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REVIEW ARTICLE

Strategies for the use of bacteriocins in Gram-negative bacteria: relevance in food microbiology

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Abstract Bacteriocins are ribosomally synthesized peptides that have bacteriostatic or bactericidal effects on other bacteria. The use of bacteriocins has emerged as an important strategy to increase food security and to minimize the incidence of foodborne diseases, due to its minimal impact on the nutritional and sensory properties of food products. Gram-negative bacteria are naturally resistant to the action of bacteriocins produced by Gram-positive bacteria, which are widely explored in foods. However, these microorganisms can be sensitized by mild treatments, such as the use of chelating agents, by treatment with plant essential oils or by physical treatments such as heating, freezing or high pressure processing. This sensitization is important in food microbiology, because most pathogens that cause foodborne diseases are Gram-negative bacteria. However, the effectiveness of these treatments is influenced by several factors, such as pH, temperature, the composition of the food and target microbiota. In this review, we comment on the main methods used for the sensitization of Gram-negative bacteria, especially *Salmonella*, to improve the action of bacteriocins produced by Gram-positive bacteria.

Keywords Bacteriocins · Biocontrol · Gram-negative bacteria · Outer membrane

Introduction

Bacteriocins are antimicrobial peptides that are ribosomally synthesized by Gram-negative and Gram-positive bacteria that have bacteriostatic or bactericidal effects on other bacteria (Héchard and Sahl 2002; Meghrous et al. 1999). These peptides show great potential for use in foods as a strategy for control of foodborne pathogens and spoilage microorganisms (Allende et al. 2007; Deegan et al. 2006). The use of bacteriocins has minimal impact on the nutritional and sensory properties of foods, thus satisfying consumer demand for products with a lower amount of chemical additives (Gálvez et al. 2007; Settanni and Corsetti 2008). In addition, bacteriocins have several desirable characteristics, such as low toxicity and stability against proteases and temperature (Dischinger et al. 2014; Garcia et al. 2010).

Usually, Gram-negative bacteria are naturally resistant to the bacteriocins produced by Gram-positive bacteria, due to their outer membrane, which acts as an effective barrier (Cao-Hoang et al. 2008; Gyawali and Ibrahim 2014). Nevertheless, agents or treatments that destabilize the outer membrane enable these peptides to affect Gram-negative bacteria (Gálvez et al. 2014; Chalón et al. 2012; Martin-Visscher et al. 2011). This sensitization is relevant in food microbiology, because most pathogens related to foodborne diseases are Gram-negative bacteria (Boziaris and Adams 1999). This strategy has been demonstrated with food additives, such as chelating agents or plant essential oils; with sanitization treatments using other antimicrobial compounds, such as sodium hypochlorite; and with conservation and food processing treatments, such as freezing, high pressure processing and pulsed electric fields, on several Gram-negative bacteria, including *Aeromonas hydrophila*, *Arcobacter butzleri*, *Citrobacter freudii*, *Enterobacter aerogenes*, *Escherichia coli*, *Salmonella*, *Shigella flexneri*, *Shigella sonnei*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas putida* and *Yersinia enterocolitica* (Tables 1 and 2).

The efficacy of these strategies has already been demonstrated in several tests both in vitro and in food models. Most such tests have used nisin (Tables 1 and 2), because of its approval for use in foods by the *Food and Drug Administration* (FDA) as a

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Table 1 Use of bacteriocins on Gram-negative bacteria associated to different treatments

Bacteriocin	Target microorganism	Concentration	Associated treatment	Reference
Nisin	Several serovars of *Salmonella*, *E. coli*, *E. aerogenes*, *S. flexneri*, *C. freudii*	50 μg.mL^{-1}	EDTA (20 mM)	Stevens et al. (1991)
	E. coli	200 to 1000 AU.mL^{-1}	EDTA (10 mM), phosphate (20 mM), citric acid (20 mM) and pyrophosphate (20 mM)	Boziaris and Adams (1999)
	P. fluorescens, *S. sonnei*, *S. enterica* Enteritidis	100 AU.mL^{-1}	High pressure processing (155 to 400 MPa, 15 min)	Masschalck et al. (2001)
	A. butzleri	500 AU.mL^{-1}	EDTA (20 mM) and phosphate trisodium (10 %)	Phillips and Duggan (2001)
	E. coli O157:H7	1066 AU.mL^{-1}	EDTA (500 and 1000 mM) and sodium lactate (800 mM)	Belfiore et al. (2007)
	E. coli	25 to 1000 AU.mL^{-1}	Fast freezing	Cao-Hoang et al. (2008)
	E. coli DH5α, *P. aeruginosa*, *S. enterica* Typhimurium	50 μM	EDTA (20 mM)	Martin-Visscher et al. (2011)
	E. coli, *S. enterica* Typhimurium	100 AU.mL^{-1}	Low temperature (6.5 °C)	Elliason and Tatini (1999)
	S. enterica Enteritidis	500 to 2500 AU.mL^{-1}	Heating (55 °C, 15 min)	Boziaris et al. (1998)
	A. butzleri	500 AU.mL^{-1}	Heating (50 °C)	Phillips and Duggan (2002)
	S. enterica Typhimurium, *Y. enterocolitica*, *A. hydrophila*, *P. putida*	4000 AU.mL^{-1}	Heating (55 °C, 10 min) or frozen (− 20 °C, 2 h)	Kalchayanand et al. (1992)
	S. enterica Typhimurium	200 AU.mL^{-1}	EDTA (1.5 mM)	Prudêncio et al. (2015)
Gallidermin	*E. coli*, *P. aeruginosa*, *S. enterica* Typhimurium	50 μM	EDTA (20 mM)	Martin-Visscher et al. (2011)
Bovicin HC5	*S. enterica* Typhimurium	50–200 AU.mL^{-1}	EDTA (1.6 mM), citric acid (7 mM), gluconic acid (100 mM) sodium citrate (100 mM), nitrile-tri-acetic acid (6.25 mM) and diethanolamine (50 mM); Tween 80 and Ramnolipid (5X CMC)	Prudêncio et al. (2014)
Lactocin 705	*E. coli* O157:H7	2133 AU.mL^{-1}	EDTA (500 and 1000 mM) and sodium lactate (800 mM)	Belfiore et al. (2007)
Cerein 8A	*S. enterica* Enteritidis,	3200 AU.mL^{-1}	EDTA (20 to 100 mM) and sodium lactate (200 mM)	Lappe et al. (2009)
Carnocyclin A	*E. coli* DH5α, *P. aeruginosa*	25 μM	EDTA (20 mM)	Martin-Visscher et al. (2011)
Carnobacteriocin BM1 Piscicolin 126	*P. aeruginosa*			

