Chapter 4
Biopreservation of Meats and Meat Products

4.1 Application of Bacteriocin Preparations

4.1.1 Raw Meats

The microbial populations most frequently associated with the meat environment are known to primarily belong to the groups Enterobacteriaceae, lactic acid bacteria (LAB), *Brochothrix thermosphacta*, and pseudomonads (Borch et al. 1996; Labadie 1999; Nychas et al. 2008). Microbial metabolism of meat during growth results in microbial spoilage, with the development of offodors which make the product undesirable for human consumption (Jackson et al. 1997). Also, pathogenic bacteria initially present at low concentrations may grow during meat spoilage may proliferate during refrigeration storage, especially *Listeria monocytogenes*.

In raw meats, bacteriocins have been tested alone or in combination with other hurdles for carcass decontamination and/or to inhibit bacterial growth on stored fresh meats (Table 4.1). Washing, spraying or dipping with bacteriocin solutions have been tested alone or in combination with other antimicrobials to potentiate bacteriocin activity. In order to increase the efficacy of treatments and/or avoid cross contamination, raw meats are chilled, packaged under different atmospheric conditions such as vacuum packaging, MAP, or active packaging with O_2 scavengers or CO_2 generating systems (Coma 2008; McMillin 2008). Additional combinations such as low dose irradiation, UV surface decontamination or HHP have been proposed (Aymerich et al. 2008). All these processing treatments have selective effects the initial microbiota, and may act in synergy with bacteriocins to increase the product safety and shelf life. Although raw meat products are further processed prior to consumption by treatments that usually destroy pathogenic bacteria, they can be a considerable source of cross contamination. Growth of toxin-producing bacteria in raw materials (such as minced meats) should also be controlled, especially for heat-stable toxins.

A. Gálvez et al., *Food Biopreservation*, SpringerBriefs in Food, Health, and Nutrition, DOI 10.1007/978-1-4939-2029-7_4

Table 4.1 Examples of applications of bacteriocin preparations in meat and poultry products

Bacteriocin preparations	Effect(s)	Reference(s)
Raw meats		
Nisin combinations (organic acids, chelators, lysozyme, vacuum packaging, MAP)	Decontamination of raw meat surfaces before processing	Thomas et al. 2000
Nisin activated film with EDTA	Inhibition of LAB, carnobacteria and *B. thermosphacta* and reduction of *Enterobacteriaceae* load on beef cuts	Ercolini et al. 2010
Pediocins	Anti-listeria protection by pediocins in raw meats	Rodríguez et al. 2002
Pentocin 31-1	Reduction of growth of *Listeria* and *Pseudomonas* and total volatile basic nitrogen production in chill-stored tray-packaged pork meat	Zhang et al. 2010
RTE meats		
Nisin activated films	Increased inactivation of *L. monocytogenes* in several vacuum-packaged products	Aymerich et al. 2008
Nisin in combination with HHP	Increased inactivation of *E. coli* and staphylococci in cooked ham, avoiding regrowth of *E. coli* and slime-forming bacteria	Garriga et al. 2002
Nisin and pulsed light	Application of a Nisaplin dip followed by exposure to pulsed light reduced the population of *L. innocua* on sausages	Uesugi and Moraru 2009
Nisin-pectin film, in combination with low-dose irradiation	Increased microbial inactivation of *L. monocytogenes* on RTE turkey meat and inhibition of survivor proliferation during storage	Jin et al. 2009
Pediocin in combination with post-packaging irradiation or thermal treatment	Effective combination with to control *L. monocytogenes* on frankfurters	Chen et al. 2004a, b
Enterocin alginate film, in combination with HHP	Prevention of *L. monocytogenes* regrowth in the treated cooked ham during cold storage as well as during cold chain break	Marcos et al. 2008a, b

At present, there is a great body of research data concerning bacteriocin trials on raw meats, many of them dealing with nisin. Nisin has been widely tested for preservation of raw meats (Thomas et al. 2000). However, the application of nisin in meats has several drawbacks such as its poor solubility, interaction with phospholipids and antagonism by glutathione (Thomas et al. 2000; Stergiou et al. 2006). Nevertheless, positive results have been reported for surface decontamination of raw meats before processing and packaging, in which antimicrobial activity was potentiated by combination with other antimicrobials or hurdles such as organic acids, chelators, vacuum packaging, or MAP. Representative examples reported on vacuum-packaged beef are the reduction in the numbers of *Listeria innocua* and *B. thermosphacta* after nisin treatment (Cutter and Siragusa 1996a) or the inhibition of *L. monocytogenes* and *Escherichia coli* O157:H7 after treatment with nisin and

EDTA (Zhang and Mustapha 1999). Similarly, dipping in solutions containing combinations of lactic or polylactic acids and nisin reduced the microbial load of meats before processing and afforded an extended shelf-life in vacuum-packaged fresh meat (Ariyapitipun et al. 1999, 2000; Barboza de Martinez et al. 2002), and treatment with a combination of nisin and lysozyme effectively inhibited *B. thermosphacta* and LAB in vacuum-packaged pork (Nattress et al. 2001; Nattress and Baker 2003). Other reports indicated that, under MAP, nisin was able to completely inhibit growth of *L. monocytogenes* in pork (Fang and Lin 1994a, b).

In raw poultry meats, application of antimicrobial treatments using nisin and EDTA to in combination with MAP or vacuum packaging (VP) reduced total aerobic plate counts and increased the product shelf-life by a minimum of 4 days when packaged under aerobic conditions and a maximum of 9 days when vacuum packaged (Cosby et al. 1999). The use of MAP (65 % CO_2, 30 % N_2, 5 % O_2) in combination with nisin–EDTA antimicrobial treatments affected the populations of mesophilic bacteria, *Pseudomonas* sp., *B. thermosphacta*, lactic acid bacteria and *Enterobacteriaceae*, and resulted in an organoleptic extension of refrigerated, fresh chicken meat for up to 14 days, decreasing the formation of volatile amines, trimethylamine nitrogen and total volatile nitrogen (Economou et al. 2009).

Treatment of raw meats and poultry meats with pediocins (especially pediocin PA-1/Ach) singly or in combination with other hurdles can inhibit or delay growth of spoilage Gram-positive bacteria (such as *B. thermosphacta*) and/or reduce *L. monocytogenes* populations (Rodríguez et al. 2002; Nieto-Lozano et al. 2006; Kalchayanand 1990; Nielsen et al. 1990; Motlagh et al. 1992; Degnan et al. 1993; Schlyter et al. 1993: Taalat et al. 1993; Goff et al. 1996; Murray and Richard 1997). For example, treatment of raw meat surfaces with 500, 1,000 or 5,000 bacteriocin units/ml (BU/ml) reduced the counts of inoculated *L. monocytogenes* after storage at 15 °C during 72 h by 1, 2 or 3 log cycles, and treatment with 1,000 or 5,000 BU/ml reduced its viable counts by 2.5 or 3.5 log cycles, respectively, after storage at 4 °C during 21 days compared to the control not treated with bacteriocin. The same bacteriocin treatments exerted a bacteriostatic effect on *Clostridium perfringens* (Nieto-Lozano et al. 2006). In poultry meats, treatment with pediocin PA-1/Ach adsorbed to heat killed *Pediococcus acidilactici* cells was very effective in the control of *L. monocytogenes* in refrigerated chicken meat (Goff et al. 1996).

