Food Microbiology, 2002, **19**, 509–518 doi:10.1006/yfmic.498
Available online at http://www.idealibrary.com on IDEAL®

ORIGINAL ARTICLE

Bactericidal synergism through bacteriocins and high pressure in a meat model system during storage

M. Garriga*, M. T. Aymerich, S. Costa, J. M. Monfort and M. Hugas

High hydrostatic pressure represents an attractive non-thermal process for meat products to avoid post-processing contamination. When combined with antimicrobials, like bacteriocins, the death rate may be increased because of sub-lethal injuries to living cells. The behaviour of several foodborne bacteria inoculated in a meat model system with added bacteriocins (enterocins A and B, sakacin K, pediocin AcH or nisin) after pressurization (400 MPa, 10 min, 17°C) and during chilled storage was investigated. Although Staphylococcus *was the genus least sensitive to pressurization, the samples including nisin displayed lower and significantly different counts during the 4°C storage than the rest of the treatments. A greater inactivation of* Escherichia coli *($>6\ log_{10}$) in the presence of nisin was recorded, the number of survivors remained unchanged during storage at 4°C for 61 days. Nisin was also the bacteriocin capable of maintaining slime-producing lactic acid bacteria below the detection limit ($<10^2\ cfu\ g^{-1}$).* Listeria monocytogenes *in treatments with sakacin, enterocins or pediocin was kept $<10^2\ cfu\ g^{-1}$ till the end of storage (61 days).* Salmonella enterica *subsp.* enterica *ser London and* Salmonella enterica *subsp.* enterica *ser Schwarzengrund counts in every treatment were kept at the level obtained after pressurization, with no significant differences between treatments during the chilled storage.*

Introduction

High-pressure technology in foods was first examined by Hite (1899). This technology has traditionally been used in the production of ceramics, steels and superalloys for high-speed and carbide tools (Bundy 1964).

High hydrostatic pressure (HHP) represents an attractive non-thermal process for meat products to avoid post-processing contamination. Slicing cooked meat products or removing the casing of cooked sausages, even with the observation of strict hygienic procedures, constitutes a permanent risk of contamination, despite the implementation of modern technologies and HACCP systems.

The mode of action of HHP is the destruction of microbial cells by destabilizing the structural and functional integrity of the cytoplasmic membrane, by inducing protein denaturation and by inhibiting genetic mechanisms (Hoover et al. 1989). The effectiveness of moderate high pressure on the destruction of foodborne pathogens in phosphate buffer and in some

*Corresponding author. Fax. +34-972-630373
E-mail: margarita.garriga@irta.es

Received: 1 November 2001

IRTA, Meat Technology Centre, Granja Camps i Armet, s/n 17121 Monells, Spain

0740-0020/02/050509+10 $35.00/0

foods has been published (Shigehisa et al. 1991, Patterson et al. 1995a, Kalchayanand et al. 1998a, Lucore et al. 2000). In general, the higher the pressure and time of pressurization, the bigger the destruction. The killing effect is usually less pronounced in foods than in buffers, because of the baroprotective effect of some food constituents, and some rich protein foods would not be suitable for consumption after treatment at pressures higher than 400 MPa because the texture and colour would be affected.

The hurdle concept proposed by Leistner (1992) could be useful for improving the efficiency of HHP treatment of foods. Kalchayanand et al. (1998b) reported that the combination of pressure and antimicrobials would increase the death rate because cells surviving pressurization also become sub-lethally injured and are then killed by bacteriocins. García-Graells et al. (1999) reported synergy of HHP and bacteriocins on the inactivation of *Escherichia coli* strains in milk.

Few reports studying the microbiota of pressurized meat products under refrigeration storage have been published (Murano et al. 1999, Yuste et al. 1999, 2000) and to our knowledge this is the first report investigating the behaviour of several foodborne bacteria inoculated in a meat model system with added bacteriocins, during storage for 61 days at 4°C.

Material and Methods

Bacterial species

The target strains were isolated from meat and/or meat products and selected as representative for pathogenic species: *Escherichia coli* CTC1018, CTC1023; *Salmonella enterica* subsp. *enterica* ser London CTC1003, *Salmonella enterica* subsp. *enterica* ser Schwarzengrund CTC1015; *Staphylococcus aureus* CTC1008, CTC1019; *Listeria monocytogenes* CTC1010, CTC1034; spoilage species: slime-producing lactic acid bacteria (LAB): *Lactobacillus sakei* CTC746 and *Leuconostoc carnosum* CTC747 and from a technological interest: *Staphylococcus carnosus* LTH2102 (commercial starter). The strains were generally grown at 30°C in Tryptic Soy Broth (TSB, DIFCO Laboratories, Detroit, Michigan, USA), whereas *Listeria* strains were grown in TSBYE (TSB supplemented with 0.6% yeast extract) and LAB in MRS (de Man et al. 1960) (DIFCO Laboratories, Detroit, Michigan, USA). All strains were maintained as frozen stocks in 20% glycerol at −80°C.

