Biopreservation, an ecological approach to improve the safety and shelf-life of foods

S. Ananou[1], **M. Maqueda**[1], **M. Martínez-Bueno**[1] and **E. Valdivia**[1,2,*]

[1]Department of Microbiology, Faculty of Sciences, University of Granada, Campus Fuente Nueva s/n, 18071 Granada, Spain.
[2]Institute of Biotechnology, University of Granada, Campus Fuente Nueva s/n, 18071 Granada, Spain.

Biopreservation, defined as the extension of shelf life and enhanced safety of foods by the use of natural or controlled microbiota and/or antimicrobial compounds, is an innocuous and ecological approach to the problem of food preservation and has gained increasing attention in recent years. Consequently, certain lactic acid bacteria (LAB), with demonstrated antimicrobial properties commonly associated with foods, are being assayed to increase the safety and/or prolong the shelf life of foods. The antagonistic properties of LAB derive from competition for nutrients and the production of one or more antimicrobial active metabolites such as organic acids (lactic and acetic), hydrogen peroxide, and antimicrobial peptides (bacteriocins). Nowadays the use of LAB bacteriocins is considered an integral part of hurdle technology. Their combined use allows most pathogenic and spoilage bacteria to be controlled and also extend their inhibitory activity spectrum to such intrinsically resistant organisms as the Gram-negative bacteria.

Keywords Biopreservation; hurdle technology; lactic acid bacteria; bacteriocins.

1. Introduction

Modern technologies implemented in food processing and microbiological food-safety standards have diminished, but not altogether eliminated, the likelihood of food-related illness and product spoilage in industrialized countries. The increasing consumption of precooked food, prone to temperature abuse, and the importation of raw foods from developing countries are among the main causes of this situation. Hence, in Europe, morbidity from foodborne illnesses is second only to respiratory diseases, with estimates of 50,000 to 300,000 cases of acute gastroenteritis per million population every year [1]. The 7th report (1993-1998) of WHO's (World Health Organization) surveillance programme for the control of foodborne infections and intoxications in Europe has documented 5517 of outbreaks of food poisoning in Spain in that period, with 69553 people affected and 6820 hospitalized [2]. In the USA, acute gastroenteritis affects 250 to 350 million people annually, and an estimated 22% to 30% of these cases are thought to be foodborne diseases with the main foods implicated including meat, poultry, eggs, seafood, and dairy products [3]. According to data from the Centres for Disease Control and Prevention, it has been estimated that approximately one in four Americans may experience some form of foodborne illness each year [4]. The bacterial pathogens that account for many of these cases include *Salmonella*, *Campylobacter jejuni*, *Escherichia coli* 0157:H7, *Listeria monocytogenes*, *Staphylococcus aureus,* and *Clostridium botulinum* [5]. Until now, approaches to seek improved food safety have relied on the search for more efficient chemical preservatives or on the application of more drastic physical treatments (e.g. high temperatures). Nevertheless, these types of solutions have many drawbacks: the proven toxicity of many of the commonest chemical preservatives (e.g. nitrites), the alteration of the organoleptic and nutritional properties of foods, and especially recent consumer trends in purchasing and consumption, with demands for safe but minimally processed products without additives.

To harmonize consumer demands with the necessary safety standards, traditional means of controlling microbial spoilage and safety hazards in foods are being replaced by combinations of innovative technologies that include biological antimicrobial systems such as lactic acid bacteria (LAB) and/or their bacteriocins. The use of LAB and/or their bacteriocins, either alone or in combination with mild

physicochemical treatments and low concentrations of traditional and natural chemical preservatives, may be an efficient way of extending shelf life and food safety through the inhibition of spoilage and pathogenic bacteria without altering the nutritional quality of raw materials and food products [6-9]. Hence, the last two decades have seen intensive investigation on LAB and their antimicrobial products to discover new bacteriocinogenic LAB strains that can be used in food preservation.

2. Biological methods for food preservation

Biopreservation, as commented above, can be defined as the extension of shelf life and food safety by the use of natural or controlled microbiota and/or their antimicrobial compounds [10]. One of the most common forms of food biopreservation is fermentation, a process based on the growth of microorganisms in foods, whether natural or added. These organisms mainly comprise lactic acid bacteria, which produce organic acids and other compounds that, in addition to antimicrobial properties, also confer unique flavours and textures to food products. Traditionally, a great number of foods have been protected against spoiling by natural processes of fermentation. Currently, fermented foods are increasing in popularity (60% of the diet in industrialized countries) [11] and, to assure the homogeneity, quality, and safety of products, they are produced by the intentional application in raw foods of different microbial systems (starter/protective cultures). Moreover, because of the improved organoleptic qualities of traditional fermented food, extensive research on its microbial biodiversity has been carried out with the goal of reproducing these qualities, which are attributed to native microbiota, in a controlled environment.

The starter cultures of fermented foods can be defined as preparations of one or several systems of microorganisms that are applied to initiate the process of fermentation during food manufacture [12], fundamentally in the dairy industry and, currently, extended to other fermented foods such as meat, spirits, vegetable products, and juices. The bacteria used are selected depending on food type with the aim of positively affecting the physical, chemical, and biological composition of foods, providing attractive flavour properties for the consumer. To be used as starter cultures, microorganisms must fulfil the standards of GRAS status (Generally Recognized As Safe by people and the scientific community) and present no pathogenic nor toxigenic potential. In addition, use must be standardized and reproducible [13]. The same cultures have been employed for different uses and under different conditions. For the starter cultures, generally LAB, metabolic activity, such as acid production in cheese, is of great technological importance, whereas antimicrobial activity is secondary. However, for the protective culture, generally LAB also, the objectives are the opposite and must always take into account an additional factor for safety as its implantation must reduce the risk of growth and survival of pathogenic microorganisms [11]. An ideal strain would fulfil both the metabolic and antimicrobial traits.