Table 2 Use of bacteriocins on Gram-negative bacteria in foods associated to different treatments

Bacteriocin	Target microorganism	Concentration	Associated treatment	Food matrix	Reference
Nisin	*E. coli*	1280 AU.g^{-1}	High pressure processing (400 MPa, 10 min)	Ham	Garrida et al. (2002)
	A. butzleri	500 AU.mL^{-1}	Sodium lactate (2 %), sodium citrate (1.5 %)	Chicken	Long and Phillips (2003)
	E. coli, *P. fluorescens*	500 AU.mL^{-1}	High pressure processing (200 to 500 MPa, 5 min)	Milk	Black et al. (2005)
	S. enterica Typhimurium	0.25 to 0.5 μg.mL^{-1}	Essential oil (5 to 30 μL.100 mL^{-1})	Barley soup	Moosavy et al. (2008)
	Pseudomonas sp.	500 to 1500 AU.g^{-1}	EDTA (10 mM)	Chicken	Economou et al. (2009)
	S. enterica Stanley, *E. coli*, *S. enterica* Newport	300 AU.mL^{-1}	EDTA (20 mM)	Apple juice	Ukuku et al. (2009)
	S. enterica Enteritidis	500 or 1000 AU.g^{-1}	Oregano essential oil (0.6 or 0.9 %)	Sheep meat	Govaris et al. (2010)
	Pseudomonas sp.	500 AU.mL^{-1}	EDTA (20 mM), potassium sorbate, sodium benzoate, sodium diacetate (3 %)	Shrimps	Norhana et al. (2012)
	S. enterica Enteritidis	500 to 2500 AU.mL^{-1}	Heating (55 °C)	Egg white	Boziaris et al. (1998)
Enterocin AS-48	*E. coli* O157:H7	50, 100 or 200 μg.mL^{-1}	EDTA (20 mM), sodium polyphosphate (0.3 or 0.5 %)	Apple juice	Ananou et al. (2005)
	S. enterica Choleraesuis	30 μg.mL^{-1}	Pulsed electric field (35 kV, 150 Hz)		Viedma et al. (2008)
	S. enterica	25 μg.mL^{-1}	Lactic acid (1.5 %) and polyphosphoric acid (0.1 %), tri-sodium phosphate (1.5 %), sodium hypochlorite (100 ppm)	Soybean sprouts	Cobo Molinos et al. (2008)
	E. coli, *S. sonnei*, *S. flexneri*, *E. aerogenes*, *Y. enterocolitica* and *A. hydrophila*	25 μg.mL^{-1}	Polyphosphoric acid (different concentrations for each species between 0.2 and 2 %)		

"Generally recognized as Safe" (GRAS) additive (Federal Register 1988). Bacteriocins can be used in several products foods, such as meat, chicken, dairy, eggs, seafoods, fruit and vegetables products (Gálvez et al. 2014). Its use increases the food security and the shelf life of foods (Allende et al. 2007; Deegan et al. 2006). They are produced in foods, by activity of bacteriocin-producing strains or are added in foods, as food preservatives (Settanni and Corsetti 2008). Its activity can be influenced by several factors, such as environmental conditions of storage of food, food components, solubility and food additives (Settanni and Corsetti 2008). The sensibility of target cell can be also altered due to changes in cell composition in response to environmental conditions (Kawasaki 2012).

Classification and mechanism of action of the bacteriocins

Bacteriocins can be subdivided into classes that reflect the similarities and differences of the molecules (Cleveland et al. 2001; Garcia et al. 2010). Class I, also known as lantibiotics, contains small peptides, of 19 to 50 amino acids and a molecular mass of less than 5 kDa (Table 3) (Cleveland et al. 2001). This group is characterized by the presence of unusual amino acids residues, such as lanthionine (Lan) and methyl lanthionine (MeLan), that result from post-translational modifications (Cleveland et al. 2001; Guder et al. 2000; Sahl and Bierbaum 1998). Lantibiotics are subdivided into subclasses or subtypes according to their chemical structure and activity (Kraaij et al. 1999). Subclass A is composed of amphipathic peptides that are elongated and flexible, with capacity to form pores, such as nisin, galidermin and bovicin HC5. Lantibiotics grouped in subclass B are smaller and globular, such as mesarcidin and cinnamicin, and can inhibit specific enzymes (Kraaij et al. 1999).

Class II bacteriocins are composed of thermostable peptides, containing 20 to 60 amino acids, without post-translational modifications and molecular mass lower than 10 kDa (Table 3) (Deegan et al. 2006; Héchard and Sahl 2002). Generally, this class is subdivided into subclasses: subclass IIa is composed of peptides that demonstrate activity against *Listeria* and exhibit a common N-terminal domain (Tyr-Gly-Asn-Gly-Val-X-Cys). Examples of this group include the carnobacteriocin BM1 and the piscicolin 126 (Cintas et al. 2001; Ennahar et al. 2000; Héchard and Sahl 2002). Subclass IIb is composed of bacteriocins that are formed by two peptides that act synergistically, such as lactocin 705, in which the active molecule is formed by the interaction of two peptides, of 33 amino acids residues each, called 705α and 705β (Castellano et al. 2003). Subclass IIc includes circular peptides that are dependent on the Sec system, such as carnocyclin A and enterocin AS-48 (Cintas et al. 2001; Héchard and Sahl 2002).