Other bacteriocins such as sakacins, carnobacteriocins, bifidocins, lactocins, lactococcins, enterocins or pentocins have shown variable inhibitory effects against spoilage or pathogenic bacteria in raw meats or poultry meats (Aymerich et al. 2000, 2008; Galvez et al. 2008). In chicken breasts, addition of enterocins A and B produced by the meat isolate *Enterococcus faecium* CTC492 (4,800 AU/cm^2) reduce the population of *Listeria* to 3.6 MNP/cm^2 during incubation at 7 °C (Aymerich et al. 2000). In vacuum-packed chicken cuts stored under refrigeration, treatment with sakacin-P caused strong inhibition of *L. monocytogenes* (Katla et al. 2002). Addition of bifidocin B (from *Bifidobacterium bifidum*) and lactococcin R (produced by *Lactococcus lactis* subsp. *cremoris*) to irradiated raw chicken breast inhibited the growth of *L. monocytogenes* or *Bacillus cereus* for 3–4 weeks at 5–8 °C or 6–12 h at 22–25 °C (Yildirim et al. 2007). Another study reported that application

of pentocin 31-1 (produced by a *Lactobacillus pentosus* strain isolated from the traditional Chinese fermented Xuan-Wei Ham) in chill-stored non-vacuum tray-packaged pork meat substantially reduced the growth of *Listeria* and *Pseudomonas* as well as the total volatile basic nitrogen (measured as an indicator of meat spoilage) during cold storage compared with the untreated control (Zhang et al. 2010).

One attractive approach to optimize the activity of bacteriocins in raw meats has been immobilisation in substrates (such as beads, liposomes, coatings or films). Nisin (alone or in combinations with citric acid, EDTA, and Tween 80) incorporated in a variety of substrates (such as calcium alginate gels, agar coatings, palmitoylated alginate-based films, polyvinyl chloride, LDPE, or nylon) showed strong inhibition of bacteria such as *L. monocytogenes*, *B. thermosphacta*, *Staphylococcus aureus*, or *Salmonella* Typhimurium on refrigerated raw meats (Chen and Hoover 2003; Aymerich et al. 2008; Gálvez et al. 2007, 2008). This approach decreases the impact of interaction with food components and enzyme inactivation of bacteriocin activity, and also decreases the amount of bacteriocin required for inhibition of target bacteria (Quintavalla and Vicini 2002). In raw meats and poultry samples packaged in bags coated with pediocin powder, the pediocin completely inhibited growth of inoculated *L. monocytogenes* through 12 weeks storage at 4 °C (Ming et al. 1997). Application of nisin immobilized in calcium alginate gel on beef carcass tissues completely suppressed *B. thermosphacta* (Cutter and Siragusa 1996b), and low density polyethylene films containing nisin prevented carcass contamination by this bacterium (Siragusa et al. 1999). Nisin bound to activated alginate beads or in a palmitoylated alginate-based film (to avoid nisin degradation) reduced the viable counts of *S. aureus* in ground beef and on sliced beef meat, respectively (Millette et al. 2007). Treatment of fresh poultry with agar coatings containing nisin achieved substantial reductions in *S.* Typhimurium growth after storage at 4 °C for 96 h (Natrajan and Sheldon 1995). The efficiency of numerous films formulation based on polyvinyl chloride, LDPE, nylon, calcium-alginate or agar containing nisin (in combinations with citric acid, EDTA, and Tween 80) to inhibit the antibiotic resistant *S.* Typhimurium on poultry drumstick skin was demonstrated by the same researchers (Natrajan and Sheldon 2000a, b). Combinations of nisin with citric acid, EDTA and Tween-80 also led to a 4–5 log reduction of psychotrophic aerobes during 72 h of storage. In another study, Ercolini et al. (2010) tested a nisin activated plastic antimicrobial packaging (developed by using a nisin, HCl and EDTA solution) on beef cuts stored at 1 °C. The combination of chill temperature and antimicrobial packaging proved to be effective in enhancing the microbiological quality of beef cuts by inhibiting LAB, carnobacteria and *B. thermosphacta* in the early stages of storage and by reducing the loads of *Enterobacteriaceae*, without affecting the species diversity according to PCR-DGGE fingerprints of DNA extracted from the treated meat cuts (Ercolini et al. 2010). Also, plastic bags activated at their internal face with a nisin-EDTA solution were used for vacuum-packaging of beef chops (Ferrocino et al. 2013). During storage in the activated films at 1 °C, *B. thermosphacta* was unable to grow for the whole storage time (46 days), while the levels of *Carnobacterium* spp. were below the detection limit for the first 9 days and reached levels below 5 log CFU/cm^2 after 46 days. The antimicrobial packaging had no

effect on *Enterobacteriaceae* or *Pseudomonas* spp., with final populations of about 4 log CFU/cm^2. Nevertheless, the active packaging reduced the release of volatile metabolites in the headspace of beef with a probable positive impact on meat quality. Recycling of industrial wastes into useful products is a growing trend not only in the food industry but in many other fields as well. In a recent and innovative study, a novel poly(lactic acid)/sawdust particle biocomposite film with anti-listeria activity was developed by incorporation of pediocin PA-1/AcH (Woraprayote et al. 2013). It was reported that sawdust particle played an important role in embedding pediocin into the hydrophobic PLA film. Application of the activated film as a food-contact antimicrobial packaging on raw sliced pork efficiently inhibited *L. monocytogenes* during chill storage.

The bacteriocin 32Y (from *Lactobacillus curvatus* 32Y) was used to develop an industrially produced activated plastic film (Mauriello et al. 2004). In experiments of food packaging with pork steak and ground beef (simulating hamburgers) contaminated by *L. monocytogenes* V7, highest antimicrobial activity was observed after 24 h at 4 °C, with a decrease of about 1 log of the *L. monocytogenes* population (Mauriello et al. 2004). The lactocins 705 and AL705 are produced by *L. curvatus* CRL705. Lactocin 705 has antagonist effect against LAB and *B. thermosphacta*, while AL705 is active against *Listeria* species (Castellano and Vignolo 2006). Both bacteriocins retained antimicrobial activity when included in polymer matrices such as LDPE (Blanco et al. 2008, 2012) and gluten (Blanco Massani et al. 2014). In trials with *L. curvatus* CRL705 immobilized bacteriocins, a bacteriostatic effect against *L. innocua* 7 was observed in both synthetic (Cryovac films) and gluten activated packages until the fourth week of storage (Blanco Massani et al. 2014).

