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Food and Chemical Toxicology 44 (2006) 1774–1781
www.elsevier.com/locate/foodchemtox

Application of the broad-spectrum bacteriocin enterocin AS-48 to inhibit *Bacillus coagulans* in canned fruit and vegetable foods

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Received 7 July 2005; accepted 20 May 2006

Abstract

The enterococcal bacteriocin (enterocin) AS-48 is a broad-spectrum cyclic peptide. Enterocin AS-48 was tested against *Bacillus coagulans* in three vegetable canned foods: tomato paste (pH 4.64), syrup from canned peaches (pH 3.97), and juice from canned pineapple (pH 3.65). When vegetative cells of *B. coagulans* CECT (Spanish Type Culture Collection) 12 were inoculated in tomato paste supplemented with 6 μg/ml AS-48 and stored at different temperatures, viable cell counts were reduced by approximately 2.37 (4 °C), 4.3 (22 °C) and 3.0 (37 °C) log units within 24 h storage. After 15-days storage, no viable cells were detected in any sample. Strain *B. coagulans* CECT 561 showed a poor survival in tomato paste, but surviving cells were also killed by AS-48. The bacteriocin was also very active against *B. coagulans* CECT 12 vegetative cells in juice from canned pineapple stored at 22 °C, and slightly less active in syrup from canned peaches. In food samples supplemented with 1.5% lactic acid, enterocin AS-48 (6 μg/ml) rapidly reduced viable counts of vegetative cells below detection limits within 24 h storage. Addition of glucose and sucrose (10% and 20%) significantly increased bacteriocin activity against vegetative cells of *B. coagulans* CECT 12. Enterocin AS-48 had no significant effect on *B. coagulans* CECT 12 spores. However, the combined application of AS-48 and heat (80–95 °C for 5 min) significantly increased the effect of thermal treatments on spores.

Keywords: *Bacillus coagulans*; Canned vegetables; Flat sour; Biopreservation; Enterocin AS-48

1. Introduction

Food canning has been one classical way to provide a continuous supply of food independently of the seasonal availability of raw materials. Acidification and thermal treatment are two widely used methods in preservation of canned foods. However, several types of bacteria can tolerate these adverse conditions and proliferate in foods causing spoilage. Endospore-forming bacteria are a common problem in the food industry both because they are frequently found in many raw materials and because endospores require more intense treatments for inactivation than vegetative cells. This results in higher processing costs and less preserved product quality. Survival of bacterial endospores during food processing is of special significance in canned foods, and other additional hurdles such as acidification are often required in order to ameliorate endospore outgrowth and food spoilage. *Bacillus coagulans* is a slightly acidophilic and thermotolerant spoilage bacterium of considerable concern during the processing of acid and acidified foods. This bacterium may cause "flat-sour" spoilage, due to the production of lactic acid without gas formation. Spores of *B. coagulans* are able to germinate and grow at pH values as low as 4, and are of significance in vegetable foods (Brackett, 2001) and in canned fruits, especially in tomato products, with a pH of 4.1–5.0. For this reason, *B. coagulans* is the microorganism most frequently isolated

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doi:10.1016/j.fct.2006.05.019

from spoiled canned vegetables acidified to pH values between 4 and 4.5 (Mallidis et al., 1990). In low pH foods, *B. coagulans* is also able to increase the food pH to values that can allow germination of surviving *C. botulinum* spores (Fields et al., 1977; Anderson, 1984). Moreover, this bacterium is also found frequently in milk and some dairy products (Shehata et al., 1983; Cosentino et al., 1997), and has been involved in alterations like "flat sour" of evaporated milk (Kalogridou-Vassiliadou, 1992) and "sweet coagulation" of canned condensed milk (Carić, 1994). In addition, it has also been described among the bacteria involved in cork taint wine spoilage (Sponholz, 1993).

The application of bacteriocins as natural antimicrobial substances in biopreservation (the use of living cells and/or their products for preservation purposes) has focused mainly on foods and foodstuffs from animal origin (Stiles, 1996; Cleveland et al., 2001; Devlieghere et al., 2004) and very little work has been done on application of bacteriocins for preservation of vegetable foods. There are scarce reports on the effects of bacteriocins against *B. coagulans*, like the lantibiotic nisin (Roberts and Hoover, 1996) and the enterococcal bacteriocin EJ97 (Gálvez et al., 1998; García et al., 2003). The broad-spectrum cyclic peptide bacteriocin enterocin AS-48 from *Enterococcus faecalis* is also active against different species of bacilli (Gálvez et al., 1986). The various studies carried out on this bacteriocin have contributed to elucidate its molecular composition and structure as well as its mode of action and its genetic determinants (reviewed by Maqueda et al., 2004). Enterocin AS-48 is heat-resistant, sensitive to digestive proteases, non-toxic to eukaryotic cells, and offers a good potential for application in food preservation (Maqueda et al., 2004). Recently, satisfactory results on application of enterocin AS-48 have been reported for dairy products, meat, and fruit juices including pathogenic and/or spoilage bacteria like *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus* and *Alicyclobacillus* sp. (Muñoz et al., 2004; Ananou et al., 2005; Grande et al., 2005a). The purpose of the present work was to test the efficacy of enterocin AS-48 in canned vegetable foods against the spoilage bacterium *B. coagulans*. Specific objectives were to determine the concentration of AS-48 required for inactivation of *B. coagulans* vegetative cells, to determine the influence of additives (lactic acid and sugars) on bacteriocin activity, and to test whether the lethal effect of heat treatments on endospores of this bacterium could be potentiated by bacteriocin addition.