Table 3 General characteristics of bacteriocins used against Gram-negative bacteria

Classification		Bacteriocin	Producer strain	Biochemical characteristics	Mode of action	Reference
Lantibiotic		Nisin	*Lactococcus lactis* subsp lactis	Amphipathic peptide, elongated, positively charged, of 34 amino acid residues and molecular mass of 3.3 kDa. Contains unusual amino acids (lanthionine and methyl-lanthionine), derived from post-translational modifications. Stable to heat and to pH (between 2.0 and 7.0).	Formation of pores in the cytoplasmic membrane and inhibition of cell wall synthesis.	Liu and Hansen (1990), Delves-Broughton et al. (1996), Breukink and Kruijff (1999)
		Gallidermin	*Staphylococcus gallinarum*	Peptide of 22 amino acid residues, similar to epidermin		Kellner et al. (1988), Gotz et al. (2014)
		Bovicin HC5	*Streptococus bovis* HC5	Peptide of 2.4 kDa, about 22 amino acid residues. Stable to heat and acidic pH.		Mantovani et al. (2002)
-[a]		Cerein 8A	*Bacillus cereus* 8A	Peptide with molecular mass of 26 kDa. Stable at temperatures below 80 °C and pH between 2.0 and 11.0	Possible formation of pores in the cytoplasmic membrane.	Bizani and Brandelli (2002)
Class II	IIc	Carnocyclin A	*Carnobacterium maltaromaticum*	Circular peptide of 60 amino acid residues and molecular mass of 5.9 kDa. Stable to heat and pH between 2.0 and 12.0.	Formation of pores in the cytoplasmic membrane.	Martin-Visscher et al. (2008; 2011)
	IIa	Carnobacteriocin BM1	*C. maltaromaticum*	Peptide of 43 amino acids residues and molecular mass of 4.5 kDa.		Quadri et al. (1994)
	IIc	Enterocin AS-48	*Enterococcus faecalis* subsp. *liquefaciens* A 48–32	Circular cationic peptide of 70 amino acids residues, molecular mass of 7.2 kDa. Presents globular structure, formed by five helices (α_1 a α_5), with pI 10.5. Stable to heat and acidic pH.		Samyn et al. (1994), Gonzalez et al. (2000), Cobos et al. (2001)
	IIb	Lactocin 705	*Lactobacillus casei* CRL705	Its activity depends of complementation of two peptides, called 705α and 705β, of 33 amino acids residues each.		Castellano et al. (2003)
	IIa	Piscicolin 126	*C. maltaromaticum*	Peptide of 44 amino acids residues and molecular mass of 4.4 kDa. Stable to heat and acidic pH, but inactivated at neutral and alkaline pH.		Jack et al. (1996)

[a] Not determined

Generally, bacteriocins act in sensitive cells by forming pores in the cytoplasmic membrane, which causes an efflux of intracellular metabolites, such as potassium and amino acids, resulting in the depolarization of the membrane and, consequently, cellular death (Helander and Mattila-Sandholm 2000; Nes et al. 2006). During the anchoring in the cytoplasmic membrane, the bacteriocins can use a specific receptor, which explain the high efficiency in vivo. Nisin, gallidermin and bovicin HC5 appear to use the same receptor molecule, lipid II (Bonelli et al. 2006; Hasper et al. 2004; McAuliffe et al. 2001; Paiva et al. 2011; Wiedemann et al. 2001). Thus, these bacteriocins also interfere with the biosynthesis of peptidoglycan (Guder et al. 2000). Because the lipid II is a molecule that is highly conserved among prokaryotes, if these peptides have access to the cytoplasmic membrane, they will act more efficiently than those peptides that require specific receptors for anchoring to the membrane, such as carnobacteriocin BM1 and piscicolin 126 that use the mannose phosphotransferase system (Martin-Visscher et al. 2011). In this case, the amino acid sequence of the receptor may vary among different bacterial species, which can lead to differences in the sensitivity of target cells (Martin-Visscher et al. 2011).

Despite some similarities among the bacteriocin molecules of the same class, the sensitivity of target cells varies. These variations are not only due to changes in the bacteriocin molecule but also to differences in the lipid composition of the target cell membrane (Nissen-Meyer and Nes 1997).

Bacteriocins combined with food preservatives

Food preservatives are substances added to products to extend the storage life, by prevent or retard the deterioration of odor, color, texture, flavor, appearance, safety and nutritive value (Richter et al. 1993). Examples including the ethylenediaminetetraacetic acid (EDTA), which is widely used as a chelating agent to minimize reactions catalyzed by metals and acids and their salts, which used to minimize the growth of microorganism and with technologic function (Beales 2004; Branen and Davidson 2004; Chalón et al. 2012). These preservatives may act in destabilize of the outer membrane, by release of components of the structure or by intercalate in the membrane, permitting the bacteriocin action (Alakomi et al. 2000).

The use combined of the bacteriocins with EDTA is one of most common strategies in the sensitization of Gram-negative bacteria (Tables 1 and 2). EDTA acted promotes the release of the LPS layer (Alakomi et al. 2003). Details of the action mechanism are not yet understood, but it is known that there is at least partial disruption of the lipopolysaccharide layer (LPS), possibly due to binding to calcium and magnesium ions, which would establish a cross-link with sugar residues and phosphate radicals, inside the core polysaccharide, reinforcing the structure of the outer membrane (Alakomi et al. 2003; Branen and Davidson 2004). Thus, EDTA acts to enhance the bacterial activity of other antimicrobials, in addition to expanding the spectrum of action of bacteriocins, particularly against Gram-negative bacteria, such as *Salmonella*, *E. aerogenes*, *S. flexneri*, *C. freudii*, *E. coli*, *P. aeruginosa* and *A. butzleri* (Table 1) (Branen and Davidson 2004). Usually, low concentrations of EDTA (10 to 20 mM) are sufficient to produce sensitization for bacteriocin activity (Tables 1 and 2).

