Chapter 8
Antimicrobial Films and Coatings Incorporated with Food Preservatives of Microbial Origin

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Abstract Food quality and safety constitute main issues for the food industry. However, in spite of several efforts that have been carried out, food preservation is still challenging. Synthetic substances have been widely applied in the food industry as preservatives, however some of them have been associated with harmful effects to human health. This fact has prompted the quest of new methods for food preservation using natural and safer agents. Biopreservatives such as lactic acid bacteria and their bacteriocins have been widely recognized as potent natural compounds able to inhibit or prevent the growth of spoilage and pathogenic microorganisms in food systems. Therefore, the incorporation of these biopreservatives into polymeric films and coatings constitutes a promising strategy to develop new antimicrobial packaging materials to ensure food safety and extend the food shelf-life. This chapter presents the main developments regarding active packaging intended for food biopreservation. Different strategies for the incorporation of biopreservatives into food packaging materials are analyzed. Finally, the challenges against the large-scale production and successful commercialization of these materials containing biopreservatives are also addressed.

Keywords Antimicrobial food packaging · Bacteriocin · Biopreservation · Lactic acid bacteria

8.1 Introduction

Global initiatives on food loss and waste reduction have gained growing attention in the last years. According to Food and Agriculture Organization of the United Nations (FAO), roughly one third of the food produced in the world for human consumption every year (i.e., approximately 1.3 billion tonnes) gets lost or wasted (FAO 2011). Food losses and waste amounts to roughly US$ 680 billion in industrialized countries and US$ 310 billion in developing countries (FAO 2011). In

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T. J. Gutiérrez (ed.), *Polymers for Food Applications*,
https://doi.org/10.1007/978-3-319-94625-2_8

addition, food losses represent a waste of resources used in production such as land, water, energy and inputs. Producing food that will not be consumed leads to unnecessary CO_2 emissions in addition to loss of economic value of the food produced.

Active packaging's constitute an actual choice for reducing food waste along the value chain (FAO 2014). Food packaging can be termed active when it performs a certain desirable role other than providing an inert barrier to external conditions (Anu Bhushani and Anandharamakrishnan 2014). Active packaging systems could include antioxidants, antimicrobial agents or oxygen scavengers (Chang-Bravo et al. 2014; López-Córdoba et al. 2017; Piñeros-Hernandez et al. 2017; Gutiérrez 2017, 2018). Other than these, moisture absorbing, flavor or odor absorbing active packaging systems are also being developed for food applications (Anu Bhushani and Anandharamakrishnan 2014). In particular, the fabrication of antimicrobial packaging's has received increasing importance in the past years because they offer slow and continuous migration of antimicrobial agents from packaging material to food surfaces, increasing their shelf-life (Blanco Massani et al. 2014a, b; Garcia et al. 2012; Woraprayote et al. 2018). Packaging's containing antimicrobial agents from natural sources are preferred, instead synthetic additives, since the latest alternatives have been associated with harmful effects on human health (Gutiérrez and Álvarez 2016, 2018a). In this context, lactic acid bacteria (LAB) have been proposed as natural preservatives to inhibit or prevent the growth of spoilage and pathogenic microorganisms in food systems and, consequently, to enhance their safety and prolong their shelf life (Aloui and Khwaldia 2016). LAB have ability to produce various types of antimicrobial compounds, the most important being bacteriocins. Bacteriocins and bacteriocin-producing cultures have the potential to increase the shelf-life of foods and contribute towards decreasing the incidence of foodborne diseases.

This chapter provides an overview of the current applications of lactic acid bacteria and their bacteriocins in food packaging (film and coatings) and highlight useful applications for these materials to extend shelf life of different food products such as meat, fish, dairy fruit, vegetables or other food products.

8.2 Lactic Acid Bacteria and Their Bacteriocins as Food Biopreservatives

Lactic acid bacteria (LAB) comprise a group of Gram-positive bacteria, non-sporulating, cocci or rods, and catalase-negative organisms with high tolerance for low pH (Calo-Mata et al. 2008). LAB are characterized by the production of lactic acid as the major end product during the fermentation of carbohydrates, lowering the pH of the food and also directly inhibiting the growth of many microorganisms.