2. Materials and methods

2.1. Canned products

The following food products were used in the present study: commercial canned tomato paste, pH 4.64 (natural paste from extra quality tomatoes; Orlando Heinz ibérica S.A., Alfaro, La Rioja, Spain), syrup from canned peaches, pH 3.97 (peach halves in syrup—17–20° Brix; Hacendado™; Alcurnia Alimentación S.L., Molina de Segura, Murcia, Spain) and juice from canned pineapple, pH 3.65 (sliced pineapple in pineapple juice; PT Great Giant Pineapple, Lampung, Indonesia).

2.2. Bacterial strains and culture conditions

B. coagulans strains CECT 12 and CECT 561 were supplied by the Spanish Type Culture Collection (CECT). *E. faecalis* A-48-32 (a cured mutant from strain *E. faecalis* S-48; Martinez-Bueno et al., 1990) was used to produce enterocin AS-48. *E. faecalis* S-47 was used as an indicator strain for determination of bacteriocin activity. Bacilli were grown in Tryptic Soya Broth (TSB, Scharlab, Barcelona) or Tryptic Soya Agar (TSA, Scharlab) at 37 °C. Enterococci were propagated in Brain Heart Infusion broth (BHI; Scharlau, Barcelona) at 37 °C.

2.3. Preparation of endospore suspensions

Cultures of *B. coagulans* CECT 12 grown in BHI broth for 24 h were surface-spread on a solid sporulation medium consisting of nutrient agar (NA, Oxoid, Madrid) supplemented with 0.05 g/l of $MnSO_4$ (NAMS agar) and incubated at 37 °C according to Beuchat et al. (1997), to obtain at least 90–95% spores (4–5 days). Spores were collected with a sterile cotton swab and resuspended in sterile distilled water (3 ml per plate). The pool of spores collected from the different plates was centrifuged at $5000 \times g$ for 15 min at 4 °C, washed two times with sterile distilled water by repeated centrifugation, and finally resuspended in sterile distilled water (6–7 log units/ml, as determined by plating on TSA) and stored in Eppendorf tubes at −20 °C until use. The number of spores was determined by serially diluting the heat-shocked spore suspensions in sterile saline solution and plating by triplicate on TSA. Plates were incubated for 48 h at 37 °C and the grown colonies were counted.

2.4. Bacteriocin preparation

Enterocin AS-48 was obtained from cultured broths of the producer strain *E. faecalis* A-48-32 after concentration by cation exchange chromatography as described by Abriouel et al. (2003). Bacteriocin concentrates were filtered through 0.22 μm pore size low protein binding filters (Millex GV; Millipore Corp., Belford, MA, USA) under sterile conditions. Samples were serially diluted and tested (100 μl) 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 (Gálvez et al., 1986). One arbitrary unit (AU) was defined as the highest dilution producing a visible (9 mm diameter) zone of inhibition. The bacteriocin concentration of samples was determined from the previously-published specific activity value of 3.5 AU/μg protein (Abriouel et al., 2003).

2.5. Bacteriocin treatment

Exponential-phase cultures of *B. coagulans* CECT 12 or *B. coagulans* CECT 561 grown overnight (12 h) in BHI broth at 37 °C were inoculated in duplicate onto food products, and then enterocin AS-48 was added at final concentrations of 3 and 6 μg/ml. Food samples were thoroughly mixed and stored in an incubator at 37 °C or at 22 °C in a refrigerated incubation chamber (Memmert, Schwabach, Germany), or stored under refrigeration at 4 °C. At different intervals of incubation at the desired temperature, food samples were serially-diluted in sterile saline solution with vigorous vortexing and plated in triplicate on TSA. Sterile 1-ml tips with approximately 0.5 cm excised ends were used to dispense samples with higher viscosity when necessary. Plates were incubated at 37 °C for 48 h and the average number of colonies was used to calculate the initial concentration of viable cells, expressed as the $\log_{10}$ colony forming units (CFU) per ml (log units). The detection limit was 10 CFU/ml. Negative controls of tomato, pineapple and peach samples were always carried out in order to corroborate that the starting food material did not contain *B. coagulans*.

2.6. Effect of lactic acid and enterocin AS-48 on B. coagulans vegetative cells

Exponential-phase cultures of *B. coagulans* CECT 12 prepared as described above were inoculated in duplicate on the food samples

described above supplemented with 1.5% lactic acid (Sigma, Madrid, Spain), either alone or in combination with enterocin AS-48 at final concentrations of 3 and 6 μg/ml. At desired intervals of incubation at 22 °C, viable counts were determined as described above.

