Effect of Immersion Solutions Containing Enterocin AS-48 on *Listeria monocytogenes* in Vegetable Foods

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The effect of immersion solutions containing enterocin AS-48 alone or in combination with chemical preservatives on survival and proliferation of *Listeria monocytogenes* CECT 4032 inoculated on fresh alfalfa sprouts, soybean sprouts, and green asparagus was tested. Immersion treatments (5 min at room temperature) with AS-48 solutions (25 µg/ml) reduced listeria counts of artificially contaminated alfalfa and soybean sprouts by approximately 2.0 to 2.4 log CFU/g compared to a control immersion treatment in distilled water. The same bacteriocin immersion treatment applied on green asparagus had a very limited effect. During storage of vegetable samples treated with immersion solutions of 12.5 and 25 µg of AS-48/ml, viable listeria counts were reduced below detection limits at days 1 to 7 for alfalfa and soybean sprouts at 6 and 15°C, as well as green asparagus at 15°C. Only a limited inhibition of listeria proliferation was detected during storage of bacteriocin-treated alfalfa sprouts and green asparagus at 22°C. Treatment with solutions containing AS-48 plus lactic acid, sodium lactate, sodium nitrite, sodium nitrate, trisodium phosphate, trisodium trimetaphosphate, sodium thiosulphate, *n*-propyl *p*-hydroxybenzoate, *p*-hydroxybenzoic acid methyl ester, hexadecylpyridinium chloride, peracetic acid, or sodium hypochlorite reduced viable counts of listeria below detection limits (by approximately 2.6 to 2.7 log CFU/g) upon application of the immersion treatment and/or further storage for 24 h, depending of the chemical preservative concentration. Significant increases of antimicrobial activity were also detected for AS-48 plus potassium permanganate and in some combinations with acetic acid, citric acid, sodium propionate, and potassium sorbate.

Listeria monocytogenes may produce a wide variety of human disease manifestations, which range from a nonspecific flu-like illness to severe diseases such as sepsis and meningitis (42). People at higher risk, particularly for the more severe diseases, are the elderly, immunocompromised individuals, and pregnant women. This bacterium has emerged as a food-borne pathogen within the past two decades. The Centers for Disease Control and Prevention have estimated that up to 2,500 cases of listeriosis, resulting in 500 deaths, occur each year in the United States (33). *L. monocytogenes* is widely distributed in the environment, where it is associated with decaying vegetation, soil, sewage, and feces of animals, and has been isolated from several types of vegetables (7, 8). *L. monocytogenes* is of particular concern for manufacturers of refrigerated ready-to-eat foods largely because of its wide distribution in the environment and its ability to grow on a variety of vegetables at refrigeration temperatures (4, 6, 10) and at the low O₂ levels often used to extend the shelf life of refrigerated ready-to-eat foods.

Cases of human listeriosis that have been associated with the consumption of raw vegetables are likely, in part, due to contamination by manure from ruminants (7). Listeriosis outbreaks have been associated with fresh produce such as raw

celery, tomatoes, lettuce, and coleslaw (7). *L. monocytogenes* has been recovered from various fresh vegetables, including bean sprouts (12, 26, 29, 39), and recalls of alfalfa sprouts have occurred due to contamination with this bacterium (24). In recent years there has been an increase in consumer demand for mung bean, alfalfa, soybean, radish, and other seed sprouts (38) that are usually eaten raw in salads or in sandwiches, and concerns for the safety of these raw foods have increased lately due to their implication as vehicles for transmission in a number of food-borne outbreaks of infection (40). To date, there are no chemical or water-rinse treatments that will effectively decontaminate sprouts and yield an edible raw product, and the U.S. government issued a warning regarding the hazard of eating raw sprouts (36). In a similar way, green asparagus destined for fresh consumption with minimal manipulation, that includes only the base cut and then packaging in bundles, has increased its presence in the market (44). Modified atmospheres packaging has been used to increase the shelf-life of asparagus, and growth of *L. monocytogenes* in packaged fresh green asparagus has been reported (15).