LAB are categorized into homofermentative and heterofermentative microorganisms, based on the products of the fermented carbohydrates. Homofermentative

LAB degrade hexoses to lactate, whereas heterofermentative LAB degrade hexoses to lactate and additional products such as acetate, ethanol, CO_2, formate, or succinate (Calo-Mata et al. 2008). LAB are widely used as starter cultures in the food industry for the production of fermented foods, including dairy (e.g. yogurt and cheese), meat (e.g. sausages), fish, cereals (e.g. bread and beverages such as beer), fruit (malolactic fermentation processes in wine production), and vegetables (e.g. sauerkraut, kimchi and silage) (Chelule et al. 2010). Most LAB are considered GRAS (generally recognized as safe) by the US Food and Drug Administration. As probiotics, LAB are increasingly being used owing to their contribution to the healthy microflora of human mucosal surfaces (Mokoena and Paul 2017; Porto et al. 2017).

The LAB group is currently classified in the phylum *Firmicutes*, class *Bacilli*, and order *Lactobacillales*, Families *Aerococcaceae*, *Carnobacteriaceae*, *Enterococcaceae*, *Lactobacillaceae*, *Leuconostocaceae*, and *Streptococcaceae*. LAB are classified based on cellular morphology, mode of glucose fermentation, range of growth temperature, and sugar utilization patterns. LAB genera include *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Aerococcus*, *Alloiococcus*, *Carnobacterium*, *Dolosigranulum, Enterococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella* (Mokoena and Paul 2017). Among LAB, *Lactobacillus* is the genus including a high number of GRAS species and many strains are among the most important bacteria in food microbiology and human nutrition, due to their contribution to fermented food production or their use as probiotics (Salvetti et al. 2012).

Biopreservation refers to the use of natural or controlled microbiota or its antibacterial metabolites to extend the shelf life and enhance the safety of foods (Hugas 1998; Stiles 1996). This strategy can help to reduce the addition of synthetic preservatives as well as the intensity of heat treatments, resulting in foods which are more naturally preserved and richer in organoleptic and nutritional properties (Gálvez et al. 2007; García et al. 2010).

LAB have widely recognized as biopreservatives because can protect foods from microbial spoilage by the lowering their pH, by competitive growth against spoilage and pathogenic bacteria and by the production of antagonistic metabolic products such as organic acids (e.g., lactic acid), diacetyl, fatty acids, CO_2, peroxide, and bacteriocins (Calo-Mata et al. 2008).

Bacteriocins are ribosomally-synthesized peptides or proteins with antimicrobial activity, produced by many Gram-positive and Gram-negative microorganisms (Abbasiliasi et al. 2017; Woraprayote et al. 2016). However, bacteriocins produced by Gram-positive microorganisms such as LAB are more frequently used in the food industry (Cotter et al. 2005; Gálvez et al. 2007; García et al. 2010).

The bacteriocins produced by LAB offer several desirable properties that make them suitable for food preservation: (i) are generally recognized as safe substances (GRAS), (ii) are not active and nontoxic on eukaryotic cells, (iii) become inactivated by digestive proteases, having little influence on the gut microbiota, (iv) are usually pH and heat-tolerant, (v) they have a relatively broad antimicrobial spectrum, against many food-borne pathogenic and spoilage bacteria, (vi) they show a

Table 8.1 Classification of bacteriocins from lactic acid bacteria

Class	Properties	Producer strains	Examples
I	Small peptides (<5 kDa) that possess the eponymous lanthionine or β-methyllanthionine residues	*Lactobacillus lactis; Streptococcus mutans*	Nisin, mersacidin, lacticin 481; lacticin 3147; cytolysin
IIa	Small (<10 kDa) heat-stable peptides, which do not undergo extensive posttranslational modification	*Lactobacillus sakei; enterococcus faecium*	Pediocin PA1, leucocin A
IIb	Consist of two different individual peptide molecules that require equal peptide ratio of each peptide to exert its optimal antimicrobial activity	*Lactobacillus plantarum; Lactococcus lactis*	Lactacin F, lactococcin G
IIc	Circular LAB bacteriocins consist of N-to-C-terminally linked antimicrobial peptides, produced by gram-positive bacteria of the phylum *Firmicutes*	*Enterococcus faecalis; enterococcus faecium*	Enteriocin AS48, reuterin 6
IId	Include the remaining well-characterized bacteriocins, combined as miscellaneous, which are now including nonpediocin like single linear peptides	*Lactococcus lactis*	Lactococcin A, divergecin A

Adapted from Cotter et al. (2005) and Woraprayote et al. (2016)

bactericidal mode of action, usually acting on the bacterial cytoplasmic membrane: no cross resistance with antibiotics, and (vii) their genetic determinants are usually plasmid-encoded, facilitating genetic manipulation (Cotter et al. 2005; Gálvez et al. 2007; Woraprayote et al. 2016).

