Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Stress enhances the sensitivity of *Salmonella enterica* serovar Typhimurium to bacteriocins

M.F. Galvão, C.V. Prudêncio and M.C.D. Vanetti

Departamento de Microbiologia, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil

Keywords
biocontrol, bovicin HC5, food industry, hurdle technology, nisin, outer membrane, *Salmonella*.

Correspondence
Maria Cristina Dantas Vanetti, Departamento de Microbiologia, Universidade Federal de Viçosa, Av. PH Rolfs, s/n, Viçosa 36570-900, Minas Gerais, Brazil.
E-mail: mvanetti@ufv.br

2014/2264: received 4 November 2014, revised 16 January 2015 and accepted 30 January 2015

doi:10.1111/jam.12776

Abstract

Aims: The aim of this study was to evaluate the potential application of bacteriocins against Gram-negative bacteria when associated with others food preservation methods.
Methods and Results: *Salmonella* was subjected to heat, cold, acid and chemical (with ethylenediaminetetracetate and trisodium phosphate) stresses. Then, the cells were recovered and subjected to treatment with bacteriocins (500 AU ml^{-1}) for 6 h. Heat and cold stress were those that promoted more sensitization to bactericidal activity of nisin. Under the same conditions, bovicin HC5 acted more rapidly than nisin reducing the number of viable cells to undetectable levels after 20 min of treatment. Similar results with use of nisin only were observed after 6 h of treatment.
Conclusions: Stress conditions used in food industry, such as temperature and pH, and use of chelating agents or membrane disruptors, sensitized *Salmonella* Typhimurium cells to bacteriocins produced by lactic acid bacteria, such as nisin and bovicin HC5.
Significance and Impact of the Study: Food preservation methods sensitized Gram-negative bacteria to bacteriocins activity, which demonstrate the potential of nisin and bovicin HC5 to inhibit the growth of *Salmonella*.

Introduction

Bacteriocins are ribosomally synthesised antimicrobial peptides that have activity against bacteria of the same species or those from other genera (Cotter *et al.* 2005). Among bacteriocins, those produced by lactic acid bacteria have received great attention due to interest regarding industrial applications and their several desirable features for use in food preservation, such as a lack of toxicity and activity against eukaryotic cells, inactivation during digestion and relative stability to heat and low pH values (Gálvez *et al.* 2007; García *et al.* 2010; Dischinger *et al.* 2014).

Nisin, a bacteriocin synthesised by *Lactococcus lactis*, which belongs to the class of lantibiotics, is the only approved as a food additive by *Codex Alimentarius* and has Generally Recognized as Safe (GRAS) status (Federal Register 1988). The mechanism of action of nisin occurs through interaction with lipid II, an important precursor of cell wall synthesis in bacteria, and results in the formation of pores in the cytoplasmic membrane. This alters the permeability of the target cell and causes the efflux of small metabolites and ions, in addition to interfering with the biosynthesis of peptidoglycan (Breukink *et al.* 2003; Hasper *et al.* 2006).

Bovicin HC5 is a bacteriocin produced by *Streptococcus bovis* HC5, a bacteria isolated from the bovine rumen. Bovicin HC5 contains post-translational modified amino acids and the same pattern of lanthionine rings found in nisin, suggesting that this peptide is also a lantibiotic (Mantovani *et al.* 2002). Indeed, this bacteriocin is able to recruit molecules of lipid II into similar pore structures and to sequester these molecules into domains, inhibiting bacterial cell wall synthesis (Paiva *et al.* 2011).

In general, the outer membrane of Gram-negative bacteria confers resistance to such antimicrobial peptides, preventing the diffusion of a bacteriocin to its site of action (Boziaris and Adams 1999). These barrier properties are

largely influenced by the presence of a specific layer of lipopolysaccharide (LPS) on the surface of the structure, the integrity of which affects the stability of the outer membrane (Alakomi *et al.* 2000; Prudêncio *et al.* 2015a). Inside the LPS layer, the negative charges of the phosphate radical and carboxyl residues of 2-keto-3-deoxyoctonate (KDO) act as sites for crosslinking with divalent cations such as calcium and magnesium, reinforcing the membrane structure and minimising the electrostatic repulsion between LPS molecules (Delcour 2009).

During the processing of food, the outer membrane of Gram-negative bacteria is commonly destabilised by stress conditions, such as temperature and pH (Thongbai *et al.* 2006). Furthermore, the use of chelating agents, such as ethylenediaminetetracetate (EDTA), or cell membrane disruptors, such as trisodium phosphate (TSP), is alternatives used to damage the outer membrane permitting the action of bacteriocins, such as nisin and bovicin HC5 (Delves-Broughton *et al.* 1993; Carneiro De Melo *et al.* 1998; Molinos *et al.* 2008; Prudêncio *et al.* 2014, 2015a, b). Knowledge of such processes is of great importance in food microbiology, because many pathogens related to foodborne diseases are Gram-negative bacteria, mainly *Salmonella* (Boziaris and Adams 1999).