Bacteriocins are natural antimicrobial substances with a high potential for food preservation. However, most studies carried out on bacteriocin applications have focused on foods from animal origin (17), and very little work has been done on the application of bacteriocins for the preservation of vegetable foods. The broad spectrum antimicrobial peptide enterocin AS-48 from *Enterococcus faecalis* (20, 21) offers a good potential for application in food preservation. The various studies carried out on this bacteriocin have served to elucidate its

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molecular composition and structure, mode of action, and genetic determinants (reviewed in reference 31). Recent studies on the application of enterocin AS-48 in food preservation have shown satisfactory results for dairy products, meat, and fruit juices (2, 23, 35). We tested here the application of immersion solutions for decontamination of raw vegetables alone or in combination with chemical preservatives.

MATERIALS AND METHODS

Bacterial strains and cultivation conditions. *Listeria monocytogenes* CECT 4032 was provided by the Spanish Type Culture Collection, and was isolated in Colindale, United Kingdom, from a patient with meningitis associated with eating contaminated cheese. *E. faecalis* A-48-32 (32) was used for production of enterocin AS-48, and *E. faecalis* S-47 from our collection was used for standard determination of bacteriocin activity. All strains were cultivated routinely on brain heart infusion (Scharlab, Barcelona, Spain) broth at 37°C and stored at 4°C.

Preparation of bacteriocin solutions and chemical preservatives. Enterocin AS-48 was obtained by cultivation of the producer strain *E. faecalis* A-48-32 in CMG medium followed by cation-exchange chromatography as described elsewhere (1, 20). Bacteriocin concentrates were filtered through 0.22- μ m-pore-size low-protein-binding filters (Millex GV; Millipore Corp., Bedford, MA) under sterile conditions and tested for bacteriocin activity against the indicator strain *E. faecalis* S-47 by the agar well diffusion method using stainless steel cylinders of 8-mm (outer) diameter (20). Immersion solutions were prepared by diluting bacteriocin concentrates (500 μ g/ml) in sterile distilled water or in aqueous solutions of the chemical preservatives to be tested in the case of combined treatments.

Solutions containing 0.1 or 0.5% (wt/vol) of the chemical preservatives acetic acid, lactic acid, or sodium lactate (all from Sigma, Madrid, Spain) or 80 mg of peracetic acid (Fluka, Madrid, Spain)/liter were prepared by diluting commercial preparations in sterile distilled water. Solutions containing 0.1 or 0.5% (wt/vol) of citric acid (Sigma), sodium propionate (Fluka), or potassium sorbate (Fluka), 25 mM potassium permanganate (Fluka), 50 or 100 mg of sodium nitrite (Panreac, Barcelona, Spain) or potassium nitrate (Panreac), 0.01 N sodium thiosulfate (Sigma), and 1.5% (wt/vol) trisodium phosphate (Fluka)/liter, as well as 0.1 or 0.5% (wt/vol) trisodium trimetaphosphate (Sigma) were prepared in distilled water and sterilized by autoclaving. Solutions containing 0.1 or 0.5% (wt/vol) *n*-propyl *p*-hydroxybenzoate or *p*-hydroxybenzoic acid methyl ester (both from Sigma) were prepared in distilled water by 50- to 100-fold dilution of concentrates dissolved in ethanol. Sodium hypochlorite solutions were prepared in sterile distilled water from commercial concentrated bleach (ConejoTM; Henkel Ibérica, Barcelona, Spain) to the desired final concentrations of free chlorine (25 to 100 ppm or mg/liter). All solutions were prepared fresh before use.