Bacteriocins preparation

The following bacteriocins were used as antimicrobials in combination with HHP: enterocins A and B produced by *Enterococcus faecium* CTC492 (Aymerich et al. 1996, 2000a); sakacin K produced by *L. sakei* CTC494 (Hugas et al. 1995, Aymerich et al. 2000b); pediocin AcH produced by *Pediococcus acidilactici* (Bhunia et al. 1988). The bacteriocins were precipitated from the supernatant of 3 l culture of the producer strains with ammonium sulphate 300 g l^{-1}. The protein precipitates were pelleted by centrifugation at 10 000 *g* for 30 min at 4°C and dissolved in 1:100 of the initial culture volume in phosphate buffer 50 mM pH 7·2.

Nisin A was obtained from Nisaplin, commercially produced by Danisco Cultor (Copenhaguen K, Denmark). One gram of liophylized product was dissolved in 5 ml of phosphate buffer 50 mM pH 7·2 and centrifuged for 1 min at 3000 rpm in a microfuge (Heraeus™) to remove the solid residues.

The titer of the bacteriocins was determined against *L. innocua* CTC1014, or *L. sakei* CTC746 for Nisaplin, as described by Barefoot and Klaenhammer (1983). The extract titer was defined as the reciprocal of the highest dilution showing inhibition of the indicator lawn and was expressed in arbitrary units (AU) per ml. The sensitivity of each of the strains to each of the bacteriocins was assessed by the method of the agar spot test (Tagg et al. 1976).

Model meat system

Commercial canned cooked ham was used to prepare the meat model. The composition of ham (Serra & Mota, Spain) was detailed in each can as follows: ham, water, salt, stabilizers (sucrose, gelatins), polyphosphates, gelatinizer (E-407), antioxidants (E-301), curing

agents (E-250) and species. The chemical composition was moisture, 73·8%; protein, 17·3%; fat, 4·8%; sodium chloride, 1·75%; sodium nitrate, 33 ppm; sodium nitrite, 8·5 ppm.

The model system consisted of cooked ham blended with distilled water (1:3) in a Waring Blendor (Lab. Humeau, Nantes, France), pH 6·5. The mixture was further treated for 10 min at 80°C and distributed (40 g) in plastic pouches containing bacteriocins (1280 AU g^{-1}) where indicated.

Five treatments were prepared: control (with no added bacteriocins), enterocins A and B, sakacin K, pediocin AcH, nisin A.

Sample preparation and pressurization

Each strain was separately grown for 24 h in the media previously described and inoculated (1:10) to 10^8 cfu g^{-1} final concentration, in plastic pouches containing the meat matrix and bacteriocin where indicated. The assay was carried out in duplicate for each treatment and repeated once. Samples were gently mixed, vacuum sealed and kept at 4°C during handling before and after pressurization and during the storage.

An industrial hydrostatic pressurization unit (Alstom, Nantes, France) capable of operating up to 400 MPa was used. The pressure level (400 MPa), time (10 min) and temperature (17°C) were set by an automatically controlled device. The reported pressurization time did not include the increase and decrease times.

Enumeration of viable cells

After high-pressure treatment the samples were transferred to sterile tubes and stored at 4°C for up to 61 days. At selected times: before (time 0) and after pressurization (24 h and 4, 7, 11, 18, 26, 46 and 61 days) each treatment was sampled as follows: 2 g was diluted in 18 ml of 0·1% peptone, 0·85% NaCl; gently mixed and serially diluted in the same solution. The plating was done in selective media: *E. coli* (VRB Agar, Merck, Darmstadt, Germany) incubated at 37°C for 24 h, *Salmonella* (XLD Agar, Merck, Darmstadt, Germany) incubated at 37°C for 2 days, *L. monocytogenes* (Palcam Agar, Merck, Darmstadt, Germany) incubated at 30°C for 3 days, *Staph. carnosus* (MSA, Difco Laboratories, Detroit, Michigan, USA) at 30°C for 3 days, *Staph. aureus* (MSA, Difco Laboratories, Detroit, Michigan, USA) at 37°C for 2 days, slime-producing strains *L. sakei* CTC746 and *Lc. carnosum* CTC747 (MRS, Difco Laboratories, Detroit, Michigan, USA) at 30°C for 3 days. Typical colonies were further confirmed by Gram staining, oxidase and catalase tests.

Bacteriocin recovery

To assess the remaining bacteriocin activity at the end of the storage period, the model meat system mixture was homogenized 1:10 in sodium acetate 50 mM EDTA 100 mM and Triton 0·2% at pH 5 with an Ultraturrax T25 (Jauke and Kunkel, IKA Labortchnik, Sweden) for 1 min, boiled for 10 min, cooled and filtered in order to obtain the liquid phase. The bacteriocins were precipitated with ammonium sulphate 300 g l^{-1} and the pellet was dissolved in phosphate buffer 50 mM pH 7·2. The suspension was heated at 80°C for 10 min and stored at −80°C. Inhibitory activity was assayed against *L. innocua* CTC1014 by the method of the agar spot test.

Statistical analyses

Analysis of variance (ANOVA) was performed using the General Linear Model procedure of the SAS software. Values below the detection limit ($< 2 \log_{10}$ cfu g^{-1}) were scored as 1.99. The treatment effect was evaluated by means comparisons using the Bonferroni test (SAS Institute Inc. 1988).