2.7. Effect of glucose and sucrose plus enterocin AS-48 on *B. coagulans* vegetative cells

Sterile saline solutions (0.85% NaCl, PanReac, Barcelona, Spain) supplemented with different concentrations (10% and 20%, wt/vol) of glucose (Fluka, Madrid, Spain) or sucrose (Sigma) were inoculated in duplicate with exponential-phase cultures of *B. coagulans* CECT 12 prepared as described above and then supplemented with enterocin AS-48 at final concentrations of 2, 4, 6, and 10 μg/ml. After 10 min incubation at 22 °C, the viable counts of samples were determined as described above.

2.8. Effect of enterocin AS-48 on *B. coagulans* endospores in combination with heat treatments

The combined effect of enterocin AS-48 and heat treatments was tested on endospores of *B. coagulans* CECT 12 inoculated on canned tomato paste as well as syrup from canned peaches and juice from canned pineapple. Aliquots (1 ml) of each inoculated food were placed in sterile glass tubes (12 × 80 mm, Corning Glass Works, Medfield, MA) with or without enterocin AS-48 (3 and 6 μg/ml, final concentration), and immersed in a water bath (Memmert) previously warmed at desired temperatures (22, 80, 85, 90 or 95 °C). After 5 min incubation, samples (in duplicate) were cooled on ice, and 0.2 ml aliquots were removed from each tube and serially-diluted in ice-cold sterile saline solution and plated on TSA for viable counts. The heat-treated samples were also incubated at 22 °C for 48 h before they were serially diluted and plated for viable cell counts.

2.9. Statistical analyses

The average data ± standard deviations were determined with Excel programme (Microsoft Corp., USA). In order to determine the statistical significance of data, a paired *t*-test was performed at the 95% confidence interval with Statgraphics Plus version 5.1 (Statistical Graphics Corp, USA). The significance of combined treatments was determined by comparison of data from the same incubation time.

3. Results

3.1. Effect of enterocin AS-48 on *B. coagulans* vegetative cells in canned tomato paste

Strains of *B. coagulans* CECT 12 and CECT 561 were inoculated on a commercial tomato paste and stored at temperatures of 37, 22 and 4 °C (Fig. 1). At 37 °C, the concentration of viable cells for strain CECT 12 was reduced significantly ($P < 0.05$) within the first 24 h by 2.3 and 3.0 log units for 3 and 6 μg/ml AS-48, respectively

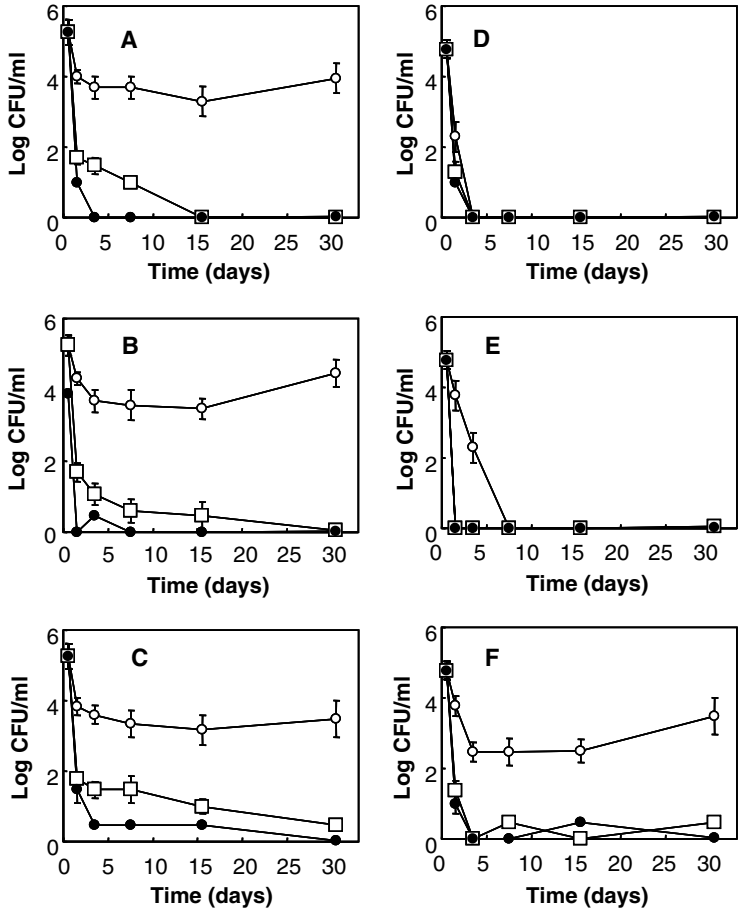

Fig. 1. Effect of enterocin AS-48 on vegetative cells of *B. coagulans* CECT 12 (A–C) and *B. coagulans* CECT 561 (D–F) inoculated on a commercial tomato paste. Samples were incubated at 37 °C (A, D), 22 °C (B, E), and 4 °C (C, F). Food samples were supplemented with enterocin AS-48 final concentrations of 0 (○), 3 (□) and 6 μg/ml (●). The average data of two determinations ± standard deviation (error flags) are shown.

(Fig. 1A). Viable counts were reduced below detection limits after 3 days for 6 μg/ml AS-48 or after 15 days for 3 μg/ml. Similar reductions were obtained in sample stored at 22 °C (Fig. 1B) and 4 °C (Fig. 1C), but the surviving fraction decreased more slowly during prolonged storage at both temperatures (Fig. 1B and C).