The process operations for manufacture of minced meats facilitate inoculation of contaminating bacteria in the meat batter. Therefore, the presence and multiplication of foodborne pathogens in minced meats should be controlled. One study showed that the single addition of nisin extended the lag phase of *L. monocytogenes* inoculated into minced buffalo meat (Pawar et al. 2000). In minced meats, the combination of bacteriocins with plant essential oils at levels where they would not impart undesirable flavour is being considered as a way to increase inactivation of *L. monocytogenes* and inhibition of *Salmonella* Enteritidis (Solomakos et al. 2008; Govaris et al. 2010). Antilisterial activity of nisin in minced beef increased greatly in combination with thyme essential oil. The combination of essential oil at 0.6 % with nisin at 1,000 IU/g decreased the population of *L. monocytogenes* below the official limit set by European Union during storage at 4 °C for at least 12 days (Solomakos et al. 2008). At that concentration, the thyme oil did not impart undesirable flavour. Promising results have also been reported on inhibition of *S*. Enteritidis in sheep minced meat by a combination of nisin and oregano essential oil (Govaris et al. 2010), while the single treatment of minced sheep meat with nisin at 500 or 1,000 IU/g had no activity against *S*. Enteritidis. The combination of the oregano essential oil at 0.6 % with nisin at 500 IU/g showed stronger antimicrobial activity against *S*. Enteritidis than the single oregano essential oil at 0.6 % but lower than the combination with nisin at 1,000 IU/g (Govaris et al. 2010). Best results were reported for the combinations of oregano essential oil at 0.9 % with nisin at 500 or

1,000 IU/g, which showed a bactericidal effect against the pathogen. The inhibitory effects were higher in samples stored at 10 °C compared to 4 °C. This could be a draw-back for cold-stored meats, but at the same time could be an advantage under episodes of cold chain break and temperature abuse. Regarding the effects of other bacteriocins in minced meats, addition of a partially-purified plantaricin preparation from *Lactobacillus plantarum* UG1 rapidly reduced the population of *L. monocytogenes* below detectable levels in minced meat stored at 8 °C, (Enan et al. 2002), and the addition of a freeze-dried whey fermentate from *C. piscicola* (containing piscicocin CS526) to a ground mixture of beef and pork meat reduced the population of *L. monocytogenes* below detectable levels for at least 4 days at 12 °C and for up to 25 days at 4 °C (Azuma et al. 2007). Furthermore, in minced pork treated with a preparation of enterocins A and B (1,600 AU/g) from *E. faecium* CTC492, the levels of listeria were reduced below 3 MNP/g after 6 days of incubation at 7 °C while the untreated control increased from 5 MNP/g to 48 CFU/g (Aymerich et al. 2000).

4.1.2 Semi-processed and Cooked Meats

Cooked meat products are widely consumed ready-to-eat (RTE) foods. They may consist of whole primary meat pieces, but usually they are made by grinding and mixing secondary meats, fat, animal organs, or blood with other ingredients, followed by stuffing/molding and cooking. The cooking process inactivates natural microbiota, paving the way for growth of post-process contaminants. The pH values of most cooked meat products are compatible with growth of pathogenic and spoilage bacteria, which can proliferate at refrigeration temperatures during the product shelf life. Some of these meats may also undergo further processing such as slicing, peeling, and packaging, which increase the risks for cross-contamination (Murphy et al. 2005). For these reasons, there has been a great interest in the application of bacteriocins (mainly pediocin and nisin) as hurdles against spoilage bacteria and pathogens (mainly *L. monocytogenes*). The main approaches tested are based on addition of bacteriocin preparations to the meat slurries before the heating process, surface application of the bacteriocins before packaging, or application of films or coatings dosed with bacteriocins. The possibility of adding bacteriocins in the meat before the cooking process due to their thermotolerance is of great interest.

Strains of LAB (mainly *Lactobacillus* and *Leuconostoc*) are the major group of spoilage bacteria developing on various types of vacuum-packed meats, where they produce typical sensory changes such as souring, gas, SH_2 and slime (Korkeala et al. 1988; Björkroth and Korkeala 1997). In one study using sakacin K, nisin and enterocins, the results obtained clearly depended on the bacteriocin and the target bacteria (Aymerich et al. 2002). Sakacin K and nisin were unable to prevent ropiness caused by *Lactobacillus sakei* CTC746 strain, but nisin was able to prevented ropiness caused by *Leuconostoc carnosum* CTC747 (Aymerich et al. 2002). Nisin was also the most effective bacteriocin on staphylococci, but did not prevent regrowth of *L. monocytogenes* (while enterocins, sakacin and pediocin did).

Ovotransferrin is the main component in the antimicrobial defense system of hens' egg. Antimicrobial activity of ovotransferrin is mainly due to its iron-binding capacity, but direct interactions with the bacterial surface also seem to play an important role in contributing to its inhibitory activity (Moon et al. 2011). Ovotransferrin, nisin, and their combinations had strong antilisterial activity in BHI broths. However, addition of ovotransferrin to frankfurters did not inhibit growth of *L. monocytogenes*. When nisin (1,000 IU/frankfurter) was applied, an early bactericidal effect followed by delayed growth was observed (Moon et al. 2011). However, no differences were reported in the antilisterial effect when the same nisin concentration was applied in combination with ovotransferrin (40 mg/frankfurter). The observed differences could be explained by the influence of factors such as interaction with food substrate or a higher iron content in meat.

Incorporation of nisin into bologna-type sausages during mixing of ingredients inhibited the growth of spoilage LAB during further storage at 8 °C of the resulting vacuum-packed sausages (Davies and Delves-Broughton 1999). The effectiveness of nisin against several bacteria (such as *B. thermosphacta*, *L. curvatus*, *Ln. mesenteroides*, *L. monocytogenes*, *Salmonella* sp. and *E. coli* O157:H7) in ham and/or bologna sausages increased in combination with lysozyme and EDTA (Gill and Holley 2000a, b). In fresh pork sausages, a combination of nisin and organic acids reduced the viable counts of *Salmonella* Kentucky and *S. aureus* (Scannell et al. 1997). The combination of sodium citrate or sodium lactate with nisin or lacticin 3147 was also reported to increase the inhibition of *Listeria* and *C. perfringens* in fresh pork sausages (Scannell et al. 2000a).

