration and packaging systems are widely used for fresh meat, and other preservation technologies have also been developed or proposed as additional hurdles to control microbial growth and extend product shelf-life. Besides physical (e.g., high pressure processing, ionizing irradiation, and ultrasound) and chemical (e.g., organic acids, peracetic acid, and nanoparticles) approaches, biopreservation has emerged as a promising option in hurdle technology due to increasing consumer demand for natural preservation methods and minimally processed food (Chen et al. 2012; Rosario et al. 2020).

Biopreservation is based on the use of natural or controlled microbiota and/or antimicrobial compounds to enhance food safety and extend product shelf-life (Ockerman and Basu 2014). The antimicrobial compounds that can be used for biopreservation (simply termed as biopreservatives) can come from a variety of sources, including plants (e.g., essential oils), animals (e.g., chitosan), and microorganisms (e.g., bacteriocins). They vary in their nature and may be used individually or in combination (Davidson et al. 2015). Microbial biopreservatives have been investigated in numerous studies against common meat-

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Table 1. Characteristics of major spoilage bacteria of chilled beef and lamb meat.

Bacteria	Gram reaction	Minimum growth temperature (°C)	Oxygen requirement	Spoilage potential	Common spoilage characteristics
Pseudomonas spp.	Negative	0	Aerobe	High	Slime, sulfurous/putrid off-odors, discoloration
Brochothrix thermosphacta	Positive	−0.8	Facultative anaerobe	High	Slime, sour/cheesy odors
Lactic acid bacteria	Positive	0–6[a]	Aerotolerant anaerobe	Low	Slime, sour/cheesy odors, greening of meat on exposure to air
Enterobacteriaceae	Negative	−2 to 7[b]	Facultative anaerobe	High	Sulfurous/putrid off-odors, cheesy odors, discoloration

References: Bautista 2014; Casaburi et al. 2015; Chattopadhyay and Adhikari 2014; da Silva et al. 2018; Dodd 2014; Fusco et al. 2015; Halkman and Halkman 2014; Mills, Donnison, and Brightwell 2014.
[a]Minimum growth temperature range of genera *Carnobacterium, Lactobacillus, Lactococcus, Leuconostoc*, and *Weissella*
[b]Minimum growth temperature range of genera *Citrobacter, Enterobacter, Hafnia, Klebsiella, Pantoea, Proteus*, and *Serratia*

borne pathogens, such as *Salmonella* spp., Shiga toxin-producing *Escherichia* (*E.*) *coli*, *Staphylococcus* (*S.*) *aureus*, and *Listeria* (*L.*) *monocytogenes*, and have shown great effectiveness. However, to date, their use to control spoilage bacteria has received much less attention, as spoilage generally causes economic losses rather than health risks (Sofos et al. 2013).

This review discusses the current state of meat (beef and lamb) biopreservation with reference to microbial biopreservatives. It begins by briefly describing bacteria associated with red meat spoilage and the level of inhibitory effect that can be achieved by the current refrigeration and packaging systems. The review then focuses on the application and effect of different microbial biopreservatives for controlling meat spoilage bacteria. The final section outlines other practical factors that must be taken into consideration for the successful application of these biopreservatives, including their often-overlooked effect on meat sensory properties.

In reviewing the application of biopreservatives of microbial origin presented in Sections "Biopreservation by antimicrobials of microbial origin" and "Other factors to consider," we screened 1613 articles and included 38 experimental studies that evaluated any type of microbial biopreservatives against at least one of the following bacteria or bacterial groups: total viable counts (TVC), lactic acid bacteria (LAB), *Brochothrix* (*B.*) *thermosphacta*, *Pseudomonas* (*P.*) spp., and *Enterobacteriaceae* on fresh beef or lamb meat. Studies of fresh meat products containing other food ingredients such as salt, spices, and herbs were excluded. Since we are not aware of any earlier reviews with this particular focus, and due to the small number of studies in this area, no time restrictions were applied for the inclusion of any relevant studies. Details of the method used for literature searching and the selection of references are provided in Appendix I.

Refrigeration and packaging technologies for controlling spoilage bacteria

Microorganisms from animals themselves, the process workers, and the processing environment are transferred onto meat during processing (Zagorec and Champomier-Vergès 2017). Since fresh meat is usually chill-stored at 1–4 °C, the growth of mesophilic microorganisms is inhibited. Under these conditions, although initially present as a small fraction of the microbial population on meat, psychrotrophic microorganisms are favored to grow with the potential for some of them becoming the spoilers (Sun and Holley 2012).

A variety of psychrotrophic microorganisms can contribute to fresh meat spoilage. Since yeasts and molds are present in low numbers and grow more slowly on chilled meat compared to bacteria, they usually do not play an important role in meat spoilage (Petruzzi et al. 2017; Sohaib et al. 2016). Among the bacterial species found on spoiled chilled meat, the ones that are likely to be present in significant numbers are from the genera *Acinetobacter*, *Brochothrix, Moraxella*, *Pseudomonas*, and *Shewanella* and members of the *Enterobacteriaceae* family and LAB (Doulgeraki et al. 2012; Mills, Donnison, and Brightwell 2014; Odeyemi et al. 2020; Sun and Holley 2012). *B. thermosphacta*, *Pseudomonas* spp., *Enterobacteriaceae*, and LAB have been commonly recognized as the main groups of meat spoilage bacteria (Pennacchia, Ercolini, and Villani 2011), and their growth and spoilage characteristics are presented in Table 1. *Acinetobacter* spp. and *Moraxella* spp. have low spoilage potential and therefore are of little significance. *Shewanella* spp., although high in spoilage potential, are only major spoilage organisms in high-pH (pH $>$ 6.0) meat (Sun and Holley 2012).

The significance of different spoilage bacteria varies depending on the gaseous environment the meat is stored in. Under aerobic conditions (e.g., over-wrapped trays or plastic bags), *Pseudomonas* spp. predominate as they have a higher affinity for oxygen due to their strict respiratory type of metabolism and thus grow faster (Palleroni 2015; Sofos et al. 2013). Control of these aerobes can be achieved by modifying the gas atmosphere in the immediate environment of the meat products, with vacuum packaging (VP) (including vacuum skin packaging) and modified atmosphere packaging (MAP) being the most common processes used around the world (Arvanitoyannis and Stratakos 2012).

In VP, meat is placed in low gas permeable films, and the air is removed while the package is sealed, creating an anaerobic environment. In MAP, meat is placed within barrier packaging materials, and the air is removed and then replaced by a mixture of gases (usually oxygen, carbon dioxide, and nitrogen). Carbon dioxide at levels greater than 20% inhibits the growth of aerobic Gram-negative bacteria (e.g., *Pseudomonas* spp.). The exact mode of bacteriostatic action is unknown but may include alteration of bacterial membrane function, inhibition of enzyme activity, and penetration of bacterial membranes causing intracellular pH change. The fact that carbon dioxide dissolves in the food (both aqueous and lipid phases) and forms carbonic acid reducing the pH may also act as a hurdle for microbial growth. As carbon dioxide becomes increasingly soluble

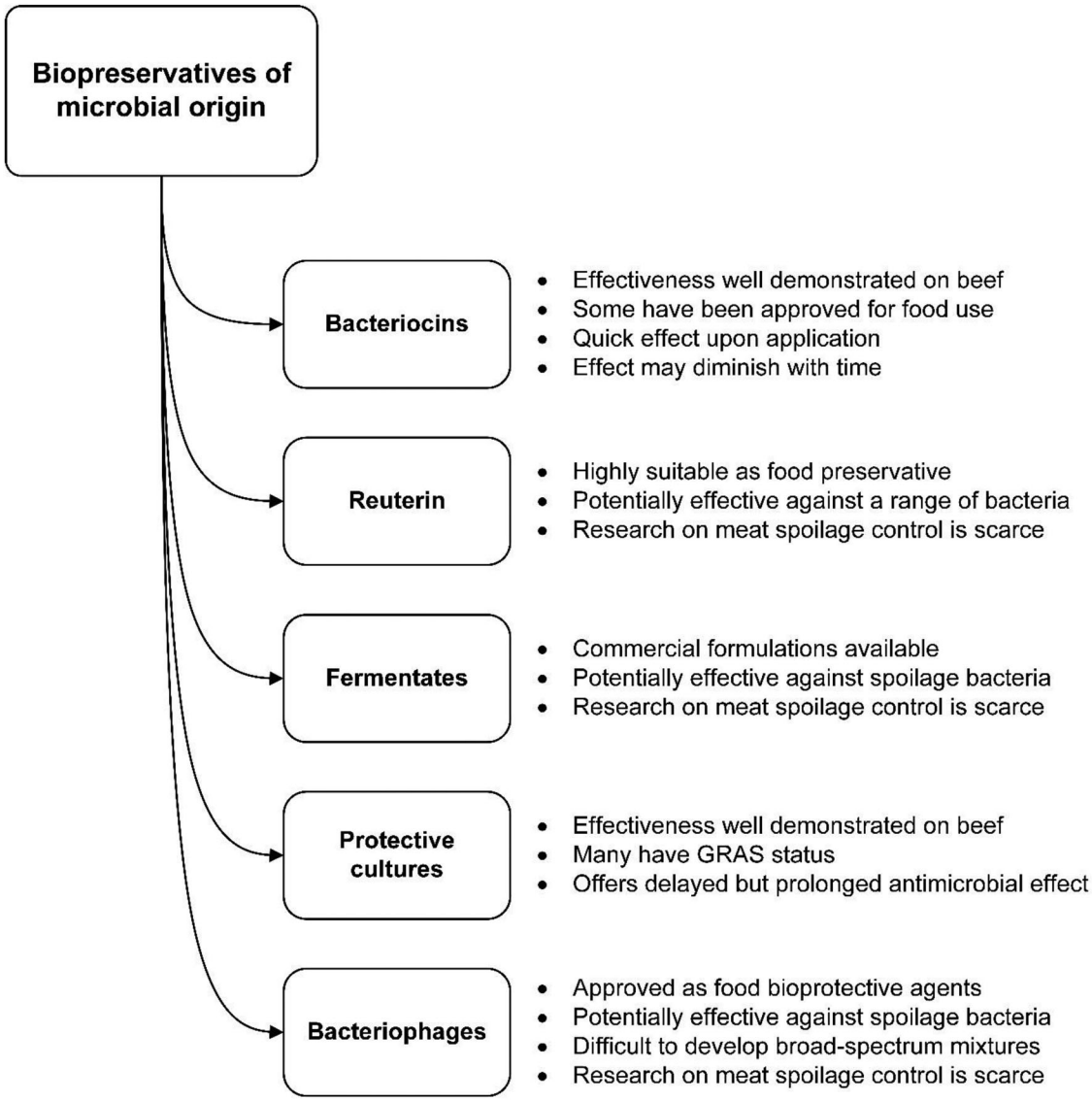

Figure 1. Different types of biopreservatives of microbial origin and their advantages and limitations in terms of meat spoilage control.

with decreasing temperature, its antimicrobial effect is enhanced under refrigeration conditions. Oxygen is typically applied at 70–80% for fresh red meat to maintain the characteristic bright red color by maintaining myoglobin in its oxygenated state (oxymyoglobin). Nitrogen may be used as a filler gas to prevent package collapse (Arvanitoyannis and Stratakos 2012; Djenane and Roncalés 2018; Kontominas 2014). Under these conditions, *Pseudomonas* spp. are inhibited, and spoilage is mainly due to the activities of *B. thermosphacta* and LAB, with *B. thermosphacta* having greater spoilage potential despite being present in lower numbers. *Enterobacteriaceae* are also present in relatively low numbers and are major spoilage organisms for high-pH meat under conditions with limited oxygen (Mills, Donnison, and Brightwell 2014; Sofos et al. 2013).