Results

Sensitivity of the bacterial strains to bacteriocins

Among the Gram-positive strains, *Staph. carnosus* LTH2102, *Staph. aureus* CTC1008, CTC1019 and *Lc. carnosum* CTC747 were sensitive to nisin A but not sensitive to enterocins A and B, sakacin K and pediocin AcH. The rest of Gram-positive strains, *L. monocytogenes* CTC1010, CTC1034 and *L. sakei* CTC746 were

sensitive to all the bacteriocins studied (Table 1).

Gram-negative strains, *E. coli* CTC1018, CTC1023, *S.* London CTC1003, *S.* Schwarzengrund CTC1015 were not sensitive to enterocins A and B, sakacin K, pediocin AcH and nisin A.

Behaviour of the strains in a model meat system after high-pressure treatment and storage at 4°C

The results obtained are detailed in Figs 1–5 and are the mean of two different experiments performed in duplicate.

E. coli displayed a 4·5 $\log_{10}$ cycle decline 24 h after pressurization (Fig. 1). However, a greater inactivation was recorded ($>6\log_{10}$) in the treatment where nisin A was included and the number of survivors remained unchanged during the chilled storage. In the other treatments,

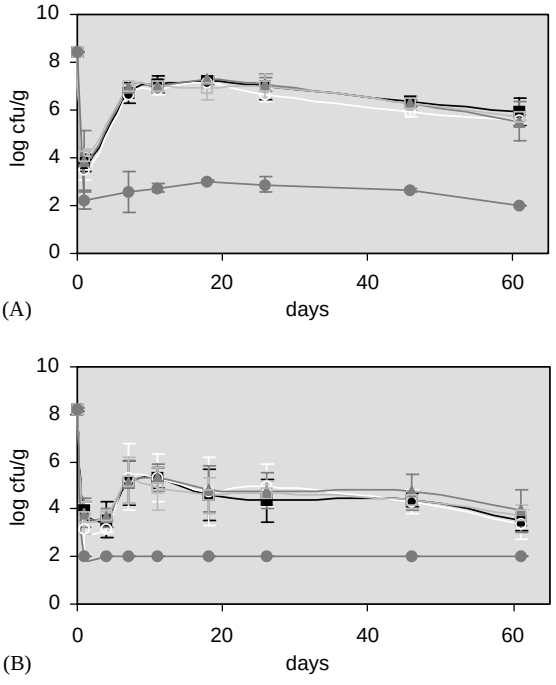

Figure 1. Behaviour of (A) *E. coli* CTC1018 and (B) *E. coli* CTC1023 after HHP at 400 MPa for 10 min at 17°C in a meat model system during storage at 4°C. Treatments: (○) enterocins A and B, (▲) sakacin K, (□) pediocin AcH, (●) nisin A, (■) control. Values are mean ± standard deviation. The minimum level of detection was 2 $\log_{10}$ cfu g^{-1}.

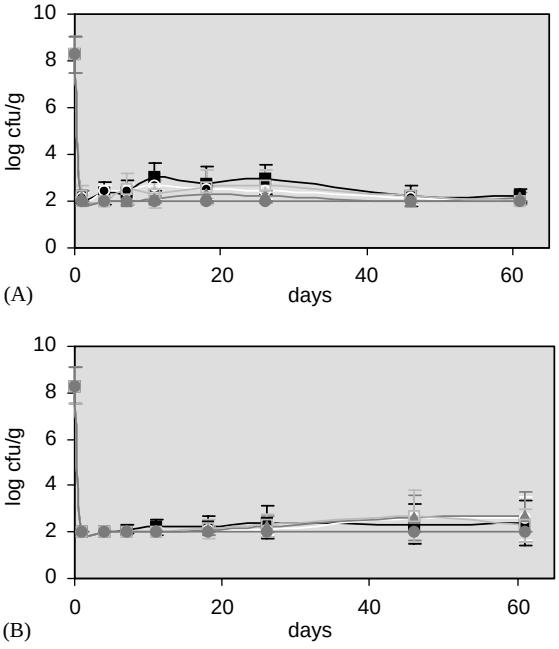

Figure 2. Behaviour of (A) *S.* London CTC1003 and (B) *S.* Schwarzengrund CTC1015, after HHP at 400 MPa for 10 min at 17°C in a meat model system during storage at 4°C. Treatments: (○) enterocins A and B, (▲) sakacin K, (□) pediocin AcH, (●) nisin A, (■) control. Values are mean ± standard deviation. The minimum level of detection was 2 $\log_{10}$ cfu g^{-1}.

both strains grew considerably during storage reaching counts of 10^6–10^7 cfu g^{-1} with no significant differences ($P>0{\cdot}05$) between control (non-added bacteriocins) and samples containing sakacin, enterocins or pediocin (Fig. 1).

S. London CTC1003 and *S.* Schwarzengrund CTC1015 counts in every treatment were kept at the level obtained after pressurization, around 10^2 cfu g^{-1}, with no significant differences between treatments (Fig. 2) during the chilled storage.

Although *Staphylococcus* was the genus least sensitive to high-pressure treatment, the samples including nisin displayed lower and significantly different ($P<0{\cdot}05$) counts during the 4°C storage than the rest of the treatments (Fig. 3). The effect of nisin was more pronounced for *Staph. carnosus* LTH2102, with a decline of 3·8 $\log_{10}$ cycles at the end of storage.

