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Potential Applications of the Cyclic Peptide Enterocin AS-48 in the Preservation of Vegetable Foods and Beverages

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Abstract Bacteriocins are antimicrobial peptides produced by bacteria. Among them, the enterococcal bacteriocin (enterocin) AS-48 stands for its peculiar characteristics and broad-spectrum antimicrobial activity. AS-48 belongs to the class of circular bacteriocins and has been studied in depth in several aspects: peptide structure, genetic determinants, and mode of action. Recently, a wealth of knowledge has accumulated on the antibacterial activity of this bacteriocin against foodborne pathogenic and spoilage bacteria in food systems, especially in vegetable foods and drinks. This work provides a general overview on the results from tests carried out with AS-48 in different vegetable food categories (such as fruit juices, ciders, sport and energy drinks, fresh fruits and vegetables, pre-cooked ready to eat foods, canned vegetables, and bakery products). Depending on the food substrate, the bacteriocin has been tested alone or as part of hurdle technology, in combination with physico-chemical treatments (such as mild heat treatments or high-intensity pulsed electric fields) and other antimicrobial substances (such as essential oils, phenolic compounds, and chemical preservatives). Since the work carried out on bacteriocins in preservation of vegetable foods and drinks is much more limited compared to meat and dairy products, the results reported for AS-48 may open new possibilities in the field of bacteriocin applications.

Keywords Bacteriocin · Preservation · Vegetable foods · Drinks

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Introduction

Bacteriocins are defined in a broad sense as ribosomally synthesised antimicrobial peptides or proteins of bacterial origin [55]. Research on bacteriocins has provided relevant information on peptide structure, biosynthesis, protein secretion, mode of action, ecological function, and applications in food preservation and health [19, 27–29, 31, 41, 42, 67, 82, 83, 88, 90, 95]. Among the different bacteriocins studied to date, AS-48 stands, together with nisin, as one of the best-studied antimicrobial peptides. AS-48 is a cyclic peptide produced by several enterococcal isolates [35], including *Enterococcus faecalis* strains (such as S-48, EFS2, INIA 4, or 39-5) [37, 57, 64, 98] and *Enterococcus faecium* strains 7C5 and RJ16 [5, 34]. The physico-chemical properties, mode of action, and genetic determinants of this bacteriocin have been reviewed [66].

The structure of AS-48 is based on a 7.14-kDa cationic cyclic peptide linked by a head-to-tail peptide bond and folded into five alpha-helix regions [43, 86, 87] (Fig. 1). Such folded structure confers bacteriocin molecules a remarkable stability to extremes of pH, heat, and denaturing agents [26], a reason why AS-48 remains stable in food environments. Interestingly, AS-48 adopts different oligomeric conformations according to physiochemical conditions, existing in monomeric form at pH below 3.0 and in dimeric forms in the pH range of 4.5–8.5 [2, 87]. The dimeric states can form either by hydrophobic interactions

Fig. 1 The cyclic structure of enterocin AS-48

(DF-I) or by hydrophilic interactions (DF-II). Bacteriocin oligomerization is thought to be responsible for the high water solubility of this peptide (which is in the mg ml^{-1} range) [4].

AS-48 is a pore-forming peptide, which disrupts the bacterial cytoplasmic membrane and leads to dissipation of proton motive force [40]. It has been proposed that transition from dimeric state DF-1 to DF-II is responsible for insertion of AS-48 molecules into the bacterial membrane [87]. By contrast, AS-48 does not have any effect on any of the eukaryotic organisms tested so far, such as *Saccharomyces cerevisiae, Naegleria fowleri* or *Acanthamoeba* sp., even at concentrations as high as 100 μg ml^{-1} [38] nor the HeLa and MCDK cell lines nor erythrocytes [66], a reason why it is considered a safe antimicrobial for application in foods.

A relevant feature of AS-48 is the broad spectrum of antibacterial activity, involving most of the Gram-positive bacteria tested and also some Gram-negatives [38, 39]. In culture broths, addition of AS-48 alone or in combination with chemical preservatives or moderate heat treatments shows a remarkable bactericidal activity against foodborne pathogens such as *Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes,* or *Salmonella choleraesuis* [1, 3, 6, 78]. Furthermore, control of foodborne pathogens has been demonstrated in several food products of animal origin, including *L. monocytogenes* and *S. aureus* in sausages [7, 8] and cooked ham [11], *B. cereus* in cheese [80], and *L. monocytogenes* and/or *S. aureus* in skim milk and dairy products [12, 81]. Extensive work has been carried out on application of AS-48 for preservation of vegetable foods and drinks, as will be described further in this review.

Compared to meat and dairy products, vegetable foods and beverages still remain an emerging field for application of bacteriocins, where most trials have been based on inoculation with bacteriocinogenic cultures (mainly in fermented foods) and much less on addition of bacteriocin preparations, mainly nisin [41, 90]. The purpose of this review is to provide an overview of the recent progress on application of AS-48 for preservation of vegetable foods and drinks and the benefits of using this bacteriocin as a natural preservative.

Fruit Juices and Beverages

The thermophilic endospore former *Alicyclobacillus acidoterrestris* can withstand pasteurisation temperatures commonly applied during food processing and spoil freshly made juices as well as processed juices [91]. Laboratory trials have shown that addition of low AS-48 concentrations (2.5 μg ml^{-1}) to juices artificially contaminated with vegetative cells and endospores of this bacterium caused complete bacterial inactivation and afforded protection for up to 14 days in freshly made orange and apple juices and for up to 60–90 days in several commercial fruit juices [45] (Table 1). Treatment of endospores with AS-48 caused inhibition of germination and disorganisation of endospore structure [44, 45] (Fig. 2). Another report indicated that AS-48 addition caused rapid inactivation of the thermophilic sporeformer *Geobacillus stearothermophilus* in coconut milk and coconut water [71] (Table 1), strengthening the value of this bacteriocin for application in the preservation of fruit juices.

AS-48 showed strong bactericidal activity against bacteria causing ropiness and other alterations in apple juice and apple cider (Table 1). Vegetative cells of the rope-forming strain *Bacillus licheniformis* LMG 19409 isolated from spoiled Normand ciders [60] were rapidly inactivated by bacteriocin addition in fresh-made apple juice and in commercial apple ciders [46]. Although *B. licheniformis* endospores were resistant to AS-48, the combination of bacteriocin and moderate heat treatments (85–100°C) increased the heat inactivation of endospores in cider, decreasing *D* and *z* values [46]. These results suggest that AS-48 could be applied in combination with mild heat treatments to inactivate *B. licheniformis* endospores in ciders when a high contamination with endospores of this bacterium is suspected.

Lactic acid bacteria (LAB) may cause ropiness of ciders and produce off flavour precursors such as 3-hydroxypropionaldehyde (3-HPA), which may derive to acrolein [17, 18, 32, 89]. Since apple cider is made from fresh apple juice, application of heat treatments to inactivate apple cider-spoilage LAB is not satisfactory. AS-48 was tested against exopolysaccharide-producing lactic acid bacteria strains of *Lactobacillus collinoides, Lb. diolivorans*, and

Table 1 Antibacterial effects of AS-48 in fruit juices and beverages

Food category		Bacteriocin treatment/microbial inactivation effects	Reference(s)
Fruit juices	*Alicyclobacillus acidoterrestris*	• 2.5 μg ml^{-1} (vegetative cells and endospores): 3.5 log cycles	[45]
	Geobacillus stearothermophilus	• 1.75 μg ml^{-1}(vegetative cells and endospores): 2.5–3 log cycles	[71]
	Bacillus licheniformis	• 3 μg ml^{-1} (vegetative cells): 3.5–4 log cycles	[46]
		• 6–12 μg ml^{-1} + heat (95°C): decreases observed for *D* and *z* values for endospores	
	Lactobacillus collinoides Lactobacillus diolivorans Pediococcus parvulus	• 2.5–5 μg ml^{-1} (single cultures): 4.5–5.5 log cycles	[69, 72, 73]
		• 12.5 μg ml^{-1} (mixed cultures): 4.5–5 log cycles	
		• 0.175 μg ml^{-1} + HIPEF treatment (35 kV/cm, 150 Hz, 4 μs): 6.6 log cycles for pediococci	
		• 2 μg ml^{-1} + HIPEF treatment (35 kV/cm, 150 Hz, 4 μs): 4.9 log cycles for lactobacilli	
	Salmonella enterica	• 60 μg ml^{-1} + HIPEF treatment (35 kV/cm, 150 Hz, 4 μs, 40°C): 4.5 log cycles	[70]
	Escherichia coli O157:H7	• 50 μg ml^{-1} + EDTA, STPP, pH 5, pH 8.6, or heat: variable reductions of 3.8–8.1 log cycles for dual or multiple treatments	[9]
Sport and energy drinks	*Listeria monocytogenes*	• 1 μg ml^{-1}: 6 log cycles	[74]
	Bacillus licheniformis	• 12.5 μg ml^{-1} (vegetative cells): 4.5 log cycles	[74]
	Bacillus cereus	• 12.5–25 μg ml^{-1} (vegetative cells): 4 log cycles	[74]
	Staphylococcus aureus	• 12.5–25 μg ml^{-1}: 5 log cycles	[74]