Bacteriocins are classified according to their chemical structure, molecular mass, enzymatic susceptibility, genetics, mechanism of microbial destruction, thermostability, producing strains, antimicrobial activities and the presence of post-translational modified amino acid residues (Kaškonienė et al. 2017; Mokoena and Paul 2017). Therefore, one only classification is not currently available and some authors distinguish two, three or four classes with subclasses/subcategories (Cotter et al. 2005; Kaškonienė et al. 2017; Mokoena and Paul 2017; Woraprayote et al. 2016). Between them, the classification proposed by Cotter et al. (2005) seems better for LAB bacteriocins at this moment. Accordingly, LAB bacteriocins can be classified into two major classes: class I lantibiotics (lanthionine-containing antibiotics) and class II which can further be grouped into four subclasses: IIa, IIb, IIc, and IId, respectively (Table 8.1). Bacteriocins from class I and IIa (pediocin-like bacteriocins) are among the best biochemically and genetically characterized antimicrobial peptides and the most likely to be used in food applications due to their target specificity. Among hundreds of bacteriocins, nisin is the most popular and extensively investigated bacteriocin, probably because it is approved for use as additive (code E234) in food products and is now available commercially (Woraprayote et al. 2016). Nisin is produced by many strains of *Lactococcus lactis*, a species widely used for cheese manufacture. It has a broad antimicrobial spectrum against a wide range of Gram-positive genera, including *Staphylococci*, *Streptococci*,

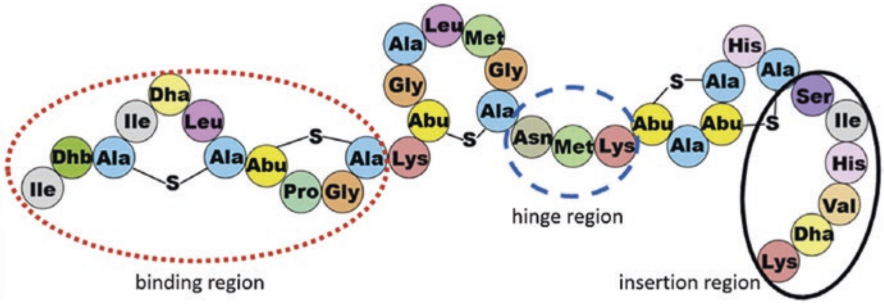

Fig. 8.1 Nisin molecular structure showing key regions responsible for its antimicrobial activity: binding region to the cell, hinge region to create cage and inhibit cell wall synthesis, and insertion region into the pore. Reprinted with permission from Han et al. (2017)

Listeria spp., *Bacilli*, and *Enterococci*, with a minimal inhibitory concentration in the nanomolar range (Woraprayote et al. 2016). To date, eight types of natural nisin were discovered: nisins A, Z, F and Q produced by *Lactococci* and nisins U, U2, P and H by some *Streptococcus* strains (Kaškonienė et al. 2017; O'Connor et al. 2015; Woraprayote et al. 2016).

Nisin acts on target bacteria by two major steps: (1) passage through the cell wall; (2) interaction with lipid II (e.g. binding to lipid II, pore formation on cell membrane), which is essential for the biosynthesis on the cell wall. Nisin's mechanism of action (see Fig. 8.1) involves first binding of the N-terminus to the lipid II complex and forming a pyrophosphate cage that inhibits cell wall synthesis. The C terminal is then responsible for pore-formation. A three amino acid hinge region exists between the N and C domains and allows conformational changes to occur upon contacting a microbe (Han et al. 2017).

Pediocins are another widely studied biopreservatives which are produced by *Pediococcus spp*. These class IIa bacteriocins are recognized because present a broad spectrum of antimicrobial activity against Gram-positive bacteria, with highlights efficient bactericidal effects against pathogenic bacteria, such as *Listeria monocytogenes* (Porto et al. 2017; Ríos Colombo et al. 2017; Rodríguez et al. 2002). It has been demonstrated that class IIa bacteriocins act on the cytoplasmic membrane of Gram-positive cells dissipating the transmembrane electrical potential, which results in an intracellular ATP depletion. These peptides induce the exit of ions, amino acids and other essential molecules by forming hydrophilic pores in the target (Ríos Colombo et al. 2017).

Among the pediocins isolated from different strains, only pediocin PA1 (*P. acidilactici PAC 1.0*) and pediocin AcH (*P. acidilactici LB42–923*) have been well characterized. Despite that pediocins have been widely studied, they have no official approved use in foods (Woraprayote et al. 2016).