However, several factors can alter the sensibility of target cells, including the manner of administration of the chelating agent, the time of treatment and the environmental conditions of treatment (Boziaris and Adams 1999; Phillips and Duggan 2001; Prudêncio et al. 2015). The inhibition of microbial growth appears to be a time-dependent process, and the method of application can be a critical factor in obtaining the desired effect, it is recommended the simultaneous use of the bacteriocin and the chelating agent (Boziaris and Adams 1999; Phillips and Duggan 2001; Stevens et al. 1991). The activity of the chelating agent is also influenced by pH: at low pH, a large proportion of the carboxylate group is in its non-ionized form, which is not a particularly effective electron donor and the EDTA-metal complex is less stable. Therefore, the combination of EDTA with bacteriocins is more effective at near-neutral pH, although several peptides, such as nisin and bovicin HC5, are more efficient to low pH value (Ananou et al. 2005; Boziaris and Adams 1999; Houlihan et al. 2004; Lappe et al. 2009; Norhana et al. 2012; Prudêncio et al. 2015).

The sensitivity to the combination of bacteriocins and EDTA varies between different species of Gram-negative bacteria, and even between different strains of the same species. The concentration of chelating agent necessary for sensitization is also variable, possibly because of the differences in the structure of the LPS layer, which interfere with the permeability (Boziaris and Adams 1999). For use in foods, large quantities of the chelating agent may be required for the removal of exogenous divalent cations that are associated with the food system and for the effective sensitization of Gram-negative cells (Boziaris and Adams 1999; Lappe et al. 2009).

Other chemical compounds can also be used for the disruption of the outer membrane, such as acids and salts (Tables 1 and 2). Lactic, citric and polyphosphoric acids and their salts, as well as tri-sodium phosphate, act as disintegrating agents of the outer membrane and have demonstrated activity in the sensitization of Gram-negative bacteria, including *E. coli*, *S. sonnei*, *S. flexneri*, *E. aerogenes*, *Y. enterocolitica*, *A. hydrophila*, *Salmonella* and *A. butzleri* (Tables 1 and 2) (Alakomi et al. 2000; Belfiore et al. 2007; Cobo Molinos et al. 2008; Long and Phillips 2003; Phillips and Duggan 2001). These associations are efficient in foods,

and different methods of application can be employed, such as in a washing solution for sanitization or addition of bacteriocin with compounds, similar to food additives (Table 2) (Belfiore et al. 2007; Cobo Molinos et al. 2008).

Bacteriocins combined with plant essential oils

Plant essential oils are volatile complex natural substances, characterized by the presence of phenolic compounds with a strong odor that are produced by aromatic plants as secondary metabolites. Essential oils may have bactericidal, fungicidal, virucidal activities and medicinal properties, and have practical applications, such as analgesics, perfumes, anti-inflammatory agents, local anesthetics and food preservatives (Bakkali et al. 2008; Burt 2004).

Components of plant essential oils such as thymol and carvacrol, act on the bacterial membrane, resulting in important morphological alterations and in the depletion of the intracellular content (Govaris et al. 2010; Moosavy et al. 2008). The mechanism of action of such agents is still poorly understood, but it is known that thymol and carvacrol act to disintegrate the outer membrane, and their activity does not involve the chelation of divalent cations from the outer membrane, because compounds such as magnesium chloride do not interfere with their activity, in contrast to the action of EDTA (Helander et al. 1998).

However, the use of essential oils and their derivatives in foods is limited by sensory changes, because high concentrations are needed to exert antimicrobial activity (Govaris et al. 2010; Gutierrez et al. 2008; Nazer et al. 2005). Furthermore, food composition may influence the action of essential oils: high protein concentrations and moderately acidic pH result in an increase in the antimicrobial activity of oregano and thyme essential oils, while concentrations of potato starch or sunflower oil greater than 5 % reduce their efficiency (Gutierrez et al. 2008).

Thus, one alternative has been an association with other antimicrobial agents, such as bacteriocins. The effectiveness of this strategy has already been demonstrated with carvacrol and pediocin on *E. coli* O157:H7 (Turgis et al. 2012), and carvacrol and thymol and nisin on *S. enterica* Enteritidis, and others (Table 2) (Govaris et al. 2010). This strategy is viable, because of the consequent reduction of the amount of antimicrobial added to foods, and such a dual application prevents possible undesirable sensory changes due to the presence of large amounts of essential oils (Govaris et al. 2010; Nazer et al. 2005; Turgis et al. 2012). This was demonstrated on *S. enterica* Typhimurium and *S. aureus*, in which the presence of nisin considerably reduced the concentration of essential oil for the inhibition of both bacteria (Moosavy et al. 2008).

Bacteriocins combined with high pressure processing (HPP)

High pressure processing has been evaluated as a food pasteurization technique for inactivating microorganisms at room temperature, and thus minimizing the loss of sensory and nutritional components of the food (Huang et al. 2014; Masschalck et al. 2001). Under normal conditions, the process preserves the original color, flavor and nutritional content because smaller molecules such as pigments, vitamins, volatile compounds and others are less affected by high pressure (Huang et al. 2014).

The mode of action of HPP depends on the level of pressure. Pressures between 30 and 50 MPa can influence gene expression, protein synthesis and reduce the number of ribosomes. A pressure of 100 MPa induces partial protein denaturation, while 200 MPa causes damage to the cytoplasmic membrane and the internal cell structure. An increase of 300 MPa or more induces irreversible damage, such as the denaturation of enzymes and proteins and the rupture of the membrane (Garrida et al. 2002; Huang et al. 2014).