Biopreservation by antimicrobials of microbial origin

Biopreservatives of microbial origin that are suitable for fresh meat generally originate from bacteria and viruses (bacteriophages). Bacteria can inhibit the growth of other bacteria by producing acids or other antimicrobial compounds (e.g., bacteriocins), competing for nutrients and space, or a combination of these (Ben Said et al. 2019; Melero et al. 2013). They can be applied in food as live cultures or in the form of crude or purified antimicrobial extracts. Bacteriophages that work by infecting target bacterial cells are another potential option. The modes of action and applications of the major microbial biopreservatives are reviewed in this section. An overview of the advantages and limitations of each of these biopreservatives is summarized in Figure 1.

Bacteriocins

Bacteriocins are ribosomally synthesized proteins or peptides with bactericidal and/or bacteriostatic activities (Verma et al. 2014). They can be narrow- or broad-spectrum, which kill other related or non-related microorganisms, respectively. They generally act by creating pores in the target cell membrane or inhibiting cell wall synthesis, while some interfere

with nucleic acid or protein synthesis (Meade, Slattery, and Garvey 2020; Negash and Tsehai 2020; Soltani et al. 2021; Yang et al. 2014). Bacteriocins are abundant, produced by almost every bacterial species that has been examined, although most are not fully characterized. They are also highly diverse, with tens to hundreds of different kinds of bacteriocins typically produced within a species (Verma et al. 2014). Despite the occurrence of so many bacteriocins in nature, relatively few have been commercialized and approved for use in food, largely due to their having a too-narrow spectrum of activity in most cases and the legal (regulatory) food safety approvals that must be obtained before they can be marketed.

According to their characteristics and properties, bacteriocins from Gram-positive bacteria were initially divided into four classes with several subclasses. However, bacteriocin classification has been periodically reviewed and is continuously evolving. Recent reports tend to categorize bacteriocins of Gram-positive bacteria into three classes (Kumariya et al. 2019; Negash and Tsehai 2020; Zimina et al. 2020). Class I bacteriocins are also known as lantibiotics. They are small (less than 5 kDa) heat-stable peptides, highly post-translationally modified, and contain the unusual amino acids lanthionine and methyllanthionine. Class II bacteriocins are also small (less than 10 kDa) heat-stable peptides but post-translationally unmodified. Class III bacteriocins are large (over 30 kDa) heat-labile proteins (Negash and Tsehai 2020). Controversies particularly exist for Class III, with some authors classifying bacteriocins produced by Gram-negative bacteria in Class III (Kumariya et al. 2019; Meade, Slattery, and Garvey 2020), while others consider bacteriocins from Gram-negative bacteria as a separate group (da Costa et al. 2019; Negash and Tsehai 2020; Zimina et al. 2020). Alternatively, Soltani et al. (2021) proposed a classification of bacteriocins from both Gram-positive and Gram-negative in two classes, the modified and unmodified bacteriocins. The previously classified Class IV bacteriocins (complex proteins with carbohydrate or lipid moieties) are generally excluded. Detailed information on the most recent classification of bacteriocins can be found in the articles mentioned above.

Bacteriocins for meat and meat products

For food applications, substantial effort has been put into studying bacteriocins produced by LAB because LAB have been consumed for thousands of years in fermented foods, such as cheese, yogurt, and salami (Erkmen and Bozoglu 2016; Mahmud and Khan 2018). This long history of consumption simplifies the process of obtaining GRAS (Generally Recognized as Safe) status. Nisin, pediocin, and sakacin are the most studied bacteriocins for meat and meat products.

Nisin is by far the most well-known and extensively studied bacteriocin. It was first approved as a food preservative in the United Kingdom in the 1950s and is now widely used around the world, permitted in over 50 countries (Müller-Auffermann et al. 2015; Shin et al. 2016). Nisin is produced by certain strains of *Lactococcus* (*Lc.*) *lactis*. It is a member of the Class I bacteriocins, which are positively charged linear flexible peptides that generally act by forming pores in the cytoplasmic membranes of sensitive target bacteria. It was discovered that nisin forms complexes with lipid II, a peptidoglycan precursor on the bacterial cell wall that acts as a docking molecule for nisin. The nisin-lipid II complexes then insert themselves into the cytoplasmic membrane causing the formation of transient pores while cell wall synthesis is also inhibited (Gharsallaoui et al. 2016; Meade, Slattery, and Garvey 2020; O'Bryan et al. 2015). The pores result in leakage of essential cellular components and depletion of proton motive force and ATP (adenosine triphosphate) and eventually lead to cell death (Kumariya et al. 2019). Nisin is effective against a wide range of Gram-positive bacteria, and activity can be extended to Gram-negative bacteria if the outer membrane is destabilized, for example, by the addition of EDTA (ethylenediaminetetraacetic acid) (Prudêncio, Santos, and Vanetti 2015).

Pediocins produced by *Pediococcus* spp. are the second most studied bacteriocins due to their pronounced anti-*Listeria* properties (da Costa et al. 2019). Pediocins are classified as Class II bacteriocins. Their bactericidal action is caused by the cationic pediocin molecules binding to the anionic lipoteichoic acid in the cell wall of sensitive Gram-positive bacteria. Adsorption of pediocin facilitates molecules to bring about changes in the integrity of the cytoplasmic membrane of these bacteria, resulting in loss of vital cellular materials and subsequent cell death. Gram-negative bacteria do not have lipoteichoic acid and are protected by an outer cell membrane. They are, therefore, resistant to pediocin. However, when the barrier function of their outer membrane is disrupted, for example, by temperature treatments, Gram-negative bacteria may become sensitive to pediocin (Espitia, Otoni, and Soares 2016; Juneja, Dwivedi, and Yan 2012; Prudêncio, Santos, and Vanetti 2015). Food-grade pediocin-containing formulations are commercially available, marketed as ALTA 2341 or MicroGARD (Garsa et al. 2014), but application in fresh meat products has not been reported, and in contrast to nisin, the use of pediocin is not approved in all countries (e.g., the European Union) (Lücke 2014).

Sakacins, produced by certain strains of *Lactobacillus* (*Lb.*) *sakei*, are also Class II bacteriocins. Their antimicrobial action results from pore formation in the cytoplasmatic membrane that causes subsequent leakage of the vital cell components and depletion of the proton motive force. Sakacins have a narrow inhibitory spectrum being active against certain strains of LAB, mainly lactobacilli (Delves-Broughton 2012). Sakacins have also shown inhibitory activities against *Listeria* and *B. thermosphacta* in meat products (Castellano et al. 2017). Purified preparations of sakacin are not commonly described in published meat studies, and instead, there has been more interest in applying live cultures of sakacin-producing Lb. sakei (see Section "Protective cultures").

Application of bacteriocins against meat spoilage bacteria

Studies conducted to evaluate the antimicrobial effect of bacteriocins use either commercially available purified

products (nisin and pediocin-containing products) or partially purified forms, such as cell-free supernatants of the bacteriocin-producing bacteria. Bacteriocins are typically applied to fresh meat in a solution form and are prepared to a defined antimicrobial activity by volume or weight. For commercial nisin, concentration or potency is usually specified by the manufacturer in IU (international units) per g, and this information can be used to determine the antimicrobial activity of the prepared bacteriocin solutions. However, for noncommercial bacteriocins, antimicrobial activities are often given in AU (arbitrary units) using nonstandardized conditions and selected indicator organisms. While this is useful for comparing multiple concentrations used within a study, comparisons between studies can be difficult as the methods for quantifying antimicrobial activity vary. The development of a standard method or kit for quantifying the antimicrobial activity of bacteriocins used for meat biopreservation would be helpful for researchers.

The prepared bacteriocin solutions can be applied to meat in several different ways, as mentioned in Table 2. Whole cuts can be dipped in the solution, or a set amount of solution can be sprayed or directly added onto the meat surface. For comminuted meat, application methods of the solutions include dipping, mixing in, application to patty surfaces, and spraying onto the meat surface before mincing, while application of the powder form also serves as an option. In addition, there are opportunities for bacteriocins to be incorporated into plastic films that are used for wrapping meat for storage (Kim, Paik, and Lee 2002) or as prewraps before the meat is placed into the final packaging (Siragusa, Cutter, and Willett 1999; Cutter, Willett, and Siragusa 2001). Although flexibility in the application method is an advantage, there is a need for more studies to evaluate the effectiveness, and possibly other factors such as the impact on meat quality, of the same bacteriocin applied in different ways to find out which method is superior.