Determination of the effectiveness of enterocin AS-48 on *L. monocytogenes* in contaminated vegetables. The effect of immersion in solutions containing different concentrations of enterocin AS-48 on survival and growth of *L. monocytogenes* CECT 4032 inoculated onto fresh alfalfa and soybean sprouts, as well as green asparagus, was investigated at different storage temperatures. Cultures of *L. monocytogenes* CECT 4032 grown overnight in brain heart infusion broth at 37°C were diluted 1:1,000 in sterile saline solution to a final cell density of approximately 5.6 log CFU/ml. This dilution was used as the *Listeria*-contaminating solution. Fresh green asparagus (Mary Washington variety, 5 to 10 mm in diameter), soybean sprouts (Alleuras Industries, Madrid, Spain), and alfalfa sprouts (Productos Fanya, Madrid, Spain) were purchased from local supermarkets. Asparagus were cut onto 2-cm pieces before treatment application. Samples of the vegetable being tested (2.5 g each) were deposited inside sterile capped 50-ml polypropylene tubes (Sterilin, Stone, United Kingdom) and dipped for 5 min in 5 ml of sterile distilled water (negative controls) or in 5 ml of *Listeria* contaminating solution at room temperature. They were then deposited on sterile filter paper to drain excess water. At this step, the concentrations of *L. monocytogenes* in the artificially contaminated vegetable samples were approximately 4.69 log CFU/g (alfalfa sprouts and green asparagus) and 4.72 log CFU/g (soybean sprouts). The artificially contaminated samples were dipped for 5 min at room temperature in 5 ml of sterile distilled water (controls) or distilled water containing enterocin AS-48 (at final concentrations of 5, 12.5, or 25 μ g/ml). Immersion solutions were held at room temperature for at least 1 h before use. After immersion treatments, excess immersion solution was removed as described above, and samples were stored in sterile capped 50-ml polypropylene test tubes placed in refrigerated storage or incubation chambers (Memmert,

Schwabach, Germany) at desired incubation temperatures (6, 15, or 22°C) for different periods of time. At each step, samples (2.5 g) were mixed with 5 ml of sterile saline solution (0.85% NaCl) and pummeled for 3 min in a Stomacher 80 (Biomaster) before they were serially diluted in sterile saline solution and spread in triplicate on plates of PALCAM agar with added *Listeria* supplement (Merck). Plates were incubated at 37°C for 48 h, and the number of colonies showing features typical of *Listeria* was determined in order to calculate viable cell counts. Confirmation of *L. monocytogenes* was done by PCR amplification of the *hlyA* gene with primers DG69 (GTGCCGCAAGAAAAGGTTA) and DG74 (CGCCACTTGAGATAT) as described by Choi and Hong (16). The expected 636-bp amplicon was visualized after agarose gel electrophoresis. Before being artificially contaminated, a control sample of the raw material was tested for the presence of *L. monocytogenes* as described above, and positive samples were discarded.

Combined treatments of enterocin AS-48 and chemical preservatives were carried out on *L. monocytogenes* artificially contaminated green asparagus at room temperature essentially as described above using immersion solutions containing enterocin AS-48 (25 μ g/ml, final concentration) and/or the corresponding chemical compounds (at the final concentrations indicated above). Viable counts of *Listeria* were determined as described above after immersion treatment (time zero) and after 24 h of incubation at 22°C.

Statistical analyses. The average data from duplicate trials $\pm$ the standard deviations were determined with Excel program (Microsoft Corp.). In order to determine the statistical significance of data, a Student *t* test was performed at the 95% confidence interval with Statgraphics Plus version 5.1 (Statistical Graphics Corp.). The significance of combined treatments was determined by comparison of data from the same incubation time.

RESULTS

Effect of immersion solutions containing enterocin AS-48 on *L. monocytogenes* CECT 4032 inoculated on vegetables. Control samples of alfalfa sprouts artificially contaminated with *L. monocytogenes* still retained approximately 2.3 log CFU of viable *Listeria*/g after the control immersion treatment with distilled water. However, in samples treated with enterocin AS-48 the concentration of viable *Listeria* after immersion treatment was approximately 1.8 log CFU/g lower ($P < 0.05$) compared to control treatment for 12.5 μ g of AS-48/ml and approximately 2.3 log CFU/ml lower ($P < 0.05$) for 25 μ g of AS-48/ml. After incubation at 6°C of samples treated with AS-48 solutions, the concentrations of viable *Listeria* were always below detection limits (3 CFU/g) from days 1 to 7 of storage for bacteriocin concentrations of 12.5 and 25 μ g/ml. A lower bacteriocin concentration (5 μ g/ml) caused a smaller but still significant ($P < 0.05$) reduction of the initial population of viable *Listeria* at days 1 to 5 of storage but failed to inhibit growth at day 7 (Fig. 1A). During storage at 15°C, no viable *Listeria* were detected from days 1 to 7 in samples treated with 12.5- or 25- μ g/ml AS-48 solutions (Fig. 1B). In samples treated with 5- μ g/ml AS-48 solutions no viable *Listeria* were detected from days 1 to 5, although proliferation was detected at day 7. At 22°C, none of the bacteriocin concentrations tested was able to inhibit *Listeria* proliferation (in spite of the significant reductions obtained at time zero). However, the concentrations of viable *Listeria* were significantly lower ($P < 0.05$) at days 1 to 5 in samples treated with enterocin AS-48 at 12.5 or 25 μ g/ml and also at days 3 and 5 for 5 μ g/ml compared to the untreated control samples (Fig. 1C).