Strain CECT 561 showed a very weak survival in tomato paste at 37 °C and 22 °C, but the surviving fraction was rapidly killed by bacteriocin (Fig. 1D and E). Survival was much higher in control samples stored at 4 °C, but addition of AS-48 reduced viable counts significantly ($P < 0.05$) to values very close to or below detection limits during further storage (Fig. 1F).

3.2. Influence of lactic acid on the effect of enterocin AS-48 against *B. coagulans* CECT 12 vegetative cells in canned foods

Enterocin AS-48 (3 and 6 μg/ml) was tested alone or in combination with 1.5% lactic acid in tomato paste, in syrup from canned peach and in juice from canned pineapple at 22 °C (Fig. 2). In tomato paste, reductions of viable cell counts at 24 h storage for samples supplemented with AS-48 alone (Fig. 2A) did not differ significantly (P, 0.32 and P, 0.10 for 3 and 6 μg/ml AS-48, respectively) from samples supplemented with AS-48 and lactic acid (Fig. 2B). Similar results were obtained in juice from canned pineapple, in which the bacteriocin was also highly effective (Fig. 2C and D).

In canned peach syrup, addition of enterocin AS-48 significantly reduced viable counts ($P < 0.05$) within the first 24 h, but the remaining viable cells were inactivated more slowly during further storage (Fig. 2E). By comparison, bacterial inactivation occurred more rapidly in samples supplemented with 1.5% lactic acid plus AS-48, and no viable cells were detected after 24 h storage (Fig. 2F).

3.3. Influence of sugar concentration on bacteriocin activity

B. coagulans CECT 12 vegetative cells were inoculated onto sterile saline solution alone or supplemented with

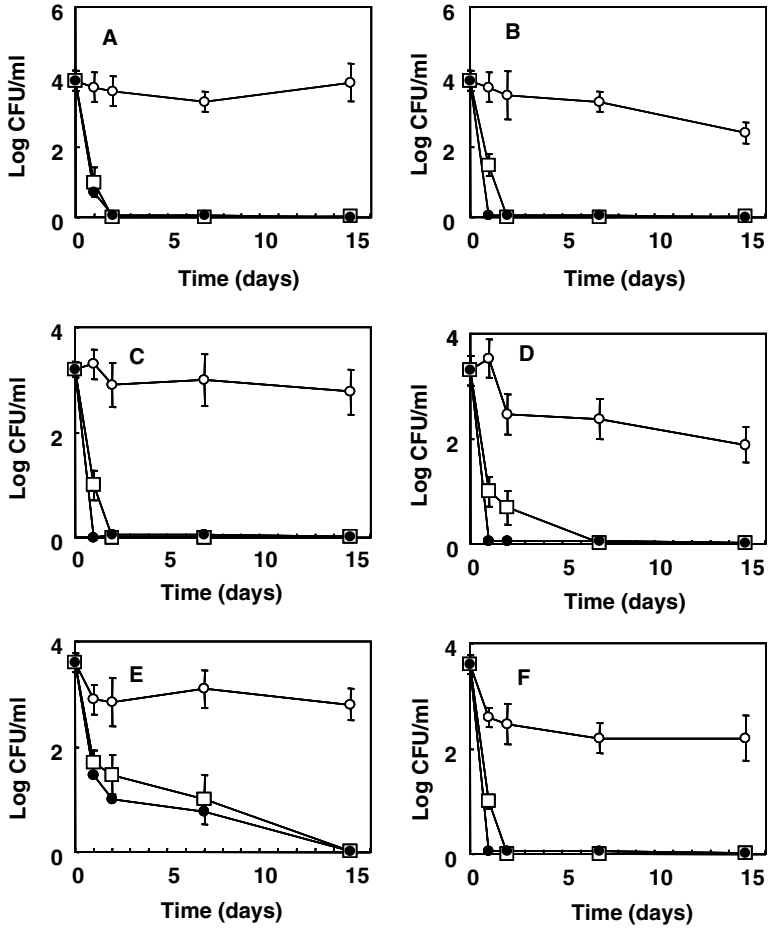

Fig. 2. Effect of enterocin AS-48 against *B. coagulans* CECT 12 vegetative cells in canned tomato paste (A, B), in juice from canned pineapple (C, D) and syrup from canned peach (E, F). Enterocin AS-48 was tested alone (A, C, E) and in combination with 1.5% lactic acid (B, D, F). Food samples were supplemented with enterocin AS-48 final concentrations of 3 (□) and 6 μg/ml (●), and incubated at 22 °C. Controls (○) were carried out without added bacteriocin (A, C, E) or were supplemented with 1.5% lactic acid alone (B, D, F). The average data of two determinations ± standard deviation (error flags) are shown.