2.1 Lactic Acid Bacteria

LAB include the genera *Lactococcus*, *Streptococcus*, *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, *Carnobacterium, Aerococcus, Oenococcus, Tetragenococcus, Vagococcus*, and *Weisella* [14]. They form a natural group of Gram-positive, nonmotile, non-sporeforming, rod- and coccus-shaped organisms that can ferment carbohydrates to form chiefly lactic acid; they also have low proportions of G+C in their DNA (< 55%). LAB present attractive physiological properties and technological applications (resistance to bacteriophages [12], proteolytic activity, lactose and citrate fermentation, production of polysaccharides, high resistance to freezing and lyophilization, capacity for adhesion and colonization of the digestive mucosa, and production of antimicrobial substances).

In general, LAB have GRAS status and play an essential role in food fermentation given that a wide variety of strains are employed as starter cultures (or protective cultures) in the manufacture of dairy, meat, and vegetable products. The most important contribution of these microorganisms is the preservation of the nutritional qualities of the raw material through extended shelf life and the inhibition of spoilage and pathogenic bacteria. This contribution is due to competition for nutrients and the presence of inhibitor agents produced, including organic acids, hydrogen peroxide, and bacteriocins [15].

There are many reviews on reported examples of spoilage and pathogenic bacteria inhibition by bacteriocin-producing LAB [8, 9, 16, 17, 18, 19].

In addition to the food applications of LAB, various strains are considered to be probiotics. Probiotics can be described as a preparation of or a product containing viable, defined microorganisms in sufficient numbers to alter the microbiota (by implantation or colonization) in a compartment of the host and that exert beneficial health effects in this host [20]. In this regard, LAB fit many of requirements for a microorganism to be defined as an effective probiotic [21]. These requirements include the ability to: (a) adhere to cells; (b) exclude or reduce pathogenic adherence; (c) persist and multiply; (d) produce acids, hydrogen peroxide, and bacteriocins antagonistic to pathogen growth; (e) be safe, noninvasive, noncarcinogenic, and nonpathogenic; and (f) coaggregate to form a normal balanced flora. Strains that are used as probiotics for man have been isolated from the human gastrointestinal tract and usually belong to species of the genera *Lactobacillus* and *Bifidobacterium*. However, strains belonging to species of other LAB have been used in the past as probiotics as well, such as *E. faecium*, *E. faecalis*, *S. thermophilus*, *L. lactis* subsp. *lactis*, *Le. mesenteroides*, and *P. acidilactici* [19].

2.2 LAB bacteriocins

The antimicrobial ribosomally synthesized peptides produced by bacteria, including members of the LAB, are called bacteriocins. Such peptides are produced by many, if not all, bacterial species and kill closely related microorganisms [22]. Due to their nature, they are inactivated by proteases in the gastrointestinal tract. Most of the LAB bacteriocins identified so far are thermostable cationic molecules that have up to 60 amino acid residues and hydrophobic patches. Electrostatic interactions with negatively charged phosphate groups on target cell membranes are thought to contribute to the initial binding, forming pores and killing the cells after causing lethal damage and autolysin activation to digest the cellular wall [23, 24].
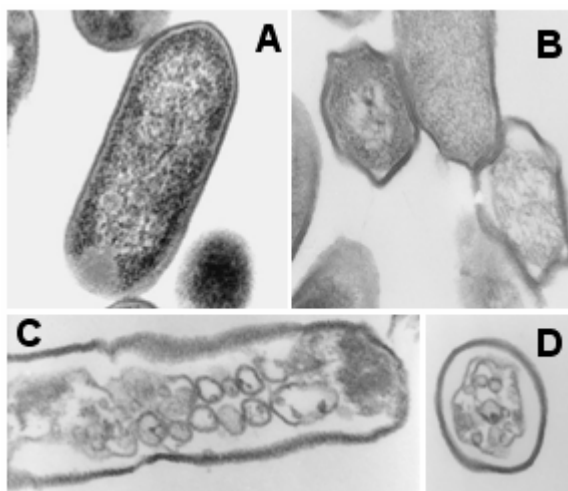

Fig. 1 Example of damage caused by bacteriocin on *L. monocytogenes* CECT 4032 cells. **(A)** cells without enterocin AS-48; **(B)** cells treated with 0.1 µg/ml of AS-48 for 2 h; **(C and D)** cells treated with 3 µg/ml of enterocin AS-48 for 10 min (adapted from [25].

The LAB bacteriocins have many attractive characteristics that make them suitable candidates for use as food preservatives, such as:

- Protein nature, inactivation by proteolytic enzymes of gastrointestinal tract
- Non-toxic to laboratory animals tested and generally non-immunogenic
- Inactive against eukaryotic cells
- Generally thermoresistant (can maintain antimicrobial activity after pasteurization and sterilization)

- Broad bactericidal activity affecting most of the Gram-positive bacteria and some, damaged, Gram-negative bacteria including various pathogens such as *L. monocytogenes, Bacillus cereus, S. aureus,* and *Salmonella*
- Genetic determinants generally located in plasmid, which facilitates genetic manipulation to increase the variety of natural peptide analogues with desirable characteristics

For these reasons, the use of bacteriocins has, in recent years, attracted considerable interest for use as biopreservatives in food, which has led to the discovery of an ever-increasing potential of these peptides. Undoubtedly, the most extensively studied bacteriocin is nisin, which has gained widespread applications in the food industry. This FDA-approved bacteriocin is produced by the GRAS microorganism *Lactococcus lactis* and is used as a food additive in at least 48 countries, particularly in processed cheese, dairy products and canned foods. Nisin is   effective against food-borne pathogens such as *L. monocytogenes* and many other Gram-positive spoilage microorganisms [26-30]. Nisin is listed in Spain as E-234, and may also be cited as nisin preservative or natural preservative. In addition to the work on nisin, several authors have outlined issues involved in the approval of new bacteriocins for food use [31-33].