The concentration of viable *Listeria* in soybean sprouts after the control water immersion treatment was approximately 2.07 log CFU/g. However, after treatment with an immersion solution containing 25 μ g of AS-48/ml, viable counts of *Listeria* were significantly lower ($P < 0.05$) by approximately 1.8 to 2.0 log CFU/g. For samples treated with AS-48 solutions of 12.5

FIG. 1. Effect of immersion treatments with solutions containing enterocin AS-48 on survival and proliferation of *L. monocytogenes* CECT 4032 inoculated on alfalfa sprouts (A, B, and C), soybean sprouts (D, E, and F), and green asparagus (G, H, and I). After immersion for 5 min at room temperature in solutions containing final bacteriocin concentrations of 0 (○), 5 (△), 12.5 (●), and 25 (□) µg/ml, samples were incubated at 6°C (A, D, and G), 15°C (B, E, and H), or 22°C (C, F, and I). The data represent the average of two independent experiments plus standard deviations (error bars).

and 25 µg/ml, no viable listeria were detected from days 1 to 7 of storage at 6°C (Fig. 1D), suggesting that the adsorbed bacteriocin was still able to kill listeria during storage. However, the lowest bacteriocin concentration tested was unable to eliminate listeria during prolonged storage. Similar results were obtained for soybean sprouts stored at 15°C, since no viable listeria were detected during storage of samples treated with AS-48 solutions of 12.5 or 25 µg/ml (Fig. 1E). At 5 µg/ml, viable counts were also reduced below detection limits for days 3 to 5 of storage. At 22°C, soybean sprouts treated with a bacteriocin solution of 25 µg/ml were also free of detectable listeria from days 1 to 7 of storage (Fig. 1F). At 12.5 µg of AS-48/ml, listeria counts decreased gradually during the first 3 days of incubation, and no listeria were detected at days 5 and 7. At 5 µg/ml, no significant reduction of viable counts was detected, but listeria proliferation was inhibited and counts remained significantly lower ($P < 0.05$) compared to the untreated controls at days 1 and 3 of storage.

Application of immersion solutions containing enterocin AS-48 on listeria-contaminated green asparagus produced a much lower effect, and differences between control viable counts (2.01 to 2.44 log CFU/g) and enterocin AS-48-treated samples were not statistically significant ($P > 0.05$) at time zero. During storage at 6°C of samples treated with 25 µg of AS-48/ml the concentration of viable listeria was reduced significantly ($P < 0.05$) at day 1, and no viable cells were detected

after day 3 (Fig. 1G). Significant reductions ($P < 0.05$) of viable counts were also detected at days 1 and 3 for bacteriocin concentrations of 5 and 12.5 µg/ml. In samples stored at 15°C, listeria counts of samples treated with enterocin AS-48 solutions of 12.5 and also 25 µg/ml remained below detection limits at days 1 to 7 (Fig. 1H). A statistically significant reduction ($P < 0.05$) was also observed for 5 µg/ml at days 1, 3, and 7. In asparagus samples stored at 22°C, listeria counts did not decrease significantly (Fig. 1I). Growth of the listeria was inhibited in proportion to the bacteriocin concentration applied in the immersion treatment, and viable counts were significantly lower compared to control counts in samples treated with 12.5 and 25 µg/ml at days 1 to 7.