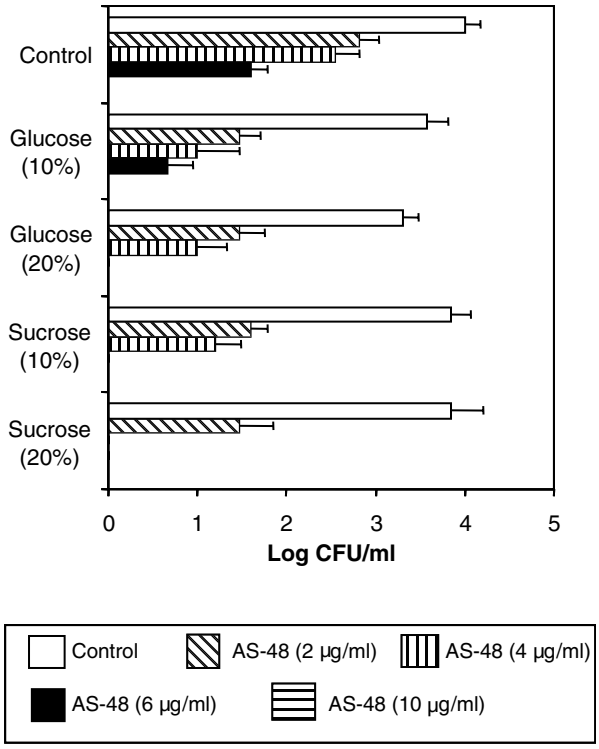

Fig. 3. Effect of enterocin AS-48 against *B. coagulans* CECT 12 vegetative cells in saline solution with or without added glucose or sucrose. Samples were incubated at 22 °C for 10 min with bacteriocin concentrations of 0 (controls), 2, 4, 6 and 10 μg/ml. The average data of two determinations ± standard deviation (error flags) are shown.

different sugar concentrations and incubated with enterocin AS-48 for 10 min (Fig. 3). In saline solution, viable counts decreased significantly ($P < 0.05$) in proportion to the bacteriocin concentration tested, and no viable cells were detected at 10 μg/ml AS-48. Reduction of viable counts by enterocin AS-48 was significantly higher ($P < 0.05$) in samples containing glucose compared to samples without added sugar. Moreover, at 20% glucose the bacteriocin concentration required to inactivate all detectable viable cells was reduced to 6 μg/ml. Similar results were obtained for sucrose. In this case, the bacteriocin concentration required to inactivate all detectable viable cells was reduced to 6 μg/ml AS-48 for 10% sucrose, and to 4 μg/ml for 20% sucrose.

3.4. Effect of enterocin AS-48 in combination with heat treatments on survival of B. coagulans CECT 12 spores

The combined effects of heat treatments and enterocin AS-48 (3 and 6 μg/ml) were tested on spores of *B. coagulans* CECT 12 inoculated in tomato paste, syrup from canned peaches, and juice from canned pineapple (Fig. 4). Incubation of *B. coagulans* CECT 12 spores with enterocin AS-48 without application of any heat treatment (22 °C in graphs) did not reduce significantly the viable counts compared to control samples without added AS-48. Application of 5-min heat treatments at temperatures from 80 °C to 95 °C in the presence of AS-48 significantly reduced viable counts ($P < 0.05$) in the three types of food tested for both bacteriocin concentrations in comparison to samples heated without added bacteriocin (Fig. 4A–C, panel 1). Reductions of viable counts were always higher as bacteriocin concentration increased.

After 48 h storage at 22 °C, viable counts of non-heated samples supplemented with AS-48 did not change significantly compared to initial values (Fig. 4A–C, panel 2). Viable counts of samples heated with AS-48 remained significantly lower compared to samples heated without bacteriocin, for both bacteriocin concentrations and all heating temperatures (Fig. 4A–C, panel 2).

Analysis of the relationship between heat temperature and survivors revealed that viable counts of samples supplemented with AS-48 decreased as heating temperature increased. After 48 h, viable counts from samples heated at 95 °C with 6 μg/ml AS-48 were significantly lower ($P < 0.05$) compared to the 80 °C combined treatment for all three food types tested.

4. Discussion

Results from the present work suggest that enterocin AS-48 can inactivate vegetative cells of *B. coagulans* in vegetable canned foods down to levels where they do not pose risk of food spoilage. Inactivation of *B. coagulans* vegetative cells by enterocin AS-48 occurred both under refrigeration conditions as well as at higher temperatures similar to the usual handling and storage temperatures of canned foods. This should be an advantage for the potential application of this bacteriocin, since control of pathogenic and food spoilage bacteria is often more difficult under temperature abuse conditions within the range of optimal growth temperature of the bacteria involved. Bacteriocin activity against *B. coagulans* did not change markedly from one food to another, although a slightly lower activity was detected in syrup from canned peaches. Little is known on the factors influencing bacteriocin activity in vegetable foods, but it has been described that the activity of bacteriocins is greatly influenced by the food composition (Gänzle et al., 1999; Aasen et al., 2003). A recent work indicated that residual enterocin AS-48 activity decreased markedly in fresh-made tomato juice, but not so much in diluted juice or in juice exposed to a mild heat treatment (Grande et al., 2005b). However, enterocin AS-48 was very active in the commercial canned tomato paste used in the present study, suggesting that the food processing operations applied to the tomato paste probably destroyed any proteases from the vegetable substrate which may inactivate AS-48. In addition, enterocin AS-48 may also interact with food components resulting in a slow release of the bacteriocin molecules, which may enhance bacteriocin efficacy during prolonged storage periods.