Though no studies known to us have examined the effect of bacteriocins against spoilage bacteria present on fresh lamb meat, published studies have shown positive results in beef (Table 2). Few authors included LAB analysis in their studies, but all studies reported decreased LAB growth after bacteriocin treatment. Ariyapitipun, Mustapha, and Clarke (1999) found nisin was able to slow down LAB growth for up to 42 days in vacuum-packaged beef, and similar findings were reported by Castellano and Vignolo (2006) using lactocin 705 (from *Lb. curvatus* CRL705) over a 36-day storage period. With aerobic packaging, Aksoy et al. (2014) found that nisin could suppress LAB for up to 7 days. Calderón-Oliver, Escalona-Buendía, and Ponce-Alquicira (2020) studied the effect of nisin in both aerobic- and vacuum-packaged beef mince and observed a significant inhibitory effect on LAB but only in the aerobic packaging system. Yildirim et al. (2016) found partially purified lactococcin BZ suppressed the growth of LAB on aerobically stored beef over 12 days, while the LAB population increased by 4.1 log colony forming units (CFU)/g on untreated samples. Since the bacteriocins used in these studies were produced by LAB strains, their inhibitory effect against the endogenous LAB was expected, as bacteriocins are generally active against species or strains that are closely related to the producer strains.

Nisin, a broad-spectrum bacteriocin, is also highly effective against *B. thermosphacta*. It can produce several log reductions in *B. thermosphacta* numbers compared to untreated meat samples (Table 2). However, in a study conducted by Cutter and Siragusa (1997), spraying nisin solution onto meat surfaces before mincing only resulted in a slight reduction (around 0.4 log units) of *B. thermosphacta* at the start of storage. In their other studies, though, both spray application and surface contact application (using a template) of nisin onto beef carcass surface tissues resulted in more significant *B. thermosphacta* reductions, regardless of tissue and packaging types (Cutter and Siragusa 1996, 1998). The reason may be that more nisin molecules adsorbed onto the meat macromolecules and became inactive due to the mincing process. Interaction between biopreservatives and food components must be considered in their application, as described in more detail in Section "Interaction with meat system constituents." It is also important to note that most of the studies on the growth of *B. thermosphacta* summarized in Table 2 used meat samples that had been inoculated with this organism, so the starting counts were significantly higher than the levels normally found on fresh meat. This raises the question of whether such inoculated samples are representative of the real-world situation. More studies need to be conducted to evaluate the efficacy of bacteriocins against natural contamination levels of spoilage bacteria on meat.

Bacteriocins have also been reported to affect the growth of Gram-negative spoilage bacteria. Nisin caused a reduction of *Pseudomonas* populations by up to 1.1 log CFU/cm^2 in vacuum-packaged beef over a 56-day storage period (Ariyapitipun, Mustapha, and Clarke 1999) and up to 2.5 log CFU/g reduction in aerobic packaging over 17 days (Aksoy et al. 2014). However, despite being a major group of spoilage bacteria with high spoilage potential, not many studies have investigated *Pseudomonas* spp. By contrast, several studies have shown that bacteriocins can control the growth of *Enterobacteriaceae* in beef (Table 2). It was even found that nisin (Shahbazi, Shavisi, and Mohebi 2016) and partially purified lactococcin BZ (Yildirim et al. 2016) could reduce *Enterobacteriaceae* or coliforms to undetectable levels in beef in aerobic packaging. Nisin also appeared to be more effective against mesophilic *Enterobacteriaceae* (effective up to 42 days) than psychrotrophic *Enterobacteriaceae* (effective up to 14 days) in vacuum-packaged beef stored at 4 °C (Ariyapitipun, Mustapha, and Clarke 1999). Since bacteriocins produced by Gram-positive bacteria are unable to act on Gram-negative bacteria unless their impermeable outer membrane is destabilized (e.g., by EDTA), reductions reported in these studies might not be the result of direct inhibition by the bacteriocins (Meade, Slattery, and Garvey 2020). The growth of *Pseudomonas* spp. and *Enterobacteriaceae* may be indirectly affected as a result of bacteriocins altering other species within the microbial community.

Table 2. Application of bacteriocins or possible bacteriocin-containing inhibitory substances produced by microorganisms to fresh beef for controlling spoilage bacteria.

Inhibitory substance	Product	Application method	Concentration	Storage conditions[a]	Target bacteria	Reduction compared to control (log_{10} CFU/g or cm^2)[b]	Reference
Bacillus cereus P9 crude bacteriocin-like substance	Beef muscle	Inhibitory substance was sprayed onto meat samples	3.3 AU/g	Aerobic, 4 ± 1 °C, 12 d	TVC TVC(P) Total coliforms	0–0.7 0.2–4.2 0.2–1	(Fangio and Fritz 2014)
BacFL31 (from *Enterococcus faecium* FL31)	Beef mince	Partially purified bacteriocin and mince were homogenized in a blender	200 AU/g	Aerobic, 4 °C, 14 d	TVC TVC(P) *Enterobacteriaceae*	0.4–1.4 0–1.4 < 1.7	(Chakchouk Mtibaa et al. 2019)
BacTN635 (from *Lb. plantarum* TN635)	Beef mince	Mince was prepared with partially purified bacteriocin	500 and 1000 AU/g	Aerobic, 4 °C, 28 d	TVC TVC(P) *Enterobacteriaceae*	0–2.6 (from 500 AU/g, over 14 d) 0–3.9 (from 1000 AU/g, over 14 d) 0–3.2 (over 21 d) 0–2.5	(Smaoui et al. 2014)
Lb. plantarum BN supernatant containing bacteriocin	Beef muscle cubes	Meat cubes were dipped in the supernatant then drained	Supernatant of 24 h culture with initial inoculum of 10^6 CFU/mL	Aerobic, 5 ± 1 °C, 15 d	TVC TVC(P)	0.4–1.6 0–2	(Fiorentini et al. 2001)
Lb. plantarum UTNCys5-4 and *Lb. plantarum* ATCC 8014 CFS and partial precipitated peptides, and nisin	Beef fillets	Fillets were immersed in the inhibitory substances then drained	6400 AU/mL (CFS and partial precipitated peptides), 200 mg/kg (commercial nisin, 2.5%)	Aerobic, refrigeration, 9 d	TVC (20 °C)	1.1–1.6 (from CFS) 1.2–2 (from partial precipitated peptides) 1.2–1.6 (from nisin)	(Tenea and Guaña 2019)
Lb. salivarius and *Lb. acidophilus* freeze-dried CFS	Beef mince	CFS powder was added individually to mince and homogenized	10 and 35 mg/g	Aerobic, 4 ± 1 °C, 9 d	TVC(P)	0–2.2	(Moradi et al. 2019)
Lc. lactis subsp. *cremoris* CTC 204 CFS containing bacteriocin-like substance	Beef mince	Mince portions were dipped in CFS then drained	500 AU/mL	Aerobic, 4 °C, time not stated	TVC	1	(Bromberg et al. 2005)
Lactocin 705 (from *Lb. curvatus* CRL705)	Beef disks	Bacteriocin was sprayed onto meat surface	2.8 μmol/L	VP, 2 °C, 36 d	LAB *B. thermosphacta*	0–2 0–3.7	(Castellano and Vignolo 2006)
Lactococcin BZ (from *Lc. lactis* spp. *lactis* BZ)	Beef pieces	Meat pieces (about 20 g) were coated with 1 mL partially purified bacteriocin	200, 400, 800, 1600, and 2500 AU/mL	Aerobic, 4–5 °C, 12 d	TVC TVC(P) LAB Total coliforms 	0.5–7.2 (from 200, 400, and 800 AU/mL) 1.2–10.1 (from 1600 and 2500 AU/mL) 0.2–7 (from 200, 400, and 800 AU/mL) 0.5–9.5 (from 1600 and 2500 AU/mL) 0.6–5.8 (from 200, 400, and 800 AU/mL) 1.3–8 (from 1600 and 2500 AU/mL) 1.2–5 (from 200 and 400 AU/mL) 1.9–4.4 (from 800 and 1600 AU/mL, over 4 d, undetectable after 4 d) 3.4–4 (from 2500 AU/mL, over 1 d, undetectable after 1 d)	(Yildirim et al. 2016)
Nisin	Beef slices	Beef slices were put in bags containing nisin solution then drained	1000 IU/mL	Aerobic, 4 ± 1 °C, 17 d	TVC TVC(P) LAB *Pseudomonas*	0–3.7 0–2.8 < 2.2 0–2.5	(Aksoy et al. 2014)

					B. thermosphacta	0–3.3	
					Enterobacteriaceae	0–0.3	
					Coliforms	< 1.2	
Nisin	Beef cubes	Beef cubes were immersed in bacteriocin solution then drip-dried	5000 IU/mL	VP, 4 °C, 25 d	*B. thermosphacta*	Completely inhibited	(Tu and Mustapha 2002)
Nisin	Beef pieces	Beef pieces were dipped in bacteriocin solution then drip-dried	200 IU/mL	VP, 4 °C, 56 d	TVC(P)	0.2–2.4	(Ariyapitipun, Mustapha, and Clarke 1999)
					LAB	0.3–1.2 (over 42 d)	
					Pseudomonas	0.2–1.1	
					Enterobacteriaceae	< 1.3	
					Enterobacteriaceae (P)[c]	< 0.9	
Nisin	Beef patties	Nisin solution was pipetted onto the two sides of each patty	250 and 500 IU/g	Aerobic, 4 ± 1 °C, 9 d	TVC	0–4	(Shahbazi, Shavisi, and Mohebi 2016)
					TVC(P)	< 3	
					Enterobacteriaceae	0–3 (over 5 d, decreased to undetectable level on treated samples after 5 d)	
Nisin	Beef patties	Nisin powder (0.5% nisin content) was added directly to beef during grinding and mixed in a mixer then molded into patties	0.3%	Aerobic, 4 °C, 10 d	TVC	1–3.4	(Gómez Cárdenas et al. 2013)
					Total coliforms	0.6–3.6	
Nisin and lacticin NK24 (from *Lc. lactis* NK24)	Beef mince	Mince was wrapped in low-density polyethylene films coated with bacteriocin	8×10^8 IU/L	Aerobic, 3 °C, 25 d or 10 °C, 15 d	TVC	0–1.6	(Kim, Paik, and Lee 2002)
					Total coliforms	0–1.2	
Nisin	Beef mince	Nisin was added directly to mince and mixed using a mixer	1000 IU/g	Aerobic or VP, 4 °C, 10 d	TVC	< 0.8	(Calderón-Oliver, Escalona-Buendía, and Ponce-Alquicira 2020)
					LAB	0.1–0.9 (aerobic) 0.1–0.3 (VP)	
					Coliforms	0–0.4 (aerobic) 0–0.2 (VP, over 3 d)	
Nisin	Beef mince	Nisin solution was added to mince and mixed to form a homogeneous mixture	500 IU/g	VP, 4 °C, 21 d	TVC	0–2	(Dhifi et al. 2020)
					TVC(P)	0–2.2	
					Enterobacteriaceae	0–2.2	
Nisin	Beef mince	Nisin solution (25 mL) was sprayed onto lean and adipose tissues (total 125 g) from beef carcass surfaces then minced	100 μg/mL	Aerobic, 4 °C, 14 d	*B. thermosphacta*	0.4 (0 d, no reduction afterward)	(Cutter and Siragusa 1997)
Nisin	Beef carcass surface tissues	Nisin solution (10 mL) was applied to lean and adipose tissues using a 7.5 cm × 7.5 cm template	100 μg/mL	Aerobic, 4 °C, 7 d	*B. thermosphacta*	1.2–2.7	(Cutter and Siragusa 1996)
Nisin	Beef carcass surface tissues (pre-rigor and post-rigor, frozen, and thawed)	Carcass surface tissues (7.5 cm × 7.5 cm) were sprayed with 7 mL nisin solution and 3 mL sterile water	10 μg/mL (nisin solution)	Aerobic, 4 °C, 7 d (pre-rigor samples); VP, 4 °C, 14 d (post-rigor, frozen, and thawed samples)	*B. thermosphacta*	4.9–7.7 (pre-rigor samples) 2.6–3.2 (post-rigor, frozen, and thawed samples)	(Cutter and Siragusa 1998)
Nisin	Beef carcass surface tissues	Carcass surfaces were wrapped in nisin-	0.1% (w/w)	VP, 4 °C, 20 d or 4 °C, 2 d then 12 °C till 20 d	*B. thermosphacta*	0–2.2 (at 4 °C) 1.2–2.7 (at 4 °C followed by 12 °C)	(Siragusa, Cutter, and Willett 1999)