2.2.1 Classification of LAB bacteriocins

LAB bacteriocins were divided into four classes by [34], with class II further divided into three subclasses. However, class IV was later eliminated and bacteriocins in class II were regrouped by different authors [35-37]. Recently, [38] have proposed four classes for Gram- positive bacteriocins that could also be applied to LAB bacteriocins.

Class I comprises the lantibiotics (lanthionine-containing peptides with antibiotic activity). They are small peptides that are differentiated from other bacteriocins by their content in dehydroamino acids and thioether amino acids. They include nisin, discovered in 1928 [39], lacticin 481 of *L. lactis* [40], citolysin of *E. faecalis* [41], and lacticin 3147 of *L. lactis* [42], among others.
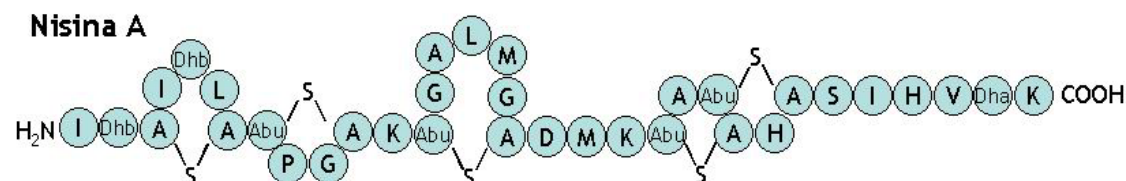

Fig. 2 Structure of nisin A.

Class II comprises the (<10 kDa) thermostable non-lantibiotic linear peptides. They are divided into three subclasses on the basis of either a distinctive N-terminal sequence, the pediocin-like bacteriocins (class II.1), the lack of leader peptide (class II.2), or neither of the above traits (class II.3). Examples of the three subclasses are pediocin PA-1/AcH produced by *Pediococcus* [43], enterocin EJ97 by *E. faecalis* [44], and enterocin L50A by *E. faecalis* [45], respectively.

Class III includes the large (> 30 KDa) heat-labile bacteriocins that encompass many bacteriolytic extracellular enzymes (hemolysins and muramidases) that may mimic the physiological activities of bacteriocins. Examples are helveticin J of *L. helveticus* [47] and bacteriocin Bc-48 of *E. faecalis* [48].
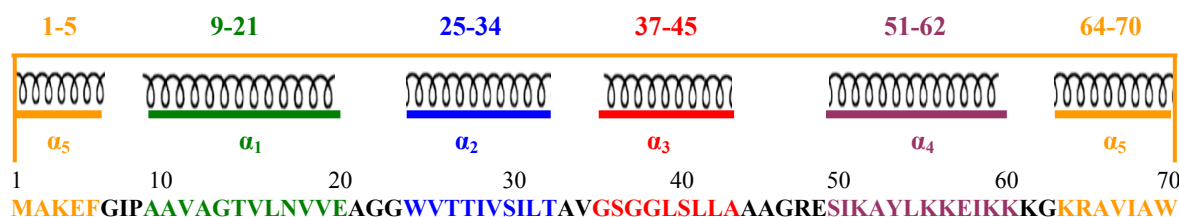
Fig. 3 Representation of primary (below) and secondary (above) structures of enterocin AS-48 with the head-to-tail ligation shown.

Class IV is a new class created to include the circular antibacterial peptide, an intriguing and novel type of antimicrobial substance produced not only by bacteria but also by plants and mammalian cells. The distinguishing characteristic is the existence of head-to-tail peptide chain ligation, which makes them molecules with neither an origin nor an end. The first circular protein described was the enterocin AS-48 (reviewed in [46]).

2.2.2 Effectiveness of bacteriocins in food systems

The application of bacteriocins, particularly nisin, in food systems has been extensively reviewed [8, 24, 27, 49, 50]. It is now known that the production and activity of bacteriocins in foods can be influenced by many factors:

- Factors negatively affecting production [50, 51] include: inadequate physical conditions and chemical composition of food (pH, temperature, nutrients, etc.); spontaneous loss in production capacity; inactivation by phage of the producing strain; and antagonism effect of other microorganisms in foods. Nisin, for example, is 228 times more soluble at pH 2 than at pH 8 [52].
- The effectiveness of bacteriocin activity in food is negatively affected by: resistance development of pathogens to the bacteriocin; inadequate environmental conditions for the biological activity; higher retention of the bacteriocin molecules by food system components (e.g. fat); inactivation by other additives; slower diffusion and solubility and/or irregular distribution of bacteriocin molecules in the meat matrix [50, 53].

2.2.3 Requirements and regulatory status for bacteriocins

In general, the following features should be considered when selecting bacteriocin-producing strains for food applications:

- The producing strain should preferably have GRAS status.
- Depending on the application, the bacteriocin should have a broad spectrum of inhibition that includes pathogens or else high specific activity.
- Thermostability.
- Beneficial effects and improved safety.
- No adverse effect on quality and flavour.

It is critical in some countries to distinguish bacteriocins from antibiotics since regulations often prohibit antibiotics in food [49]. The use of bacteriocin-producing starter cultures as ingredients may not require special consideration in many countries (e.g. USA) if the microorganism is GRAS. However, if a purified bacteriocin is used as a food preservative, the substance must be approved as GRAS, and for approval to be granted, the bacteriocin must be genetically and chemically identified and characterised, and its use and efficacy must be shown; the manufacturing process must be described and assays used for

quantification and standardization of the peptide must be shown as well. Toxicological data and the fate of the molecule after ingestion are also required.