Microorganisms demonstrate differences in their resistance to pressure, depending on the species, strain, physiological state, processing temperature and substrate (Huang et al. 2014; Patterson 2005). As the different levels of pressure exert distinct effects, it is necessary to evaluate the response of each microorganism in each food system (Huang et al. 2014). In low-acid foods, vegetative cells exhibit great resistance to this process, requiring high pressure for the inactivation of microorganisms, which is not economically feasible and furthermore may causes changes in the sensory characteristics of foods, such as texture and color (Black et al. 2005; Garrida et al. 2002; Masschalck et al. 2001).

Generally, HPP treatment does not completely inactivate microorganisms, allowing the recovery of injured cells, but such recovery is dependent upon the treatment conditions and of the presence of other antimicrobial compounds (Patterson 2005). Food constituents may protect microbial cells against the increase in hydrostatic pressure or facilitate their recovery after treatment. Thus, to prevent the emergence of resistant cells, this technology has been used in conjunction with other antimicrobial compounds to ensure food safety. One of the alternatives is the use of bacteriocins, because high pressure destabilizes the outer membrane, increasing the activity of bacteriocins in Gram-negative cells. Furthermore, Gram-negative bacteria are more sensitive to HPP, whereas Gram-positive bacteria are more sensitive to bacteriocins. Thus, combined use is complementary (Garrida et al. 2002; Masschalck et al. 2001; Patterson 2005).

Several works have demonstrated the efficacy of this strategy and report an increase in bactericidal activity against important pathogens, such as *Salmonella* and *E. coli*, in addition to the spoilage microorganisms that are measured by the

total plate count (Tables 1 and 2) (Ponce et al. 1998; Rodriguez et al. 2005; Zhao et al. 2013). Moreover, pressure-resistant strains of *E. coli* have also demonstrated sensitivity to nisin, when treated under high pressure (Masschalck et al. 2000).

However, the process of sensitization can be transient, this is occurs only during the period in which the Gram-negative cells are subjected to high pressure. Therefore, the simultaneous administration of bacteriocin with high pressure is recommended (Black et al. 2005; Masschalck et al. 2001). The sensitivity of different species is variable and *P. fluorescens* demonstrated more sensitivity than *E. coli*, in milk (Black et al. 2005).

Bacteriocins combined with a pulsed electric field

The application of a pulsed electric field is considered a non-thermal technology that acts by forming reversible or irreversible pores in the cell membrane. This process improves the sensitization of Gram-negative bacteria to the action of bacteriocins, which work synergistically, increasing the damage to the cytoplasmic membrane (Table 2) (Viedma et al. 2008).

There is little information available about the effect of this strategy in Gram-negative bacteria. However, it is known that the efficiency of the treatment increases with temperature, possibly due to the increase in cell membrane fluidity, which facilitates the process of disorganization promoted by these treatments. However, this parameter can be maintained in mild temperatures. For example: for apple juice, a temperature of 40 °C is adequate, and such a temperature ensures sensory and nutritional qualities and reduces the costs of the process, which is of great interest for the food processing industry (Viedma et al. 2008).

As with other treatments, the method of application is an important parameter, with the simultaneous use of pulsed electric fields with bacteriocins being more efficient. This is primarily due to the high resistance of Gram-negative bacteria to treatments with pulsed electric fields, particularly at acidic pH, a condition under which bacteriocins demonstrate greater efficiency (Boziaris and Adams 1999; Houlihan et al. 2004; Viedma et al. 2008).

Bacteriocins combined with temperature treatments

Temperature treatments may promote perturbations in the outer membrane, either at low or high temperatures, favoring the action of bacteriocins.

A reduction in temperature promotes a change in the structure of the outer membrane. These alterations may permeabilize the cell to bacteriocin, allowing the nisin to act on *S.* Typhimurium and *E. coli* at refrigeration temperatures (Elliason and Tatini 1999; Prudêncio et al. 2015). The chilling process only allows the effective sensitization of Gram-negative bacteria to the action of bacteriocins, when the temperature drops rapidly, because there is not enough time for the reorganization of the outer membrane, altering its permeability. It has been demonstrated that the growth phase of *E. coli* influences the concentration of bacteriocin required for inhibition. Thus, cells in stationary phase under rapid chilling require higher concentrations of nisin (Cao-Hoang et al. 2008). As observed with other strategies, simultaneous treatment with bacteriocins has a more significant result than the sequential use (Cao-Hoang et al. 2008).

The bactericidal effect varies depending on the strains, bacteriocins and methodologies used, as demonstrated by the results of Kalchayanand et al. (1992), wherein *S. enterica* Typhimurium and *Y. enterocolitica* were more sensitive to nisin, while *A. hydrophila* and *Pseudomonas putida* were more sensitive to pediocin during freezing.

Heat also sensitizes Gram-negative bacteria to the action of bacteriocins (Boziaris et al. 1998; Phillips and Duggan 2002). The application of bacteriocin after heat treatment resulted in a synergistic effect, and unlike other treatments, simultaneous use only slightly increases the reduction in viability (Ueckert et al. 1998). Differences related to strains and bacteriocins were also observed with the use of heat, and pediocin was generally more efficient than nisin (Kalchayanand et al. 1992).

Conclusions

Currently, there is a large number of characterized bacteriocins with potential use in food. However, the effectiveness of this strategy depends on several factors, such as pH, temperature, food composition and target microbiota. Therefore, it is necessary to establish effective conditions for the use of each bacteriocin in each food matrix.

Although several agents and/or treatments used in combination with bacteriocins inhibit bacterial growth alone, the presence of these peptides provides an additional level of protection by preventing the growth of cells affected by sublethal damage, thus ensuring greater safety for the food product.

The use of bacteriocins in association with chemical compounds or physical treatments allows us to extend the spectrum of action of these peptides on Gram-negative bacteria, in addition to minimizing the emergence of resistant cells. However, for effective application of this technology, more studies are necessary, with different food matrices and in mixed cultures, to understand how bacteria can survive and adapt in a complex environment.