In addition to nisin and pediocin, many other LAB bacteriocins have characteristics that make them ideal candidates for the preservation of food products including Lactococcin G, lactacin F, lactocin 705, enteriocin AS-48, lacticin Q and others.

More detailed information about LAB bacteriocins can be found in the recently published works by Garsa et al. (2014), Kaškonienė et al. (2017) and Mokoena and Paul (2017).

8.3 Incorporation of Lactic Acid Bacteria and Their Bacteriocins into Films and Coatings

Lactic acid bacteria and their bacteriocins have been applied in food products using mainly two different strategies: (i) direct inoculation of bacteriocin producing LAB culture into food products (i*n situ* production) and (ii) direct application of purified or semi-purified bacteriocin as a food additive (*ex situ* production) (Castro et al. 2017). *Ex situ* preparations are obtained by growing the producer strain at industrial scale and then concentration and purification processes are needed to obtain a pure form of the bacteriocin. Both *in situ* and *ex situ* strategies have been reported to have limitations associated with interactions of biopreservatives with other food components (e.g., lipids and proteins) and to the loss of activity due to enzymatic degradation (Aloui and Khwaldia 2016). In order to overcome these disadvantages, biopreservatives-containing films and coatings has been evaluated obtaining feasible results. This strategy allows to combine the preservative function of antimicrobials with the protective function of packaging. For this purpose, a wide range of non-edible polypropylene- and polyethylene-based packaging materials and several biodegradable protein- and polysaccharide-based edible films have been used (Garcia et al. 2012; Muriel-Galet et al. 2015; Woraprayote et al. 2018).

Antimicrobial packaging offers slow and continuous migration of antimicrobial agent from packaging material to food surfaces which enables antimicrobial agents to maintain at high concentration over a long period. Antimicrobial coatings can be obtained by incorporation of the antimicrobials into an edible polymer blend that is then applied by dipping, brushing or spraying onto the food (Guo et al. 2014). Compared to direct application, edible coatings containing LAB and/or their bacteriocins may impart a highly localized functional effect without affecting the food organoleptic properties (Campos and others 2011). Moreover, edible coatings may act as a semipermeable barrier providing an additional protection for foods against moisture loss, solute migration, gas exchange, respiration, and oxidative reactions (Aloui and Khwaldia 2016).

Biopreservatives containing polymer films have been also used as antimicrobial packaging. Several film-forming methods have been evaluated, including casting and heat-pressing. It has been found that bacteriocin activity of cast film (solvent compounding) retained three times greater than that of heat-pressed films (Dawson et al. 2003).

In this context, several studies have investigated the application of films and coatings incorporating LAB and their bacteriocins as antimicrobial packagings to extend the shelf life of different food systems, including meat, fish, dairy fruit, vegetables or other food products.

8.4 Antimicrobial Films and Coatings for Meat and Meat Products

Meat and meat products are consumed extensively throughout the world because they are an important source of nutrients including fats, proteins, vitamin B12, zinc and iron. However, these products are perishable and susceptible to microbial contamination, leading to an increased health risk to consumers as well as to the economic loss in the meat industry (Woraprayote et al. 2016). Microorganisms commonly involved in spoilage of meat and meat products include *Pseudomonas* (*P. fragi, P. uorescens, P. putida and P. lundensis*), *Shewanella putrefaciens*, *Photobacterium phosphereum*, *Brochothrix thermosphacta*, cold-tolerant *Enterobacteriaceae* (e.g., *Hafnia alvei, Serratia liquefaciens* and *Enterobacter agglomerans*), *Acinetobacter* spp., *Alcaligenes* spp., *Moraxella* spp., *Flavobacterium* spp., *Staphylococcus* spp., *Micrococcus* spp., coryneforms, fecal streptococci, lactic acid bacteria (LAB), among others (Sofos 2014). In addition, meat and meat products are also susceptible to contamination by pathogenic microorganisms such as *Salmonella* spp., thermophilic *Campylobacter jejuni*, enterohemorrhagic *Escherichia coli* O157:H7, *Clostridium perfringens*, anaerobic *Clostridium botulinum*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, and *Yesinia enterocolitica*. Among the meat-borne pathogens, *Listeria monocytogenes* consider one of the main causes of food borne illness and has been associated with cooked, ready-to-eat (RTE) meat and poultry product (Buchanan et al. 2017; Guo et al. 2014).