Pediocin activity was increased when added in combinations with sodium diacetate or sodium lactate against *L. monocytogenes* on frankfurters or *L. monocytogenes* and *Yersinia enterocolitica* on cooked poultry cuts stored under MAP at 3.5 °C (O'Sullivan et al. 2002; Chen and Hoover 2003; Aymerich et al. 2008). The antilisterial activity of pediocin in slurries prepared from ready-to-eat turkey breast meat increased greatly when tested in combination with diacetate, due to synergistic effects between the two antimicrobials (Schlyter et al. 1993). When commercial beef franks were dipped for 5 min in three antimicrobial solutions: pediocin (6,000 AU), 3 % sodium diacetate and 6 % sodium lactate combined, and a combination of the three antimicrobials, reductions of *L. monocytogenes* populations ranged between 1 and 1.5 log units and 1.5–2.5 log units after 2 and 3 weeks of storage, respectively, at 4 °C (Uhart et al. 2004). These results indicated that the use of combined antimicrobial solutions for dipping treatments is more effective at inhibiting *L. monocytogenes* than treatments using antimicrobials such as pediocin separately (Uhart et al. 2004). In another study, the effects and interactions of temperature (56.3–60 °C), added sodium lactate (0–4.8 %) and sodium diacetate (0–0.25 %) and dipping in pediocin (0–10,000 AU) on *L. monocytogenes* in bologna were studied by Maks et al. (2010). Combination treatments increased or decreased *D*-values, depending on the temperature. Pediocin (2,500 and 5,000 AU) and heat decreased *D*-values, but pediocin exhibited a protective effect at higher concentrations (≥7,500 AU). The results showed that interactions between additives in formulations can vary at different temperatures/concentrations, thereby affecting thermal inactivation of foodborne pathogens in meat products.

Enterocins have also been tested in cooked meat products. Addition of a partially-purified preparation of enterocins A and B (4,800 AU/g) reduced the numbers of *L. innocua* by 7.98 log cycles in cooked ham and by 9 log cycles in pork liver paté stored at 7 °C for 37 days (Aymerich et al. 2000). In vacuum packaged sliced cooked pork ham, added enterocins A and B (128 AU/g) inhibited the production of slime by *Lactobacillus sakei* CTC746 strain, but not by *Leuconostoc carnosum* CTC747 strain (Aymerich et al. 2002).

Results from studies on the synergistic activities of bacteriocins with other antimicrobials and on the effect of immobilized preparations or application of bacteriocins by dipping solutions, together with the technical advances in the development of activated supports opened the doors for application of immobilized bactericin preparations or activated packagings containing cocktails of antimicrobial substances on RTE meats (Coma 2008). Bacteriocin-activated films may be quite useful for cooked meat products, not only because they can prolong the product shelf life by decreasing the risks of spoilage and growth of pathogens from cross-contamination during processing, but also because the film itself acts as a barrier against external contamination of the processed product. Among the various kinds of edible coatings tested on vacuum-packaged products (hot dogs, frankfurters, or ham) best results have been reported for coatings containing nisin in combination with other antimicrobials under refrigeration storage.

Application of zein coatings containing nisin, sodium lactate, and sodium diacetate completely eliminated *L. monocytogenes* on turkey frankfurters during refrigeration storage (Lungu and Johnson 2005). In hot dogs that were vacuum-packaged in films coated with nisin, *L. monocytogenes* counts decreased during refrigeration storage (Franklin et al. 2004). Hot dogs were placed in control and nisin-containing pouches and inoculated with a five-strain *L. monocytogenes* cocktail (approximately 5 log CFU per package), vacuum sealed, and stored for intervals of 2 h and 7, 15, 21, 28, and 60 days at 4 °C. In hot dogs packaged in films coated with 2,500 IU/ml nisin solution, nisin significantly decreased ($P<0.05$) *L. monocytogenes* populations on the surface of hot dogs by greater than 2 log CFU per package throughout the 60-days study. However, *L. monocytogenes* populations still remained at approximately 4 log CFU per package after 60 days of refrigerated storage (Franklin et al. 2004). This study reported similar results when using a cellulose-based coating solution (based on methylcellulose/hydroxypropyl methylcellulose) containing nisin. However, in another study nisin-coated cellulose casings showed only moderate antilisterial activity in vacuum-sealed frankfurters, unless additional antimicrobials, such as potassium lactate and sodium diacetate, were employed (Luchansky and Call 2004). Nguyen et al. (2008) carried out similar experiments using an edible bacterial cellulose film containing nisin to control *L. monocytogenes* and total aerobic bacteria on the surface of vacuum-packaged frankfurters. The frankfurters packaged in films activated with 2,500 IU/ml showed significantly lower counts of *L. monocytogenes* and total aerobic plate counts during refrigerated storage for 14 days as compared to the controls. The authors concluded that activated cellulose films had potential applicability as antimicrobial packaging films or inserts for processed meat products. Another study reported that polythene films activated with

bacteriocin 32Y from *L. curvatus* were effective in reducing the population of listeria in vacuum-packaged frankfurters during storage at 4 °C (Ercolini et al. 2006). By using viable staining and fluorescence microscopy, the authors corroborated that the activated film caused an immediate reduction of live and appearance of dead cells just after 15 min from the packaging.

Another suggested application of nisin is the preservation of natural sausage casings. Casings derived from animal intestines can be one possible route for transmission of *C. perfringens* spores and other sulphite-reducing anaerobic spores, since the brining process of intestines does not inactivate bacterial endospores. In one study, it was shown that nisin was partly reversibly bound to casings and can reduce the outgrowth of *Clostridium sporogenes* spores in the model used by approximately 1 log cycle (Wijnker et al. 2011). This could open new possibilities to combat the entry of pathogens in the food chain.

In vacuum-packaged cooked ham, application of a gelatine coating gel containing a combination of lysozyme, nisin and EDTA in showed bactericidal activity for *B. thermosphacta*, *L. sakei*, *L. mesenteroides*, *L. monocytogenes* and *S. enterica* serovar Typhimurium (Gill and Holley 2000b). In sliced cooked ham packaged under MAP and stored at 4 °C, the inclusion of polyethylene/polyamide inserts coated with nisin (approximately 2,560 AU cm^2) reduced the levels of LAB, *Listeria innocua* and *Staphylococcus aureus*, and partially inhibited growth of total aerobic bacteria on the ham during storage (Scannell et al. 2000b). However, in ham steaks packaged in chitosan-coated plastic films containing 500 IU/cm^2 of nisin, the low bacteriocin concentration tested was ineffective in inhibiting *L. monocytogenes* (Ye et al. 2008).

Pediocin immobilization has also shown variable results. Encapsulation of pediocin AcH in liposomes enhanced its antimicrobial activity in meat slurries (Degnan and Luchansky 1992). However, in another study, when pediocin adsorbed to its heat-killed producer cells was used to treat sliced frankfurters before packaging, the number of *L. monocytogenes* decreased during 6 days of storage, but remained at constant levels for the remaining storage period (up to 21 days), indicating that the pediocin preparation was not efficient enough to kill all *L. monocytogenes* (Mattila et al. 2003). The efficacy of cellulose films containing pediocin PA-1/Ach (ALTA® 2351) against *L. innocua* and *Salmonella* sp. was tested on sliced ham packaged under vacuum and stored at 12 °C simulating abusive temperatures that can occur in supermarkets (Santiago-Silva et al. 2009). The antimicrobial films were more effective inhibiting growth of *L. innocua* (with a growth reduction of 2 log cycles compared to control treatment after 15 days of storage) than *Salmonella* (0.5 log cycle reduction in relation to control, after 12 days). However, the viable cell concentrations of the inoculated bacteria were not reduced for any of the treatments.