(*continued*)

Table 2. Continued.

Inhibitory substance	Product	Application method	Concentration	Storage conditions[a]	Target bacteria	Reduction compared to control (log_{10} CFU/g or cm^2)[b]	Reference
		impregnated low-density polyethylene films					
Nisin	Beef carcass surface tissues	Carcass surfaces were wrapped in nisin-incorporated low-density polyethylene films	0.1%	VP, 4 °C, 21 d	*B. thermosphacta*	0.7–1.8	(Cutter, Willett, and Siragusa 2001)

AU = arbitrary units; *B.* = *Brochothrix*; CFS = cell-free supernatant; CFU = colony forming units; IU = international units; LAB = lactic acid bacteria; *Lb.* = *Lactobacillus*; *Lc.* = *Lactococcus*; TVC = total viable count; TVC(P) = psychrotrophic total viable count; VP = vacuum packaging.

[a]Packaging type, temperature, and time (days).

[b]Approximate reduction; analyses of multiple time points during storage are presented in ranges; "<" indicates reduction up to the indicated value including negative numbers (meaning an increase in microbial counts).

[c]Psychrotrophic *Enterobacteriaceae*.

The TVC, although not targeting a particular group of spoilage bacteria, is determined in most studies as the TVC test is widely used to assess the overall microbial quality of many non-fermented food products, including fresh meat. The TVC is usually determined using 30 °C as the incubation temperature, and therefore it is sometimes referred to as mesophilic TVC. However, since spoilage of meat stored at refrigeration temperatures is mainly caused by psychrotrophic bacteria, psychrotrophic TVC (where plates are incubated at 7 °C) can be of more significance and thus should be included in more chilled meat studies. Bacteriocins have been reported to reduce both mesophilic and psychrotrophic TVC in beef, and the degree of suppression varied largely among studies, ranging from less than 0.5 to several log reductions. Although a reduction in total bacterial population is an overall positive effect, TVC does not provide information on specific spoilage populations and, therefore, may not be a reliable indicator of the spoilage status.

Reuterin

Reuterin is a non-proteinaceous antimicrobial compound produced by *Lb. reuteri*, which is found in a range of foods, including meat. It is a by-product of glycerol fermentation. As reuterin is both water- and lipid-soluble, stable over a wide pH range, and not sensitive to heat and proteolytic and lipolytic enzymes, it has a high potential to be used as a food preservative (Delves-Broughton 2012; Garde et al. 2014). It is a broad-spectrum antimicrobial compound, effective against a variety of Gram-positive and Gram-negative bacteria, including *S. aureus*, *L. monocytogenes*, *E. coli*, *Salmonella* spp. (Juneja, Dwivedi, and Yan 2012; Pisoschi et al. 2018). Because reuterin can be converted into various compounds in aqueous solutions and is an equilibrium mixture of monomeric, hydrated monomeric, and dimeric forms of β-hydroxypropionaldehyde, it has been difficult to determine its antimicrobial mode(s) of action (Mishra et al. 2012; Schaefer et al. 2010). Nevertheless, one of the main hypotheses is that the aldehyde groups of reuterin react with thiol groups and primary amines, inactivating bacterial proteins and small molecules containing these groups. Another hypothesis suggests that the dimeric form of reuterin, which is structurally similar to a ribose sugar, blocks the enzyme ribonucleotide reductase, thus interfering with DNA synthesis (Schaefer et al. 2010).

Some studies have been conducted on the antimicrobial effect of reuterin on meat products. Montiel et al. (2016) reported that reuterin inhibited the growth of total aerobic bacteria on cooked ham for 35 days at both 4 and 10 °C. El-Ziney et al. (1999) found reuterin decreased *L. monocytogenes* and *E. coli* O157:H7 numbers on the surface of cooked pork and in raw pork mince. Kuleaşan and Çakmakçı (2002) showed that although reuterin was able to reduce the growth of *L. monocytogenes* on the surface of beef sausages, it was ineffective against *Salmonella*. A study by Muthukumarasamy, Han, and Holley (2003) showed that *Lb. reuteri* in the presence of glycerol was able to suppress the growth of both total bacteria and LAB and also reduced

inoculated *E. coli* O157:H7 to undetectable levels in beef mince over a 25-day storage period. On the other hand, in another study (Khalili Sadaghiani et al. 2019), when glycerol, the substrate for reuterin production, was not added, *Lb. reuteri* only caused a maximum 0.5 log reduction in *L. monocytogenes* inoculated in beef mince over 12 days. These studies (while mostly on pathogens) suggest the potential of reuterin to be used for meat biopreservation that can be explored in future studies.

Fermentates

Microbial fermentates are fermented food ingredients. They are produced from the fermentation of food materials (e.g., milk, whey, sugars, and other plant-derived materials) by food-grade microorganisms that produce antimicrobial metabolites, such as organic acids, diacetyl, and bacteriocins. The commonly used microbial cultures for fermentate production are *Lc. lactis*, *Propionibacterium freudenreichii* subsp. *shermanii*, and species of *Lactobacillus* and *Pediococcus*. The culture(s) used in a fermentate formulation determines its antimicrobial activity (Davidson et al. 2015; Elsser-Gravesen and Elsser-Gravesen 2014).

Although there are limited scientific reports on fermentates' effectiveness against spoilage bacteria in fresh red meat, several commercial fermentates are currently available to food manufacturers as antimicrobials or preservatives. For example, various products marketed by DuPont under the MicroGARD trademark have been developed to target microorganisms in a range of food applications, including raw meat (www.dupontnutritionandbiosciences.com). Prolong II (BSA) is a cultured dextrose product containing organic acids. It can be added directly to a range of food products, including fresh sausage and ground meat. The manufacturer claims that application at concentrations of 0.25%–0.75% can slow down the growth of total bacteria in fresh sausage and extend the shelf-life by up to 50% (BSA 2019). Verdad fermentates (Corbion) and DuraFresh (Kerry) are other commercial fermentates developed for applications including meat products, but their effects on fresh meat spoilage control are not known.

Protective cultures

Protective cultures are microorganisms that have an inhibitory effect against other microorganisms. They can become the dominant microbiota in food as a result of competitive exclusion and/or production of antagonistic compounds (e.g., bacteriocins, organic acids, and hydrogen peroxide). Many protective cultures have been included in the Qualified Presumption of Safety list for use in food and feed chain in the European Union and gained GRAS status in the United States (Bartkiene et al. 2016; Davidson et al. 2015) and many are commonly used in food fermentations (Bourdichon et al. 2012), although recently this has been complicated by an emended nomenclature introduced for the previously well-recognized *Lactobacillus* genus (Zheng et al. 2020). Protective cultures associated with food applications are generally species of LAB (e.g., *Lactococcus*, *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Carnobacterium*, *Enterococcus*, and *Streptococcus*) and *Bifidobacterium* (Ben Said et al. 2019; Davidson et al. 2015). For application on fresh red meat, protective cultures used are mainly *Lb. sakei* and *Lb. curvatus*, which are naturally found in meat products and have long been employed as starter cultures for fermented sausages (da Costa et al. 2019; Yost 2014). Some studies also investigated other *Lactobacillus* species, as well as *Lactococcus*, *Pediococcus*, and *Staphylococcus* cultures (Table 3).

Protective cultures are typically applied in high concentrations in the form of culture suspensions and commonly contain bacteriocin-producing strains. Concentrations used in published studies range from 10^3 to 10^9 CFU per g or cm^2 of meat or meat surface, though most authors applied around 10^5–10^7 CFU per g or cm^2 (Table 3). The application method depends on the form of the meat product. The cultures can be easily mixed into comminuted meat (Chaillou et al. 2014; Comi et al. 2015; Trabelsi et al. 2019). For whole cuts, application methods include dipping the meat samples in culture suspensions followed by a brief period of draining (Zhang et al. 2018) and spraying culture suspensions onto the meat surface (Castellano et al. 2010; Castellano and Vignolo 2006; Djenane et al. 2005). Alternatively, a defined volume of culture suspensions can be inoculated onto the meat surface before (Katikou et al. 2005; Hilgarth, Nani, and Vogel 2018; Senne and Gilliland 2003) or during packaging (Jones et al. 2009; Stella et al. 2016). As protective cultures are selected strains that can grow well on meat, differences in inoculation methods are not likely to greatly affect their effectiveness, as long as the cultures are well distributed within or on the product. However, more studies are needed to determine the effect of different concentrations of protective cultures on the overall meat quality. For example, Trabelsi et al. (2019) compared beef mince formulated with 10^7, 10^8, and 10^9 CFU/g *Lb. plantarum* TN8 and found that while higher concentrations could provide stronger antimicrobial effects, 10^8 CFU/g was more desirable when considering physicochemical and sensory properties of the meat.