Lactic acid is often used as an acidulant with antimicrobial properties, and it has been shown to presensitize *B. cereus* to low dose gamma irradiation (Bhide et al., 2001)

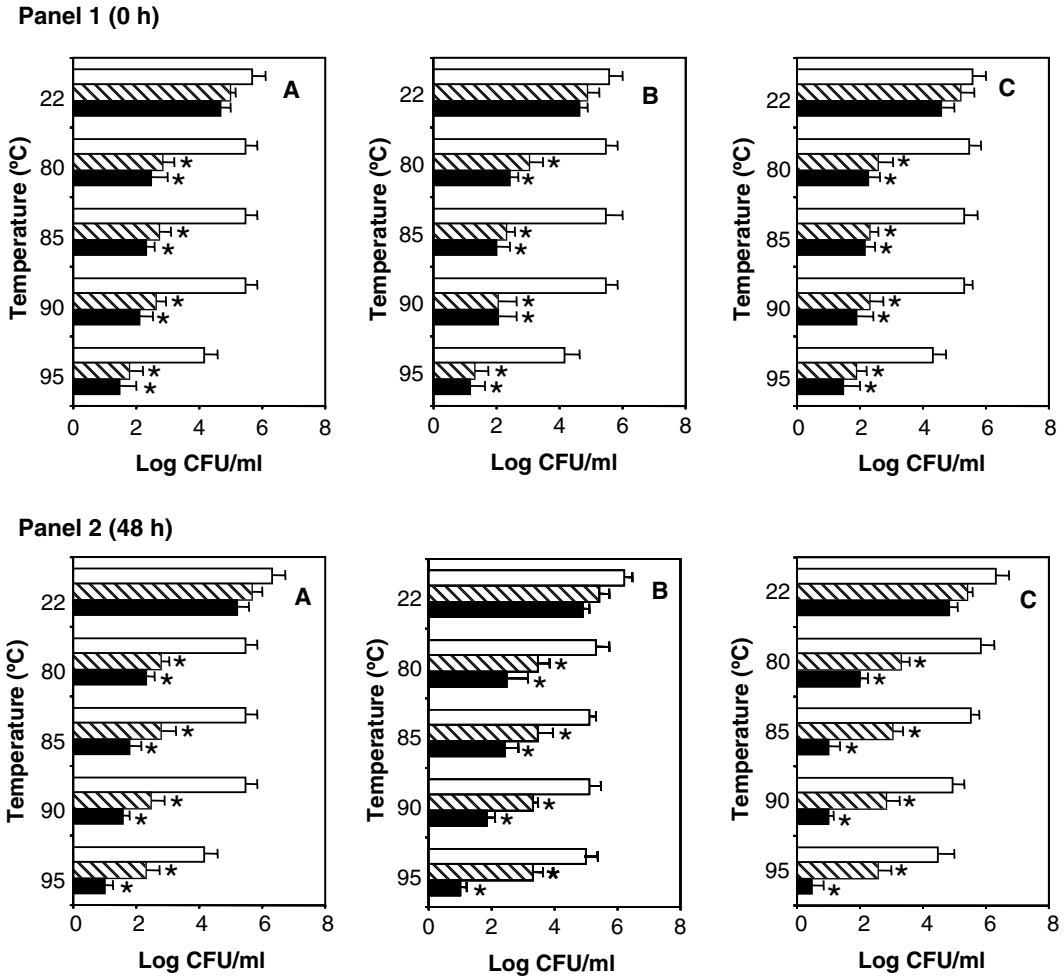

Fig. 4. Effect of enterocin AS-48 against *B. coagulans* CECT 12 endospores in canned tomato paste (A), syrup from canned peach (B) and juice from canned pineapple (C) in combination with 5-min heat treatments. Enterocin AS-48 was added at final concentrations of 3 (striped bars) and 6 μg/ml (closed bars) right before application of heat treatments. Controls without added bacteriocin are represented by empty bars. Counts were determined right after application of heat treatment (panel 1) or after 48 h incubation at 22 °C (panel 2). The average data of two determinations ± standard deviation (error flags) are shown. Asterisks denote statistically significant reductions of viable counts compared to samples heated without bacteriocin.

and to lower the number of survivors for heat-treated spores of *B. coagulans* (Palop et al., 1997). Activity of enterocin AS-48 against *B. coagulans* CECT12 only increased very slightly in combination with 1.5% lactic acid, especially in peach syrup. Since *B. coagulans* is a lactic acid producing bacterium (Heriban et al., 1993; Ohara and Yahata, 1996; Payot et al., 1999) it may also show increased resistance to this organic acid.

Sugar addition is a common practice in several canned fruit and vegetable foods not only to improve the organoleptic properties but also to increase osmolarity and decrease water activity, providing an additional hurdle to microbial growth (Jay, 2000). The activity of enterocin AS-48 against *B. coagulans* CECT 12 was clearly potentiated both by glucose and sucrose in a concentration-dependent way. At higher sugar concentrations, lower bacteriocin concentrations were required to reduce viable cell counts below detection limits. Nevertheless, these results are in disagreement with data from canned peach syrup also containing a high sugar concentration. Other factors such as bacteriocin interaction with food components or other undeclared additives of the canned peaches may account for the observed differences. Once more, these results indicate that the ultimate antibacterial effect of enterocin AS-48 in foods is a complex balance between different factors that may potentiate or counteract bacteriocin activity.