Pediococcus parvulus as well as 3-HPA-producing *Lb. collinoides* strains isolated from spoiled apple ciders from the Basque country region of Spain [32, 69]. In fresh-made apple juice, most strains were rapidly inactivated by low bacteriocin concentrations (Table 1), although two strains (*Lb. collinoides* 5 and *Lb. diolivorans* 29) as well as mixed cultures of the different strains showed higher bacteriocin resistance.

Representative strains of cider-spoilage LAB were tested in apple juice with sub-inhibitory concentrations of AS-48 in combination with high-intensity pulsed electric field (HIPEF) treatment, a non-thermal process that is gaining acceptance in liquid foods [53, 54, 68, 79]. Based on the original article authors' assumption (no FIC numbers provided) AS-48 and HIPEF acted synergistically against lactobacilli and pediococci (Table 1). The combined treatments offered better results compared to single treatments, since no viable LAB were detected during storage of the processed samples [72, 73].

Although Gram-negative bacteria are partially or completely resistant to AS-48 because of the protective effect of the bacterial outer membrane, they can be inactivated by application of combined treatments. Addition of AS-48 alone had no effect on the viability of *Sal. enterica* in apple juice. By contrast, bactericidal activity of HIPEF treatments increased in combination with AS-48 (Table 1). Survival fraction was affected by different parameters such as treatment time and temperature, and AS-48 concentration. The maximum inactivation of 4.5-log cycles was achieved with HIPEF treatment for 1,000 μs in combination with AS-48 at a treatment temperature of 40°C [70]. The synergism reported by authors depended on the simultaneous action of AS-48 and HIPEF, while bacteriocin addition to HIPEF-treated cells had no killing effect [70]. This was attributed to resealing of transient membrane pores after the HIPEF treatment.

Several other combined treatments with AS-48 showed an increased bactericidal activity against Gram-negative bacteria in fruit juices (Table 1). *Escherichia coli* O157:H7 cells sublethally injured by outer membrane permeabilizing treatments (EDTA, sodium tripolyphosphate, pH 5, and moderate heat) became sensitive to AS-48. Highest bactericidal activity was observed when the bacteriocin was applied in multiple treatments [9].

Other beverage categories in which AS-48 could find potential applications are sport and energy drinks. The low pH and acid content of drinks has been shown to be detrimental for tooth health, causing enamel softening and tooth decay [96, 100, 102]. Therefore, drinks with lower acidity and higher pH values should be recommended. However, raising the pH of drinks may increase the risk of bacterial spoilage, requiring mild preservation treatments to preserve the bioactive components of drinks (such as vitamins and others). In trials carried out with low-acidity drinks, AS-48 rapidly inactivated *L. monocytogenes*, *B. cereus, B. licheniformis*, and *S. aureus* cells [74] (Table 1).

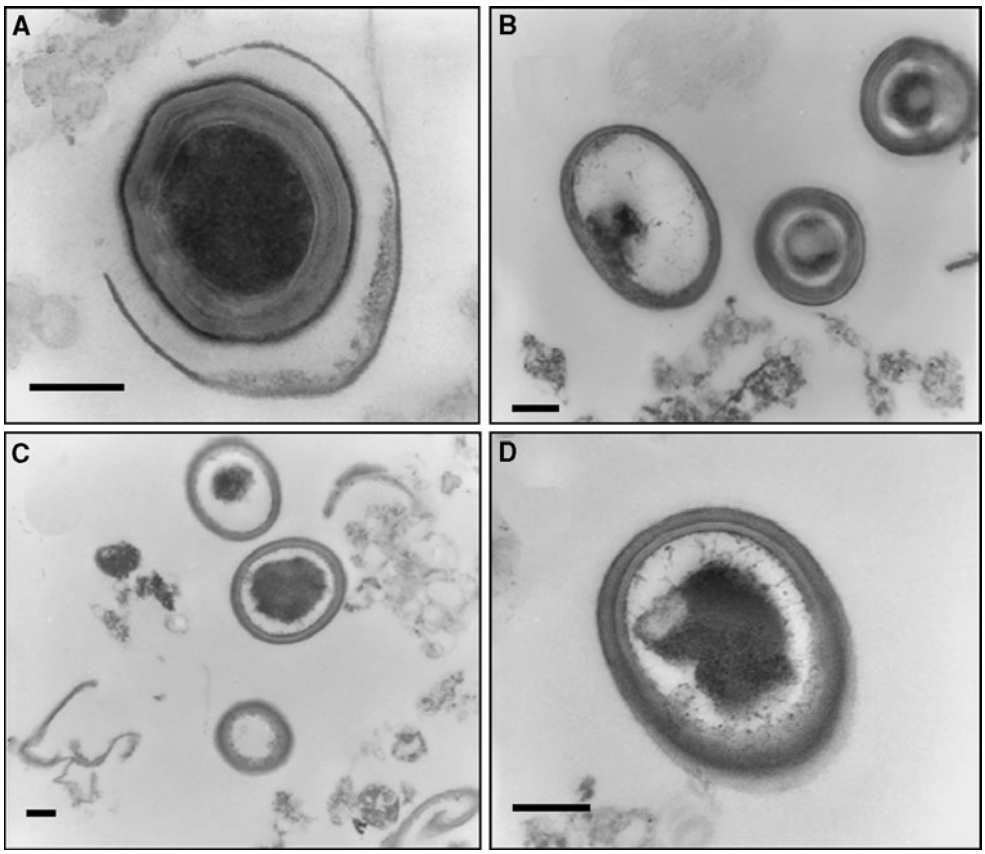

Fig. 2 Electron microscopy examination of *Alicyclobacillus acidoterrestris* DSMZ 2498 endospores treated with AS-48. Untreated control endospores (**a**), and samples of bacteriocin-treated preparations after 8 h (**b**) or 24 h of incubation (**c**, **d**). Bar = 0.2 μm [44]

The results suggested that AS-48 could be used as a natural preservative in less acidic sport and energy drinks.

Fresh-Cut Produce

Freshly cut vegetables and fruits may be involved in the transmission of pathogenic bacteria [30, 33, 52, 63, 84]. Since most of the fruits and a large percentage of the vegetables are consumed without further cooking, it is very important to apply disinfection treatments that, while having little or no effect on the food organoleptic properties, decrease the microbial load, inactivate microbial pathogens, and contribute to prolong the product shelf life. Application of washing treatments with AS-48 solutions caused significant inactivation ($P < 0.05$) of *L. monocytogenes* inoculated on whole or sliced fruits (strawberries, raspberries and blackberries, melon, watermelon, pear, and kiwi), but did not avoid proliferation of survivors during storage at abuse temperature [21]. Antilisteria activity of washing treatments increased greatly when AS-48 was applied in combination with several other antimicrobials (Table 2). The combinations of AS-48 (25 μg ml^{-1}) and carvacrol (12 mM) or n-propyl *p*-hydroxybenzoate (50 mM) also avoided regrowth of the listeria during storage of fruit slices at 22°C.