The application of LAB and their bacteriocins in meat and meat products has received a considerable attention in the last years. As above mentioned, several LAB produce microbial antagonists, such as bacteriocins, which are active against pathogens microorganism, including *Listeria monocytogenes*. The most-studied bacteriocins in meat and meat products are nisin, enterocin AS-48, enterocins A and B, sakacin, leucocin A and especially pediocin PA-l/AcH. These biopreservatives have been used alone or in combination with other hurdle treatments such as modified atmosphere packaging, high hydrostatic pressure (HHP), heat and chemical food preservatives (Castro et al. 2017; Cleveland et al. 2001; Delves-Broughton et al. 1996; Paul Ross et al. 2002).

The incorporation of LAB and/or their bacteriocins in food packaging's have been proposed as a useful strategy to prevent their degradation and achieved their controlled release towards the food products. Woraprayote et al. (Woraprayote et al. 2013) developed a poly(lactic acid) (PLA)/sawdust particle biocomposite film with anti-listeria activity by incorporation of pediocin PA-1/AcH. The addition of sawdust particle promoted the embedding of pediocin into the hydrophobic PLA film.

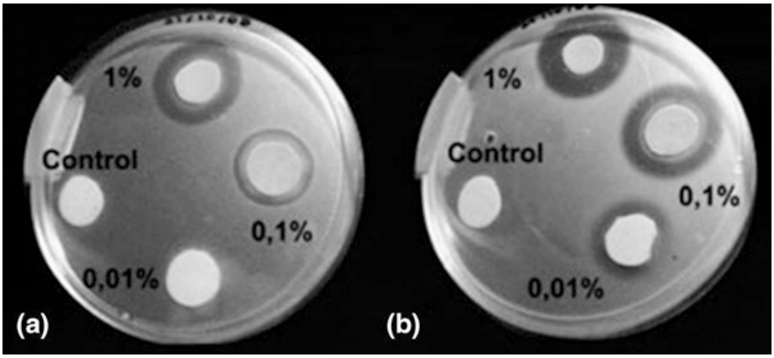

Fig. 8.2 Lactocin 705 (**a**) and AL705 (**b**) antimicrobial activity on wheat gluten films doped with bacteriocin crude extract (0.01%, 0.1% and 1%) against *L. plantarum CRL691* and *L. innocua 7*. Control film had no antimicrobials in its formulation. Reprinted with permission from Blanco Massani et al. (2014a, b).

The films significantly reduced the listerial population by about 1.5–2 log cycles from 1 to 14 days. This effect was enhanced when a film pre-conditioned by dry-heat treatment was carried out. Blanco Massani et al. (2014a, b) developed antimicrobial wheat gluten film containing *Lactobacillus curvatus* CRL705 bacteriocins (lactocin 705 and lactocin AL705). In order to determine the antimicrobial minimum inhibitory concentration, different bacteriocin crude extract concentrations were added to the film-forming solution (0.01%, 0.1% and 1% v/v) and the obtained films were assayed for antimicrobial activity by the agar well diffusion method (Fig. 8.2). *Lactobacillus plantarum* CRL691 and *Listeria innocua* 7 were used as indicators of lactocin 705 and lactocin AL705, respectively. It was found that wheat gluten films containing a bacteriocin crude extract concentration above 0.1% were active against both *L. innocua 7* and *L. plantarum* CRL691 bacteria (Fig. 8.2).

In other work, Blanco Massaniet al. (2014a, b) assayed the antimicrobial effectiveness of gluten film using Wieners inoculated with *Lactobacillus plantarum* CRL691 and *Listeria innocua* 7 (10^4 CFU/g) stored at 5 °C during 45 days. The wieners were separately inoculated under sterile conditions by immersion (30 s) in a solution containing *L. innocua* 7 (10^4 CFU/g) and *L. plantarum* CRL691 (10^4 CFU/g). After drying, three Wieners (42 g) were placed into each active and control packagings previously prepared. In parallel, control (without bacteriocins) uninoculated Wieners packages were included. Typical growth of both inoculated microorganisms was observed in control packages which reached 10^6–10^7 CFU/g at the end of storage period. In the active packages, *L.innocua* 7 was effectively inhibited (2.5 log cycles reduction at day 45), while *L. plantarum* CRL691 was only slightly inhibited (0.5 log cycles) up to the second week of storage, then counts around 10^6–10^7 CFU/g were reached. More recently, Correa et al. 2017 worked on the development of polyhydroxybutyrate/polycaprolactone (PHB/PCL) nisin activated films with and without the addition of organo-clays (Cloisite1 30 B and 10A).