Endospores surviving heat treatments applied during or after food processing represent the highest risk for spoilage of canned foods. Since endospore-forming bacteria are widely distributed in nature, especially in soil and vegetable materials, canned vegetable foods are also of higher risk for spore contamination. For this reason, the combined effect of mild heat treatments and enterocin AS-48 was tested. While application of heat treatments alone of up to 95 °C for 5 min had very limited effect on viability of *B. coagulans* CECT 12 endospores, the significant reductions of viable counts obtained after application of combined treatments of heat and AS-48, and the observed decrease in the concentrations of the remaining viable spores during

prolonged storage are of great relevance for application of less intense heat treatments in canned fruit and vegetable foods. Bacterial endospores are usually resistant to bacteriocins and other antimicrobial agents. Exceptions are endospores from *Alicyclobacillus acidoterrestris*, which are sensitive to enterocin AS-48 (Grande et al., 2005a). Endospores of *B. cereus* are resistant to enterocin AS-48, but they become sensitive gradually during the course of germination and outgrowth (Abriouel et al., 2002). Furthermore, application of sublethal heat treatments in combination with nisin has been reported to reduce the viability of *B. stearothermophilus* and *B. licheniformis* endospores (Beard et al., 1999; Wandling et al., 1999). A recent work has shown that enterocin AS-48 can drastically reduce the viable population of *B. cereus* spores in food in combination with heat treatments (Grande et al., 2006) in agreement with results reported in the present work for *B. coagulans*.

In conclusion, this study suggests that, although *B. coagulans* endospores are resistant to enterocin AS-48, the added bacteriocin increases the efficacy of thermal treatments. Therefore, application of less intense heat treatments in combination with AS-48 could lower the processing costs in terms of energy input while improving the preservation of the food nutritional value and organoleptic properties, and increasing acceptance by consumers. Furthermore, added at concentrations as low as 6 μg/ml, enterocin AS-48 can suppress *B. coagulans* vegetative cells in canned vegetable foods. Depending on the food type, the efficacy of AS-48 could be potentiated by other additives like lactic acid, glucose or sucrose. Since vegetative cells of *B. coaglans* are responsible for flat-sour spoilage, enterocin AS-48 could be used as an additional hurdle against spoilage of canned vegetables caused by this bacterium.

Acknowledgements

This work was supported by the Spanish Ministry of Science and Technology and FEDER funds (research project AGL2001-3315-CO2-02). We also acknowledge the Research Plan of the Junta de Andalucía (research group AGR230) from which M[a] J. Grande received a fellowship, and the Research Programme of the University of Jaén.

References

Aasen, I.M., Markussen, S., Møretrø, T., Katla, T., Axelsson, L., Naterstad, K., 2003. Interactions of the bacteriocins sakacin P and nisin with food constituents. International Journal of Food Microbiology 87, 35–43.

Abriouel, H., Maqueda, M., Gálvez, A., Martínez Bueno, M., Valdivia, E., 2002. Inhibition of bacterial growth, enterotoxin production, and spore outgrowth in strains of *Bacillus cereus* by bacteriocin AS-48. Applied and Environmental Microbiology 681, 1473–1477.

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. Journal of Microbiological Methods 55, 599–605.

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. International Journal of Food Microbiology 103, 179–190.

Anderson, R.E., 1984. Growth and corresponding elevation of tomato juice pH by *Bacillus coagulans*. Journal of Food Science 49, 647–649.

Beard, B.M., Sheldon, B.W., Foegeding, P.M., 1999. Thermal resistance of bacterial spores in milk-based beverages supplemented with nisin. Journal of Food Protection 62, 484–491.

Beuchat, L.R., Clavero, M.R., Jaquette, C.B., 1997. Effects of nisin and temperature on survival, growth, and enterotoxin production characteristics of psychrotrophic *Bacillus cereus* in beef gravy. Applied and Environmental Microbiology 63, 1953–1958.

Bhide, M.R., Paturkar, A.M., Sherikar, A.T., Waskar, V.S., 2001. Presensitization of microorganisms by acid treatments to low dose gamma irradiation with special reference to *Bacillus cereus*. Meat Science 58, 253–258.

Brackett, R.E., 2001. Fruits, vegetables, and grains. In: Doyle, M., Beuchat, L.R., Montville, T.J. (Eds.), Food Microbiology, Fundamentals and Frontiers. ASM Press, Washington, DC, pp. 127–138.

Carić, M., 1994. Concentrated and Dried Dairy Products. VCH Publ., Inc., New York,NY.

Cleveland, J., Montville, T.J., Nes, I.F., Chikindas, M.L., 2001. Bacteriocins: safe, natural antimicrobials for food preservation. International Journal of Food Microbiology 71, 1–20.

Cosentino, S., Mulargia, A.F., Pisano, B., Tuveri, P., Palmas, F., 1997. Incidence and biochemical characteristics of *Bacillus* flora in Sardinian dairy products. International Journal of Food Microbiology 38, 235–238.