Effect on total and lactic acid bacteria populations

Since protective cultures applied to meat are mostly LAB, it is expected that microbiological analysis will show higher LAB counts at the start of storage compared to untreated samples. This has been observed for most studies listed in Table 3 that analyzed LAB, and the differences were generally in the order of several log units. During storage, though, the growth of the total LAB population tends to be slower in culture-treated samples as substrates are depleted more rapidly, and growth typically stops when the population reaches around 8 log CFU per g or cm^2 (Hernández-Macedo, Barancelli, and Contreras-Castillo 2011). An exception to this was *Lb. plantarum* TN8 added to beef mince, which increased from an inoculum level of 9 log CFU/g to over 14 log CFU/g after 10 days of storage in refrigerated conditions (Trabelsi et al. 2019).

As the added LAB cultures also contribute significantly to the TVC, many previous studies have reported similar growth patterns for both groups of bacteria. However, this effect was observed only for mesophilic TVC. Analyses of psychrotrophic TVC have shown different growth patterns. Senne and Gilliland (2003) found that beef steaks and carcass surface samples inoculated with *Lb. delbrueckii* subsp. *lactis* had around 1.5 and 0.5 log units lower psychrotrophic TVC, respectively, than untreated samples throughout storage. Trabelsi et al. (2019) reported similar levels of psychrotrophic aerobic bacteria in *Lb. plantarum* TN8-treated and untreated beef mince samples at the start of storage but a lower growth rate in treated samples during storage. Because of the difference in incubation temperature, the mesophilic TVC is likely to be mostly made up of the added protective cultures leading to the high numbers, whereas the psychrotrophic TVC is expected to consist of larger proportions of the endogenous psychrotrophic spoilage bacteria. This again reinforces that psychrotrophic TVC for chilled meat products is a better indicator of spoilage inhibition and should be included in more studies, possibly with microbial community analysis, to distinguish between the spoilage bacteria and added protective cultures. Researchers may also be able to determine the growth of both mesophilic and psychrotrophic TVC using 20 °C incubation temperature, as performed by Tenea and Guaña (2019) who reported 1–1.6 log reduction by *Lb. plantarum* strains inoculated onto beef fillets.

Although most studies found a slower increase of the LAB numbers and TVC on culture-treated meat samples, it cannot be assumed that protective cultures always suppress the growth of the endogenous spoilage bacteria. Several approaches could be used to address this problem. Hilgarth, Nani, and Vogel (2018) and Zhang et al. (2018) analyzed microbial diversity of beef steaks at various time points during refrigerated storage using MALDI-TOF MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) and 16S rRNA gene sequencing, respectively, to confirm the dominance of the added protective cultures. Using a completely different approach, Castellano and Vignolo (2006) compared the effect of *Lb. curvatus* culture with the bacteriocin it produced. They found that the purified bacteriocin did not contribute to higher starting counts of LAB, and it retarded the growth of LAB during storage.

It should be noted that when protective cultures are applied, spoilage cannot be concluded based merely on the TVC and LAB numbers. LAB have low spoilage potential, and when they become the dominant flora, particularly toward the end of storage, high numbers do not necessarily indicate spoilage from a consumer sensory perspective. High concentrations of added LAB protective cultures, such as *Lb. curvatus* and *Lb. sakei*, may contribute to the deterioration of the meat by producing acidic or cheesy odor, but it is not considered to be particularly undesirable because the odor disappears after opening the package (Ray and Bhunia 2013). In addition, since spoilage changes tend to be strain-dependent, it is possible that some LAB strains can offer bioprotective functions without contributing significantly to spoilage (Pothakos et al. 2015). Therefore, analyses of other spoilage bacteria such as *B. thermosphacta, Pseudomonas* spp., and *Enterobacteriaceae* become more relevant and meaningful in these instances.

Effect against meat spoilage bacteria

B. thermosphacta, either naturally contaminated or artificially inoculated, can be reduced by protective cultures in general by at least 0.5 log units in beef (Table 3). Castellano et al. (2010) and Castellano and Vignolo (2006) even showed complete inhibition of *B. thermosphacta* by *Lb. curvatus* CRL705. However, Chaillou et al. (2014) reported that *B. thermosphacta* numbers in ground beef treated with a *Lb. sakei* strain mixture were only 0.1–0.5 log units lower than in untreated samples, and Jones et al. (2009) also found similar levels of *B. thermosphacta* on *Lc. lactis* 75-treated and untreated lamb meat samples. These findings may be attributed to the inability of the low concentrations of the applied protective cultures (10^3–10^4 CFU/g *Lb. sakei* in ground beef and 3×10^3 and 3×10°CFU/cm^2 *Lc. lactis* 75 on lamb) to produce any significant inhibitory effect. Furthermore, as both of these studies had meat samples inoculated with specific *B. thermosphacta* strains, it is unknown whether natural *B. thermosphacta* populations on meat would have a similar response to these protective cultures. This highlights the importance of considering the concentration of applied protective cultures and the need and appropriateness for testing their efficacy on naturally contaminated meat.

For Gram-negative meat spoilage bacteria, *Pseudomonas* spp. and *Enterobacteriaceae*, some protective cultures appear to exhibit stronger inhibitory effects than others. Zhang et al. (2018) and Katikou et al. (2005) both found that the growth of *Pseudomonas* and *Enterobacteriaceae* was retarded by *Lb. sakei* and *Lb. curvatus* in vacuum-packaged beef, and *Lb. sakei* was more effective than *Lb. curvatus*. The inhibitory effect of these species on *Enterobacteriaceae* was also shown by Stella et al. (2016). Conversely, *Lb. delbrueckii* subsp. *lactis* did not show any significant effect against coliforms at the end of storage on either beef steaks (9 days) or carcass surface sections (8 days), though this protective culture was able to suppress the growth of coliforms on the steaks in the first 6 days (Senne and Gilliland 2003). *Lb. curvatus* CRL705, as tested by Castellano et al. (2010), caused no significant inhibition of *Pseudomonas* or coliforms. As bacteriocins produced by Gram-positive protective cultures are generally not active against Gram-negative bacteria, the inhibitory effect observed in these studies may be the result of competitive exclusion. There is also the possibility that other antagonistic compounds were produced by the protective cultures, though inactivation by organic acids produced by fermentation seems unlikely, as none of these studies reported a significant pH drop in the meat samples.

The efficacy of protective cultures can also be affected by the packaging conditions of the meat product. Djenane et al. (2005) tested the effect of *Lb. sakei* CTC 372 and uncharacterized *Lb.* CTC 711 on beef packaged under two different gas compositions in MAP. It was found that both cultures could slow down the growth of *Pseudomonas*, and the effect

Table 3. Application of protective cultures to fresh beef and lamb meat for controlling spoilage bacteria.

Culture	Product	Application method	Concentration	Storage conditions[a]	Target bacteria	Reduction compared to control (log_{10} CFU/g or cm^2)[b]	Reference
Lb. curvatus CRL705	Beef steaks	Culture cells diluted in saline solution were sprayed onto meat surface	10^6 CFU/g	VP, 2 °C, 60 d	TVC LAB *Pseudomonas* *B. thermosphacta* Total coliforms	No reduction No reduction < 0.4 < 2.2 < 0.6	(Castellano et al. 2010)
Lb. curvatus CRL705	Beef disks	Cultures were sprayed onto meat surface	10^6 CFU/cm^2	VP, 2 °C, 36 d	LAB *B. thermosphacta*	No reduction 0–3.7	(Castellano and Vignolo 2006)
Lb. curvatus and *Lb. sakei*	Beef steaks	Steaks were dipped into individual culture solutions and drained	7.5 log CFU/g	VP, 4 °C, 38 d	TVC LAB *Pseudomonas* *B. thermosphacta* *Enterobacteriaceae*	No reduction No reduction 0–1.1 < 0.6 (from *Lb. curvatus*) 0.2–2.4 (from *Lb. sakei*) < 0.9 (from *Lb. curvatus*) 0.1–2.6 (from *Lb. sakei*)	(Zhang et al. 2018)
Lb. curvatus CECT 904^T and *Lb. sakei* CECT 4808	Beef slices	Individual or combined culture suspensions were pipetted onto each side of the beef slices and massaged to ensure good contact	At least 10^8 CFU/mL	VP, 4 ± 1 °C, 28 d	LAB *Pseudomonas* *B. thermosphacta* *Enterobacteriaceae*	No reduction 0–1.3 0–1.4 0–2	(Katikou et al. 2005)
Lb. curvatus 2 strain mixture and *Lb. sakei* 6 strain mixture	Beef slices	Beef slices were inserted into individual vacuum bags and culture mixtures were then added	5 log CFU/g	VP, 4 °C, 60 d	TVC LAB *Enterobacteriaceae*	< 1.3 (from *Lb. curvatus*) < 1.7 (from *Lb. sakei*) No reduction 0–1.3 (from *Lb. curvatus*); 0–2.2 (from *Lb. sakei*)	(Stella et al. 2016)
Lb. sakei strain mixtures	Beef mince (10% fat)	Mince was inoculated with cultures and homogenized	10^3–10^4 CFU/g	VP, 4 and 8 °C, 14 d; MAP (70% O_2:30% CO_2), 4 and 8 °C, 7 d	LAB *B. thermosphacta*	No reduction 0.1–0.5 (effect not reproducible)	(Chaillou et al. 2014)
Lb. sakei CTC 372 and *Lb.* CTC 711	Beef steaks	Cultures were sprayed onto meat surface	10^4–10^5 CFU/cm^2	MAP (70% O_2:20% CO_2:10% N_2 or 60% O_2:40% CO_2), 1 ± 1 °C, 28 d	*Pseudomonas* *B. thermosphacta*	0–0.3 (from *Lb.* CTC 711 under 60% O_2:40% CO_2) 0–1.1 (from other treatment and storage conditions) < 0.7 (from *Lb.* CTC 711 under 60% O_2:40% CO_2) < 1.7 (from other treatment and storage conditions)	(Djenane et al. 2005)
Lb. sakei, *Lb. sakei* subsp. *carnosus*, *Lc. lactis* ssp. *lactis*, and *S. xylosus* mixtures	Beef patties	Ground meat was inoculated with cultures and then formed into patties	10^5 CFU/g	MAP (70% O_2:30% CO_2), 4 ± 2 °C, 12 d	TVC LAB *B. thermosphacta*	< 0.4 No reduction < 2.2	(Comi et al. 2015)
Lb. delbrueckii subsp. *lactis* RM2-5	Beef steaks or carcass surfaces	Concentrated cell suspension and sterile water were added to the geometric center of the top of each piece of meat	10^7 CFU/cm^2	Aerobic, 5 °C, 9 (steaks) or 8 d (carcass surfaces)	TVC (15 °C) Coliforms	1.4–1.7 (on steaks) 0.5–0.7 (on carcass surfaces) 0–0.9 (on steaks) 0.3–0.5 (on carcass surfaces)	(Senne and Gilliland 2003)
Lb. plantarum TN8	Beef mince			VP, 4 °C, 10 d	TVC(P)	< 2.7	(Trabelsi et al. 2019)

(*continued*)

Table 3. Continued.