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References

Alakomi HL, Saarela M, Helander IM (2003) Effect of EDTA on *Salmonella enterica* serovar Typhimurium involves a component not assignable to lipopolysaccharide release. Microbiology 149: 2015–2021

Alakomi HL, Skytta E, Saarela M, Mattila-Sandholm T, Latva-Kala K, Helander IM (2000) Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. Appl Environ Microbiol 66(5):2001–2005

Allende A, Martínez B, Selma MV, Gil MI, Suárez JE, Rodríguez A (2007) Growth and bacteriocin production by lactic acid bacteria in vegetable broth and their effectiveness at reducing *Listeria monocytogenes* in vitro and in fresh-cut lettuce. Food Microbiol 24:759–766

Ananou S, Gálvez A, Martínez-Bueno M, Maqueda M, Valdivia E (2005) Synergistic effect of enterocin AS-48 in combination with outer membrane permeabilizing treatments against *Escherichia coli* O157:H7. J Appl Microbiol 99:1364–1372

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils—a review. Food Chem Toxicol 46:446–475

Beales N (2004) Adaptation of microorganisms to cold temperatures, weak acid preservatives, low pH and osmotic stress: a review. Compr Rev Food Sci F 3:1–20

Belfiore C, Castellano P, Vignolo G (2007) Reduction of *Escherichia coli* population following treatment with bacteriocins from lactic acid bacteria and chelators. Food Microbiol 24:223–229

Bizani D, Brandelli A (2002) Characterization of a bacteriocin produced by a newly isolated *Bacillus* sp. strain 8A. J Appl Microbiol 93:512–519

Black EP, Kelly AL, Fitzgerald GF (2005) The combined effect of high pressure and nisin on inactivation of microorganisms in milk. Innov Food Sci Emerg Technol 6:286–292

Bonelli RR, Schneider T, Sahl HG, Wiedemann I (2006) Insights into in vivo activities of lantibiotics from gallidermin and epidermin mode of action studies. Antimicrob Agents Chemother 50(4):449–1457

Boziaris IS, Adams MR (1999) Effect of chelators and nisin produced in situ on inhibition and inactivation of Gram-negatives. Int J Food Microbiol 53:105–113

Boziaris IS, Humpheson L, Adams MR (1998) Effect of nisin on heat injury and inactivation of *Salmonella enteritidis* PT4. Int J Food Microbiol 43:7–13

Branen JK, Davidson MP (2004) Enhancement of nisin, lysozyme, and monolaurin antimicrobial activities by ethylenediaminetetraacetic acid and lactoferrin. Int J Food Microbiol 90:63–74

Breukink E, Kruijff B (1999) The lantibiotic nisin, a special case or not? Biochim Byophis Acta 1462:223–234

Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods - a review. Int J Food Microbiol 94:223–253

Cao-Hoang L, Marechal PA, Le-Thanh M (2008) Synergistic action of rapid chilling and nisin on the inactivation of *Escherichia coli*. Appl Microbiol Biotechnol 79:105–109

Castellano P, Raya R, Vignolo G (2003) Mode of action of lactocin 705, a two-component bacteriocin from *Lactobacillus casei* CRL705. Int J Food Microbiol 85:35–43

Chalón MC, Acuña L, Morero RD, Minahk CJ, Bellomio A (2012) Membrane-active bacteriocins to control of *Salmonella* in foods are they the definitive hurdle? Food Res Int 45:735–744

Cintas LM, Casaus MP, Herranz C, Nes IF, Hernández PE (2001) Review: bacteriocins of lactic acid bacteria. Food Sci Technol Int 7(4):281–305

Cleveland J, Montville TJ, Nes IF, Chikindas ML (2001) Bacteriocins: safe, natural antimicrobial for food preservation. Int J Food Microbiol 71:1–20

Cobo Molinos A, Abriouel H, López RL, Valdivia E, Omar NB, Gálvez A (2008) Combined physico-chemical treatments based on enterocin AS-48 for inactivation of Gram-negative bacteria in soybean sprouts. Food Chem Toxicol 46(8):2912–2921

Cobos ES, Filimonov VV, Gálvez A, Maqueda M, Valdivia E, Martinez JC, Mateo PL (2001) AS-48: a circular protein with an extremely stable globular structure. FEBS Lett 505:379–382

Deegan LH, Cotter PD, Hill C, Ross P (2006) Bacteriocins: biological tools for bio-preservation and shelf-life extension. Int Dairy J 16: 1058–1071

Delves-Broughton J, Blackburn P, Evans RJ, Hugenholtz J (1996) Applications of the bacteriocin, nisin. Antonie Van Leeuwenhoek 69:193–202

Dischinger J, Chipalu SB, Bierbaum G (2014) Lantibiotics: promising candidates for future applications in health care. Int J Med Microbiol 304:51–62

Economou T, Pournis N, Ntzimani A, Savvaidis IN (2009) Nisin-EDTA treatments and modified atmosphere packaging to increase fresh chicken meat shelf-life. Food Chem 114:1470–1476

Elliason DJ, Tatini SR (1999) Enhanced inactivation of *Salmonella* typhimurium and verotoxigenic *Escherichia coli* by nisin at 6.5 °C. Food Microbiol 16:257–267

Ennahar S, Sashihara T, Sonomoto K, Ishizaki A (2000) Class IIa bacteriocins: biosynthesis, structure and activity. FEMS Microbiol Rev 24:85–106

Federal Register (1988) Nisin preparation: affirmation of GRAS status as a direct human food ingredient. Fed Regist 54:11247–11251

Gálvez A, Abriouel H, López RL, Omar NB (2007) Bacteriocin-based strategies for food preservation. Int J Food Microbiol 120:51–70

Gálvez A, Burgos MJG, López RL, Pulido RP (2014) Food Biopreservation. Springer, London