Consumption of seed sprouts is considered to have health-promoting effects, but at the same time, sprouts have been shown to act as vehicles for pathogenic bacteria [33, 52, 84]. For this reason, sprouts were chosen as a model food system to test the effects of AS-48 washing treatments against several bacterial species, alone or in combination with chemical preservatives. Immersion treatments (5 min at room temperature) with AS-48 solutions reduced viable counts of *L. monocytogenes* on artificially contaminated alfalfa and soybean sprouts by approximately 2.0–2.4 log CFU g^{-1} compared to a control treatment in distilled water [20] (Table 2). After bacteriocin treatments, no viable listeria were detected in most of the samples stored under refrigeration, but listeria proliferation was observed for samples stored at room temperature [20]. Antilisterial activity increased significantly when AS-48 was applied in combination with other antimicrobials, providing several options for decontamination treatments (Table 2).

Washing treatments with AS-48 solutions reduced the viable counts of *B. cereus* and *Bacillus weihenstephanensis*

Table 2 Antibacterial effects of washing treatments with AS-48 in fresh-cut produce

Food category		Bacteriocin treatment/microbial inactivation effects	Reference(s)
Fruit surfaces and sliced fruits	*Listeria monocytogenes*	• 25 μg ml^{-1}: 1.5–3 log cycles, depending on substrate • 25 μg ml^{-1} + chemical antimicrobials[a]: 2–2.5 log cycles; inhibition of regrowth during storage	[21]
Seed sprouts, green asparagus	*Listeria monocytogenes*	• 25 μg ml^{-1}: 2–2.5 log cycles • 25 μg ml^{-1} + chemical antimicrobials[b]: 2–2.5 log cycles; inhibition of regrowth during storage	[20]
	Bacillus cereus	• 25 μg ml^{-1} (vegetative cells): 1–1.5 log cycles 25 μg ml^{-1} + chemical antimicrobials[c] (vegetative cells): increased antimicrobial activity (up to 2.5 log cycles); inhibition of regrowth during storage	[22]
	Bacillus weihenstephanensis	• 25 μg ml^{-1} (vegetative cells: 1.5–2.4 log cycle reduction 25 μg ml^{-1} + chemical antimicrobials[d] (vegetative cells increased antimicrobial activity (up to 2.5 log cycles); inhibition of regrowth during storage	[22]
	Salmonella enterica	• 25 μg ml^{-1} + heat (65°C, pH 9): 4.7 log cycles • 25 μg ml^{-1} + chemical antimicrobials[e]: up to 4.5–5 log cycles	[23]
	Salmonella enterica *Escherichia coli* O157:H7 *Shigella* spp. *Enterobacter aerogenes* *Yersinia enterocolitica* *Aeromonas hydrophila* *Pseudomonas fluorescens*	• 25 μg ml^{-1} + polyphosphoric acid (0.1 to 2%): 4–6 log cycles; inhibition of regrowth during storage	[23]

[a] Trisodium trimetaphosphate, sodium lactate, lactic acid, polyphosphoric acid, carvacrol, hydrocinnamic acid, *p*-hydroxybenzoic acid, n-propyl *p*-hydroxybenzoate, or 2-nitropropanol

[b] Acetic acid, citric acid, lactic acid, sodium lactate, sodium nitrite, sodium nitrate, sodium propionate, potassium sorbate, tri-sodium phosphate, tri-sodium tri-metaphosphate, sodium thiosulphate, n-propyl *p*-hydroxybenzoate, *p*-hydoxybenzoic acid methyl ester, hexadecylpyridinium chloride, peracetic acid, or sodium hypochlorite

[c] Synergism with cinnamic and hydrocinnamic acids, carvacrol, polyphosphoric acid, peracetic acid, hexadecylpyridinium chloride, or sodium hypochlorite; complete inactivation/regrowth inhibition for the combinations of AS-48 and sodium hypochlorite, peracetic acid, or hexadecylpyridinium chloride

[d] Complete inactivation/regrowth inhibition for the combinations of AS-48 and sodium hypochlorite, peracetic acid, or hexadecylpyridinium chloride

[e] EDTA, lactic acid, peracetic acid, polyphosphoric acid, sodium hypochlorite, hexadecylpyridinium chloride, propyl-*p*-hydroxybenzoate, or hydrocinnamic acid

cells on sprouts by 1.0–1.5 and by 1.5–2.4 log units, respectively [22]. In both cases, no viable bacilli were detected in samples stored at 6°C although bacterial growth was observed in samples stored at 15 or 22°C (Table 2). Microbial inactivation was enhanced greatly when AS-48 was combined with various antimicrobials or sanitizers (Table 2). The combinations of AS-48 (25 μg ml^{-1}) and sodium hypochlorite (100 ppm), peracetic acid (40 ppm) or hexadecylpyridinium chloride (0.5%) provided the best results [22]. After application of the combined treatments on alfalfa sprouts contaminated with *B. cereus* or with *B. weihenstephanensis* cells, no viable bacilli were detected or remained at very low concentrations during one-week storage of samples at 15°C. Results from this study indicate that application of washing treatments containing AS-48 alone can reduce viable cell counts of bacilli in samples stored under refrigeration, while application of combined treatments should be recommended to avoid proliferation of the surviving bacilli under temperature abuse conditions.

Soybean sprouts spiked with Gram-negative bacteria were treated with AS-48 [23]. Washing solutions with AS-48 alone at neutral pH had no effect on *Sal. enterica*. However, increased bactericidal activity was detected for alkaline bacteriocin solutions and moderate heat [23]. Greatest inactivation (4.7 log cycles) was achieved for sprouts heated for 5 min at 65°C in AS-48 solution

adjusted to pH 9.0. Inactivation of *Sal. enterica* cells increased greatly for washing solutions containing AS-48 in combination with various chemical compounds (Table 2). The combined treatment of AS-48 (25 μg ml^{-1}) and polyphosphoric acid (0.1–2%) was tested against several other Gram-negative bacteria inoculated on sprouts. The bacteria tested showed great differences in sensitivity to polyphosphoric acid, but based on the original article authors' assumption (no FIC numbers provided) the bacteriocin synergized in its antimicrobial action with polyphosphoric acid in all cases [23]. Combinations of AS-48 and polyphosphoric acid significantly inhibited the populations of *Sal. enterica, E. coli* O157:H7, *Shigella* spp., *Enterobacter aerogenes, Yersinia enterocolitica, Aeromonas hydrophila*, and *Pseudomonas fluorescens* on sprouts [23]. Therefore, this combined treatment could be applied to reduce the risks of Gram-negative pathogenic as well as spoilage bacteria in sprouts.

Vegetable Salads

Ready to eat salads are prone to bacterial contamination because of the extensive manipulation of ingredients during manufacture, as well as by cross-contamination [14, 56, 99]. In a Russian-type salad, the concentrations of AS-48 required for inactivation of *L. monocytogenes* during one week storage at 10°C (Table 3) were much higher compared to other food substrates, most probably due to interaction of bacteriocin molecules with the complex food matrix of the salad [24]. Nevertheless, antilisterial activity of AS-48 in salad was enhanced greatly by addition of various essential oils, bioactive components from essential oils and plant extracts as well as other related antimicrobials of natural origin or derived from chemical synthesis, and by food preservatives (Table 3). Isobolograms for combined treatments clearly indicated synergism for AS-48 and citric acid, lactic acid, and *p*-hydroxybenzoic acid methyl ester [24].

AS-48 acted synergistically with *p*-hydroxybenzoic acid methyl ester (FIC value, 0.33) and with 2-nitropropanol (FIC value, 0.40) against *Sal. enterica* serovar Enteritidis in Russian-type salad [25]. In tests carried out in salads stored at 10°C challenged with a cocktail of *Salmonella* strains (*Sal. enterica* ssp. *enterica* serotype Typhi, *Sal. enterica* ssp. *enterica* serovar Choleraesuis, *Sal. enterica* ssp. *enterica* serovar Enteritidis, *Sal. enterica* ssp. *arizonae* serovar Arizonae, and *Sal. enterica* ssp. *salamae*), the combinations of AS-48 and *p*-hydroxybenzoic acid methyl ester or 2-nitropropanol reduced the concentrations of viable *Salmonella* by up to 4.75 log CFU g^{-1} during a 7-day storage period (Table 3) [25].