Devlieghere, F., Vermeiren, L., Debevere, J., 2004. New preservation technologies: pssibilities and limitations. International Dairy Journal 14, 273–285.

Fields, M.L., Zamora, A.F., Bradsher, M., 1977. Microbiological analysis of home-canned tomatoes and green beans. Journal of Food Science 42, 931–934.

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*. Canadian Journal of Microbiology 32, 765–771.

Gálvez, A., Valdivia, E., Abriouel, H., Camafeita, E., Méndez, E., Martínez-Bueno, M., Maqueda, M., 1998. Isolation and characterization of enterocin EJ97, a bacteriocin produced by *Enterococcus faecalis* EJ97. Archives of Microbiology 171, 59–65.

Gänzle, M.G., Weber, S., Hammes, W.P., 1999. Effect of ecological factors on the inhibitory spectrum and activity of bacteriocins. International Journal of Food Microbiology 46, 207–217.

García, M[a] T., Ben Omar, N., Lucas, R., Pérez-Pulido, R., Castro, A., Grande, M[a] J., Martínez-Cañamero, M., Gálvez, A., 2003. Antimicrobial activity of enterocin EJ97 on *Bacillus coagulans* CECT 12. Food Microbiology 20, 533–536.

Grande, M[a] J., Lucas, R., Abriouel, H., Ben Omar, N., Maqueda, M., Martínez-Bueno, M., Martínez-Cañamero, M., Valdivia, E., Gálvez, A., 2005a. Control of *Alicyclobacillus acidoterrestris* in fruit juices by enterocin AS-48. International Journal of Food Microbiology 104, 289–297.

Grande, M[a] J., Lucas, R., Valdivia, E., Abriouel, H., Maqueda, M., Martínez-Cañamero, M., Ben Omar, N., Gálvez, A., 2005b. Stability of enterocin AS-48 in fruit and vegetable juices. Journal of Food Protection 68, 2085–2094.

Grande, M[a] J., 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. International Journal of Food Microbiology 106, 185–194.

Heriban, V., Sturdik, E., Zalibera, L., Matus, P., 1993. Process and metabolic characteristics of *Bacillus coagulans* as a lactic acid producer. Letters in Applied Microbiology 16, 243–246.

Jay, J.M., 2000. Modern Food Microbiology, sixth ed. Aspen Publishers, Inc., Gaithersburg, MD.

Kalogridou-Vassiliadou, D., 1992. Biochemical activities of *Bacillus* species isolated from flat sour evaporated milk. Journal of Dairy Science 75, 2681–2686.

Mallidis, C.G., Frantzeskakis, P., Balatsouras, G., Katsabotxakis, C., 1990. Thermal treatment of aseptically processed tomato paste. International Journal of Food Science and Technology 25, 442–448.

Maqueda, M., Gálvez, A., Martínez Bueno, M., Sanchez-Barrena, M[a] J., González, C., Albert, A., Rico, M., Valdivia, E., 2004. Peptide AS-48: prototype of a new class of cyclic bacteriocins. Current Protein and Peptide Science 5, 399–416.

Martinez-Bueno, M., Galvez, A., Valdivia, E., Maqueda, M., 1990. A transferable plasmid associated with AS-48 production in *Enterococcus faecalis*. Journal of Bacteriology 172, 2817–2818.

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. Journal of Food Protection 67, 1517–1521.

Ohara, H., Yahata, M., 1996. L-Lactic acid production by *Bacillus* sp. in anaerobic and aerobic culture. Journal of Fermentation and Bioengineering 81, 272–274.

Palop, A., Marco, A., Raso, J., Sala, F.J., Condón, S., 1997. Survival of heated *Bacillus coagulans* spores in a medium acidified with lactic or citric acid. International Journal of Food Microbiology 38, 25–30.

Payot, T., Chemaly, Z., Fick, M., 1999. Lactic acid production by *Bacillus coagulans*—kinetic studies and optimization of culture medium for batch and continuous fermentations. Enzyme and Microbial Technology 24, 191–199.

Roberts, C.M., Hoover, D.G., 1996. Sensitivity of *Bacillus coagulans* spores to combinations of high hydrostatic pressure, heat, acidity and nisin. Journal of Applied Bacteriology 81, 363–368.

Shehata, A.E., Magdoub, M.N., Sultan, N.E., El-Samgray, Y.A., 1983. Aerobic mesophilic and psychrotrophic sporeforming bacteria in buffalo milk. Journal of Dairy Science 66, 1228–1231.

Sponholz, W.R., 1993. Wine spoilage by microorganisms. In: Fleet, G.H. (Ed.), Wine Microbiology and Biotechnology. Harwood Acad. Publ., Chur, Switzerland, pp. 395–420.

Stiles, M.E., 1996. Biopreservation by lactic acid bacteria. Antonie van Leeuwenhoek 70, 221–245.

Wandling, L.R., Sheldon, B.W., Foegeding, P.M., 1999. Nisin in milk sensitizes *Bacillus* spores to heat and prevents recovery of survivors. Journal of Food Protection 62, 492–498.