Films activated with enterocin 496K1 (from *Enterococcus casseliflavus* IM 416K1) and enterocins A and B have been tested in ready to eat meat products (Iseppi et al. 2008; Marcos et al. 2007). Enterocin 416K1 activated films reduced the levels of *L. monocytogenes* in contaminated frankfurters by ca. 1.5 to 0.5 log cycles within 24 h of storage at temperatures of 4 and 22 °C, but did not avoid expo-

nential growth of the pathogen during further storage of samples (Iseppi et al. 2008). Marcos et al. (2007) tested the antilisterial effects of enterocins A and B immobilized in different supports (alignate, zein and polyvinyl alcohol) on air-packed and vacuum-packed sliced cooked ham stored at 6 °C. The most effective treatment for controlling *L. monocytogenes* during storage was vacuum-packaging of ham with alginate films containing 2,000 AU/cm^2 of enterocins, with no increase from inoculated levels of *L. monocytogenes* until day 15.

Pre-surface application of bacteriocins in combination with post-packaging treatments is another approach of recent interest. Bacteriocin application followed by in-package thermal treatments can provide an effective combination to control *L. monocytogenes* on products such as frankfurters or turkey bologna, as shown for pediocin, nisin, nisin-lysozyme, or combinations of these bacteriocins with sodium lactate/sodium diacetate (Chen et al. 2004a; Mangalassary et al. 2008). Mangalassary et al. (2008) studied the efficacy of in-package pasteurization (65 °C for 32 s.) combined with pre-surface application of nisin and/or lysozyme to reduce and prevent the subsequent recovery and growth of *L. monocytogenes* during refrigerated storage on the surface of low-fat turkey bologna. In-package pasteurization in combination with nisin or nisin–lysozyme treatments was effective in reducing the population below detectable levels by 2–3 weeks of storage. In bologna manufactured with different sodium lactate/sodium diacetate combinations, dipping in pediocin solution followed by heat treatment decreased the *D*-values for inactivation of *L. monocytogenes* at low pediocin concentration, but exhibited a protective effect at higher concentrations, indicating that interactions between additives in formulations can vary at different temperatures/concentrations (Maks et al. 2010). In a previous study, treatments of frankfurters with 3,000 AU or 6,000 AU pediocin (in ALTA 2341) followed by heating in hot water reduced the populations of inoculated *Listeria* in proportion to the intensity of treatments (Chen et al. 2004a). The combination of pediocin (6,000 AU) with post-packaging thermal treatment (81 °C or more for at least 60 s), achieved a 50 % reduction of initial inoculation levels. Little or no growth of *L. monocytogenes* was observed on the treated frankfurters for 12 weeks at 4 or 10 °C, and for 12 days at 25 °C. This treatment did not affect the sensory qualities of frankfurters. The authors of this study concluded that pediocin (in ALTA 2341) in combination with postpackaging thermal treatment offers an effective treatment combination for improved control of *L. monocytogenes* on frankfurters.

Another example of a combined treatment is the application of nisin with pulsed light. Application of a Nisaplin dip followed by exposure to pulsed light (PL; 9.4 J/cm^2) reduced the population of *L. innocua* on sausages by 4 log cycles and inhibited its growth during refrigeration storage for 24–48 days (Uesugi and Moraru 2009). Since application of PL is approved for decontamination of food and food surfaces, the combined treatment could be applied as a post-processing step to reduce surface contamination and increase the safety of RTE meat products.

Bacteriocins have been proposed for use in packaged foods to increase the efficacy of irradiation treatments. One study reported that irradiation acted synergistically with pediocin on *L. monocytogenes* inoculated in packaged frankfurters (Chen et al. 2004b). Combination of pediocin with postpackaging irradiation at

1.2 kGy or more was necessary to achieve a 50 % reduction of *L. monocytogenes* on frankfurters. The combination of 6,000 AU of pediocin and irradiation at 2.3 kGy or more was the most effective treatment for inhibition of the pathogen for 12 weeks at 4 or 10 °C. Best results were reported on samples stored at 4 °C, with little or no growth of the pathogen during 12 weeks of storage and no adverse effects on the sensory quality of frankfurters. Similarly, bacteriocin-activated films have been tested as a way to increase the radiation sensitivity of the target pathogens, aimed at reducing radiation doses and impact on product quality. In ready-to-eat turkey meat vacuum-packaged with a pectin-nisin film and treated by low dose irradiation (2 kGy), the reduction obtained for the *L. monocytogenes* population (5.36 log CFU/cm^2) were greater compared to irradiation and pectin film single treatments. In addition, pectin-nisin films did significantly slow the proliferation of *L. monocytogenes* cells that survived irradiation during 8 weeks of storage at 10 °C (Jin et al. 2009). The authors concluded that the combined treatment could serve to prevent listeriosis due to postprocessing contamination while reducing radiation doses and impact on product quality, or to prevent *L. monocytogenes* growth in accidentally recontaminated packages of irradiated RTE meats.

High hydrostatic pressure processing (HHP) is now being used more frequently as a food processing technology that is applied on packaged foods. Several reports indicate that bacteriocins can enhance the antibacterial effects of HHP treatments. In one study, the efficacy of enterocins added to cooked ham increased in combination with a HHP treatment at 400 MPa for 10 min (Garriga et al. 2002). The combined treatment avoided overgrowth of *L. sakei* CTC746 strain during storage, improving the results compared to HHP treatment alone (Garriga et al. 2002). *L. monocytogenes* was also kept at levels <10 CFU/g for 61 days at 4 °C (Garriga et al. 2002). However, the bacteriocins had no effect on regrowth of other survivors (*Ln. carnosum* CTC747, *Staphylococcus carnosus* and *S. aureus* strains, *E. coli* or *S. enterica* strains). A combined treatment of enterocins (2,400 AU/g) and HHP (400 MPa, 10 min) avoided overgrowth of surviving listeria upon a simulated cold-chain break event when the samples were stored at 1 °C, but not at 6 °C (Marcos et al. 2008a), indicating the influence of storage temperature on the delicate balance between inhibited proliferation of survivors and repair of sublethal damage and cell growth.

Protective coatings in the form of activated films have also been tested to increase the efficacy of HHP in ready-to-eat meat products (Aymerich et al. 2008). The efficacy immobilized enterocins in combination with HHP to control *L. monocytogenes* growth during the shelf life of artificially inoculated cooked ham was investigated (Jofré et al. 2007). The antilisterial activity of enterocins immobilised in plastic interleaves was strongly potentiated by application of HHP treatment (400 MPa, 10 min), reducing viable counts by about 4 log units and holding the levels of *L. monocytogenes* in the treated sliced ham below 1.5 log CFU/g at the end of storage for 30 days at 6 °C (Jofré et al. 2007). Storage of samples at a lower temperature of 1 °C extended the protective effect of the combined treatment for at least 60 °C, even in the event of a simulated cold chain break (Marcos et al. 2008b). In a separate study (Marcos et al. 2008a), sliced cooked ham was packaged in alginate films containing or not enterocins A and B, and then was pressurized (400 MPa, 10 min, 17 °C).