Vegetable Sauces

Vegetable sauces are sold as commercially sterile products, but they are also prepared at home or at restaurants for direct consumption. After handling, contaminated sauces may act as vehicles for *S. aureus* and lead to food

Table 3 Antibacterial effects of AS-48: vegetable salads and vegetable sauces

Food category		Bacteriocin treatment/microbial inactivation effects	Reference(s)
Vegetable salads	*Listeria monocytogenes*	• 30–60 μg g^{-1}: 2–4 log cycles	[24]
		• 30 μg g^{-1} + essential oils, bioactive oil components, chemical antimicrobials, food preservatives[a]: up to 4.5 log cycles; regrowth inhibition during storage	
	Salmonella strains (*Sal. enterica* ssp. *enterica* serotype Typhi, *Sal. enterica* ssp. *enterica* serovar Choleraesuis, *Sal. enterica* ssp. *enterica* serovar Enteritidis, *Sal. enterica* ssp. *arizonae* serovar Arizonae, *Sal. enterica* ssp. *salamae*)	• 40–80 μg g^{-1} + *p*-hydroxybenzoic acid methyl ester or 2-nitropropanol: synergistic effects on *Sal. enterica* serovar Enteritidis (FIC numbers; 0.33, 0.40, respectively); no viable cells detected for the cocktail of *Salmonella* strains during storage (4.3–4.7 log cycles)	[25]
Vegetable sauces	*Staphylococcus aureus*	• 80 μg ml^{-1}: partial inactivation or no viable cells detected (4 log cycles), depending on sauce	[48]
		• 15 μg ml^{-1} + phenolic compounds[b]: increased antimicrobial activity (4–4.5 log cycles)	

[a] Essential oils: thyme verbena, thyme red, Spanish oregano, ajowan, tea tree, clove, sage and rosemary oil; bioactive components from essential oils and plant extracts, other related antimicrobials of natural origin or derived from chemical synthesis: carvacrol, eugenol, thymol, terpineol, tyrosol, hydroxytyrosol, caffeic, ferulic and vanillic acids, luteolin, geranyl butyrate, geranyl phenylacetate, pirocatechol, hydrocinnamic acid, tert butylhydroquinone, phenylphosphate, isopropyl methyl phenol, coumaric acid, and 2-nitropropanol; food preservatives: citric and lactic acid, sucrose palmitate, sucrose stearate, *p*-hydroxybenzoic acid methyl ester, and Nisaplin

[b] Carvacrol, geraniol, eugenol, terpineol, caffeic acid, *p*-coumaric acid, citral, or hydrocinnamic acid

poisoning [58, 61]. When tested in vegetable sauces, AS-48 showed variable effects against *S. aureus* (Table 3), depending on the bacteriocin concentration, type of sauce and storage temperature [48]. Anti-staphylococcal activity of AS-48 was potentiated by phenolic compounds such as carvacrol, geraniol, eugenol, terpineol, caffeic acid, *p*-coumaric acid, citral, and hydrocinnamic acid, although the efficacy of the combined treatments depended both on the phenolic compound and the type of sauce (Table 3).

Inactivation of Endospore-Forming Bacteria in Rice-Based Foods and Purees

Endospore-forming bacteria are the main cause of food poisoning and spoilage of vegetable pre-cooked foods, given the abundance of bacterial endospores in the raw materials and the sometimes insufficient heat treatment during cooking process [36, 50, 56, 94]. In boiled rice and in a commercial infant rice-based gruel dissolved in whole milk artificially contaminated with a psychrotrophic enterotoxigenic strain of *B. cereus* cells, AS-48 addition caused complete bacterial inactivation and avoided enterotoxin production within a temperature range of 6–37°C [47] (Table 4). Activity of AS-48 against *B. cereus* cells in rice gruel was potentiated by sodium lactate. The heat sensitivity of endospores increased markedly in food samples supplemented with bacteriocin. No survivors were detected after heating with AS-48 for one min at 90°C in boiled rice or at 95°C in rice-based gruel (Table 4).

The effect of AS-48 against aerobic mesophilic endospore-forming bacterial cells was tested in commercial soups and purees (Table 4). *B. cereus* was completely inhibited by AS-48 in all six vegetable foods tested (natural vegetable cream, asparagus cream, traditional soup, homemade style traditional soup, vegetable soup, and vichyssoise) for up to 30 days at 6, 15, and 22°C [49]. Strains isolated from spoiled purees [51] were slightly more resistant to AS-48, showing a slower inactivation over time. Bacteriocin resistance increased greatly in purees inoculated with cocktails of strains from *Bacillus* and *Paenibacillus* species. In the mixed cultures, paenibacilli (along with some *B. cereus* cells) were the predominant survivors after bacteriocin treatment. Bactericidal activity against the cocktail of strains was greatly enhanced by phenolic compounds (carvacrol, eugenol, geraniol, and hydrocinnamic acid), achieving a fast inactivation of bacilli [49].

Table 4 Antibacterial effects of AS-48 against endospore-forming bacteria in cooked and canned foods

Food category		Bacteriocin treatment/microbial inactivation effects	Reference(s)
Rice-based foods	*Bacillus cereus*	• 20–35 μg ml^{-1} (vegetative cells, germinating endospores): 3.5–6 log cycles	[47]
		• 16 μg ml^{-1} + heat (intact endospores): increased inactivation of endospores; decrease of *D* values	
Soups and purees	*Bacillus cereus*	• 10 μg ml^{-1} (vegetative cells): 3.5–4 log cycles	[49]
	Bacillus macroides	• 10 μg ml^{-1} (vegetative cells): 3.5–4 log cycles	[49]
	Paenibacillus sp.	• 10 μg ml^{-1} (vegetative cells): 3.5–4 log cycles	[49]
	Paenibacillus polymyxa		
	Paenibacilus amylolyticus		
	Cocktail of 8 strains: *B. cereus* (three strains), *B. macroides* (two strains), *Paenibacillus* sp., *P. polymyxa* and *P. amylolyticus*	• 50 μg ml^{-1} (vegetative cells): 4–5 log cycles	[49]
		• 20 μg ml^{-1} + Nisaplin (vegetative cells): additive effect	
		• 20 μg ml^{-1} + phenolic compounds[a] (vegetative cells): increased and rapid inactivation (3.5–4 log cycles)	
Canned foods	*Bacillus coagulans*	• 6 μg ml^{-1} (vegetative cells): 3-5 log cycles	[62]
		• 6 μg ml^{-1} + lactic acid, glucose or sucrose (vegetative cells): increased bacterial inactivation	
		• 6 μg ml^{-1} + heat treatments (endospores): increased inactivation	
	Geobacillus stearothermophilus	• 1.75 to 7 μg g^{-1} (vegetative cells): 2.5–3 log cycles; no viable cells detected during storage at 45°C	[71]
		• 1.75 μg g^{-1} (endospores): 2.5–3 log cycles; regrowth inhibition	

[a] Carvacrol, eugenol, geraniol, or hydrocinnamic acid

Canned Foods

Low-acid canned foods can be spoiled by endospore-forming bacteria if heat treatments fail to inactivate the bacterial endospores [56, 65]. Addition of AS-48 in three low-acid vegetable canned foods (tomato paste, syrup from canned peaches, and juice from canned pineapple) caused complete or partial inactivation of *Bacillus coagulans* cells [62] (Table 4). Microbial inactivation increased upon addition of lactic acid, glucose, or sucrose. Although AS-48 had no significant effect on *B. coagulans* spores, the combined application of AS-48 and heat (80–95°C for 5 min) significantly increased endospore inactivation. These results were similar to those reported previously for *B. cereus* spores [47].

Thermophilic spoilage bacteria could also be controlled by bacteriocin addition. In samples from canned corn and peas supplemented with AS-48 and inoculated with vegetative cells of two *G. stearothermophilus* strains, no viable bacilli were detected during storage of samples at 45°C for 30 days [71]. In the canned food samples inoculated with intact *G. stearothermophilus* endospores, no viable cells were detected shortly after bacteriocin addition and during storage (Table 4). Through trypsin rescue experiments, it was shown that intact endospores were in fact resistant to AS-48, but they avidly adsorbed bacteriocin molecules which acted at later stages during germination [71].