Organo-clays were able to act as a filler, increasing the thermal stability and the barrier and mechanical properties of the nanocomposites. PHB/PCL nisin activated films were effective against *L. plantarum* CRL691 (used as processed meat spoilage bacterium model) inoculated on sliced ham. Lag phase was extended from 7.03 to 22.39 days due to the nisin effect in the active packages, avoiding LAB counts to reach more than 6 log units, thus extending the ham shelf life up to 28 days at 5 °C.

Several authors have suggested promising ways to enhance antimicrobial effectiveness of bacteriocin in meat products by the combination with another hurdle technology. For example, Guo et al. 2014 suggesting edible antimicrobial coating solutions incorporating chitosan, lauric arginate ester and nisin to reduce foodborne pathogen contamination on ready-to-eat (RTE) meats. Two different approaches were evaluated: RTE deli meat samples were directly coated with the solutions, or treated with a solution-coated polylactic acid (PLA) films. The antimicrobial efficacy of the coatings and films against *Listeria innocua* inoculated onto the surface of RTE meat samples was investigated. It was found that the addition of nisin to chitosan coating solutions significantly reduced more *Listeria* than the chitosan coating solutions without nisin. However, chitosan coatings with nisin exhibited less anti-listerial activity than lauric arginate ester and the combination of nisin with this agent did not contribute to a synergistic or additional anti-listerial effect (Guo et al. 2014).

Huq et al. (2015) evaluated the synergistic effect of gamma (γ)-irradiation and microencapsulated antimicrobials (nisin and oregano and cinnamon essential oils), alone or in combination, against *Listeria monocytogenes* on ready-to-eat (RTE) ham. Microencapsulation of essential oils and nisin showed a synergistic anti-listerial effect with γ-irradiation on RTE meat products. These combinations led to a lag phase of bacterial growth and provoked a reduction in the bacterial growth rate of 32%, compared to microencapsulated combined antimicrobials without irradiation.

8.5 Antimicrobial Films and Coatings for Fish and Fish Products

Fishery products have a high economic importance and they are one of the most important protein sources in human nutrition. However, these products are perishable and, if left unpreserved, spoil rapidly (Calo-Mata et al. 2008). Main spoilage causes of fishery products include enzymatic, microbial and chemical action.

Films and coatings incorporating LAB and/or their bacteriocins have been used to extend the shelf life and to maintain the quality of fresh and processed fish. Recently, Woraprayote et al. (2018) developed an antimicrobial biodegradable food packaging for control of pathogens in pangasius fish fillets. Bacteriocin 7293, a new found antimicrobial peptide produced by *W. hellenica* BCC 7293, was chosen as a biopreservative because its broad antimicrobial spectrum against both Gram-positive

Table 8.2 Application of bacteriocin and bacteriogenic strains in dairy products

Bacteriocin	Bacteriocin-producing culture	Application	Pathogen	Product
Lacticin 3147	*Lc. lactis* DPC 3147	Spray-dried powder	*L. monocytogenes*	Cottage cheese
Pediocin	*P. acidilactici* PAC1.0	Dry powder	*L. monocytogenes*	Cottage cheese and yogurt
Piscicolin 126	*C. piscicola* JG 126	Concentrated supernatant	*L. monocytogenes*	Camembert cheese
Enterocin CRL35	*E. faecium* CRL 35	Concentrated supernatant	*L. monocytogenes*	Goat milk cheese
Nisin	*Lc. lactis* CNRZ 150	Starter culture	*L. monocytogenes*	Camembert cheese
Nisin	*Lc. lactis* TAB 50	Starter culture	*L. monocytogenes*	Semihard cheese
Lacticin 481	*Lc. lactis* TAB 24	Starter culture	*L. monocytogenes*	Semihard cheese
Lacticin 3147	*Lc. lactis* DPC 4275	Starter culture	*L. monocytogenes*	Cottage cheese
Enterocin AS-48	*E. faecalis* TAB 28	Starter culture	*L. monocytogenes*	Semihard cheese
Enterocin AS-48	*E. faecalis* INIA 4	Starter or adjunct culture	*L. monocytogenes*	Manchego cheese
Pediocin	*Lc. lactis* MM 217	Starter culture	*L. monocytogenes*	Cheddar cheese
Pediocin	*Lb. plantarum* WHW 92	Surface sprayed cell suspension	*L. monocytogenes*	Munster cheese
Pediocin	*Lc. lactis* CL1	Adjunct culture	*L. monocytogenes*	Semihard cheese
Pediocin	*Lc. lactis* CL1	Adjunct culture	*S. aureus*	Semihard cheese
Nisin	*Lc. lactis* ESI 515	Adjunct culture	*S. aureus*	Semihard cheese

Reprinted from Arqués et al. (2015)

and Gram-negative bacteria (Woraprayote et al. 2015). The antimicrobial effectiveness of the produced PLA/sawdust particle films impregnated with Bac7293 on raw pangasius fish fillet was evaluated and it was found that these films effectively inhibited both Gram-positive (*Listeria monocytogenes* and *Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Escherichia coli* and *Salmonella Typhimurium*) which have been considered as a reason for the rejection of pangasius fish fillets in worldwide markets.