3. Applications of bacteriocin-producing LAB in food

The strategies for the application of LAB and/or bacteriocins in food are diverse:

- Inoculation of food with LAB (starter cultures or protective cultures) where bacteriocins are produced *in situ*
- Use of food previously fermented with the bacteriocin-producing strains as an ingredient in the food processing (Nisaplin™, Microgard™, Alta™ 2341)
- Addition of purified or semipurified bacteriocins. The purified bacteriocins are considered additives and always require express authorization for their use [31]

The potential of bacteriocin-producing LAB and their bacteriocins, especially lactococci, pediococci, lactobacilli, and enterococci, to control undesirable microorganisms in food has been evaluated by a number of research groups. Although most bacteriocins have been isolated from food-associated LAB, they are not necessarily effective in all food systems. However, several bacteriocins certainly do have potential in food applications when used under the proper conditions. The following section will review examples where bacteriocin-producing cultures or their bacteriocins, which show potential for future applications, have successfully been employed to inhibit pathogenic microorganisms in a variety of food systems.

3.1 Application of bacteriocins in dairy products

Several researchers have demonstrated the effectiveness of nisin and/or nisin-producing strains against pathogenic bacteria such as *Clostridium butulinum* in cheese [54] and against *L. monocytogenes* in cheeses such as Camembert [55, 56], Ricotta [57], and Manchego [58].

Other bacteriocins have been tested in milk and dairy products, such as pediocin AcH in milk and Cheddar and Munster cheeses against *L. monocytogenes, S. aureus,* and *E. coli* O157:H7 [59-62], lacticin 3147 against undesirable LAB, *L. monocytogenes* and *B. cereus* in Cheddar, Cottage cheese and yogurt [9, 63-65], and enterocin AS-48 against *B. cereus, S. aureus* and *L. monocytogenes* in milk and Manchego cheese [66-68].

3.2 Applications in meat products

When evaluating a bacteriocin-producing culture for sausage fermentation and/or biopreservation, one must bear in mind that meat and meat products are complex systems with a number of factors influencing microbial growth and metabolite production. Therefore, the influence of formula and fermentation technology on the performance of bacteriocin-producing cultures needs to be assayed.

The most-studied bacteriocins in meat and meat products include nisin, enterocin AS-48, enterocins A and B, sakacin, leucocin A, and especially pediocin PA-l/AcH, alone or in combination with several physicochemical treatments, modified atmosphere packaging, high hydrostatic pressure, (HHP), heat, and chemical preservatives, as an additional hurdle to control the proliferation of *L. monocytogenes* and other pathogens [50, 69-74]. Furthermore, several bacteriocinogenic LAB have been used as bioprotective cultures for food manufacturing processes in attempts to control these pathogens [16, 73-79].

The data available on the use of nisin in cured and fermented meat are equivocal [80]. Compared to dairy products, nisin use in meat products has not been very successful because of its low solubility, irregular distribution, and lack of stability. Pediocin PA-l/AcH is more suitable for use in meat and meat products than nisin; however, *P. acidilactici* is not an indigenous meat strain [16].

3.3 Applications in vegetable products

Tests of bacteriocins in vegetable products include nisin in tinned vegetables and fruit juices [26, 61, 81], pediocin PA-1/AcH in salad and fruit juice [50, 61, 82], and enterocin AS-48 against *B. cereus* in rice and vegetables [83, 84] and in fruit juices against other pathogens such as *E. coli* O157:H7, *S. aureus,* and the spoilage bacterium *Alicyclobacillus acidoterrestris* [84-86].

3.4 Applications in fish

The deterioration of fresh fish is generally caused by Gram-negative microorganisms; however, in vacuum-packed fresh fish and seafood, pathogenic organisms such as *Clostridium botulinum* and *L. monocytogenes* can also cause problems. Scant work has focused on incorporating live bacteriocin-producing cultures into these products or on the addition of concentrated bacteriocin preparations. The combination of nisin and Microgard reduced the total aerobic bacteria populations of fresh chilled salmon, increased its shelf-life, and also reduced the growth of innoculated *L. monocytogenes* in frozen-thawed salmon [87]. The inhibition of *L. monocytogenes* was also confirmed with other bacteriocin-producer cultures such as *Carnobacterium divergens* [88]. [89] demonstrated the synergistic effect of combination lactic acid, sodium chloride, and/or nisin in rainbow trout, and more recently [90] showed the effect of LAB cultures on pathogenic microorganism control in fish.

4. Hurdle technology for food preservation

4.1 Hurdle concept and hurdle technology

The hurdle concept was introduced by Leistner in 1978 [91] and stated that the microbial safety, stability, sensorial, and nutritional qualities of foods are based on the application of combined preservative factors (called hurdles) that microorganisms present in the food are unable to overcome. Thus, hurdle technology refers to the combination of different preservation methods and processes to inhibit microbial growth. An intelligent application of this technology requires a better understanding of the occurrence and interaction of different hurdles in foods as well as the physiological responses of microorganisms during food preservation. Using an adequate mix of hurdles is not only economically attractive; it also serves to improve not only microbial stability and safety, but also the sensory and nutritional qualities of a food [92].

A novel concept-multitarget food preservation has emerged in relation to hurdle technology, based on the proven fact that, at times, different hurdles in food have not just an additive effect on microbial stability, but a synergistic one [93]. This approach may afford a nonagressive but more effective preservation of foods by the application of multiple soft treatments that disturb homeostasis and metabolic exhaustion and avoid stress reactions by bacteria. In practical terms, this means that it is more effective to employ different small-intensity preservation factors than one large-intensity preservation factor because the combined use of several preservation factors may produce a synergistic effect.

The principal hurdles employed in food safety are temperature (higher or lower), a_w, pH, Eh, chemical preservatives, vacuum packaging, modified atmosphere, HHP, UV, and competitive flora (LAB producing antimicrobial compounds).