While the single antimicrobial packaging treatment was able to inhibit growth of *L. monocytogenes* during the first 8 days of storage at 6 °C, and the single HHP pretreatment attained a ca. 3.4 logs reduction of viable counts for about the same period followed by regrowth of the listeria in both cases, the combined treatment extended the lag phase of listeria to 22 days, and the slight growth observed afterwards did not exceed 1.8 log CFU/g by the end of storage (day 60). For samples stored at 1 °C, the combined treatment of HHP and enterocin film caused a faster decline of *L. monocytogenes* counts compared to HHP alone, but no regrowth was observed in either case for 60 days, suggesting that at the lower temperature of storage, antimicrobial packaging did not give additional protection against *L. monocytogenes* to pressurized samples. However, after a simulated cold-chain break event at day 60, there was a dramatic increase in the *L. monocytogenes* population for single HHP treatments (8.5 log CFU/g), indicating the capacity of pressure-injured *L. monocytogenes* cells to recover under favourable conditions. By contrast, for the combined treatment of HHP and enterocin films, the temperature abuse resulted in a slight increase until 1.7 log CFU/g at 90 days. The authors concluded the combination of antimicrobial packaging with HPP could be useful to control and reduce the numbers of *L. monocytogenes* and to overcome temperature abuse. In a similar study, Jofré et al. (2008) tested the effectiveness of the application of interleavers (composed by polypropylene/polyamide layers) containing enterocins A and B, sakacin K, nisin A, potassium lactate and nisin plus lactate alone or in combination with a 400 MPa HHP treatment in sliced cooked ham spiked with *Salmonella* spp. It was concluded that nisin was the only treatment that produced absence of *Salmonella* 24 h after pressurisation and the application of nisin through interleavers and combined with an HHP treatment appears as the most effective treatment to achieve absence of *Salmonella* in 25 g samples during refrigeration storage of the sliced ham (Jofré et al. 2008).

4.1.3 Fermented Meats

Bacteriocin preparations can be added to meat batters for reduction of the initial levels of bacteriocin-sensitive populations and inactivation of microbial pathogens in fermented meat products. The lower pH attained in sausages compared to fresh meats may increase the solubility of some bacteriocins like nisin, and probably their antimicrobial activity as well. Microbial inactivation by bacteriocin addition may also be an attractive hurdle for slightly fermented sausages, in which the higher pH and water content may facilitate survival and proliferation of certain pathogenic bacteria.

Several bacteriocins such as nisin, enterocins (CCM 4231, A, B and AS-48) or leucocins improved the reduction of *L. monocytogenes* or *S. aureus* populations in fermented meats (Rodríguez et al. 2002; Chen and Hoover 2003; Aymerich et al. 2008; Galvez et al. 2008). Addition of nisin alone was effective in preservation of bologna-type sausages against LAB spoilage (Davies and Delves-Broughton 1999) and in the inhibition of *L. monocytogenes* in sucuk, a Turkish fermented sausage

(Hampikyan and Ugur 2007). The effectiveness of nisin in fermented meats increased in combination with other antimicrobials, such as organic acids (reducing the viable counts of *S.* Kentucky and *S. aureus*; Scannell et al. 1997), lysozyme-EDTA (inhibiting the growth of *B. thermosphacta*, *L. curvatus*, *Ln. mesenteroides*, *L. monocytogenes* and *E. coli* O157:H7; Gill and Holley 2000a) or grape seed extract (Sivarooban et al. 2007). Enterocins can inhibit *Listeria* in fermented meats, as shown for enterocin CCM 4231 in dry fermented Hornád salami (Lauková et al. 1999) or enterocins A and B in espetec (traditional Spanish sausage; Aymerich et al. 2000). Addition of enterocin CCM 4231 (12,800 AU/g) from *E. faecium* CCM 4231 to Hornád salami meat mixture resulted in a reduction of *L. monocytogenes* by 1.67 log cycle immediately after addition of the bacteriocin (Lauková et al. 1999). Although the added bacteriocin did not prevent growth of the listeria during storage of samples in drying rooms at temperatures between 24 and 15 °C, viable counts were significantly lower that the controls. In espetec (a Spanish slightly-fermented sausage), addition of enterocins A and B (648 AU/g) reduced the viable counts of *L. innocua* below 50 CFU/g from the fifth day until the end of the process (12 days) of manufacturing (Aymerich et al. 2000).

In Italian sausages ("cacciatore"), enterocin 416K1 (10 AU/g, in the form of a concentrated culture supernatant) decreased the levels of *L. monocytogenes* in sausages by ca. 2.5 log CFU/g during the drying period (3 days), but failed to suppress the pathogen during ripening (Sabia et al. 2003). Regarding enterocin AS-48, after addition of this bacteriocin at 450 AU/g in a meat sausage model system, it was observed that no viable listeria were detected after 6 and 9 days of incubation at 20 °C (Ananou et al. 2005a), and also that viable counts of *S. aureus* were reduced below detectable levels at the end of storage (Ananou et al. 2005b). Also bacteriocins from leuconostocs have been tested in fermented meats. Addition of semi-purified bacteriocin of *Ln. mesenteroides* E131 improved the reduction of *L. monocytogenes* viable counts in challenge experiments during fermented sausage manufacturing (Drosinos et al. 2006).

4.2 Application of Protective Cultures

4.2.1 Raw Meats

Many LAB naturally associated with meats can grow at refrigeration temperatures. Therefore, bacteriocin-producing strains of these LAB that do not have adverse effects on meats can be selected as protective cultures for raw meat preservation (Table 4.2). Previous works have demonstrated the effectiveness of bacteriocin-producing *L. sakei* and *L. curvatus* strains in inhibiting *L. monocytogenes* or *B. thermosphacta* in raw meat products. When *L. sakei* CWBI-B1365 and *L. curvatus* CWBI-B28 (producers of sakacin G and P, respectively) were tested as protective cultures on raw beef and poultry meat challenged with *L. monocytogenes* and stored at 5 °C in sealed bags, inhibition of the listeria was found to depend greatly on the

Table 4.2 Examples of applications of bacteriocin-producing cultures in meat and poultry products