L. monocytogenes in treatments with sakacin, enterocins or pediocin was kept below or equal to the detection limit ($<10^2$ cfu g^{-1}) until the

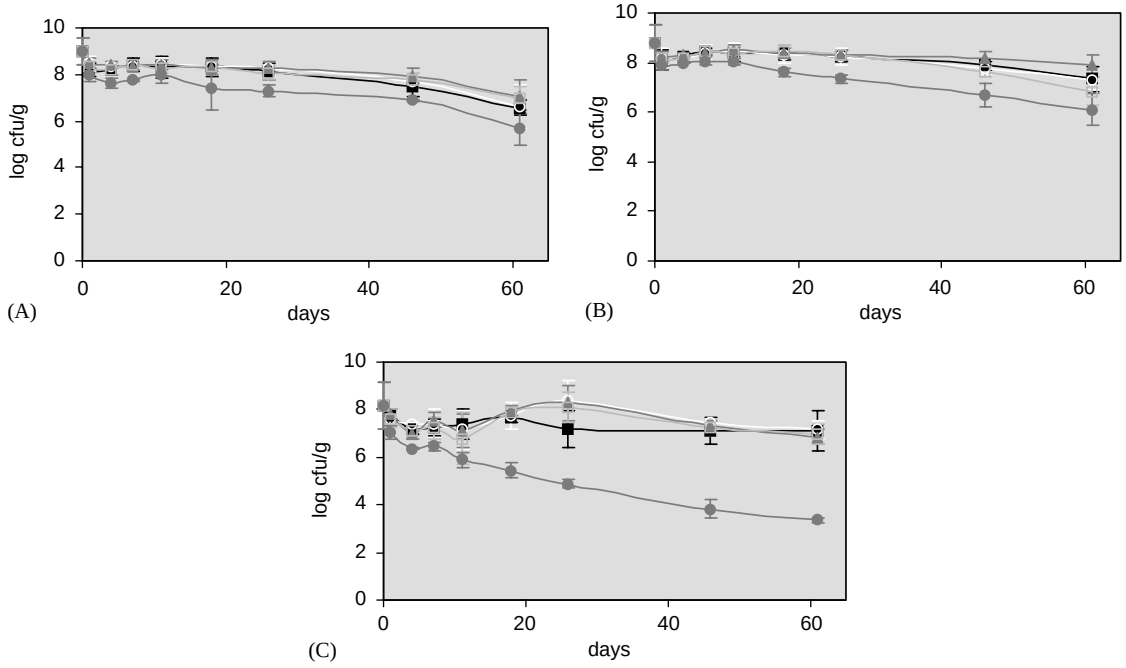

Figure 3. Behaviour of (A) *Staph. aureus* CTC1008, (B) *Staph. aureus* CTC1019 and (C) *Staph. carnosus* LTH2102 after HHP at 400 MPa for 10 min at 17°C in a meat model system during storage at 4°C. Treatments: (○) enterocins A and B, (▲) sakacin K, (□) pediocin AcH, (●) nisin A, (■) control. Values are mean ± standard deviation. The minimum level of detection was 2 $\log_{10}$ cfu g^{-1}.

end of storage with no significant differences ($P > 0{\cdot}05$). However, the pathogen reached the same values as before pressurization (10^9 cfu g^{-1}) (Fig. 4) in the control and in nisin treatments during storage. Large standard deviations were recorded for both treatments because the counts of several samples were below the detection limit ($< 10^2$ cfu g^{-1}).

Slime-producing lactic acid bacteria, *L. sakei* CTC746 and *Lc. carnosum* CTC747, decreased more than 6 $\log_{10}$ cycles after high-pressure treatment (Fig. 5). However, the counts increased during storage, reaching the initial inoculum level in the control samples (non-added bacteriocin), sakacin and pediocin treatments when *L. sakei* CTC746 was the target strain. Nisin A was the only bacteriocin capable of keeping *Lc. carnosum* CTC747 counts below 10^2 cfu g^{-1} throughout the storage.

Bacteriocin recovery

In order to assess any remaining bacteriocin activity, several treatments showing counts below the detection limit at the end of the experiment (61 days) were sampled, as described, for detection of bacteriocin activity in the meat mixture. All the samples assayed showed inhibition of the indicator strain *L. innocua* CTC1014 indicating there was bacteriocin remaining in the samples.

Discussion

A meat model system consisting of cooked ham homogenized with water was chosen in order to assess the effect of high hydrostatic pressure on several spoilage and pathogenic bacteria. As some food constituents are known to exert a protective effect on bacteria in pressure processing, the use of this model was considered to be more realistic as a first approach to meat products than the use of buffered solutions.