Effect of enterocin AS-48 in combination with chemical preservatives. The less favorable conditions for inhibition of *L. monocytogenes* CECT 4032 by AS-48 (green asparagus incubated at 22°C) were chosen for assay of combined treatments with chemical preservatives (Fig. 2). Application of immersion treatments consisting of enterocin AS-48 alone (25 µg/ml) did not reduce listeria counts significantly compared to counts obtained after the control water treatment (approximately 2.67 to 2.79 log CFU/g). Solutions containing up to 0.5% acetic acid, citric acid, sodium propionate, or potassium sorbate, as well as 0.1% lactic acid, had no effect on survival and further proliferation of *L. monocytogenes* inoculated on green asparagus, while sodium lactate only had limited effects (data not

FIG. 2. Effect of immersion treatments with solutions containing enterocin AS-48 (25 μ g/ml) and/or acetic acid, citric acid, sodium propionate, potassium sorbate, lactic acid, sodium lactate, sodium nitrite, potassium nitrate, trisodium phosphate (TSP), trisodium trimetaphosphate (TSTMP), sodium thiosulfate, potassium permanganate, *n*-propyl-*p*-hydroxybenzoate (Propyl-*p*-HB), *p*-hydroxybenzoic acid methyl ester (*p*-HBME), peroacetic acid, hexadecylpyridinium chloride (HDP), or sodium hypochlorite on survival and proliferation of *L. monocytogenes* CECT 4032 inoculated on green asparagus. Immersion treatments were applied for 5 min at room temperature. Viable cell counts were determined right after application of treatment (□) and after 24 h of incubation at 22°C (■). The data represent the average of two independent experiments plus standard deviations (error bars). Statistically significant reduction of viable counts is indicated by asterisk.

shown). When used in combination with enterocin AS-48, viable counts were significantly ($P < 0.05$) lower compared to samples treated with bacteriocin alone for 0.5% acetic acid (at 24 h of incubation), 0.5% citric acid (0 and 24 h of incubation), 0.1% sodium propionate (0 and 24 h of incubation), 0.5% propionate (24 h of incubation), 0.1% potassium sorbate (24 h of incubation), and 0.1% lactic acid (time zero) (Fig. 2). Moreover, no viable listeria were detected after 24 h of incubation of

samples treated with combinations of AS-48 and 0.1% lactic acid or 0.1% sodium lactate, as well as in samples treated with AS-48 plus 0.5% lactic acid or plus 0.5% sodium lactate for incubation times of 0 and 24 h (Fig. 2).

Solutions containing sodium nitrite as well as potassium nitrate (50 to 100 ppm) had no effect or almost no effect on viable counts of listeria inoculated on green asparagus (data not shown). The combination of AS-48 and 50 ppm sodium nitrite reduced viable listeria counts below detection limits at time zero of incubation and significantly ($P < 0.05$) reduced the number of survivors after 24 h of incubation, while 100 ppm nitrite plus AS-48 only caused a significant reduction ($P < 0.05$) at 24 h of incubation, indicating that there was no clear correlation between the nitrite concentration tested and reduction of viable counts. However, the combination of AS-48 and potassium nitrate was much more effective, reducing viable counts significantly ($P < 0.05$) at time zero and keeping listeria counts below detection limits at 24 h of treatment for both concentrations of nitrate tested (Fig. 2).

Treatment with trisodium phosphate alone did not decrease the viability of listeria (data not shown). However, solutions containing 1.5% trisodium phosphate and enterocin AS-48 reduced the population of viable listeria below detection limits at time zero and also achieved a significant reduction of viable counts ($P < 0.05$) at 24 h (Fig. 2). Treatment with solutions containing trisodium trimetaphosphate did not lower the initial counts of listeria but still reduced growth during the following 24 h of incubation (data not shown). At the lowest concentration tested, trisodium trimetaphosphate in combination with AS-48 exhibited an increased antimicrobial activity, as shown by the reduction of viable counts below detection limits at time zero and the significant ($P < 0.05$) reduction detected at 24 h of incubation (Fig. 2). Furthermore, no viable listeria were detected in all samples treated with enterocin AS-48 plus 0.5% trisodium trimetaphosphate (Fig. 2).