4.2 Applications of hurdle technology

In the past and often still today, hurdle technology has been applied empirically without knowledge of the governing principles in the preservation of a particular food. In industrialized countries, hurdle technology is of great interest in the food industry for extending the shelf life and safety of minimally processed foods, such as those that display low fat contents and/or salt [94]. Similarly, it is applied in fermented or refrigerated foods in which low temperature is often the only hurdle to be overcome (e.g.

during distribution), which can lead to the alteration and intoxication of the foods. In developing countries, most foods are stored without refrigeration and are stabilized by the empiric use of hurdle technology. Several traditional foods have already been optimized by the intentional application of hurdles for safety and stability enhancement [95]. In addition, this technology is used for making new products and for reducing energy-consuming hurdles (e.g. refrigeration) or chemical preservatives (e.g. nitrites). The need to incorporate novel and effective combinations has spurred interest for natural and biological preservatives [96] such as LAB and their antimicrobial compounds.
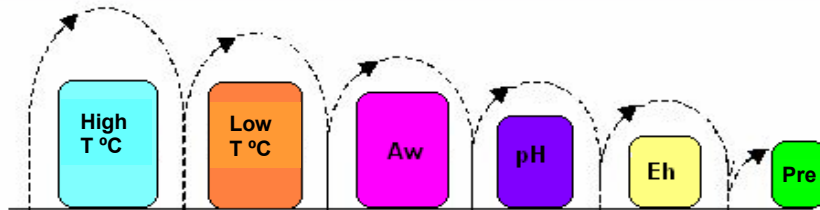

Fig. 4 Hurdle technology in food preservation (adapted from [96]). Example of food model with 6 hurdles: high temperature during processing (High T ºC), low temperature during storage (Low T ºC), limited water activity, acidity (pH), potential redox (Eh), and preservatives (Pre). LAB can contribute in two of these hurdles, a significant decrease in pH and the production of antimicrobial compounds (bacteriocins).

4.3 Applications of LAB bacteriocins in hurdle technology

Several authors [92, 95-97] have recommended the use of bacteriocins combined with other preservation methods to create a series of hurdles during the manufacturing process to reduce food spoilage by microorganisms. In fact, it has been proven that the application of chemical preservatives, physical treatments (heat), or new mild non-thermal physical methods (pulsed electric field, HHP, vacuum, or modified atmosphere packaging), which increase the permeability of cell membranes, positively affects the activity of many bacteriocins [72, 85, 98-100]. Notably, combined treatments of bacteriocins with selected hurdles affecting outer-membrane (OM) permeability increase the effectiveness of some LAB bacteriocins against Gram-negative cells, which are generally resistant. Concretely, the growth of Gram-negative pathogens such as *E. coli* O157:H7 and *Salmonella* can also be controlled when metal chelators, such as EDTA, sodium tripolyphosphate (STPP) or physical methods such as heat and HHP, are used in combination with bacteriocins [24, 61, 85, 98]. Table 1 presents several examples of the successful applications of LAB bacteriocins to control pathogen and spoilage microorganisms in different food systems.

Table 1 Examples of bacteriocins used as a part of hurdle technology to control pathogen and spoiling microorganisms in foods.

Bacteriocin	Other hurdles	Results	Reference
Nisin	HHP	Combination of HHP and nisin was effective to inactivate cheese indigenous microbiota. This combination was also effective against *S. carnosus* and *B. subtilis* spores, although a part of population survived the treatment	[101]
	pH and low temperature	A significant reduction in *L. innocua* was observed with a combination of low pH 5.5 and nisin at 20 ºC. However, nisin-resistant cells regrew. Additional hurdles, such as refrigeration temperature, caused a dramatic reduction in population and allowed an increase of storage time to 10 days in liquid cheese whey.	[102]
	Pulsed electric fields (PEF)	The addition of nisin prior to PEF treatment increased the susceptibility of *L. innocua* to PEF treatment in whey.	[103]
	Sodium citrate and sodium lactate	The combination of low temperature, sodium lactate and/or sodium citrate with nisin controls *Arcobacter butzleri* on chicken.	[104]
Pediocin AcH	HHP and high temperature	The combination of HHP , higher temperature, and pediocin acts synergistically, causing reduction of viability of *S. aureus*, *L. monocytogenes*, *E. coli* O157:H7, *Lb. sakei*, *Le. mesenteroides*	[105]
	Sodium diacetate	Combination of pediocin and sodium diacetate works synergistically against *L. monocytogenes* at room and low temperature	[106]
Enterocins A and B	HHP	Enterocins A and B were used in combination with HHP to the enhancement of safety in cooked ham against *L. monocytogenes*. Pathogen counts were below detection limits at the end of storage.	[72]
Enterocin AS-48	Heat treatment	The efficacy of AS-48 against *S. aureus* was greatly enhanced by combination with a moderate heat treatment in milk.	[68]
	STPP, lactic, acetic and citric acids	The combination of AS-48 and STPP or lactate acts synergistically against *S. aureus*. The activity of AS-48 increases in the presence of organic acids at pH 4.5. The combination with lactate reduces *S. aureus* population by 6 log units under neutral pH.	[107]
	Mild heat treatment, OM-permeabilizing agents or acidic/alkaline pH	The antimicrobial activity of AS-48 against *E. coli* O157:H7 enhanced by combination with mild heat treatment, OM-permeabilizing agents (EDTA and STPP), or under acidic or alkaline conditions in buffer and in apple juice.	[85]
	NaCl and low temperature	Highest effectiveness of AS-48 against *S. aureus* was obtained at 4 ºC in combination with high concentrations of NaCl (6 and 7%).	[100]

5. Conclusions

The use of bacteriocins and/or bacteriocin-producing strains of LAB are of great interest as they are generally recognized as safe organisms and their antimicrobial products as biopreservatives. However, it is desirable to continue to expand our understanding of the influences that environmental factors have on the implantation and survival of bacteriocinogenic strains and the activity of their bacteriocins in order to quantitatively estimate their efficacy for future applications in food model systems and establish adequate means of application of these biopreservatives.