Bakery Products and Ingredients

Wheat doughs are a frequent source of bacterial endospores which, after germination, may cause bread defects such as ropiness and produce enterotoxins [85, 92, 93]. AS-48 was tested against rope-forming *Bacillus subtilis* and *B. licheniformis*, as well as on *B. cereus* and *Bacillus pumilus* strains in experimental dough from wheat flour [77] (Table 5). In doughs supplemented with AS-48 and inoculated with *B. subtilis* cells, no viable bacilli were detected after 24 h provided that initial bacterial contamination levels were kept below 4 log CFU g^{-1}. However, samples inoculated with endospores activated to germinate required higher bacteriocin concentrations to achieve the same effects. *B. cereus* and *B. licheniformis* cells were inactivated by AS-48 in doughs, but only partial inactivation was observed for *B. pumilus* strains.

In bakery ingredients, inhibition of *S. aureus* by AS-48 greatly depended on the food substrate, ranging from complete inactivation in liquid caramel to non-significant inhibition in vanilla or chocolate creams [76]. Significant reductions ($P < 0.05$) of viable counts were also achieved in substrates like pumpkin comfiture or diluted almond cream (Table 5). Anti-staphylococcal activity increased markedly when AS-48 (50 μg ml^{-1}) was applied in combination with eugenol (0.1%), 2-nitropropanol (0.5%), or Nisaplin (3%) [76].

Various types of desserts were contaminated with *S. aureus, B. cereus,* and *L. monocytogenes* and then treated with AS-48 [75]. Greatest inactivation of *S. aureus* was observed in baker cream, while lowest activity was detected in soy-based desserts and in gelatin puddings, in which the efficacy of AS-48 greatly depended on inoculum size (Table 5). For *L. monocytogenes*, added AS-48 caused rapid bacterial inactivation and avoided regrowth of survivors. The lowest activity was also detected in soy-based desserts. In tests carried out in instant pudding, no viable

Table 5 Antibacterial effects of AS-48 in bakery products

Food category		Bacteriocin treatment/microbial inactivation effects	Reference(s)
Dough from wheat flour	*Bacillus subtilis*	• 50 μg g^{-1} (vegetative cells): up to 4 log cycles • 80 μg g^{-1} (germinating spores): 3 log cycles	[77]
	Bacillus licheniformis *Bacillus cereus*	• 50 μg g^{-1} (vegetative cells): 3.5–4 log cycles	[77]
	Bacillus pumilus	• 50 μg g^{-1} (vegetative cells): 1–1.5 log cycles	[77]
Bakery ingredients	*Staphylococcus aureus*	• 50 μg g^{-1}: partial inactivation (1.8–2.7 log cycles) or no effect observed, depending on substrate and microbial load • 50 μg g^{-1} + eugenol, 2–nitropropanol or Nisaplin: increased inactivation (3–5.5 log cycles)	[76]
Desserts	*Staphylococcus aureus*	• 50 μg g^{-1}: 1.5–5.5 log cycles, depending on substrate and microbial load	[75]
	Listeria monocytogenes	• 15–25 μg g^{-1}: 4.5–6 log cycles	[75]
	Bacillus cereus	• 15–50 μg g^{-1}(vegetative cells): 4–4.5 log cycles; inhibition of proteolytic gelatin degradation	[75]

B. cereus cells were detected after AS-48 treatment, although an increased bacteriocin resistance was observed in the soy pudding. Bacteriocin addition in gelatin pudding inactivated *B. cereus* cells and inhibited gelatin liquefaction caused by the proteolytic activity of this bacterium (Table 5). According to these results, AS-48 could find applications in desserts in combination with good hygienic practices to reduce the food microbial load. Bacteriocin activity seems to be negatively affected by fat content and by certain other components of raw materials (like soy derivatives). Under those circumstances, the efficacy of treatments could improve considerably in combination with other antimicrobial substances.

Concluding Remarks

AS-48 is a promising candidate for preservation of vegetable foods and drinks, either alone or in combination with other hurdles. In fruit juices, the bacteriocin is highly active against endospore-forming spoilage bacteria as well as spoilage lactic acid bacteria. These effects could be achieved at low bacteriocin concentrations similar to those reported in studies carried out with nisin against endospore formers [59, 101]. The efficacy of other bacteriocins (such as pediocin PA-1/Ach) in fruit juices has not been tested so far, nisin being the only licensed biopreservative for this purpose. Therefore, AS-48 could be used as an alternative to nisin avoiding proliferation of specific nisin resistant strains. Of particular interest is the effect of AS-48 against 3-HPA and EPS-producing spoilage bacteria in apple cider, where no published studies are yet available on the activities of other bacteriocins such as nisin or pediocins. Depending on the target bacteria, AS-48 could be used in drinks alone or in combination with physico-chemical treatments such as chelators or HIPEF treatment, increasing inactivation of Gram-negative bacteria and decreasing the risks for proliferation of survivors during the commercial shelf life of the product. Bacteriocin addition could ameliorate the problems associated to heat treatments, achieving a better preservation of nutrients, vitamins and organoleptic properties. It could also help the food industry to design new food products such as less acidic drinks or more naturally preserved beverages.

Decontamination of fresh-cut fruits and vegetables is an interesting field for application of AS-48. Fresh-cut products are highly perishable and can only withstand limited types of treatments for decontamination. Washing treatments with AS-48 are useful to reduce the microbial load of listeria and bacilli, and they can also afford protection for samples stored under refrigeration. A broad range of combinations of AS-48 and other antimicrobial substances provided increased bactericidal effects, affording protection under temperature abuse conditions. Among them, the combinations of AS-48 and polyphosphoric acid are of greatest interest, since they also afforded protection against Gram-negative bacteria. The results obtained for washing treatments with AS-48 against *L. monocytogenes* are superior to those reported for nisin (50 μg ml^{-1}) and pediocin (240 μg ml^{-1}) [13] because of the lower concentrations of enterocin required (25 μg ml^{-1}). Protection of treated samples during storage has not been reported for nisin or pediocin, being this a key issue for the safety of the treated produce. There are no results available on the activity of nisin or pediocin on other bacteria of concern in fresh produce such as endospore-forming bacilli or pathogenic enterobacteria. Application of AS-48 in combined treatments that avoid transmission of both Gram-positive and Gram-negative bacteria in fresh-cut produce would be beneficial to the vegetable food industry.

Application of essential oils and their bioactive phenolic compounds in food preservation has been pursued for a long time, but it has been limited by the strong impact they have on the food organoleptic properties [15]. Nevertheless, the strong bactericidal effects reported for these antimicrobials in combination with AS-48 opens new possibilities for application in vegetable foods, such as vegetable salads or sauces, where special flavours may be desired. Also, because of the lower concentrations of antimicrobials required for inhibition of target bacteria in the combined treatments, the impact on food is decreased.

Control of endospore-forming bacteria in processed vegetable foods (such as rice-based foods, soups, puree, and canned vegetables) is an interesting field for application of this bacteriocin. The studies carried out have shown that AS-48 can inactivate different species of endospore-forming bacteria in foods substrates, at varying concentrations depending on the food and bacterial strain. Most interesting, it has been shown that addition of AS-48 reduces the intensity of heat treatments necessary for inactivation of endospores in vegetable foods. Nisin is also highly effective against endospore formers in canned vegetable foods [97], but there are scarce or no reports on the effects of other bacteriocins in this field [16]. Since contamination of raw material of vegetable origin with bacterial endospores is one of the main problems in the food industry, AS-48 could find interesting applications as an additional hurdle alternative to nisin in processed vegetable foods not only to increase the efficacy of heat treatments (allowing a reduction of heat intensity) but also to control germinating spores in the finished product.

Endospore-forming bacteria may cause several problems in the bread and bakery industry, since they are often present in flours. Although the effect of AS-48 in flours is limited by the low water availability, this bacteriocin could be incorporated in doughs to prevent proliferation of bacilli

responsible for ropiness and enterotoxin production. Similarly, AS-48 could find applications in the bakery industry, controlling the proliferation of foodborne pathogens such as *L. monocytogenes, B. cereus,* or *S. aureus*. However, the strong interference observed for high-fat components such as chocolate or soy products with bacteriocin activity is a clear limitation as to the type of substrates where it could be used, unless other antimicrobials acting synergistically with AS-48 are included.