A higher resistance to pressure in Gram-positive bacteria than in Gram-negative has been generally demonstrated (Hoover et al. 1989)

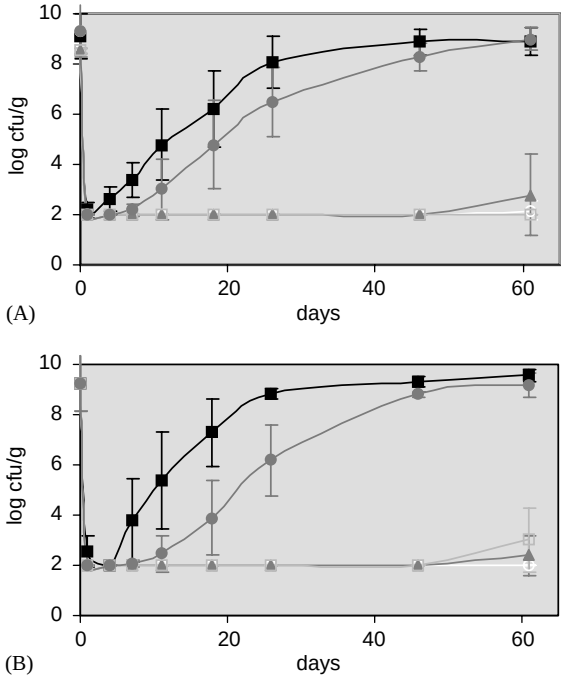

Figure 4. Behaviour of (A) *L monocytogenes* CTC1010 and (B) *L. monocytogenes* CTC1034, after HHP at 400 MPa for 10 min at 17°C in a meat model system during storage at 4°C. Treatments: (○) enterocins A and B, (▲) sakacin K, (□) pediocin AcH, (●) nisin A, (■) control. Values are mean ± standard deviation. The minimum level of detection was 2 $\log_{10}$ cfu g^{-1}.

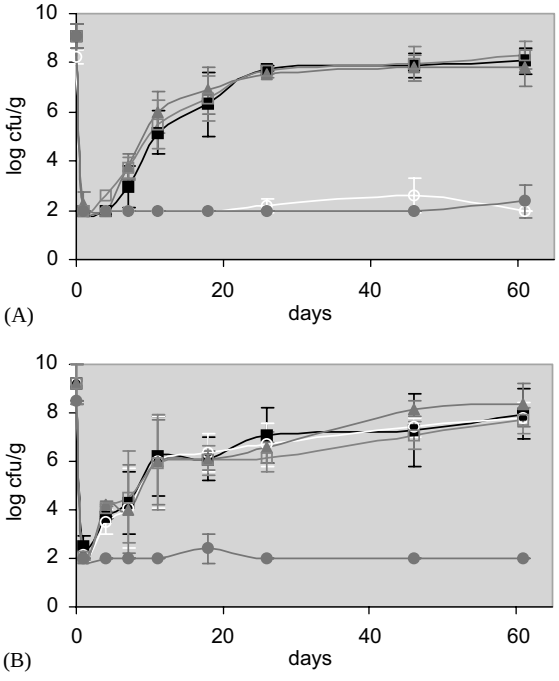

Figure 5. Behaviour of LAB (A) *L. sakei* CTC746 and (B) *L. carnosum* CTC747, after HHP at 400 MPa for 10 min at 17°C in a meat model system during storage at 4°C. Treatments: (○) enterocins A and B, (▲) sakacin K, (□) pediocin AcH, (●) nisin A, (■) control. Values are mean ± standard deviation. The minimum level of detection was 2 $\log_{10}$ cfu g^{-1}.

although some authors stated that the sensitivity is more correlated to cell morphology, cocci being the most pressure resistant (Ludwig and Schreck 1997). The results obtained in this study agree with the latter observations, *Staphylococcus* being the most resistant among the strains tested, followed by the *E. coli* strains which displayed a short rod morphology under the microscope.

The variation in sensitivity between different strains from the same species has also been described (Patterson et al. 1995b, Alpas et al. 1999). This fact was observed in both *E. coli* strains during the 4°C storage, which suggests the importance of using a cocktail of strains as target bacteria in further studies on food products. Alpas et al. (1999) assayed seven different *Staph. aureus* strains in 10% peptone solution. After pressurization (345 MPa, 5 min, 25°C) six of them recorded a viability loss average of 1·93 log cycles, whereas one particular strain was extremely sensitive, showing a 7·80 log decline. The results reported by different authors after pressurization are greater than those obtained in this study for *Staph. aureus*. Moreover, neither the suspension media, the pressure parameters nor the strain were the same. In reconstituted skimmed milk (10%) at 250 MPa for 30 min at 25°C *Staph. aureus* ATCC6538 showed a 2·2 log kill (Morgan et al. 2000). In peptone water at 345 MPa for 10 min at 25°C, *Staph. aureus* 582 suffered a viability loss of 4.8 log cycles (Kalchayanand et al. 1998a). These results indicate the necessity to validate the efficiency of HHP in real food systems.

Gram-negative bacteria are insensitive to bacteriocins of lactic acid bacteria as they do not have specific receptors (Bhunia et al. 1991) although their inner membrane can be destabilized by these bacteriocins (Gao et al. 1991). In the treatment with nisin, although both strains

of *E. coli* were not sensitive to bacteriocins in the agar spot test, the population of survivors after pressurization remained below the detection limit ($<10^2$ cfu g^{-1}) throughout the 4°C storage period, suggesting that the injured survivors after pressure became sensitive to nisin resulting in the loss of viability. According to Ray (1989) following sub-lethal stress, barrier functions of cell wall structures are impaired in the injured survivors. The wall no longer prevents the surface-active compounds from entering the cells, and destabilizing of the cytoplasmatic (or inner) membrane results in the loss of viability. Following sub-lethal treatments such as freezing, heating, exposure to weak acids or high-pressure treatments, sensitivity to bacteriocins like nisin and pediocin has been reported by several authors (Kalchayanand et al. 1992, 1994, 1998b, García-Graells et al. 1999).