Although sodium thiosulfate had no effect on the viability or growth of listeria (data not shown), the activity of AS-48 was enhanced by this chemical preservative as shown by reduction of counts below detection limits after application of the combined treatment, and the significant ($P < 0.05$) reduction observed after 24 h of incubation (Fig. 2). A potassium permanganate solution (25 ppm) did not have any effect on survival and further growth of the listeria (data not shown), while the combination of AS-48 and permanganate significantly ($P < 0.05$) reduced viable counts after 24 h of incubation (Fig. 2).

Immersion in *n*-propyl *p*-hydroxybenzoate solutions had a marked effect on viability of listeria, in proportion to the concentration of the antimicrobial being used (data not shown). This effect was significantly enhanced in combination with enterocin AS-48, reducing viable counts below detection limits after 24 h (for 0.1% *n*-propyl *p*-hydroxybenzoate) or both at time zero and 24 h for 0.5% *n*-propyl *p*-hydroxybenzoate (Fig. 2). The effect of *p*-hydroxybenzoic acid methyl ester was comparatively lower when tested alone (data not shown). However, it had a marked antilisterial effect when used in combination with enterocin AS-48, as shown by the significant reductions ($P < 0.05$) of viable counts obtained for the lower concentration of this antimicrobial tested and the elimination of detectable listeria in all samples for the highest concentration tested (Fig. 2).

A solution containing peracetic acid (80 ppm) was insufficient to completely eliminate listeria (data not shown). Immersion in a solution containing peracetic acid plus enterocin AS-48 caused a significant ($P < 0.05$) reduction of viable counts right after treatment, and no viable listeria were detected at 24 h of incubation (Fig. 2).

Hexadecylpyridinium chloride showed a marked antilisterial effect at 0.5% but not at 0.1% (data not shown). Solutions of this compound plus enterocin AS-48 caused a significant ($P < 0.05$) reduction of viable counts or a complete elimination of detectable listeria both at time zero and in 24 h stored samples (Fig. 2).

Hypochlorite solutions (25 to 100 ppm free chlorine) reduced counts of viable listeria below detectable levels at time zero but afforded very limited protection during further incubation, as shown by growth of listeria in all treated samples (data not shown). Immersion of asparagus samples in solutions containing enterocin AS-48 and hypochlorite significantly reduced proliferation of listeria during the following 24 h of incubation, in proportion to the hypochlorite concentration tested (Fig. 2). In the case of asparagus samples treated with AS-48 plus 100 ppm hypochlorite, no viable listeria were detected in any of the treated samples.

Confirmation of *L. monocytogenes* in treated samples. The identity of *L. monocytogenes* in vegetable samples after treatments with enterocin AS-48 was confirmed by PCR amplification of the *hlyA* gene. Typically, PCR amplification of colonies isolated at random from PALCAM agar plates yielded a DNA band with the expected size of 635 bp (data not shown).

DISCUSSION

In agreement with previous reports (7, 11, 15, 19, 30, 37), results from the present study clearly indicate that *L. monocytogenes* is able to grow on alfalfa and soybean sprouts, as well as on green asparagus at temperatures of 6 to 22°C and to reach high cell numbers during storage. Therefore, it would be desirable to apply decontamination treatments to reduce the viable counts of listeria and avoid proliferation of this bacterium during further storage of the treated product before its consumption while having minimal effects on the vegetable organoleptic and physicochemical properties. According to previous studies carried out in culture media under laboratory conditions, *L. monocytogenes* is highly sensitive to enterocin AS-48 (34). Results from the present study indicated that application of immersion solutions containing AS-48 reduced viable counts of listeria considerably when the bacteriocin concentration applied was sufficiently high (25 µg/ml) in comparison with a control treatment in distilled water. However, at a lower bacteriocin concentration, it was also possible to reduce listeria counts during storage of sprouts under refrigeration conditions or under temperature abuse conditions of 15°C for up to 1 week. These results indicate that, after immersion treatment, sprouts became impregnated with sufficient amounts of AS-48 to kill the remaining viable listeria. Therefore, application of solutions containing AS-48 may provide an effective treatment to decontaminate sprouts and still yield an edible raw product.