In conclusion, AS-48 could find different applications in vegetable foods either as an additive or as a natural sanitizer. At present, the bacteriocin can be obtained at pilot scale by cultivation of producer strains on food-grade by products such as whey or whey permeates, and can be concentrated and stabilised in different ways, e.g. by cation exchange chromatography, tangential flow ultrafiltration, or spray drying [10]. Hopefully, the availability of commercial preparations of AS-48 together with the body of knowledge already available on the efficacy of this antimicrobial peptide in vegetable food systems should pave the way for its commercial application in food industries.

References

1. Abriouel H, Valdivia E, Gálvez A, Maqueda M (1998) Response of *Salmonella choleraesuis* LT2 spheroplasts and permeabilized cells to the bacteriocin AS-48. Appl Environ Microbiol 64:4623–4626
2. Abriouel H, Valdivia E, Gálvez A, Maqueda M (2001) Influence of physico-chemical factors on the oligomerization and biological activity of bacteriocin AS-48. Curr Microbiol 42:89–95
3. Abriouel H, Maqueda M, Gálvez A, Martínez-Bueno M, Valdivia E (2002) Inhibition of bacterial growth, enterotoxin production and spore outgrowth on strains of *Bacillus cereus* by bacteriocin AS-48. Appl Environ Microbiol 68:1473–1477
4. Abriouel H, Valdivia E, Martínez-Bueno M, Maqueda M, Gálvez A (2003) A simple method for semi-preparative-scale production and recovery of enterocin AS-48 derived from Enterococcus faecalis subsp. liquefaciens A-48–32. J Microbiol Methods 55:599–605
5. Abriouel H, Lucas R, Ben Omar N, Valdivia E, Maqueda M, Martínez-Cañamero M, Gálvez A (2005) Enterocin AS-48RJ: a variant of enterocin AS-48 chromosomally encoded by *Enterococcus faecium* RJ16 isolated from food. System Appl Microbiol 28:383–397
6. Ananou S, Valdivia E, Martínez-Bueno M, Gálvez A, Maqueda M (2004) Effect of combined physico-chemical preservatives on enterocin AS-48 activity against the enterotoxigenic *Staphylococcus aureus* CECT 976 strain. J Appl Microbiol 97:48–56
7. Ananou S, Garriga M, Hugas M, Maqueda M, Martínez-Bueno M, Gálvez A, Valdivia E (2005) Control of *Listeria monocytogenes* in model sausages by enterocin AS-48. Int J Food Microbiol 103:179–190
8. Ananou S, Maqueda M, Martínez-Bueno M, Gálvez A, Valdivia E (2005) Control of *Staphylococcus aureus* in sausages by enterocin AS-48. Meat Sci 71:549–576
9. 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
10. Ananou S, Muñoz A, Gálvez A, Martínez-Bueno M, Maqueda M, Valdivia E (2008) Optimization of the production of enterocin AS-48 on a whey-based substrate. Int Dairy J 18: 923–927
11. Ananou S, Baños A, Maqueda M, Martínez-Bueno M, Gálvez A, Valdivia E (2009a) Effect of combined physico-chemical treatments based on enterocin AS-48 on the control of *Listeria monocytogenes* and *Staphylococcus aureus* in a model cooked ham. Food Control (in press)
12. Ananou S, Muñoz A, Martínez-Bueno M, González-Tello P, Gálvez A, Maqueda M, Valdivia E (2009b) Evaluation of an enterocin AS-48 containing bioactive powder obtained by spray-drying. Food Microbiol (in press)
13. Bari ML, Ukuku DO, Kawasaki T, Inatsu Y, Isshiki K, Kawamoto S (2005) Combined efficacy of nisin and pediocin with sodium lactate, citric acid, phytic acid, and potassium sorbate and EDTA in reducing the *Listeria monocytogenes* population of inoculated fresh-cut produce. J Food Prot 68:1381–1387
14. Bornemeier VL, Albrecht JA, Sumner SS (2003) Survey of mayonnaise-based salads for microbial safety and quality. Food Prot Trends 23:387–392
15. Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods-a review. Int J Food Microbiol 94:223–253
16. Cabo ML, Torres B, Herrera JJ, Bernárdez M, Pastoriza L (2009) Application of nisin and pediocin against resistance and germination of *Bacillus* spores in sous vide products. J Food Prot 72:515–623
17. Claisse O, Lonvaud-Funel A (2000) Assimilation of glycerol by a strain of *Lactobacillus collinoides* isolated from cider. Food Microbiol 17:513–519
18. Claisse O, Lonvaud-Funel A (2001) Detection of lactic acid bacteria producing 3-hydroxypropionaldehyde (acrolein precursor) from glycerol by molecular tests. Lait 81:173–181
19. Cleveland J, Montville TJ, Nes IF, Chikindas ML (2001) Bacteriocins: safe, natural antimicrobials for food preservation. Int J Food Microbiol 71:1–20
20. Cobo Molinos A, Abriouel H, Ben Omar N, Valdivia E, Lucas R, Maqueda M, Martínez Cañamero M, Gálvez A (2005) Effect of immersion solutions containing enterocin AS-48 on *Listeria monocytogenes* in vegetable foods. Appl Environ Microbiol 71:7781–7787
21. Cobo Molinos A, Abriouel H, Ben Omar N, Lucas R, Valdivia E, Gálvez A (2008) Inactivation of *Listeria monocytogenes* in raw fruits by enterocin AS-48. J Food Prot 71:2460–2467
22. Cobo Molinos A, Abriouel H, Lucas R, Ben Omar N, Valdivia E, Gálvez A (2008) Inhibition of *Bacillus cereus* and *B. weihenstephanensis* in raw vegetables by application of washing solutions containing enterocin AS-48 alone and in combination with other antimicrobials. Food Microbiol 25:762–770
23. Cobo Molinos A, Abriouel H, Lucas R, Valdivia E, Ben Omar N, Gálvez A (2008) Combined physico-chemical treatments based on enterocin AS-48 for inactivation of Gram-negative bacteria in soybean sprouts. Food Chem Toxicol 46:2912–2921
24. Cobo Molinos A, Abriouel H, Ben Omar N, Lucas R, Valdivia E, Gálvez A (2009) Enhanced bactericidal activity of enterocin AS-48 in combination with essential oils, natural bioactive compounds, and chemical preservatives against *Listeria monocytogenes* in ready-to-eat salads. Food Chem Toxicol 47: 2216–2223
25. Cobo Molinos A, Lucas R, Abriouel H, Ben Omar N, Valdivia E, Gálvez A (2009) Inhibition of *Salmonella enterica* cells in deli-type salad by enterocin AS-48 in combination with other antimicrobials. Probiotics & Antimicrob Prot 1:85–90