Starter or protective cultures		
Raw meats		
Bacteriocin producer *L. curvatus* CRL705	Effective inhibition of *L. innocua* and *B. thermosphacta* and indigenous contaminant LAB in fresh beef; contribution to meat ageing by limited proteolysis	Fadda et al. 2008
BLIS-producing *L. sakei*	Delayed blownpack spoilage caused by *C. estertheticum* and reduced survival of *C. jejuni* on meat	Jones et al. 2009
BLIS-producing *L. fermentum* ACA-DC179	Growth inhibition of *S. Enteritidis* in refrigerated chicken ground meat	Maragkoudakis et al. 2009
RTE meats		
Bacteriocin-producing *P. acidilactici* strains	Inhibition of *L. monocytogenes* in cooked meats	Rodríguez et al. 2002
Sakacin K-producing *L. sakei* CTC494	Inhibition of *L. monocytogenes* in cooked meat products	Hugas et al. 1998
Bacteriocin-producing *L. sakei*	Growth inhibition of *L. monocytogenes* and *E. coli* O157.H7 in cooked, sliced, vacuum-packaged meats	Bredholt et al. 1999
Fermented meats		
Bacteriocin-producing *L. sakei* starter cultures	Reduction of *Listeria* populations in fermented sausages	Ravyts et al. 2008
Curvacin-producing *L. curvatus*	Antilisterial effects in meat fermentation	Dicks et al. 2004
Pediocin-producing *P. acidilactici*	Commercial starter cultures for fermentation of meat products to reduce the numbers of *L. monocytogenes* in the final product	Amezquita and Brashears 2002
E. faecalis CECT7121 (producer of enterocin MR99)	Reduction of viable counts of *Enterobacteriaceae*, *S. aureus* and other Gram-positive cocci in craft dry-fermented sausages	Sparo et al. 2008

meat substrate (Dortu et al. 2008). On raw beef, *L. curvatus* CWBI-B28 was more effective in reducing *L. monocytogenes* cell concentrations below detectable levels (7 days) than *L. sakei* CWBI-B1365 (21 days). In poultry meat, the application of the LAB strains separately showed much lower inhibitory activities, but their addition in combination led to growth inhibition of the listeria. This is an interesting example of a synergistic effect between two sakacin-producing strains in a food system.

Lactobacillus curvatus CRL705 used as a protective culture in fresh beef was effective in inhibiting *L. innocua* and *B. thermosphacta* as well as the indigenous contaminant LAB at low temperatures and had a negligible effect on meat pH (Castellano et al. 2008). It was observed that meat inoculation with *L. curvatus* CRL705 showed a net increase of free amino acids, due to the complementary activity of the bacterial and meat proteases on meat sarcoplasmic proteins (Fadda et al. 2008).

It was proposed that *L. curvatus* CRL705 protective cultures could contribute to meat ageing by generating small peptides and free amino acids, while improving shelf life (Fadda et al. 2008).

Inoculation with a sakacin A producer *L. sakei* strain reduced the population of *L. monocytogenes* on vacuum-packed lamb during 12 week storage. Similarly, inoculation with BLIS-producing *L. sakei* strains delayed blownpack spoilage caused by *Clostridium estertheticum* and reduced the survival of *Campylobacter jejuni* on beef meat (Jones et al. 2009). In vacuum-packaged chicken cuts, inoculation with sakacin-P producing *L. sakei* achieved a growth inhibition of *L. monocytogenes* (Katla et al. 2002). Plantaricin-producing *L. plantarum* showed anti-listerial effects in uncooked and cooked chicken meat (Enan 2006; Gamal 2006). Enterococci have also been tested as protective cultures in raw meats. In chicken ground meat stored at 8–10 °C, growth of *L. monocytogenes* and *S*. Enteritidis was adversely affected by the respective presence of protective cultures consisting of strain *E. faecium* PCD71 (carrying the genetic determinants for enterocins A, P, L50A and L50B) and strain *L. fermentum* ACA-DC179, producer of BLIS against *Salmonella* (Maragkoudakis et al. 2009; Zoumpopoulou et al. 2008). Strain *E. faecium* PCD71 inhibited the growth of *L. monocytogenes* by at least 0. 7 log CFU/g after 7 days storage, while strain *L. fermentum* ACA-DC179 inhibited the growth of *S*. Enteritidis by up to 1.3 log CFU/g compared to the control (Maragkoudakis et al. 2009). In addition, none of these two strains caused detrimental effects on biochemical parameters related to spoilage of the chicken meat.

4.2.2 Semi-processed and Cooked Meats

Lactic acid bacteria are the prevalent spoilage microorganisms in cooked meat products (Mataragas et al. 2006, Audenaert et al. 2010, Chenoll et al. 2007). The shelf life of most heat processed meats is limited by *Lactobacillus* and *Leuconostoc* strains that rapidly recontaminate the product during handling and slicing (Lücke 2000). These LAB also tend to displace pathogenic bacteria. In the absence of competing microbiota, *L. monocytogenes* will proliferate more easily. Specific bacteriocin-producing LAB strains could be used as protective cultures for semi-processed and cooked meats provided that they cause only a minimal change in the desired sensory properties of the products while inhibiting *Listeria* and displacing other LAB involved in spoilage (Hugas et al. 1998; Lücke 2000; Chen and Hoover 2003; Aymerich et al. 2008; Galvez et al. 2008). Bacteriocin-producing protective cultures have been shown to inhibit *L. monocytogenes* in vacuum-packaged processed meats, such as *Lactobacillus bavaricus* MN in minimally heat-treated beef cubes (Winkowski et al. 1993), *P. acidilactici* JBL 1095 in wieners (Degnan et al. 1992), or *P. acidilactici* JD1-23 in frankfurters (Berry et al. 1991). In Brazillian raw sausage lingüiça, bacteriocin-producing *Lactobacillus sake* 2a also inhibited growth of *L. monocytogenes* (Liserre et al. 2002). The bacteriocinogenic strains *L. sakei* CTC494

and *E. faecium* CTC492 (producer of enterocins A and B) prevented slime formation in cooked pork by *Lb. sakei* but not by *Leuconostoc mesenteroides* (Aymerich et al. 1998). In sliced, vacuum-packaged cooked ham, the same enterococcal strain partially prevented ropiness by *L.sakei* (Aymerich et al. 2002). Inoculation of strains producing sakacin P or leucocin in cooked meat products was shown to inhibit growth of listeria (Katla et al. 2002; Jacobsen et al. 2003), and protective *L. sakei* cultures were also shown to inhibit *L. monocytogenes* and *E. coli* O157:H7 in vacuum-packed cooked meat products (Bredholt et al. 1999). The bacteriocinogenic strain *L. curvatus* CWBI-B28 reduced *L. monocytogenes* levels below detection limits in bacon meat within 1 or 2 weeks in absence or presence of nitrites, respectively (Ghalfi et al. 2006). Anti-listerial effect was also observed with a plantaricin producing *L. plantarum* strain in cooked chicken meat (Enan 2006). There are already several LAB cultures in the market introduced as starter or bioprotective culture with the aim of contributing to microbiological safety of semi-processed and cooked meats (Aymerich et al. 2008).

4.2.3 Fermented Meats

Certain lactic acid bacteria play key roles in meat fermentations. Therefore, bacteriocin-producing strains have been proposed as starter cultures to combat pathogens such as *L. monocytogenes* (Työppönen et al. 2003; Leroy et al. 2006; Aymerich et al. 2008). Bacteriocin-producing lactobacilli (mainly *L. sakei* and *L. curvatus*, but also *Lactobacillus rhamnosus* and *L. plantarum*) have demonstrated anti-listerial effects in sausage or salami fermentations, depending to a great extent on strain and type of meat (Erkkilä et al. 2001; Leroy et al. 2005; Dicks et al. 2004; Benkerroum et al. 2005; Todorov et al. 2007) (Table 4.2).