The efficacy of solutions containing enterocin AS-48 for decontamination of *L. monocytogenes* inoculated on green aspar-

agus was comparatively much lower, especially at incubation temperatures of 6 and 22°C. However, it still was possible to suppress detectable listeria during storage at 15°C using low bacteriocin concentrations. Several factors may account for the temperature-dependent effect of enterocin AS-48, including a lower sensitivity of the listeria at refrigeration temperature as well as bacteriocin inactivation at the higher temperature by vegetable enzymes or by the accompanying mesophilic microbiota of vegetables. Conceivably, other key factor contributing to the observed differences in the efficacy of enterocin AS-48 in the different vegetable foods tested can be the interaction with food components, which may influence not only the amount of bacteriocin adsorbed on the vegetable surfaces but also diffusion and release of the adsorbed bacteriocin. Furthermore, in contrast to sprouts, green asparagus contained cut surfaces that may release nutrients and enzymes and also provide a different type of substrate on which the listeria may grow. Therefore, the different results obtained here illustrate the great influence of environmental conditions on the efficacy of bacteriocins in food systems, as suggested by other authors (22), and indicate that specific conditions must be established for particular applications of bacteriocins in order to achieve highest efficacy against *L. monocytogenes*.

Several chemical preservatives have also been used for decontamination of vegetable foods, although their efficacy is always limited when tested alone. As shown here, solutions of chemical preservatives that caused a remarkable reduction of viable counts were unable to prevent further growth of listeria during prolonged storage under temperature abuse conditions (the only exception being 0.5% hexadecylpyridinium chloride). Although there are several reports on the activity of chemical disinfectants against *L. monocytogenes* (5, 9, 18, 46), the lack of chemical or water-rinse treatments that will effectively decontaminate vegetables and prevent regrowth of listeria strengthens the need for alternative or combined decontamination treatments. Interestingly, results presented in the present study clearly indicate that combinations of enterocin AS-48 and several chemical preservatives showed increased antimicrobial activity, and solutions containing AS-48 in combination with selected chemical preservatives can effectively decontaminate green asparagus and avoid listeria proliferation during storage under extreme temperature abuse conditions. Therefore, depending on the type of food, immersion solutions containing enterocin AS-48 alone or in combination with chemical preservatives could provide an efficient method for decontamination of selected raw vegetable foods, such as added-value foods or special foods prepared for individuals that are more susceptible to listeria infections.

This is the first report on the application of enterocin AS-48 for decontamination of vegetable foods. In previous studies, the application of the bacteriocin nisin for biocontrol of bacterial pathogens in fresh-cut produce has been examined (27, 41). Furthermore, competitive exclusion techniques, where nonpathogenic microorganisms are used to repress the growth of pathogenic bacteria during sprouting have been suggested previously. Isolation of naturally occurring microbes that produce antimicrobial substances against pathogens in fresh produce products has been reported (11, 14, 28, 45), and several strains of lactic acid bacteria have been shown to be effective in suppressing the growth of pathogens on ready-to-use vegeta-

bles (43). When coinoculated with *L. monocytogenes*, nisin-producing lactococci isolated from bean sprouts reduced the levels of the pathogen by one log (13). However, Bennik et al. (3) reported that a bacteriocinogenic *Enterococcus mundtii* isolate inhibited *L. monocytogenes* on sterile vegetable medium but not on fresh mung bean sprouts and Harp and Gilliland (25) also reported that a hydrogen peroxide-producing strain of *Lactobacillus delbrueckii* was unable to control *E. coli* O157:H7 and *L. monocytogenes* in fresh-cut vegetables, indicating that bacterial antagonism is largely influenced by environmental conditions and especially the food microbiota. Therefore, application of *ex situ* produced bacteriocins seems a reasonable alternative to avoid problems of *in situ* bacteriocin production such as slow or limited growth of the producer strain and production of insufficient bacteriocin amounts (especially at low storage temperatures), poor survival under environmental stress condition, or antagonism by competing bacteria. Because of its broad spectrum of inhibition and increased stability due to its cyclic structure (31), enterocin AS-48 may be a sound candidate for decontamination of vegetable foods containing *L. monocytogenes* and other food-borne bacteria sensitive to this bacteriocin. Therefore, the spectrum of applications of AS-48 for decontamination of vegetable foods should be further investigated in future studies.

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