8.6 Antimicrobial Films and Coatings for Dairy Products

Milk and dairy products have been an important part of the human diet for some 8000 years and are part of the official nutritional recommendations in many countries worldwide. However, it is well known, that these products provide a potential growth medium for the development of spoilage and pathogen microorganisms such as *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, and pathogenic *Escherichia coli*. Several bacteriocin and bactioricin-producing strain have been used in dairy foods (Table 8.2). Moreover, different strategies of incorporation of biopreservatives into dairy foods have been considered in order to prevent the loss of antimicrobial activity of these agents.

LAB and their bacteriocins have been included in polymers for the production of active packaging for dairy foods. Recently, Marques et al. (2017) evaluated the effectiveness of a biodegradable film, with antimicrobial metabolites produced by *Lactobacillus curvatus* P99 incorporated, targeting the control of *Listeria monocytogenes* in sliced "Prato" cheese. *Lactobacillus curvatus* is part of the microbiota of many fermented products and stands out for its bacteriocinogenic activity, due to the production of different antimicrobial metabolites, especially bacteriocins, known as curvacins and sakacins and characterized by their antilisterial activity (de Souza Barbosa et al. 2015). Starch films incorporating cell-free supernatant containing bacteriocins from *Lactobacillus curvatus* P99 were prepared by casting. Films with added minimum bactericidal concentration (62.5 μL/mL) showed activity against different indicator microorganisms and were able to control *L. monocytogenes* Scott A when used in sliced "Prato" cheese. During 10 days of storage at 4 °C, the target microorganism count remained below the limit of detection (2.7 Log CFU/g).

Ollé Resa et al. (2016) proposed an innovative approach to prevent the contamination of an Argentinian Port Salut cheese with mixed cultures (bacteria, molds and yeast). Nisin was used as an antibacterial agent while natamycin was employed to prevent yeasts and moulds contamination. Both active compounds were incorporated together within tapioca starch edible films and the effectiveness of the active films was evaluated, at 7 ± 1 °C, in relation to the improvement of the microbiological stability of Argentinian Port Salut cheese. The films inhibited the growth of yeasts and moulds and controlled the growth of psychrotrophic bacteria originally present in the Port Salut cheese stored at refrigeration temperature. It also inhibited the development of a mixed culture (*Saccharomyces cerevisiae* and *Listeria innocua*) present in the cheese due to a superficial contamination, along a storage of 8 days at 7 ± 1 C.

8.7 Antimicrobial Films and Coatings for Fruits and Vegetables

The promotion of greater consumption of fruits and vegetables constitutes a worldwide challenge. This is due to that the low fruit and vegetable intake is estimated to cause some 5.2 million deaths each year, and was among the top 10 risk factors contributing to mortality (FAO 2017). Fruits and vegetables can be consumed either fresh or processed. Production and consumption of minimally processed foods, such as fresh-cut fruits and vegetables, is gaining popularity due to consumer preferences towards healthier foods. However, challenge for fresh-cut industry is to maintain fresh like characteristics of fresh-cut produce for a prolonged storage time. Fresh-cut products have much larger cut surface and consequently much shorter shelf-life. Loss of quality parameters such as color, firmness, juiciness, flavor and excessive moisture loss results in limited shelf-life and increased chances of rejection of the produce by the consumers (Yousuf et al. 2018).

Different approaches have been used to preserve the quality of fresh-cut fruits and vegetables (Barbosa et al. 2017; Yousuf et al. 2018). Between them, the application of antimicrobial packaging's containing plant extracts, antimicrobial polymers (e.g. chitosan), enzymes (e.g. lysozyme) and other agents has been proposed as a useful strategy to extend the shelf life of these products (Álvarez et al. 2018; Gutiérrez et al. 2018b). However, despite that LAB and their bacteriocins have been extensively studied to preserve foods of animal origin, little information is available for their use in vegetable products, especially in minimally processed ready-to-eat fruits (Barbosa et al. 2017). Some studies deal with the application of bacteriocins to extend the shelf life of pineapple pulps, apple juice and minimally processed fruits and vegetables (e.g., sliced apples and lamb's lettuce) have been reported (Leite et al. 2016; Pei et al. 2017; Siroli et al. 2015). Antimicrobial packagings containing LAB and/or their bacteriocins also have been developed. Narsaiah et al. (2015) developed pediocin-containing calcium alginate coating for preservation of minimally processed papaya fruit. Fruit quality parameters such as firmness, weight loss, color, head space gas composition, acidity, total soluble solids and microbial load were evaluated for 21 days of refrigerated storage. It was found that the pediocin-containing alginate coating prolonged the shelf-life of minimally processed papaya by maintaining physicochemical properties and microbial safety along 21 days of refrigerated storage.