L. sakei CTC 494 (producing sakacin K) is a promising functional starter culture with antilisterial activity, being capable to successfully suppress *L. monocytogenes* in Spanish-style and German-style fermented sausages (Aymerich et al. 2008) or to reduce listeria populations in Belgian-style sausages, Italian salami, and Cacciatore salami (Ravyts et al. 2008). The efficacy of *L. sakei* is influenced by environmental factors such as sausage ingredients, salt, fat and nitrite content, acidification level, and temperature (Leroy et al. 2006). Since *L. sakei* and *L. curvatus* can hydrolyze muscle sarcoplasmic proteins and, in a lesser extent, myofibrillar proteins, they can contribute to the generation of small peptides and amino acids which contribute as direct flavour enhancers or as precursors of other flavour compounds during the ripening of dry-fermented sausages (Leroy et al. 2006). Exploitation of these activities may lead to the use of a new generation starter cultures with industrial or nutritional important functionalities (Leroy et al. 2006). Another, yet unexplored possible application of these functional properties would be the generation of bioactive peptides from the meat proteins by selected LAB with adequare proteolytic activities.

Bacteriocin-producing pediococci can reduce *L. monocytogenes* populations in fermented meats (Amezquita and Brashears 2002; Rodríguez et al. 2002; Aymerich

et al. 2008). Pediococci are preferred as starters in certain products (rather than lactobacilli), e.g. in American-style sausages fermented at higher temperatures. Bacteriocin-producing pediococci were proposed as indigenous starter cultures in the fermentation of Urutan, a Balinese traditional dry fermented sausage (Antara et al. 2004). One advantage is that pediocin PA-1 producers do not inhibit bacteria relevant to the fermentation such as staphylococci and micrococci (Gonzalez and Kunka 1987).

Enterococci are often part of the normal microbiota in meat fermentations, and have demonstrated to be effective as antilisteria agents in fermented meats, being also able to inhibit *S. aureus* (Foulquié Moreno et al. 2003; Aymerich et al. 2008; Galvez et al. 2008). However, their application in foods is controversial because of their potential virulence as opportunistic pathogens and also as carriers of antimicrobial resistance genes. The bacteriocinogenic strains *E. faecium* CCM 4231 and *E. faecium* RZS C13 strongly inhibited the growth of *Listeria* spp. in sausage fermentations (Callewaert et al. 2000), and *Enterococcus casseliflavus* IM 416K1 (producer of enterocin 416K1) was able to suppress *L. monocytogenes* in artificially inoculated "cacciatore" Italian sausages (Sabia et al. 2003). During sausage fermentation, inoculated *Enterococcus faecalis* A-48-32 (producer of the broad-spectrum cyclic enterocin AS-48) or its transconjungant *E. faecium* S-32-81, reduced the concentration of *L. monocytogenes* down to undetactable levels within 7 or 6 days of incubation at 20 °C (Ananou et al. 2005a). Similarly, strain A-48-32 inhibited growth of *S. aureus* and reduced viable cell counts to 1 log CFU/g at the end of fermentation (Ananou et al. 2005b). Strain *E. faecalis* CECT 7121 (isolated from natural corn silage, and producer of the broad-spectrum enterocin MR99) is interesting because it is devoid of the genes for haemolysin and gelatinase production, and does not produce biogenic amines (Sparo et al. 2008). When tested in the manufacture of craft dry-fermented sausages, the sausages inoculated with *E. faecalis* CECT 7121 had lower viable counts of *Enterobacteriaceae*, *S. aureus* and other Gram-positive cocci at the end of fermentation (2 days), with no detectable enterobacteria and *S. aureus* at the end of drying (21 days). *E. faecalis* CECT7121 did not affect the growth of *Lactobacillus* spp. but it displaced the autochthonous populations of enterococci (Sparo et al. 2008).

The potential of bacteriocin-producing lactococci in meat fermentations has been studied to a much less extent. Nisin-producing lactococcal strains isolated from fermented sausages were suggested as adjunct cultures for improving the food safety of meat fermented products manufactured under poor hygienic conditions such as indigenous fermentations (Rodriguez et al. 1995; Noonpakdee et al. 2003). Furthermore, it was reported that a transformant *L. lactis* strain producing lacticin 3417 significantly reduced the populations of *L. innocua* and *S. aureus* in sausages, although growth of the bacteriocin producer was markedly influenced by sausage ingredients (Scannell et al. 2001). In another study on manufacture of merguez, a dry-fermented beef meat sausage, inoculation with the Bac+ strain *L. lactis* subsp. *lactis* M significantly reduced the levels of *L. monocytogenes* during the fermentation phase (Benkerroum et al. 2003). However, inoculation with a lyophilized culture of the bacteriocin-producing strain *L. lactis* LMG21206 decreased *Listeria* counts to

below the detectable limit after 15 days of drying, but it had no effect on the viability of the listeria during sausage fermentation. By comparison, the results obtained with the Bac + strain *L. curvatus* LBPE were superior, with highly significant reductions during fermentation and ripening (Benkerroum et al. 2005).

Several LAB strains may antagonise growth o *E. coli* O157:H7 in fermented sausages. This inhibitory effect has been attributed to the production of small antimicrobial compounds (such as reuterin, 3-hydroxy fatty acids, phenyllactic acid, and 4-hydroxyphenyllactic acid and novel bacteriocins; Leroy et al. 2006). It was shown that inoculation of salami with strains of *Lactobacillus* spp. as well as bifidobacteria reduced the levels of *L. monocytogenes* and *E. coli* O111 during fermentation of sausage batter (Pidcock et al. 2002). Similar results were reported for *Lactobacillus reuteri* and *Bifidobacterium longum* in dry fermented sausages. In the treatment containing *L. reuteri* (producer of reuterin), a 3 log CFU/g reduction in *E. coli* O157:H7 numbers was found at the end of drying, while *B. longun* was reported to have lower effects (1.9 log CFU/g reduction) (Muthukumarasamy and Holley 2007).

Staphylococci and micrococci may also be exploited as sources for antibacterial substances applicable in sausage fermentations. The introduction of the lysostaphin gene (an endopeptidase that specifically cleaves the glycine–glycine bonds unique to the interpeptide cross-bridge of the *S. aureus* cell wall) into meat starter lactobacilli (Cavadini et al. 1998) is an interesting approach to prevent the growth of *S. aureus*. Furthermore, one *Staphylococcus xylosus* sausage isolate that produces an antilisterial substance increased the microbial inactivation of *L. monocytogenes* in Naples-type sausage (Villani et al. 1997). *Kocuria varians* (formerly *Micrococcus varians*) produces the lantibiotic variacin (Pridmore et al. 1996). Strains producing this lantibiotic were isolated form Italian-type raw salami fermentations. Bacteriocinogenic *Kocuria* strains could be very interesting as adjunct protective cultures in meat fermentations.

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