References

[1] J. B. Luchansky, Antonie van Leeuwenhoek, **76:** 335 (1999).

[2] K., Schmidt, C. Tirado (Eds). Seventh report 1993-1998. WHO Surveillance programme for control of foodborne infections and intoxications in Europe Berlin, 2001, pp 321-333.

[3] P.S., Mead, L., Slutsker, V., Dietz, L.F., McCaig, J.S., Bresee, C., Shapiro, P.M., Griffin, R.V., Tauxe, Emerging Infectious Diseases **5**: 607-625 (1999).

[4] B.J. McCabe-Sellers, S.E. Beattie, Journal of American Dietetic Association **104**, 1708-1717 (2004).

[5] J.C., Buzby, T., Roberts, T., Jordan, C.T., Lin, and J.M., MacDonald, (Eds) Bacterial Foodborne Diseases: Medical Costs and Productivity Losses, Agricultural Economic Report Number 741, US Department of Agriculture, Washinton, DC, 1996.

[6] T.J. Montville, and K., Wikowski, In: M.P., Doyle, L.R. Beuchat, and T.J., Montville, (Eds), Food Microbiology Fundamentals and Frontiers. ASM Press, Washington DC, 1997, pp. 557-577.

[7] M., Hugas, M., Garriga, M., Pascual, M.T., Aymerich and J.M., Monfort, Food Microbiology **19**, 519-528. (2002).

[8] L., O'Sullivan, R.P., Ross, and C., Hill, Biochemistry **84,** 593- 604 (2002).

[9] R.P., Ross, S., Morgan, and C., Hill, International Journal of Food Microbiology **79,** 3-16 (2002).

[10] M.E. Stiles, Antonie van Leeuwenhoek **70,** 331-345 (1996).

[11] W.H., Holzapfel, R., Geisen, and U., Schillinger, International Journal of Food Microbiology **24,** 343-362 (1995).

[12] R.C., Wigley, In: R.K., Robinson, C.A., Batt, and P.D., Patel, (Eds). Encyclopaedia of Food Microbiology, Academic Press, Oxford, U.K., 1999, pp 2084-2095.

[13] C.R., Dass, In: R.K, Robinson, C.A., Batt, P.D., Patel, (Eds). Encyclopedia of Food Microbiology. Academic Press, Oxford, U.K., 1999, pp. 2095-2100.

[14] M.R., Adams Journal of Biotechnology **68,** 171-178 (1999).

[15] B., Ray, In: Ray, B. and Daeschel, M. (Eds). Food Biopreservatives of Microbial Origin, CRC Press. Boca Raton, 1992.

[16] M., Hugas, Meat Science **49,** 139-150 (1998).

[17] L.H., Deegan, P.D., Cotter, C., Hill, and P. Ross, International Dairy Journal **16,** 1058-1071 (2006).

[18] D. Drider, G. Fimland, Y. Héchard, L. M. McMullen, and H. Prévost Microbiology and Molecular Biology Reviews **70,** 564–582 (2006)

[19] M.R. Foulquié Moreno, P. Sarantinopoulos, E. Tsakalidou, L. De Vuyst, International Journal of Food Microbiology **106**, 1-24 (2006).

[20] De Vuyst, L., Avonts, L., Makras, E., C., Remacle, B., Reusens, (Eds.), Functional Foods, Ageing and Degenerative Disease. Woodhead Publishing Ltd., Cambridge, U.K., 2004, pp. 416-482.

[21] Salminen, S., Isolauri, E., Salminen, E., 1996. Antonie van Leeuwenhoek **70**, 347– 358.

[22] R.W., Jack, J.R., Tagg, and B., Ray, Microbiology Reviews **59**, 171-200 (1995).

[23] A., Gálvez, E., Valdivia, M., Martínez-Bueno, and M., Maqueda, Journal of Applied Bacteriology **69,** 406-413 (1990).

[24] T. Abee, FEMS Microbiology Letters **129,** 1-10(1995).

[25] F., Mendoza, M., Maqueda, A., Gálvez, M., Martínez-Bueno, and E., Valdivia, Applied and Environmental Microbiology **65,** 618-625 (1999).

[26] J., Delves-Broughton, Food Technology **44,** 100-117 (1990).

[27] J., Delves-Broughton, P., Blackbum, R.J., Evans, and J., Hugenholtz, Antonie van Leeuwenhoek **69,** 193-202 (1996).

[28] A., Turtell, and J., Delves-Broughton, Bulletin of International Dairy Federation **329,** 20-23 (1998).

[29] L.V., Thomas, M.R., Clarkson, and J., Delves-Broughton, In: L.V., Thomas, M.R. Clarkson, and J. Delves-Broughton (Eds). Natural food antimicrobial systems, CRC Press, A.S. Naidu, USA, 2000, pp. 463- 524.

[30] L.V., Thomas, and J., Delves-Broughton, Research Advances in Food Science **2,** 11-22 (2001).

[31] F.O., Fields, Journal of Food Protection **S,** 72-77 (1996).

[32] S.K., Harlander, Bacteriocins of Lactic Acid Bacteria, Academic Press, San Diego, CA, 1993, pp. 233–247.

[33] R., Post, Journal of Food Protection **Sup,** 78–81 (1996).

[34] T.R., Klaenhammer, FEMS Microbiology Reviews **12,** 39-86 (1993).

[35] I.F., Nes, D.B., Diep, L.S., Havarstein, M.B., Brurberg, V., Eijsink, and H., Holo, Antonie van Leeuwenhoek **70,** 113-128 (1996).

[36] M.J., Van Belkum, and M.E. Stiles, Natural Product Reports **17,** 323-335 (2000).

[37] D.B., Diep, and I.F., Nes, Current Drug Targets **3,** 107-122 (2002).