Garcia P, Rodríguez L, Rodríguez A, Martínez B (2010) Food biopreservation: promising strategies using bacteriocins, bacteriophages and endolysins. Trends Food Sci Technol 21:373–382

Garrida M, Aymerich MT, Costa S, Monfort JM, Hugas M (2002) Bactericidal synergism through bacteriocins and high pressure in a meat model system during storage. Food Microbiol 19:509–518

Gonzalez C, Langdon GM, Bruix M, Gálvez A, Valdivia E, Maqueda M, Rico M (2000) Bacteriocin AS-48, a microbial cyclic polypeptide structurally and functionally related to mammalian NK-lysin. Proc Natl Acad Sci U S A 97(21):11221–11226

Gotz F, Perconti S, Popella P, Werner R, Schlag M (2014) Epidermin and gallidermin: staphylococcal lantibiotics. Int J Med Microbiol 304:63–71

Govaris A, Solomakos N, Pexara A, Chatzopoulou PS (2010) The antimicrobial effect of oregano essential oil, nisin and their combination against *Salmonella* Enteritidis em minced sheep meat during refrigerated storage. Int J Food Microbiol 137:175–180

Guder A, Wiedemann I, Sahl HG (2000) Posttranslationally modified bacteriocins—the lantibiotics. Pept Sci 55:62–73

Gutierrez J, Barry-Ryan C, Bourke P (2008) The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. Int J Food Microbiol 124:91–97

Gyawali R, Ibrahim SA (2014) Natural products as antimicrobial agents. Food Control 46:412–429

Hasper HE, Kruijff B, Breukink E (2004) Assembly and stability of nisin-lipid II pores. Biochemistry 43:11567–11575

Héchard Y, Sahl H (2002) Mode of action of modified and unmodified bacteriocins from gram-positive bacteria. Biochimie 84:545–557

Helander IM, Mattila-Sandholm T (2000) Permeability barrier of the gram-negative bacterial outer membrane with special reference to nisin. Int J Food Microbiol 60:153–161

Helander IM, Alakomi HL, Latva-Kala K, Mattila-Sandholm T, Pol I, Smid EJ, Gorris LGM, Wrigh A (1998) Characterization of the action of selected essential oil components on Gram-negative bacteria. J Agric Food Chem 46:3590–3595

Houlihan AJ, Mantovani HC, Russell JB (2004) Effect of pH on the activity of bovicin HC5, a bacteriocin from *Streptococcus bovis* HC5. FEMS Microbiol Lett 231:27–32

Huang HW, Lung HM, Yang BB, Wang CY (2014) Responses of microorganisms to high hydrostatic pressure processing. Food Control 40:250–259

Jack RW, Wan J, Gordon J, Harmark K, Davidson BE, Hillier AJ, Wettenhall REH, Hickey MW, Coventry MJ (1996) Characterization of the chemical and antimicrobial properties of piscicolin 126, a bacteriocin produced by *Carnobacterium piscicola* JG126. Appl Environ Microbiol 62(8):2897–2903

Kalchayanand N, Hanlin MB, Ray B (1992) Sublethal injury makes gram-negative and resistant gram-positive bacteria sensitive to the bacteriocins, pediocin AcH and nisin. Lett Appl Microbiol 15:239–243

Kawasaki K (2012) Complexity of lipopolysaccharide modifications in *Salmonella enterica*: its effects on endotoxin activity, membrane permeability, and resistance to antimicrobial peptides. Food Res Int 45:493–501

Kellner R, Jung G, Horner T, Zahner H, Schnell N, Entian KD, Gotz F (1988) Gallidermin: a new lanthionine-containing polypeptide antibiotic. Eur J Biochem 177:53–59

Kraaij C, Vos WM, Siezen RJ, Kuipers OP (1999) Lantibiotics: biosynthesis, mode of action and applications. Nat Prod Rep 16:575–587

Lappe R, Motta AS, Sant'anna V, Brandelli A (2009) Inhibition of *Salmonella* Enteritidis by cerein 8A, EDTA and sodium lactate. Int J Food Microbiol 135:312–316

Liu W, Hansen JN (1990) Some chemical and physical properties of nisin, a small-protein antibiotic produced by *Lactococcus lactis*. Appl Environ Microbiol 56(8):2551–2558

Long C, Phillips CA (2003) The effect of sodium citrate, sodium lactate and nisin on the survival of *Arcobacter butzleri* NCTC 12481 on chicken. Food Microbiol 20:495–502

Mantovani HC, Hu H, Worobo RW, Russel JB (2002) Bovicin HC5, a bacteriocin from *Streptococcus bovis* HC5. Microbiology 148: 3347–3352

Martin-Visscher LA, van Belkum MJ, Garneau-Tsodikova S, Whittal RM, Zheng J, McMullen LM, Veredas RM (2008) Isolation and characterization of carnocyclin A, a novel circular bacteriocin produced by *Carnobacterium maltaromaticum* UAL307. Appl Environ Microbiol 74(15):4756–4763

Martin-Visscher LA, Yoganathan S, Sit CS, Lohans CT, Vederas JC (2011) The activity of bacteriocins from *Carnobacterium maltaromaticum* UAL307 against Gram-negative bacteria in combination with EDTA treatment. FEMS Microbiol Lett 317(2):152–159

Masschalck B, Garcia-Graells C, Haver EV, Michiels CW (2000) Inactivation of high pressure resistant *Escherichia coli* by lysozyme and nisin under high pressure. Innov Food Sci Emerg 1:39–47

Masschalck B, Houdt RV, Michiels CW (2001) High pressure increases bactericidal activity and spectrum of lactoferrin and nisin. Int J Food Microbiol 64:325–332

McAuliffe O, Ross RP, Hill C (2001) Lantibiotics: structure, biosynthesis and mode of action. FEMS Microbiol Rev 25:285–308