The behaviour of *Staphylococcus* strains assayed in this study showed significantly lower ($P<0{\cdot}05$) counts during storage, in the presence of nisin, compared with the other treatments and this was specially important when *Staph. carnosus* LTH2102 was the target strain. Kalchayanand et al. (1998a) obtained an additional viability loss of *Staph. aureus* 582 when cells were pressurized in the presence of a mixture of pediocin AcH and nisin, with practically no survivors. The combination of those bacteriocins is known to have a greater bactericidal effect than their single use (Hanlin et al. 1993).

The baroprotective effect of water activity also needs to be taken into account. According to Lanciotti et al. (1996), *L. monocytogenes* was resistant to pressure only at high A_w. In this study, despite the higher water activity of the meat model used, both listeria strains diminished more than 6 $\log_{10}$ cycles after HHP. When sakacin, enterocins or pediocin was included in the meat mixture, the pathogen was kept below the detection limit during the chilled storage. In the control and nisin treatments HHP survivors recovered rapidly during storage. In the agar spot test both listeria strains were sensitive to nisin, but to a lesser extent when compared with pediocin, sakacin or enterocins (Table 1). It has been observed (Ritz et al. 2001) that after pressurization the population is heterogenous and cellular damage is not equally withstood by all the cells, suggesting that less injured cells are present and that cellular repair under favourable conditions, such as the presence of essential nutrients, should not be ruled out. *Listeria* is a psychrotrophic microorganism, and in consequence it can recover during the 4°C storage. The phenomenon of microbial stress might over-estimate the effect of pressurization; although after treatment the lethality recorded is high, the stressed bacteria are capable of growing during storage as depicted in almost all the strains assayed in this study.

The reported inactivation of *Listeria* is very different depending on the matrix used. Styles et al (1991) reported more than 7 $\log_{10}$ decline in phosphate buffer at 340 MPa compared to 1·5 $\log_{10}$ in UHT milk, suggesting the protective effect of fat. Similar pressure treatments, 325 MPa, in minced beef muscle (Carlez et al. 1993) or ewe's milk (Gervilla et al. 1997) presented a negligible decrease ($<0{\cdot}5$ log). When 400 MPa was applied, more than 5 log decline of listeria was recorded in minced beef muscle (Carlez et al. 1993).

In the present study lactic acid bacteria showed a similar evolution as listeria strains did in the control samples. The survivors after HHP grew during storage and reached the initial level, except when nisin was included. Some authors reported the complete inactivation of some LAB strains after treatment at 200 MPa in Hepes buffer at pH 5·3 (Sonoike et al. 1992) and in minced meat vacuum stored at 3°C after HPP at 450 MPa at 20°C for 20 min (Carlez et al. 1994). Other authors (Ulmer et al. 2000) showed that 400 MPa at 15°C for 12 min was not enough to inactivate *L. plantarum* in a model beer.

Lc. carnosum CTC747 which in the agar spot test was only sensitive to nisin, displayed the same behaviour in the meat model, nisin being capable of maintaining the survivors after pressurization below the detection limit. This bacteriocin was also effective in the prevention of ropiness caused by *Lc. carnosum* CTC747 in sliced cooked pork (Aymerich et al. 2001). For *L. sakei* CTC746 no significant differences were observed between nisin and enterocins, as the population after pressurization was kept stable

Table 1. Sensitivity of the bacterial strains to bacteriocins

Strain	Bacteriocins[a]			
	Enterocins A,B	Sakacin K	Pediocin AcH	Nisin A
Staph. carnosus LTH2102	<100	<100	<100	102 400
Staph. aureus CTC1008	<100	<100	<100	2263
Staph. aureus CTC1019	<100	<100	<100	1600
L. monocytogenes CTC1010	144 815	144 815	102 400	6400
L. monocytogenes CTC1034	102 400	102 400	289 631	4525
L. sakei CTC746	12 800	3200	4525	25 600
Lc. carnosum CTC747	<100	<100	<100	102 400

[a]Bacteriocin activity was expressed as arbitrary units (AU) per ml.

throughout the storage. In the agar spot test this strain showed a higher sensitivity to nisin, followed by enterocins, than to the other bacteriocins (Table 1).

Class IIa pediocin like bacteriocins have significant potential as biopreservatives in foods because of their antilisterial effectiveness and it is generally accepted that they should be taken into account for future approval. As bacteriocins are proteins their presence in foods would be, in general, safe for consumers because they would be inactivated by pancreatic or gastric enzymes.

To our knowledge, this is the first report studying the bactericidal effect of enterocins and sakacin, in combination with high-pressure processing, as hurdles to the enhancement of safety in a meat model system. Further research is in progress assessing its effectiveness in sliced cooked and cured meat products.

Acknowledgements

The authors thank M. Saco (Animal Health Laboratory, Barcelona) for serotyping the *Salmonella* strains. This work was supported by a CICYT project ALI98-0709.