[38] N.C.K., Heng, P.A., Wescombe, J.P., Burton, R.W., Jack, and J.R., Tagg, In: M.A. Riley and M.A. Chavan (Eds.). Bacteriocins - ecology and evolution, Springer, Heidelberg, Germany, 2007, pp. 45-92.

[39] L.A., Rogers, and E.D., Whittier, Journal Bacteriology **16,** 211-229 (1928).

[40] J.C., Piard, P.M., Muriana, M.J., Desmazeaud, T.R., Klaenhammer, Applied and Environmental Microbiology **58**, 279-284 (1992).

[41] M.S., Gilmore, R.A., Segarra, and M.C., Booth, Infection and Immunity **58,** 3914-3923 (1990).

[42] M.P., Ryan, R.W., Jack, M., Josten, H.G., Sahl, G., Jung, R.P., Ross, and C., Hill, Journal of Biological Chemistry **247,** 37544-57550 (1999).

[43] J.T., Henderson, A.L., Chopko, and P.D., Wassenaar, Archives of Biochemistry and Biophysics **295,** 5-12 (1992).

[44] M., Sánchez-Hidalgo, M., Maqueda, A., Gálvez, H., Abriouel, E., Valdivia, and M., Martínez-Bueno, Applied and Environmental Microbiology **69,** 1633-1641 (2003).

[45] L.M., Cintas, P. Casaus, C. Herranz, L.S. Håvarstein, H. Holo, P.E. Hernández, and I.F. Nes. Journal of Bacteriology **182,** 6806-6814 (2000).

[46] M., Maqueda, A., Gálvez, M., Martínez-Bueno, M.J., Sánchez-Barrena, C., González, A., Albert, M., Rico, and E., Valdivia, Current Protein and Peptide Science **5,** 399-416 (2004).

[47] M.C., Joerger, and T.R., Klaenhammer, Journal of Bacteriology **167,** 439-446 (1986).

[48] I., López-Lara, A., Gálvez, M., Martínez-Bueno, M., Maqueda, and E., Valdivia, Canadian Journal of Microbiology **37,** 769-774 (1991).

[49] S., Wessels, B., Jelle, and I., Nes, Reports of the Danish Toxicology Centre, Denmark (1998).

[50] J., Cleveland, T.J., Montville, I.F., Nes, and M.L., Chikindas, International Journal of Food Microbiology **71,** 1-20 (2001).

[51] M.A. Daeschel, In: D.G. Hoover, and L.R. Steenson (Eds.). Bacteriocins of lactic acid bacteria. Academic Press Inc., New York, 1993, pp. 63-91.

[52] W., Liu, and J.N., Hansen, Applied and Environmental Microbiology **56,** 2551-2558. (1990).

[53] U., Schillinger, R., Geisen, and W.H., Holzapfel, Trends in Food Science and Technology **7,** 158-164 (1996).

[54] A., Hirsch, E., Grinsted, H.R., Chapman, and A.T.R., Mattick, Journal of Dairy Science **18,** 205-206 (1951).

[55] G., Sulzer, and M., Busse, International Journal of Food Microbiology **14,** 287-296 (1991).

[56] S., Maisnier-Patin, N., Deschamps, S.R., Tatini, and J., Richard, Le Lait **72,** 249-263 (1992).

[57] E.A., Davies, H.E., Bevis, and J., Delves-Broughton, Letters in Applied Microbiology **24,** 343-346 (1997).

[58] M., Núñez, J.L., Rodríguez, E., Garcia, P., Gaya, and M., Medina, Journal of Applied Microbiology **83,** 671-677 (1997).

[59] N., Buyong, J., Kok, and J.B., Luchansky, Applied and Environmental Microbiology **64,** 4842- 4845 (1998).

[60] S., Ennahar, O., Assobhei, and C., Hasselmann, Journal of Food Protection **61,** 186-191 (1998).

[61] H., Alpas, and F., Bozoglu, World Journal of Microbiology and Biotechnology **16,** 387-392, (2000).

[62] E., Rodríguez, J., Calzada, J.L., Arqués, J.M., Rodríguez, M., Núñez, and M., Medina, International Dairy Journal **15,** 51-57 (2005).

[63] M.P., Ryan, M.C., Rea, C., Hill, and R.P., Ross, Applied and Environmental Microbiology **62,** 612-619 (1996).

[64] O., McAuliffe, C., Hill, and R.P., Ross, Journal of Applied Microbiology **86,** 251-256 (1999).

[65] S.M., Morgan, M., Galvin, R.P. Ross, and C., Hill, Letters in Applied Microbiology **33,** 387-391 (2001).

[66] J.L., Rodríguez, P., Gaya, M., Medina, and M., Núñez, Journal of Food Protection **60,** 28- 32 (1997).

[67] A., Muñoz, M., Maqueda, A., Gálvez, M., Martínez-Bueno, A., Rodríguez, and E. Valdivia, Journal of Food Protection **67,** 1517-1521 (2004).

[68] A., Muñoz, S., Ananou, A., Gálvez, M., Martínez-Bueno, A., Rodríguez, M., Maqueda and E., Valdivia, International Dairy Journal, **17,** 760-769 (2007).

[69] J.W., Nielsen, J.S., Dickson, J.D. Crouse, Applied and Environmental Microbiology **56,** 2142-2145 (1990).

[70] C.N., Cutter, and G.R., Siragusa, Journal of Food Protection **57,** 97-103 (1994).

[71] T., Aymerich, M., Garriga, J., Ylla, J., Vallier, J.M. Monfort, and M. Hugas, Journal of Food Protection **63,** 721-726 (2000).

[72] M., Garriga, M.T., Aymerich, S., Costa, J.M. Monfort, and M., Hugas, Food Microbiology **19,** 509-518 (2002).