Culture	Product	Application method	Concentration	**Storage conditions**[a]	Target bacteria	**Reduction compared to control (log_{10} CFU/g or cm^2)**[b]	Reference
		Culture suspensions were added to mince and vacuum-stuffed into plastic casings after homogeneous mixtures were produced	7, 8, and 9 log CFU/g		LAB *Enterobacteriaceae*	No reduction 0–2.8	
Lb. plantarum UTNCys5-4 and *Lb. plantarum* ATCC 8014	Beef fillets	Fillets were immersed in the inhibitory substances then drained	8.97 log CFU/mL (UTNCys5-4) 9.03 log CFU/mL (ATCC 8014)	Aerobic, refrigeration, 9 d	TVC (20 °C)	1–1.6	(Tenea and Guaña 2019)
Lc. lactis 75	Lamb slices	Culture suspension was inoculated into vacuum bags each containing a slice of lamb	3 and 3000 CFU/cm^2	VP, −1.5 °C, 84 d	*B. thermosphacta*	No reduction	(Jones et al. 2009)
Lc. piscium strains TMW2.1612/ 2.1614/2.1615	Beef steaks	Steaks were inoculated on top and bottom with individual culture suspensions	6 log CFU/cm^2	MAP (>70% O_2:>20% CO_2), 4 °C, 8 d	TVC	No reduction	(Hilgarth, Nani, and Vogel 2018)
P. pentosaceus LIV01 and *P. acidilactici* FLE01	Beef slices	Slices were dipped in sterile glucose solution (10% w/v) and then inoculated with cultures	10^6 CFU/g	Packaging unspecified, 30 °C, 7 d	LAB *Enterobacteriaceae*	No reduction 0–4.6	(Olaoye and Onilude 2010)

B. = *Brochothrix*; CFU = colony forming units; LAB = lactic acid bacteria; *Lb.* = *Lactobacillus*; *Lc.* = *Lactococcus*; *P.* = *Pediococcus*; MAP = modified atmosphere packaging; *S.* = *Staphylococcus*; TVC = total viable count; TVC(P) = psychrotrophic total viable count; VP = vacuum packaging.

[a]Packaging type, temperature, and time (days).

[b]Approximate reduction; analyses of multiple time points during storage are presented in ranges; "<" indicates reduction up to the indicated value including negative numbers (meaning an increase in microbial counts).

of *Lb. sakei* CTC 372 was stronger in the pack with 60% O_2:40% CO_2, while the two cultures exhibited similar effects under 70% O_2:20% CO_2:10% N_2. Similarly, a protective culture is also likely to perform differently in aerobic and in vacuum packaging. As a result, there is a need for researchers or developers to test protective cultures in different packaging systems to provide information to users in the meat industry who usually have multiple packaging options for their products.

As many protective cultures are bacteriocin-producers, their effects are often compared with the direct addition of extracted bacteriocins. In general, protective cultures appear to offer a delayed but more persistent and milder antimicrobial effect in a food environment that supports their growth. On the other hand, the inhibitory effect of bacteriocins can be observed from the early stage of storage. While some bacteriocins are reported to have long-lasting effects, many become ineffective toward the end of storage.

Bacteriophages

Bacteriophages (or simply phages) are viruses that infect and lyse bacterial cells. Following a localized interaction between a bacteriophage and a susceptible bacterium, reversible and irreversible bacteriophage binding and bacteriophage genome transfer into the host cell occurs. The bacteriophage then replicates within the bacterial cell leading to lysis and subsequent death of the host cell and release of bacteriophage progeny (Davidson et al. 2015; Fieseler, Loessner, and Hagens 2011). The antibacterial activity of bacteriophages has been evaluated against a range of Gram-positive and Gram-negative bacteria in various food applications, which has been summarized in a review recently published by O'Sullivan et al. (2019).

There are a limited number of studies that have reported the application of bacteriophage for controlling spoilage bacteria on fresh red meat. A study conducted by Greer (1986) showed that bacteriophage resulted in 1–2 log reduction of inoculated *Pseudomonas* on beef steaks wrapped in polyvinyl film stored for 4 days at 7 °C. Meat surface discoloration also decreased with phage treatment. Retail case life was found to be positively correlated with phage concentration. Further, Greer (1988) found that the ability of bacteriophage to increase retail case life was significantly influenced by the interrelationship between bacteriophage concentration and the initial bacterial density. It was also found that storage temperature within the 1–10 °C range did not affect the effectiveness of bacteriophage for controlling beef spoilage by inoculated *Pseudomonas*.

A number of more recent studies have reported significant effect of bacteriophages against various pathogenic bacteria on beef. O'Flynn et al. (2004) found that a cocktail of bacteriophages was able to eliminate artificially inoculated *E. coli* O157:H7 completely. Abuladze et al. (2008) and Carter et al. (2012) reported significant reductions of *E. coli* O157:H7 by bacteriophage in ground beef, while Shebs et al. (2020) observed a similar effect on beef cuts stored under both vacuum and aerobic conditions. Significant inhibition of *Salmonella* by bacteriophage application was reported by Yeh et al. (2018) in ground beef and by Bigwood et al. (2008) on cooked and raw beef slices. However, Dykes and Moorhead (2002) found that bacteriophages applied to vacuum-packaged beef had no effect on artificially inoculated *Listeria* during a 4-week storage period, but a synergistic effect was observed when bacteriophages were used in combination with nisin.

Some commercial bacteriophage formulations that offer protection against pathogenic bacteria (*E. coli* O157:H7, *L. monocytogenes*, and *Salmonella*) have received GRAS status. These products include PhageGuard E, PhageGuard Listex, and PhageGuard S developed by Micreos (www.phageguard.com), and EcoShield PX, ListShield, and SalmoFresh produced by Intralytix (www.intralytix.com). In addition to the United States, health and regulatory agencies in several other countries, such as Australia, Canada, and Switzerland, have issued approvals of bacteriophage preparations for use as bioprotective agents in food (Moye, Woolston, and Sulakvelidze 2018). Since spoilage is complex and involves many different species and strains compared with known single species of pathogenic bacteria, and because phages are species- and strain-specific, commercial applications of bacteriophages to control spoilage bacteria in meat are expected to be more difficult to succeed. Greer and Dilts (1990) observed a reduction in *Pseudomonas* growth in beef steaks with a phage cocktail but without any improvement in the retail shelf-life of the steaks, presumably due to the growth of other spoilage bacteria. The challenge is to develop and acquire approval for phage mixtures with broad host-specificity for the target bacteria (Davidson et al. 2015).

Other factors to consider

The successful application of microbial biopreservatives is not only determined by their effectiveness against the undesirable bacteria but also depends on several other factors, as illustrated in Figure 2. Their safety, sensory effects, interaction with meat constituents, and cost must be carefully considered.

Safety for consumption

Safety is a critical aspect of any compound proposed to be used as a food additive. While many microbial biopreservatives have gained GRAS status, the research focusing on identifying new potential antimicrobials is ongoing. Because "natural" does not necessarily mean "safe," all new naturally occurring or naturally derived antimicrobial agents must undergo strict regulatory processes and often substantial toxicological testing to ensure their safety for human consumption. The evaluation steps for a new bacteriocin to be approved as an additive is described by Soltani et al. (2021) and illustrates the complexity of the approval process. Unfortunately, the development and commercial application of most novel antimicrobial agents remains limited for this reason, as the process of collecting data to determine their toxicity and thresholds for human consumption is extremely

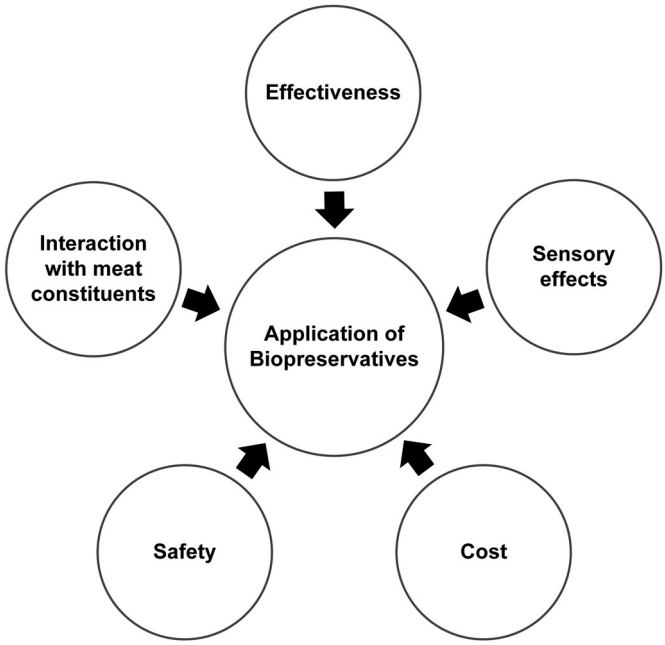

Figure 2. Factors to consider for the successful application of biopreservatives.

costly and time-consuming (Davidson et al. 2015; Soltani et al. 2021; Verma et al. 2014).

The potential allergenic effect must also be considered when choosing a food ingredient. Many microbial biopreservatives are associated with LAB, and cultivation media often contain dairy ingredients, a common allergen. Fortunately, recent studies on the production of LAB antimicrobials have shown the potential for alternatives, including plant-based media (Bali, Panesar, and Bera 2016; Bali et al. 2016; Taskila 2017).