References

Alpas, H., Kalchayanand, N., Bozoglu, F., Sikes, A., Dunne, C. P. and Ray, B. (1999) Variation in resistance to hydrostatic pressure among strains of foodborne pathogens. *Appl. Environ. Microbiol.* **65,** 4248–4251.

Aymerich, M. T., Holo, H., Havarstein, L. S., Hugas, M., Garriga, M. and Nes, I. F. (1996) Biochemical and genetic characterization of enterocin A from *Enterococccus faecium* CTC492, a new antilisterial bacteriocin in the pediocin family of bacteriocins. *Appl. Environ. Microbiol.* **62,** 1676–1682.

Aymerich, M. T., Artigas, M. G., Garriga, M., Monfort, J. M. and Hugas, M. (2000a) Effect of sausage ingredients and additives on the production of enterocins A and B by *Enterococcus faecium* CTC492. Optimization of *in vitro* production and anti-listerial effect in dry fermented sausages. *J. Appl. Microbiol.* **88,** 686–694.

Aymerich, M. T., Garriga, M., Monfort, J. M., Nes, I. F. and Hugas, M. (2000b) Bacteriocin-producing lactobacilli in Spanish-style fermented sausages: characterization of bacteriocins. *Food Microbiol.* **17,** 33–45.

Aymerich, M. T., Garriga, M., Costa, S. Monfort, J. M. and Hugas, M. (2001) Prevention of ropiness in cooked pork by bacteriocinogenic cultures. *Int. Dairy J,* in press.

Barefoot, S. F. and Klaenhammer, T. R. (1983) Detection and activity of Lactacin B, a bacteriocin produced by *Lactobacillus acidophilus. Appl. Environ. Microbiol.* **45,** 1808–1815.

Bhunia, A. K., Johnson, M. C. and Ray, B. (1988) Purification, characterization and antimicrobial spectrum of a bacteriocin produced by *Pediococcus acidilactici*. *J. Appl. Bacteriol.* **65,** 261–268.

Bhunia, A. K., Johnson, M. C., Ray, B. and Kalchayanand, N. (1991) Mode of action of pediocin AcH from *Pediococcus acidilactici* H on sensitive bacterial strains. *J. Appl. Bacteriol.* **70,** 25–31.

Bundy, F. P. (1964) Formation of new materials and structures by high pressure treatment. In '*Irreversible Effects of High Pressure and Temperature on Materials*'. ASTM Special Technical Publication no. 374, Philadelphia, Panama, USA, American Society for Testing and Materials.

Carlez, A., Rosec, J. P., Richard, N. and Cheftel, J. C. V. (1993) High pressure inactivation of *Citrobacter freundii*, *Pseudomonas fluorescens*, and *Listeria*