26. Cobos E, Filimonov VV, Gálvez A, Maqueda M, Valdivia E, Martínez JC, Mateo PL (2001) AS-48: a circular protein with an extremely stable globular structure. FEBS Lett 505:379–382
27. Cornut G, Fortin C, Soulières D (2008) Antineoplastic properties of bacteriocins: revisiting potential active agents. Am J Clin Oncol 31:399–404
28. 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
29. Diez-Gonzalez F (2007) Applications of bacteriocins in livestock. Curr Issues Intestinal Microbiol 8:15–24
30. Doyle MP, Erickson MC (2008) Summer meeting 2007—the problems with fresh produce: an overview. J Appl Microbiol 105:317–330
31. Drider D, Fimland G, Héchard Y, McMullen LM, Prévost H (2006) The continuing story of class IIa bacteriocins. Microbiol Mol Biol Rev 70:564–582
32. Dueñas M, Irastorza A, Fernández K, Bilbao A (1995) Heterofermentative lactobacilli causing ropiness isolated from Basque Country ciders. J Food Prot 58:76–80
33. DuPont HL (2007) The growing threat of foodborne bacterial enteropathogens of animal origin. Clin Infect Dis 45:1353–1361
34. Folli C, Ileana Ramazzina I, Arcidiaco P, Stoppini M, Berni R (2003) Purification of bacteriocin AS-48 from an *Enterococcus faecium* strain and analysis of the gene cluster involved in its production. FEMS Microbiol Lett 221:143–149
35. Franz CMAP, van Belkum MJ, Holzapfel WH, Abriouel H, Gálvez A (2007) Diversity of enterococcal bacteriocins and their grouping into a new classification scheme. FEMS Microbiol Rev 31:293–310
36. From C, Pukall R, Schumann P, Hormazábal V, Granum PE (2005) Toxin-producing ability among *Bacillus* spp. outside the Bacillus cereus group. Appl Environ Microbiol 71:1178–1183
37. Gálvez A, Maqueda M, Valdivia E, Quesada A, Montoya E (1986) Characterization and partial purification of a broad spectrum antibiotic AS-48 produced by *Streptococcus faecalis*. Can J Microbiol 32:765–771
38. Gálvez A, Maqueda M, Martínez-Bueno M, Valdivia E (1989) Bactericidal and bacteriolytic action of peptide antibiotic AS-48 against Gram-positive and Gram-negative bacteria and other organisms. Res Microbiol 140:57–68
39. Gálvez A, Valdivia E, Martínez M, Maqueda M (1989) Bactericidal action of peptide antibiotic AS-48 against *Escherichia coli* K-12. Can J Microbiol 35:318–321
40. Gálvez A, Maqueda M, Martínez-Bueno M, Valdivia E (1991) Permeation of bacterial cells, permeation of cytoplasmic and artificial membrane vesicles, and channel formation on lipid bilayers by peptide antibiotic AS-48. J Bacteriol 173:886–892
41. Gálvez A, Lucas López R, Abriouel H, Valdivia E, Ben Omar N (2008) Application of bacteriocins in the control of food borne pathogenic and spoilage bacteria. Crit Rev Biotechnol 28: 125–152
42. Gillor O, Etzion A, Riley MA (2008) The dual role of bacteriocins as anti- and probiotics. Appl Microbiol Biotechnol 81:591–606
43. González C, Langdon GM, Bruix M, Gálvez A, Valdivia E, Maqueda M, Rico M (2000) Bacteriocin AS-48, a cyclic polypeptide structurally and functionally related to mammalian NK-lysin. Proc Natl Acad Sci USA 97:11221–11226
44. Grande MJ (2007) Actividad de la enterocina AS-48 frente a bacterias Gram-positivas alternates o productoras de enterotoxinas en alimentos vegetales. PhD thesis. University of Jaen
45. Grande MJ, Lucas R, Abriouel H, Ben Omar N, Maqueda M, Martínez-Bueno M, Martínez-Cañamero M, Valdivia E, Gálvez A (2005) Control of *Alicyclobacillus acidoterrestris* in fruit juices by enterocin AS-48. Int J Food Microbiol 104:289–297
46. Grande MJ, Lucas R, Abriouel H, Valdivia E, Ben Omar N, Maqueda M, Martínez-Cañamero M, Gálvez A (2006) Inhibition of *Bacillus licheniformis* LMG 19409 from ropy cider by enterocin AS-48. J Appl Microbiol 101:422–428
47. Grande MJ, Lucas R, Abriouel H, Valdivia E, Ben Omar N, Maqueda M, Martínez-Bueno M, Martínez-Cañamero M, Gálvez A (2006) Inhibition of toxicogenic *Bacillus cereus* in rice-based foods by enterocin AS-48. Int J Food Microbiol 106: 185–194
48. Grande MJ, Lucas R, Abriouel H, Valdivia E, Ben Omar N, Maqueda M, Martínez-Cañamero M, Gálvez A (2007) Treatment of vegetable sauces with enterocin AS-48 alone or in combination with phenolic compounds to inhibit proliferation of *Staphylococcus aureus*. J Food Prot 70:405–411
49. Grande MJ, Abriouel H, Lucas R, Valdivia E, Ben Omar N, Martínez-Cañamero M, Gálvez A (2007) Efficacy of enterocin AS-48 against bacilli in ready-to-eat vegetable soups and purees. J Food Prot 70:2339–2345
50. Granum PE (2007) *Bacillus cereus* pp. 445–455. In: Doyle MP, Beuchat LR (eds) Food microbiology. Fundamentals and frontiers, 3rd edn. ASM Press, Washington, DC
51. Guinebretiere MH, Berge O, Normand P, Morris C, Carlin F, Nguyen-The C (2001) Identification of bacteria in pasteurized zucchini purées stored at different temperatures and comparison with those found in other pasteurized vegetable purées. Appl Environ Microbiol 67:4520–4530
52. Harris LJ, Farber JN, Beuchat LR, Parish ME, Suslow TV, Garrett EH, Busta FF (2003) Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh- cut produce. Comp Rev Food Sci Food Safety 2(Suppl):79–141
53. Heinz V, Alvarez I, Angersbach A, Knorr D (2002) Preservation of liquid foods by high intensity pulsed electric fields-basic concepts for process design. Trends Food Sci Technol 12: 103–111
54. Ho SY, Mittal GS (2000) High voltage pulsed electrical field for liquid food pasteurization. Food Rev Int 16:395–434
55. Jack RW, Tagg JR, Ray B (1995) Bacteriocins of Gram-positive bacteria. Microbiol Rev 59:171–200
56. Jay JM, Loessner MJ, Golden AA (2005) Modern food microbiology. Aspen Publishers Inc, Gaithersburg
57. Joosten HMLJ, Nuñez M, Devreese B, van Beeumen J, Marugg JD (1996) Purification and characterization of enterocin 4, a bacteriocin produced by *Enterococcus faecalis* INIA4. Appl Environ Microbiol 62:4220–4223
58. Jørgensen HJ, Maticen T, Løvseth A, Oboe K, Qvale KS, Loncarevic S (2005) An outbreak of staphylococcal food poisoning caused by enterotoxin H in mashed potato made with raw milk. FEMS Microbiol Lett 252:267–272
59. Komitopoulou E, Boziaris IS, Davies EA, Delves-Broughton J, Adams MR (1999) *Alicyclobacillus acidoterrestris* in fruit juices and its control by nisin. Int J Food Sci Technol 34:81–85
60. Larpin S, Sauvageot N, Pichereau V, Laplace J-M, Auffray Y (2002) Biosynthesis of exopolysaccharide by a *Bacillus licheniformis* strain isolated from ropy cider. Int J Food Microbiol 77:1–9
61. Le Loir Y, Baron F, Gautier M (2003) *Staphylococcus aureus* and food poisoning. Genet Molec Res 2:63–76
62. Lucas R, Grande MJ, Abriouel H, Maqueda M, Ben Omar N, Valdivia E, Martínez-Cañamero M, Gálvez A (2006) Application of the broad-spectrum bacteriocin enterocin AS-48 to inhibit *Bacillus coagulans* in low-pH canned fruit and vegetable foods. Food Chem Toxicol 44:1774–1781
63. Lynch MF, Tauxe RV, Hedberg CW (2009) The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. Epidemiol Infect 137:307–315