[73] S., Ananou, M., Garriga, M., Hugas, M., Maqueda, M., Martínez-Bueno, A., Gálvez, and E., Valdivia, International Journal of Food Microbiology **103,** 179-190 (2005a).

[74] S., Ananou, M., Maqueda, M., Martínez-Bueno, A., Gálvez, and E., Valdivia, Meat Science **71,** 549-556 (2005b).

[75] P.M., Foegeding, A.B., Thomas, D.H. Pilkington, and T.R., Klaenhammer, Applied and Environmental Microbiology **58,** 884-890 (1992).

[76] M., Campanini, I., Pedrazzoni, S., Barbuti, and P., Baldini, International Journal of Food Microbiology **20,** 169-175 (1993).

[77] M., Hugas, M., Garriga, M.T., Aymerich, and J.M., Montfort, Journal of Applied Bacteriology **79,** 322-330 (1995).

[78] M., Mataragas, E.H., Drosinos and J., Metaxopoulos, Food Microbiology **20,** 259-265 (2003).

[79] B.B., Budde, T., Hornbæk, T., Jacobsen, V. Barkholt, and A.G. Koch, International Journal of Food Microbiology **83,** 171-184 (2003).

[80] P.A. Vandenbergh, FEMS Microbiology Reviews. **12,** 221-238 (1993).

[81] E., Komitopulou, I.S., Boziris, E.A., Davies, J., Delves-Broughton, and M.R., Adams, International Journal of Food Science and Technology **34,** 81-85 (1999).

[82] E.R., Vedamuthu, J., Henderson, J., Marugg, and P., Van Wassear, In: Quest International Flavor and Food Ingredient Company, Bridgewater, NJ, USA, 1992, pp. 173-292.

[83] A., Cobo Molinos, H., Abriouel, N., Ben Omar, E., Valdivia, R., Lucas, M., Maqueda, M., Martínez-Cañamero, and A., Gálvez, Applied and Environmental Microbiology **71,** 7781-7787 (2005).

[84] M.J., Grande, R., Lucas, H., Abriouel, E., Valdivia, N., Ben Omar, M., Maqueda, M., Martínez-Bueno, M., Martínez-Cañamero and A., Gálvez, International Journal of Food Microbiology **106,** 185-194 (2006).

[85] S., Ananou, M., Maqueda, M., Martínez-Bueno, A., Gálvez, and E., Valdivia, Journal of Applied Microbiology **99,** 1364-1372 (2005c).

[86] M.J., Grande, R., Lucas, H., Abriouel, N., Ben Omar, M., Maqueda, M., Martínez-Bueno, M., Martínez-Cañamero, E., Valdivia, and A., Gálvez, International Journal of Food Microbiology **104,** 289-297 (2005).

[87] H. Zuckerman, and R. Ben Avraham, Lebensmittel-Wissenschaft und-Technologie **35,** 543-548 (2002).

[88] F., Duffesa, F., Leroib, P., Boyavalc, and X., Dousset, International Journal of Food Microbiology **47,** 33-4 (1999).

[89] A., Nykänen, A., Lapveteläinen, R.M., Heitanen, and H., Kallio, Lebensmittel-Wissenschaft und-Technologie **35,** 543-548 (1998).

[90] J.A., Vázquez, M.P., González, and M.A., Murado, Aquaculture **245,** 149-161 (2005).

[91] L., Leistner, In: W.K. Downey, (Ed.), Food Quality and Nutrition, Applied Science Publishers, London, U.K., pp. 553-557 (1978).

[92] L., Leistner, International Journal of Food Microbiology **55,** 181-186 (2000).

[93] L., Leistner, Principles and applications of hurdle technology, new methods of food preservation. G.W. Could, (ed.), Blackie Academic & Professional, London, 1995, pp. 1.

[94] L., Leistner, In: Pearson, A.M., Dutson, T.R. (Eds.), Production and Processing of Healthy meat, Poultry and Fish Products, Blackie Academic and Professional, London. U.K., 1997, pp 347-360.

[95] L., Leistner, In: B.M., Lund, A.C., Baird-Parker, and G.W. Gould, (Eds.). The Microbiological Safety of Foods, Aspen Publishers, Gaithersburg, Maryland. 1999a.

[96] L., Leistner, In: M., Shafiur Rahman, (Ed.), Handbook of Food Preservation, Marcel Dekker, New York, 1999b, pp. 457-485.

[97] S.P., Chawla, and R., Chander, Food Control **15,** 559-563 (2004).

[98] H., Abriouel, E., Valdivia, A., Gálvez, and M. Maqueda, Applied and Environmental Microbiology **64,** 4623-4626 (1998).

[99] S., Zhang, and A., Mustapha, Journal of Food Protection **62,** 1123–1127 (1999).

[100] S., Ananou, E., Valdivia, M., Martínez Bueno, A., Gálvez, and M., Maqueda, Journal of Applied Microbiology **97,** 48-56 (2004).

[101] M. Capellas, M. Mor-Mur, R. Gervilla, J. Yuste and B. Guamis, Food Microbiology **17,** 633-641 (2000).

[102] L.I. Gallo, A.M.R. Pilosof and R.J. Jagus, Food Control **18,** 1086-1092 (2007a).

[103] L.I. Gallo, A.M.R. Pilosof and R.J. Jagus, Journal of Food Engineering **79,** 188-193 (2007b).

[104] C. Long and C. A. Phillips, Food Microbiology **20,** 495-502 (2003).

[105] N. Kalchayanand, A. Sikes, C.P. Dunne and B. Ray, Food Microbiology **15,** 207-214 (1998).

[106] J.H. Schlyter, K.A. Glass, J. Loeffelholz, A.J. Degnan and J.B. Luchansky, International Journal of Food Microbiology **19,** 271-281 (1993).

[107] S., Ananou, M., Maqueda, M., Martínez Bueno, A., Gálvez, and E., Valdivia, Letters in Applied Microbiology *in press* (2007).