Barbosa et al. (2013) studied the effects of nisin-incorporated films on the microbiological and physicochemical quality of minimally processed mangoes. Films were produced using a blend of cellulose acetate and nisin by the casting method and the antimicrobial activity of the films was tested against *S. aureus* ATCC 8095, *B. cereus* ATCC 4504, *A. acidoterrestris* DSMZ 2498 and *L. monocytogenes* ATCC 7644 using the diffusion method. In addition, the antimicrobial activity of the films on *S. aureus* or *L. monocytogenes* inoculated mango slices was evaluated. Antimicrobial tests using the diffusion method showed that the antimicrobial film inhibited *S. aureus*, *L. monocytogenes, B. cereus* and *A. acidoterrestris* strains *in*

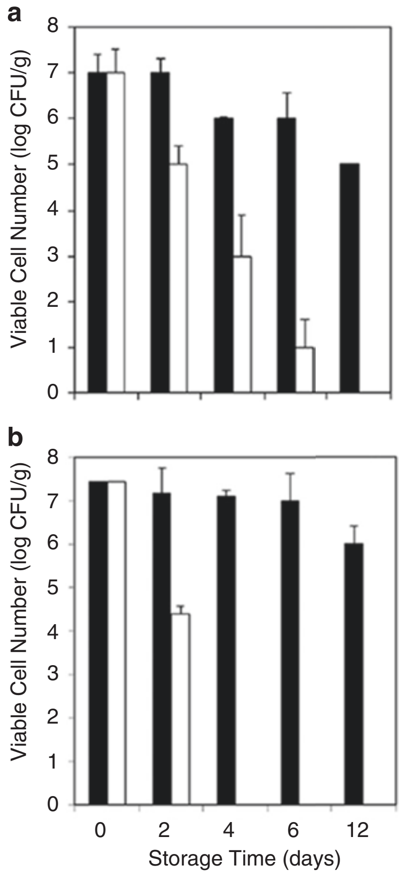

Fig. 8.3 Effects of nisin on the viability of *S. aureus* (**a**) and *L. monocytogenes* (**b**) on minimally processed mangoes. A total of 25 g of mango slices were inoculated with 10^7 CFU/g of each microorganism and packed with (white bars) and without (black bars) nisin. Reprinted with permission from Barbosa et al. (2013)

vitro. Moreover, cellulose films incorporated with nisin were efficient in eliminating *S. aureus* and *L. monocytogenes* contamination from minimally processed mango slices (Fig. 8.3), without interfering in the organoleptic characteristics of mangoes.

8.8 Conclusion

Lactic acid bacteria and their bacteriocins constitute an actual choice to decrease the use of synthetic additives in foods and also the application of thermal treatments, allowing to obtain more healthier foods. Despite that, these biopreservatives can be direct incorporated in foods, some disadvantages have reported regarding its loss of antimicrobial activity due to the interaction with food components and to its enzymatic degradation.

The incorporation of LAB and/or their bacteriocins in polymeric packaging (films or coatings) constitute a useful strategy to overcome these difficulties, improving the effectiveness of the biopreservatives. Moreover, these delivery systems allow to increase the shelf-life of foods and contribute towards decreasing the incidence of food-borne diseases, as it has been substantiated by the diversity of researches described in the current chapter.

The application of bacteriocinogenic LAB strains or their bacteriocins combined with other hurdle methods such as chemical compounds or physical processes can make use of synergies to increase microbial inactivation, without altering nutritional value and organoleptic properties of food.

Prior to successful commercialization of any of the effective films and coatings containing biopreservatives described in this study, large scale-production assays, shelf-life studies and, quality and sensory analyses will be needed. Moreover, close linkages between the scientific community and the industrial sector are highly required.

Acknowledgement The author would like to thank the Universidad Pedagógica y Tecnológica de Colombia (UPTC) and the Escuela de Administracion de Empresas Agropecuarias (UPTC Facultad Seccional Duitama) for their support.

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