An ideal biopreservative should also not contribute to the development of resistance in target microorganisms (Davidson et al. 2015). Nisin has been used in the food industry for more than 50 years without inducing widespread microbial resistance (O'Connor et al. 2015). There is no evidence that the proper use of antimicrobials in food manufacturing leads to the development of resistant microorganisms. Although researchers have recovered bacteria resistant to, for example, bacteriocin or bacteriophage treatments (Kumariya et al. 2019; Moye, Woolston, and Sulakvelidze 2018), causality is yet to be proved (Donaghy et al. 2019). Notwithstanding this, the development of resistance should be monitored closely to ensure that the applied biopreservatives remain effective. The risk of target microorganisms developing resistance may be reduced by the use of hurdle technology, including the combined use of different biopreservatives (Kumariya et al. 2019; Yost 2014). It is also important to be aware and make sure that the application of biopreservatives with specific activity against particular microorganisms does not result in positive selection for others (Davidson et al. 2015).

Effect on sensory and other non-microbial meat qualities

When evaluating potential biopreservatives, although their antimicrobial effect is the primary focus, it is equally important and necessary to take into account their effect on the non-microbial quality of the food. To be suitable for application in food products, the biopreservatives must not have any negative impact on the sensory properties. Sensory evaluation by human panelists is probably the most acceptable and appropriate method for assessing food quality, but it is costly and time-consuming (Sofos et al. 2013). As a result, only a limited number of sensory studies have been performed on meat treated with microbial biopreservatives. In most of these studies, the sensory properties (e.g., color, odor, and texture) of meat treated with biopreservatives were rated higher by the panelists, which may be associated with the delayed deterioration. In other cases, the treated samples were considered no different from the untreated samples or at least of acceptable quality (Table 4). In terms of panel size and experience, most of these studies relied on experienced or trained panels with a small number of assessors. Untrained consumer panels should be employed in more studies to understand consumer response to biopreservative-treated meat products.

Instrumental and chemical analyses are often preferred in studies involving meat quality as they are more convenient than using human sensory panels. Texture is an important meat quality attribute, but it has only been investigated in a few studies involving microbial biopreservatives. Instrumentally, texture can be analyzed by various methods, including Warner-Bratzler shear force and texture profile analysis (TPA). Castellano et al. (2010) found that the addition of the protective culture *Lb. curvatus* CRL705 had no effect on cooked beef tenderness measured using the Warner-Bratzler shear method. Trabelsi et al. (2019) used TPA to assess the texture of beef mince containing protective culture *Lb. plantarum* TN8. They reported an increase in hardness, a decrease in springiness and chewiness, and no change in cohesiveness and adhesiveness. Smaoui et al. (2014) also used TPA and found that adding bacteriocin BacTN635 to beef mince reduced hardness, springiness, and rigidity, increased adhesiveness and chewiness, and produced no change in cohesiveness. Recent work (unpublished) by us has shown that protective cultures did not affect the hardness of lamb meat measured by TPA instrument. However, with such a limited number of studies, the general effect of biopreservatives on meat texture remains inconclusive.

The pH of meat is highly correlated with its quality attributes, such as color, water-holding capacity, and tenderness (Andrés-Bello et al. 2013). During storage, meat pH may increase as a result of protein breakdown leading to the formation of ammonia and amines, which signifies spoilage (Karabagias, Badeka, and Kontominas 2011). However, a decrease in pH can also occur due to the growth of acid-producing bacteria, such as LAB and *B. thermosphacta* (Michalczyk et al. 2012). Bacteriocins and protective cultures, in general, have no adverse effect or only cause slight changes in meat pH and, therefore, are not likely to have any significant impact on meat quality (Table 4). While the addition of protective cultures might be expected to lower the pH due to the high concentrations of applied LAB, the

lower pH from some bacteriocin applications was believed to be related to the presence of acidic compounds used for dissolving the bacteriocin in the treatment solutions (Ariyapitipun, Mustapha, and Clarke 1999; Tu and Mustapha 2002). This effect can be avoided in certain applications by replacing the acid compounds, for example, with vigorous vortex mixing to assist in dissolving the bacteriocin.

Applications of biopreservatives have been shown to have a positive effect on meat odor. The concentration of thiobarbituric acid reactive substances, an index of lipid oxidation, was decreased by many biopreservative treatments (Table 4). Similarly, total volatile basic nitrogen is another indicator for evaluating meat freshness/spoilage, and its level can also be reduced by the application of microbial biopreservatives, as shown by Comi et al. (2015) and Zhang et al. (2018). Further, microbial biopreservatives generally did not negatively affect the instrumentally measured meat color (Table 4).

Interaction with meat system constituents

It has been observed that microbial biopreservatives often show great efficacy in vitro but have reduced or sometimes little effect when incorporated into food systems. This is likely to be caused by their interaction with food components. Proteins, lipids, carbohydrates, proteolytic enzymes, and ions in food have all been shown to interact with biopreservatives resulting in reduced antimicrobial activities (Ben Said et al. 2019; Davidson et al. 2015; Gharsallaoui et al. 2016). For instance, while the hydrophilic portions of amphiphilic antimicrobials, such as some LAB bacteriocins, are required for them to solubilize in the water phase of the food product where microbial growth occurs, the hydrophobic (lipophilic) portions enable interaction of these antimicrobial compounds with the microbial cell membranes comprised of phospholipids. However, the hydrophobic portions can also result in these antimicrobials being bound or solubilized by lipids or hydrophobic portions of proteins in the food system, thus rendering them unavailable (Davidson et al. 2015). The intrinsic properties of the food system can also affect the efficacy of the biopreservatives (Davidson, Critzer, and Taylor 2013). For example, the solubility and antimicrobial activity of nisin are lower at the pH of meat than in more acidic environments (da Costa et al. 2019; Gharsallaoui et al. 2016).

Fresh meat and meat products are one of the most challenging food systems for biopreservative applications. They are nonhomogeneous, with only a mildly acidic pH, and are rich in protein and fat. These factors are unfavorable for supporting the activity of most biopreservatives but favorable for the undesirable microorganisms (Davidson et al. 2015). In addition, because most of the biopreservatives have a narrow antimicrobial spectrum, it cannot be guaranteed that all the spoilage and pathogenic bacteria in a food product can be controlled solely by biopreservation (Verma et al. 2014). Therefore, although proven to be effective, biopreservation is not likely to be used alone for fresh meat preservation. Its efficacy in combination with other preservation technologies needs to be further explored, with MAP and VP technologies being the promising approaches, as these have already gained wide consumer acceptability. The essential issue that remains to be addressed is the overall efficacy of biopreservatives in meat in commercial environments and the appropriate application method to be used (Favaro and Todorov 2017; Gharsallaoui et al. 2016). Tests or challenge studies must be performed to assess the antimicrobial efficacy under conditions representative of commercial meat processing systems before they can be used effectively on a large scale (Davidson et al. 2015; Davidson, Critzer, and Taylor 2013).

Cost

The addition of antimicrobials increases the cost of manufacturing a food product. For a successful application, the antimicrobial must have a reasonable "cost in use" and be able to justify the improved microbiological stability and quality as well as sensory attributes of the product (David, Steenson, and Davidson 2013). In many cases, an additional 2–3 days of shelf-life would help significantly in offsetting the cost of using an antimicrobial (Davidson et al. 2015). The cost of applying biopreservatives of microbial origin is estimated to be up to 8 US cents per kg of food treated, which is a small amount of extra cost for meat products considering their high value. Biopreservatives are more expensive than synthetic chemical antimicrobials (Ben Said et al. 2019; Moye, Woolston, and Sulakvelidze 2018), but clean labeling is highly sought after by consumers.

The cost of biopreservatives may be reduced through optimization of the formulation and production process. For example, the media used for growing bacterial strains and producing biopreservatives, which accounts for a large proportion of the cost, can be optimized by using agro-industrial by-products, which are less expensive (Bali, Panesar, and Bera 2016; Taskila 2017). Furthermore, it should also be noted that although highly purified biopreservatives may exhibit greater activity, isolation of active antimicrobial components involves more processing steps increasing the cost and defeating the objective of biopreservatives being perceived as "natural." Therefore, the use of biopreservatives in a form as close to their sources as possible would be desirable for clean-label food products (Davidson et al. 2015).

Conclusion

Biopreservatives of microbial origin, mainly bacteriocins and protective cultures, in general, have been shown to be effective against spoilage bacteria on fresh beef. They also do not seem to negatively affect meat quality or sensory properties, showing their great potential as natural antimicrobials. On the other hand, information on the efficacy of microbial biopreservatives on lamb meat is scarce, despite the shelf-life of lamb being shorter than the equivalent cut or type of beef product. This reveals the need for more biopreservation research on lamb meat specifically, although the knowledge

Table 4. The effect of biopreservatives on physicochemical and sensory properties of beef compared to untreated samples.

Biopreservative	Product	Physicochemical properties[a]	Sensory properties (panel size, experience)	Reference
Bacillus cereus P9 crude bacteriocin-like substance	Beef	pH: slightly more increase during storage	—[b]	(Fangio and Fritz 2014)
BacFL31 (partially purified bacteriocin from *Enterococcus faecium* FL31)	Beef mince	TBARS: decreased	Higher ratings for odor, texture, color, and overall acceptability (25, unspecified)	(Chakchouk Mtibaa et al. 2019)
BacTN635 (partially purified bacteriocin from *Lb. plantarum* TN635)	Beef mince	—	Higher ratings for odor, texture, color, and overall acceptability (28, unspecified)	(Smaoui et al. 2014)
Lb. plantarum BN supernatant containing bacteriocin	Beef	pH: slightly lower	—	(Fiorentini et al. 2001)
Lb. plantarum UTNCys5-4 and *Lb. plantarum* ATCC 8014 cell cultures, supernatant, and partial precipitated peptides, and nisin	Beef fillets	pH: less increase	—	(Tenea and Guaña 2019)
Lb. salivarius and *Lb. acidophilus* freeze-dried supernatant	Beef mince	pH: less increase TBARS: decreased	Higher ratings for odor, color, and overall acceptability (21, unspecified)	(Moradi et al. 2019)
Nisin	Beef	pH: slightly lower at the start; no significant effect during storage	—	(Tu and Mustapha 2002)
Nisin	Beef	pH: slightly lower at the start; no significant effect during storage	—	(Ariyapitipun, Mustapha, and Clarke 1999)
Nisin	Beef	pH: no significant effect	Nisin did not affect meat odor or color; development of spoilage characteristics same for treated and untreated samples (4, unspecified)	(Aksoy et al. 2014)
Nisin	Beef patties	pH: less change Color: no significant effect	Lower but acceptable ratings for flavor, texture, and general aspect of cooked samples (50, untrained)	(Gómez Cárdenas et al. 2013)
Nisin	Beef mince	pH: less change TBARS: decreased	—	(Calderón-Oliver, Escalona-Buendía, and Ponce-Alquicira 2020)
Nisin	Beef mince	pH: less increase TBARS: decreased	Higher ratings for odor, color, and overall acceptability (18, experienced)	(Dhifi et al. 2020)
Nisin and Lacticin NK24 (from *Lc. lactis* NK24)	Beef mince	pH: no significant effect Color: no significant effect TBARS: decreased	—	(Kim, Paik, and Lee 2002)
Lb. curvatus CRL705	Beef	pH: no significant effect	—	(Castellano and Vignolo 2006)
Lb. curvatus CRL705	Beef	pH: slightly lower	Protective culture did not modify flavor or aroma of cooked samples; treated samples reached the end of storage period with higher sensory scores (8, trained)	(Castellano et al. 2010)
Lb. curvatus and *Lb. sakei*	Beef	pH: slightly lower Color: no negative effect; maintained redness until end of storage	—	(Zhang et al. 2018)
Lb. curvatus CECT 904^T and *Lb. sakei* CECT 4808	Beef	pH: slightly lower Color: no significant effect TBARS: Decreased (effect of *Lb. curvatus* did not last until end of storage)	Higher odor ratings (3, trained)	(Katikou et al. 2005)