64. Maisnier-Patin S, Forni E, Richard J (1996) Purification, partial characterisation and mode of action of enteroccocin EFS2, an antilisterial bacteriocin produced by a strain of *Enterococcus faecalis* isolated from a cheese. Int J Food Microbiol 30: 255–270
65. Mallidis CG, Frantzeskakis P, Balatsouras G, Katsabotxakis C (1990) Thermal treatment of aseptically processed tomato paste. Int J Food Sci Technol 25:442–448
66. Maqueda M, Gálvez A, Sánchez-Barrena MJ, González C, Albert A, Rico M, Valdivia E (2004) Peptide AS-48: prototype of a new class of cyclic bacteriocins. Curr Prot Pept Sci 5: 399–416
67. Maqueda M, Sánchez-Hidalgo M, Fernández M, Montalbán-López M, Valdivia E, Martínez-Bueno M (2008) Genetic features of circular bacteriocins produced by Gram-positive bacteria. FEMS Microbiol Rev 32:2–22
68. Martín-Belloso O, Elez-Martínez P (2005) Food safety aspects of pulsed electric fields. In: Sun DW (ed) Emerging technologies for food processing. Elsevier, Amsterdam, pp 183–217
69. Martínez-Viedma P, Abriouel H, Ben Omar N, Valdivia E, Lucas López R, Gálvez A (2008) Inactivation of exopolysaccharide and 3- hydroxypropionaldehyde-producing lactic acid bacteria in apple juice and apple cider by enterocin AS-48. Food Chem Toxicol 46:1143–1151
70. Martínez-Viedma P, Sobrino A, Ben Omar N, Abriouel H, Lucas López R, Valdivia E, Martín Belloso O, Gálvez A (2008) Enhanced bactericidal effect of High-Intensity Pulsed-Electric Field treatment in combination with enterocin AS-48 against *Salmonella enterica* in apple juice. Int J Food Microbiol 128:244–249
71. Martínez-Viedma P, Abriouel H, Ben Omar N, Lucas R, Valdivia E, Gálvez A (2009) Inactivation of *Geobacillus stearothermophilus* in canned foods and drinks by addition of enterocin AS-48. Food Microbiol 26:289–293
72. Martínez-Viedma P, Abriouel H, Sobrino A, Ben Omar N, Lucas López R, Valdivia E, Martín Belloso O, Gálvez A (2009) Effect of enterocin AS-48 in combination with High-Intensity Pulsed-Electric Field treatment against the spoilage bacterium *Lactobacillus diolivorans* in apple juice. Food Microbiol 26:491–496
73. Martínez-Viedma P, Sobrino A, Abriouel H, Ben Omar N, Lucas López R, Martín Belloso O, Gálvez A (2009c) Increased inactivation of exopolysaccharide-producing *Pediococcus parvulus* in apple juice by combined treatment with enterocin AS-48 and High-Intensity Pulsed-Electric Field. J Food Prot (in press)
74. Martínez-Viedma P, Abriouel H, Ben Omar N, Lucas López R, Valdivia E, Gálvez A (2009) Antibacterial protection by enterocin AS-48 in sport and energy drinks with less acidic pH values. J Food Prot 72:881–884
75. Martínez-Viedma P, Abriouel H, Ben Omar N, Lucas R, Valdivia E, Gálvez A (2009) Assay of enterocin AS-48 for inhibition of foodborne pathogens in desserts. J Food Prot 72: 1654–1659
76. Martínez-Viedma P, Abriouel H, Ben Omar N, Lucas R, Gálvez A (2009f) Anti-staphylococcal effect of enterocin AS-48 in bakery ingredients of vegetable origin, alone and in combination with selected antimicrobials. J Food Sci (in press)
77. Martínez-Viedma P, Abriouel H, Ben Omar N, Lucas R, Gálvez A (2009g) Effect of enterocin AS-48 against spoilage and toxinogenic *Bacillus* species in dough from wheat flour. LWT Food Sci Technol (submitted)
78. Mendoza F, Maqueda M, Gálvez A, Martínez-Bueno M, Valdivia E (1999) Antilisterial activity of peptide AS-48 and study of changes induced in the cell envelope properties of an AS-48-adapted strain of *Listeria monocytogenes*. Appl Environ Microbiol 65:618–625
79. Mosqueda-Melgar J, Elez-Martínez P, Raybaudi-Massilia RM, Martín-Belloso O (2008) Effects of pulsed electric fields on pathogenic microorganisms of major concern in fluid foods: a review. Crit Rev Food Sci Nutr 48:747–759
80. Muñoz A, Maqueda M, Gálvez A, Martínez-Bueno M, Rodriguez A, Valdivia E (2004) Biocontrol of psychrotrophic enterotoxigenic *Bacillus cereus* in a non fat hard type cheese by an enterococcal strain-producing enterocin AS-48. J Food Prot 67:1517–1521
81. Muñoz A, Ananou S, Gálvez A, Martínez-Bueno M, Rodríguez A, Maqueda M, Valdivia E (2007) Inhibition of *Staphylococcus aureus* in dairy products by enterocin AS-48 produced in situ and ex situ: bactericidal synergism with heat. Int Dairy J 17:760–769
82. Nes IF, Yoon S-S, Diep DB (2007) Ribosomally synthesized antimicrobial peptides (bacteriocins) in lactic acid bacteria: a review. Food Sci Biotechnol 16:675–690
83. Oppegård C, Rogne P, Emanuelsen L, Kristiansen PE, Fimland G, Nissen-Meyer J (2007) The two-peptide class II bacteriocins: structure, production, and mode of action. J Mol Microbiol Biotechnol 13:210–219
84. Pao S, Khalid MF, Kalantari A (2005) Sprouting seeds and potential hazards associated with enterotoxigenic *Bacillus* spp. in homegrown sprouts. J Food Prot 68:1648–1653
85. Rosenquist H, Hansen Å (1995) Contamination profiles and characterisation of *Bacillus* species in wheat bread and raw materials for bread production. Int J Food Microbiol 26:353–363
86. Samyn B, Martínez-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
87. Sánchez-Barrena M, Martínez-Ripoll G, Gálvez A, Valdivia E, Maqueda M, Cruz V, Albert A (2003) Structure of bacteriocin AS-48: from soluble state to membrane bound state. J Mol Biol 334:541–549
88. Sang Y, Blecha F (2008) Antimicrobial peptides and bacteriocins: alternatives to traditional antibiotics. Anim Health Res Rev 9:227–235
89. Sauvageot N, Gouffi K, Laplace JM, Auffray Y (2000) Glycerol metabolism in *Lactobacillus collinoides*: Production of 3-hydroxypropionaldehyde, a precursor of acrolein. Int J Food Microbiol 55:167–170
90. Settanni L, Corsetti A (2008) Application of bacteriocins in vegetable food biopreservation. Int J Food Microbiol 121: 123–138
91. Silva FVM, Gibbs P (2001) *Alicyclobacilus acidoterrestris* spores in fruit products and design of pasteurization processes. Trends Food Sci Technol 12:68–74
92. Smith JP, Daifas DP, El-Khoury W, Koukoutsis J, El-Khoury A (2004) Shelf life and safety concerns of bakery products—a review. Crit Rev Food Sci Nutr 44:19–55
93. Sorokulova IB, Reva ON, Smirnov VV, Pinchuk IV, Lapa SV, Urdaci MC (2003) Genetic diversity and involvement in bread spoilage of *Bacillus* strains isolated from flour and ropy bread. Lett Appl Microbiol 37:169–173
94. Stenfors Arnesen LP, Fagerlund A, Granum PE (2008) From soil to gut: *Bacillus cereus* and its food poisoning toxins. FEMS Microbiol Rev 32:579–606
95. Tagg JR (2009) Streptococcal bacteriocin-like inhibitory substances: some personal insights into the bacteriocin-like activities produced by Streptococci good and bad. Probiotics & Antimicro Prot 1:60–66

96. Tahmassebi JF, Duggal MS, Malik-Kotru G, Curzon MEJ (2006) Soft drinks and dental health: A review of the current literature. J Dent 34:2–11
97. Thomas LV, Clarkson MR, Delves-Broughton J (2000) Nisin. In: Naidu AS (ed) Natural food antimicrobial systems. CRC-Press, Florida, pp 463–524
98. Tomita H, Fujimoto S, Tanimoto K, Ike Y (1997) Cloning and genetic and sequence analyses of the bacteriocin 21 determinant encoded on the *Enterococcus faecalis* pheromone-responsive conjugative plasmid pPD1. J Bacteriol 179:7843–7855
99. Unicomb L, Bird P, Dalton C (2003) Outbreak of *Salmonella* Potsdam associated with salad dressing at a restaurant. Commun Dis Intellig 27:508–512
100. Wongkhantee S, Patanapiradej V, Maneenut C, Tantbirojn D (2006) Effect of acidic food and drinks on surface hardness of enamel, dentine, and tooth-coloured filling materials. J Dent 34:214–220
101. Yamazaki K, Murami M, Kawai Y, Inoue N, Matsuda T (2000) Use of nisin for inhibition of *Alicyclobacillus acidoterrestris* in acidic drinks. Food Microbiol 17:210–315
102. Zero DT (1996) Etiology of dental erosion—extrinsic factors. Eur J Oral Sci 104:162–177