innocua in inoculated minced beef muscle. *Lebensm. Wiss. U. Technol.* **26,** 357–363.
Carlez, A., Rosec, J. P., Richard, N. and Cheftel, J. C. V. (1994) Bacterial growth during chilled storage of pressure treated minced meat. *Lebensm. Wiss. U. Technol.* **27,** 48–54.
De Man, J. C., Rogosa, M. and Sharpe, M. E. (1960) A medium for the cultivation of lactobacilli. *J. Appl. Bacteriol.* **23,** 130–138.
García-Graells, C., Masschalk., B. and Michiels, C. (1999) Inactivation of *Escherichia coli* in milk by high hydrostatic pressure treatment in combination with antimicrobial peptides. *J. Food Prot.* **62,** 1248–1254.
Gao, F. A., Abee, T. L. and Koonings, W. N. (1991) Mechanism of action of the peptide antibiotic nisin in liposomes and cytochrome C oxidase containing proteolyposomes. *Appl. Environ. Microbiol.* **57,** 2164–2170.
Gervilla, R., Capellas, M., Ferragut, V. and Guamis, B. (1997) Effect of high hydrostatic pressure on *Listeria innocua* 910 CECT inoculated into ewe's milk. *J. Food Prot.* **60,** 33–37.
Hanlin, M. B., Kalchayanand, N., Ray, P. and Ray, B. (1993) Bacteriocins of lactic acid bacteria in combination have greater antibacterial activity. *J. Food Prot.* **56,** 252–255.
Hite, B. H. (1899) The effect of pressure in the preservation of milk. Bulletin 58 p. 15. West Virginia University Agriculture Experiments Station, Morgantown.
Hoover, D. G., Metrick, C., Papineau, A. P., Farkas, D. A. and Knorr, D. (1989) Biological effects of high hydrostatic pressure on food microorganisms. *Food Technol.* **43,** 99–107.
Hugas, M., Garriga, M., Aymerich, M. T. and Monfort, J. M. (1995) Inhibition of *Listeria* in dry fermented sausages by the bacteriocinogenic *Lactobacillus sake* CTC494. *J. Appl. Bacteriol.* **79,** 322–330.
Kalchayanand, N., Hanlin, M. B. and Ray, B. (1992) Sublethal injury makes Gram-negative and resistant Gram-positive bacteria sensitive to bacteriocins, pediocin AcH and nisin. *Lett. Appl. Microbiol.* **15**, 239–243.
Kalchayanand, N., Sikes, A., Dunne, C. P. and Ray, B. (1994) Hydrostatic pressure and electroporation have increased bactericidal efficiency in combination with bacteriocins. *Appl. Environ. Microbiol.* **60,** 4174–4177.
Kalchayanand, N., Sikes, A., Dunne, C. P. and Ray, B. (1998a) Factors influencing death and injury of foodborne pathogens by hydrostatic pressure-pasteurization. *Food Microbiol.* **15,** 207–214.
Kalchayanand, N., Sikes, A., Dunne, C. P. and Ray, B. (1998b) Interaction of hydrostatic pressure, time and temperature of pressurization and pediocin AcH on inactivation of foodborne bacteria. *J. Food Protect.* **61,** 425–431.
Lanciotti, F., Gardini, M., Sinigaglia, M. and Guerzoni, M. E. (1996) Effects of growth conditions on the resistance of some pathogenic and spoilage species to high pressure homogenization. *Lett. Appl. Microbiol.* **22,** 165–168.
Leistner, L. (1992) Linkage of hurdle-technology with HACCP. In *Proceedings of the 45th Annual Reciprocal Meat Conference* pp. 1–3 American Meat Science Association , 14–17 June, Colorado State University, Fort Collins, Colorado, USA.
Lucore, L. A., Shellhammer, T. H. and Yousef, A. E. (2000) Inactivation of *Listeria monocytogenes* Scott A on artificially contaminated frankfurters by high pressure processing. *J. Food Prot.* **63,** 662–664.
Ludwig, H. and Schreck, C. (1997) The inactivation of vegetative bacteria by pressure. In *High Pressure Research in the Biosciences and Biotechnology* (Ed. K. Heremans) pp. 221–224. Leuven, Belgium, Leuven University Press.
Morgan, S. M., Ross, R. P., Beresford, T. and Hill, C. (2000) Combination of hydrostatic pressure and lacticin 3147 causes increase killing of *Staphylococcus* and *Listeria*. *J. Appl. Microbiol.* **88,** 414–420.
Murano, E. A., Murano, P. S., Brennan, R. E., Shenoy, K. and Moreira, R. G. (1999) Application of high hydrostatic pressure to eliminate *Listeria monocytogenes* from fresk pok sausage. *J. Food Prot.* **62,** 480–483.
Patterson, M. F., Quinn, M., Simpson, R. and Gilmore, A. (1995a) Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate buffered saline and foods. *J. Food Prot.* **58,** 524–529.
Patterson, M. F., Quinn, M., Simpson, R. and Gilmore, A. (1995b) Effects of high pressure on vegetative pathogens. In *High Pressure Processing of Foods* (Eds D. A. Ledward, D. E. Johnston, R. G. Earnshow and A. P. M. Hastings) pp. 47–63. Leicestershire, Nottingham University Press.
Ray, N. (1989) Enumeration of injured indicator bacteria from foods. In *Injured Index and Pathogenic Bacteria* (Ed. B. Ray) pp. 9–54. Boca Ratón, CRC Press Inc.
Ritz, M., Tholozan, J. L., Federighi, M. and Pilet, M. F. (2001) Morphological and physiological characterization of *Listeria monocytogenes* subjected to high hydrostatic pressure. *Appl. Environ. Microbiol.* **67,** 2240–2247.
SAS Institute Inc. (1988) SAS^{R} Language Guide. Release 6.03 edn. Cary, North Carolina, USA, SAS.
Shigehisa, T., Ohmori, T., Saito, A., Taji, S. and Hayashi, R. (1991) Effects of high hydrostatic pressure on characteristics of pork slurries and inactivation of microorganisms associated with meat and meat products. *Int. J. Food Microbiol.* **12,** 207–216.
Sonoike, K., Setoyama, T., Kuma, Y. and Kobayashi, S. (1992) Effect of pressure and temperature on the death rates of *Lactobacillus casei* and *Escherichia coli*. In *High Pressure and Biotechnology* (Eds C. Balny, R. Hayashi, K. Heremans and P. Mason)

pp. 297–301. Colloque INSERM/ John Libbey Eurotext, Montrouge, France.

Styles, M., Hoover, D. and Farkas, D. (1991) Response of *Listeria monocytogenes* and *Vibrio parahaemolyticus* to high hydrostatic pressure. *J. Food Sci*. **56,** 1404–1407.

Tagg, J. R., Dajani, A. S. and Wannamaker, L. W. (1976) Bacteriocins of Gram-positive bacteria. *Bacteriol. Rev.* **40,** 722–756.

Ulmer, H. M., Gänzle, M. G. and Vogel, R. F. (2000) Effects of high pressure on survival and metabolic activity of *Lactobacillus plantarum* TMW1.460. *Appl. Environ. Microbiol.* **66,** 3966–3973.

Yuste, J., Mor-Mur, M., Capellas, M. and Pla, R. (1999) Pressure vs. heat-induced bacterial stress in cooked poultry sausages: a preliminary study. *Lett. Appl. Microbiol.* **29,** 233–237.

Yuste, J., Pla, R., Capellas, M., Ponce, E. and Mor-Mur, M. (2000) High-pressure processing applied to cooked sausages: bacterial populations during chilled storage. *J. Food Prot.* **63,** 1093–1099.