Lb. curvatus 2 strain mixture and *Lb. sakei* 6 strain mixture	Beef	pH: no significant effect Color: no significant effect	—	(Stella et al. 2016)
Lb. CTC 711 and *Lb. sakei* CTC 372	Beef	pH: no significant effect Color: no significant effect TBARS: low levels for all samples	Protective cultures did not affect meat odor (6, trained)	(Djenane et al. 2005)
Lb. plantarum TN8	Beef mince	pH: slightly lower Color: no significant effect TBARS: decreased	—	(Trabelsi et al. 2019)
Lb. sakei, *Lb. sakei* subsp. *carnosus*, *Lc. lactis* ssp. *lactis*, and *S. xylosus* mixtures	Beef patties	pH: slightly lower Color: no significant effect	Higher ratings for odor, taste, and flavor parameters of cooked samples; higher concentrations of protective cultures prevented slime formation (12, non-professional)	(Comi et al. 2015)
Lc. piscium strains TMW2.1612/ 2.1614/2.1615	Beef	pH: slightly lower	Treated samples were overall acceptable; protective cultures did not cause discoloration or off-odor (unspecified, untrained)	(Hilgarth, Nani, and Vogel 2018)
P. pentosaceus LIV01 and *P. acidilactici* FLE01	Beef slices	pH: lower TBARS: decreased	—	(Olaoye and Onilude 2010)

Lb. = *Lactobacillus*; *Lc.* = *Lactococcus*; *P.* = *Pediococcus*; *S.* = *Staphylococcus*.
[a]Include pH, color (instrumental), and TBARS (thiobarbituric acid reactive substances) where reported.
[b]"—" indicates that the result was not reported.

that has been gained in the beef studies would be helpful because of the similarities of these products.

Many researchers have produced antimicrobial compounds in their own laboratories using isolated microorganisms with the aim of identifying and developing potential new biopreservatives. While it is useful to continue searching for new biopreservatives, the regulatory process and toxicological testing required for a new biopreservative to be approved in the market are costly and time-consuming. Further, human sensory trials of certain attributes such as the taste of the treated meat usually cannot be performed prior to regulatory approval, which means the consumer acceptability of these biopreservatives remains unclear. For those microbial biopreservatives that have gained GRAS status or otherwise been approved for commercial use, research focused on identifying better ways to utilize them, especially as part of hurdle technology, will help accelerate industrial-scale applications of biopreservatives for the spoilage control of fresh red meat.

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Appendix I

search strategy

Literature searches were performed mainly in Web of Science Core Collection and Scopus. Search terms (as entered in the databases) are shown below. Search fields included article title, abstract, and keywords.

Search terms:

- Bacteriocins
  (meat* OR beef OR lamb) AND (bacteriocin* OR nisin) AND (biopreserv* OR "bio-preserv*" OR preserv* OR "shelf-life" OR shelflife OR spoil*)
- Protective cultures
  (meat* OR beef OR lamb) AND (protective OR bioprotective OR "bio-protective" OR culture*) AND (biopreserv* OR "bio-preserv*" OR preserv* OR "shelf-life" OR shelflife OR spoil*) NOT (ferment*)
- Bacteriophages
  (meat* OR beef OR lamb) AND (bacteriophage* OR phage*) AND (biopreserv* OR "bio-preserv*" OR preserv* OR "shelf-life" OR shelflife OR spoil*)
- Reuterin
  (meat* OR beef OR lamb) AND (reuterin OR reuteri) AND (biopreserv* OR "bio-preserv*" OR preserv* OR "shelf-life" OR shelflife OR spoil*)
- Fermentates
  (meat* OR beef OR lamb) AND (fermentate* OR "cultured dextrose")

Filters:

- Web of Science
- Language: English
- Search areas: Food science technology, microbiology, biotechnology applied microbiology, agriculture
- Scopus
  - Language: English
  - Subject area: Agricultural and biological sciences, immunology and microbiology, biochemistry, genetics and molecular biology, environmental science

The final search of the databases was conducted on November 23, 2020. The reference lists of relevant previous reviews and research articles were also examined for possible relevant studies that could have been missed during the database search. The literature search and selection processes were conducted following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (Moher et al. 2009). The PRISMA flow chart is shown in Figure A1.

The following inclusion and exclusion criteria were applied for study selection:

- Inclusion criteria
  - Experimental studies
  - Studies on fresh beef or lamb meat samples

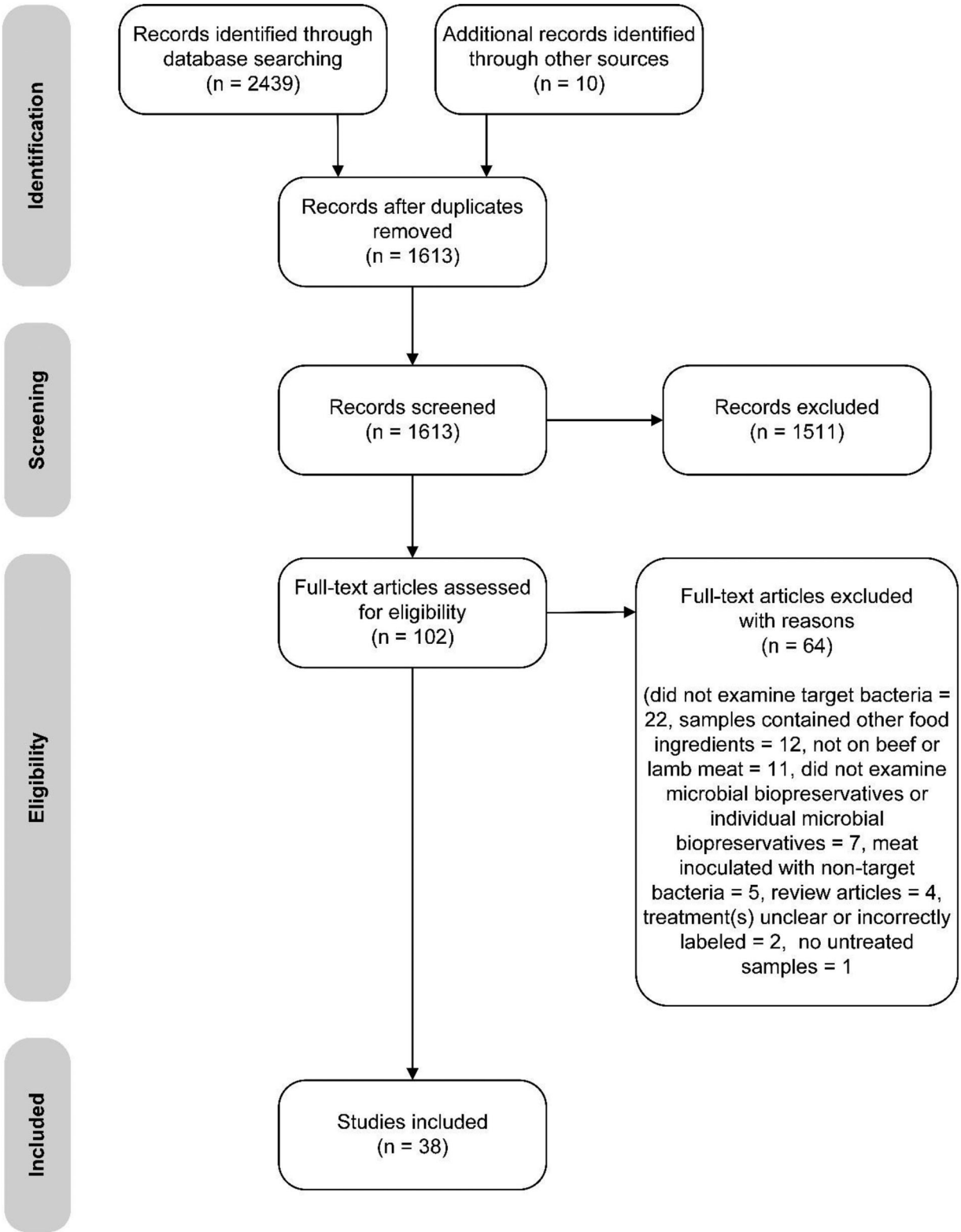

Figure A1. PRISMA flow chart of literature search and selection process.

- Studies included evaluation of any of the microbial biopreservatives (bacteriocins, protective cultures, bacteriophages, reuterin, and fermentates) against any of the following bacteria or bacterial groups: total viable counts, lactic acid bacteria, *Brochothrix thermosphacta*, *Pseudomonas* spp., and *Enterobacteriaceae*
- Written in English

- Exclusion criteria
  - Studies on fermented, cured, or cooked meat products
  - Fresh meat products containing other food ingredients
  - Meat samples inoculated with bacteria other than those stated in the inclusion criteria
  - Lack of untreated or control group
  - Treatment groups did not include microbial biopreservatives individually