Meghrous J, Lacroix C, Simard RE (1999) The effects on vegetative cells and spores of three bacteriocins from lactic acid bacteria. Food Microbiol 16:105–114

Moosavy MH, Basti AA, Misaghi A, Salehi TZ, Abbasifar R, Mousavi HAE, Alipour M, Razavi NE, Gandomi H, Noori N (2008) Effect of *Zataria multiflora* Boiss. essential oil and nisin on *Salmonella* Typhimurium and *Staphylococcus aureus* in a food model system and on the bacterial membranes. Food Res Int 41:1050–1057

Nazer AI, Kobilinsky A, Tholozan J, Dubois-Brissonnet F (2005) Combinations of food antimicrobials at low levels to inhibit the growth of *Salmonella* sv. Typhimurium: a synergistic effect? Food Microbiol 22:391–398

Nes IF, Brede DA, Holo H (2006) The nonlantibiotic heat-stable bacteriocins in gram-positive bacteria. In: Kastin AJ (ed) Handbook of biologically active peptides. Academic, USA, pp 107–114

Nissen-Meyer J, Nes IF (1997) Ribosomally synthesized antimicrobial peptides: their function, structure, biogenesis, and mechanism of action. Arch Microbiol 167:67–77

Norhana MN, Poole SE, Deeth HC, Dykes GA (2012) Effects of nisin, EDTA and salts of organic acids on *Listeria monocytogenes*, *Salmonella* and native microflora on fresh vacuum packaged shrimps stored at 4 °C. Food Microbiol 31:43–50

Paiva AD, Breukink E, Mantovani HC (2011) Role of lipid II and membrane thickness in the mechanism of action of the lantibiotic bovicin HC5. Antimicrob Agents Chemother 55(11):5284–5293

Patterson MF (2005) Microbiology of pressure-treated foods. J Appl Microbiol 98:1400–1409

Phillips CA, Duggan J (2001) The effect of EDTA and trisodium phosphate, alone and in combination with nisin, on the growth of *Arcobacter butzleri* in culture. Food Microbiol 18:547–554

Phillips CA, Duggan J (2002) The effect of temperature and citric acid, alone, and in combination with nisin, on the growth of *Arcobacter butzleri* in culture. Food Control 13:463–468

Ponce E, Pla R, Sendra E, Guamis B, Mor-Mur M (1998) Combined effect of nisin and high hydrostatic pressure on destruction of *Listeria innocua* and *Escherichia coli* in liquid whole egg. Int J Food Microbiol 43:15–19

Prudêncio CV, Mantovani HC, Cecon PR, Vanetti MCD (2015) Differences in the antimicrobial activity of nisin and bovicin HC5 against *Salmonella* Typhimurium under different temperature and pH conditions. J Appl Microbiol 118:18–26

Prudêncio CV, Mantovani HC, Vanetti MCD (2014) Inhibition of *Salmonella* Typhimurium by bovicin HC5 associated with chelating agents and surfactants. Afr J Microbiol Res 8(1):12–18

Quadri LE, Sailer M, Roy KL, Veredas JC, Stiles ME (1994) Chemical and genetic characterization of bacteriocinas produced by *Carnobacterium piscicola* LV17B. J Biol Chem 269(16):12204–12211

Richter FL, Pedersen DE, Fredell DL, Reinhardt DJ (1993) Octanoic acid or derivatives. United States Patents US5234719 A

Rodriguez E, Arques JL, Nunez M, Gaya P, Medina M (2005) Combined effect of high-pressure treatments and bacteriocin-producing lactic acid bacteria on inactivation of *Escherichia coli* O157:H7 in raw-milk cheese. Appl Environ Microbiol 71(7):3399–3404

Sahl HG, Bierbaum G (1998) Lantibiotics: biosynthesis and biological activities of uniquely modified peptides from Gram-positive bacteria. Annu Rev Microbiol 52:41–79

Samyn B, Martinez-Bueno M, Devreese B, Maqueda M, Gálvez A, Valdivia E, Coyette J, Van Beeumen J (1994) The cyclic structure of the enterococcal peptide antibiotic AS-48. FEBS Lett 352:87–90

Settanni L, Corsetti A (2008) Application of bacteriocins in vegetable food biopreservation. Int J Food Microbiol 121:123–138

Stevens KA, Sheldon BW, Klapes NA, Klaenhammer TR (1991) Nisin treatment for inactivation of *Salmonella* species and other gram-negative bacteria. Appl Environ Microbiol 57(12):3613–3615

Turgis M, Vu KD, Dupont C, Lacroix M (2012) Combined antimicrobial effect of essential oils and bacteriocins against foodborne pathogens and food spoilage bacteria. Food Res Int 48:696–702

Ueckert JE, Steeg PF, Coote PJ (1998) Synergistic antibacterial action of heat in combination with nisin and magainin II amide. J Appl Microbiol 85:487–494

Ukuku DO, Zhang H, Huang L (2009) Growth parameters of *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes* and aerobic mesophilic bacteria of apple cider amended with nisin-EDTA. Foodborne Pathog Dis 6(4):487–494

Viedma PM, López AS, Omar NB, Abriouel H, López RL, Valdivia E, Belloso OM, Gálvez A (2008) Enhanced bactericidal effect of

enterocin AS-48 in combination with high intensity pulsed electric field treatment against *Salmonella enterica* in apple juice. Int J Food Microbiol 128:244–249

Wiedemann I, Breukink E, Van Kraaij C, Kuipers OP, Bierbaum G, Kruijff B, Sahl H (2001) Specific binding of nisin to the peptidoglycan precursor lipid II combines pore formation and inhibition of cell wall biosynthesis for potent antibiotic activity. J Biol Chem 276(1):1772–1779

Zhao L, Wang S, Liu F, Dong P, Huang W, Xiong L, Liao X (2013) Comparing the effects of high hydrostatic pressure and thermal pasteurization combined with nisin on the quality of cucumber juice drinks. Innov Food Sci Emerg 17:27–36