In this article, we evaluate the effect of stress conditions that generally occur during food preservation steps in the sensitization of *Salmonella* Typhimurium cells to the activity of nisin and bovicin HC5.

Materials and methods

Bacterial strains and culture conditions

Salmonella enterica serovar Typhimurium ATCC 14028 was cultured in Brain Heart Infusion broth (BHI; Difco, Sparks) at 36 ± 1°C for 18–20 h and reactivated under the same conditions for 2 h. The cells were centrifuged at 2500 ***g*** (Sorvall RT 6000D; DuPont, Wilmington, DL), washed and resuspended in 0·85% saline. The bovicin HC5-producing strain, *Strep. bovis* HC5, was cultivated under anaerobic conditions in EC medium at 39 ± 1°C overnight (Mantovani and Russell 2001). *Lactococcus lactis* ATCC 19435 was grown in Man, Rogosa and Sharpe broth (MRS; Himedia, Mumbai, India) under aerobic conditions for 16 h at 37 ± 1°C (De Man *et al.* 1960).

Preparation and activity of bacteriocins

Nisin solution (Chrisin C; CHL Hansen, Horsholm, Denmark) was prepared in sodium phosphate solution (PBS) (5 mmol l^{-1}) at pH 6·5. Extracts of bovicin HC5 were prepared as described by Mantovani and Russell (2003). The antimicrobial activity was determined by the diffusion method in agar (Tagg *et al.* 1976) using *Lc. lactis* as the indicator organism and quantified by the critical dilution method (Hoover and Harlander 1993). One arbitrary unit (AU) was defined as the reciprocal of the highest dilution that showed a zone of inhibition with at least 5 mm diameter.

Preparation of stressed *Salmonella* Typhimurium cells

Treatments under different conditions of stress were performed in BHI broth using a concentration of approximately 10^8 CFU ml^{-1} of *Salmonella* cells in the exponential phase.

For acid stress, *Salmonella* cells were transferred to BHI broth adjusted to pH 4·5 with HCl (5 mol l^{-1}) and incubated at 37°C for 2 h (Thongbai *et al.* 2006).

For stress with chemical agents, the cells were added to BHI broth with 20 mmol l^{-1} of EDTA (Reagen, Colombo, Brazil) or 20 mmol l^{-1} of TSP (Carlos Erba, São Paulo, Brazil) and incubated at 37 ± 1°C for 30 min (Phillips and Duggan 2001).

Heat stress was applied by heating to 55 ± 1°C for 10 min in a water bath; the tubes were then cooled in running water for 2 min at room temperature (Boziaris and Adams 2001). For cold stress, the cell suspensions were placed at −26 ± 1°C for 2 h and then warmed to room temperature in running water for 4 min (Boziaris and Adams 2001).

Cell viability was determined by plating in Plate Count Agar (PCA; Difco, Sparks) using the microdrop method (Morton 2001) in triplicate or the pour-plate technique in duplicate. The plates were incubated at 36 ± 1°C for 8–10 h in the case of the microdrop technique and for 24 h when using the pour-plate technique before counting using a colony counter (Mod. CE 550A; Phoenix, Araraquara, Brazil).

Treatment of pre-stressed cells with bacteriocins

Salmonella Typhimurium cells subjected to stresses were collected by centrifugation at 2500 ***g*** for 15 min at 4°C, washed and resuspended in 0·85% saline. Suspensions of 10^5 CFU ml^{-1} of stressed cells were inoculated in tubes containing 0·85% saline plus glucose (22 mmol l^{-1}) to energise the membrane, and bacteriocins (nisin or bovicin HC5, 500 AU ml^{-1}) (Mantovani *et al.* 2002). For treatments with bovicin HC5, only the heat and cold-stressed cells were chosen due to good results obtained with nisin.

The tubes were incubated at 37°C for 0, 20, 40, 60 min and 6 h, and aliquots were collected for evaluation of the number of viable cells. For each treatment, controls consisted of previously stressed cells treated with PBS instead

of the bacteriocin solutions. In addition, non-stressed cells were evaluated in absence and presence of nisin.

Statistical analyses

Each treatment was performed using at least two biological replicates. Statistical analyses were performed with the 9.1 SAEG program (Federal University of Viçosa, 2007), using the Tukey's test with a significance level of 0·05.

Results

Stress effect on *Salmonella* Typhimurium cell viability

The viability of *Salm.* Typhimurium before and after treatments of acid, chemical, heat and cold stresses is shown in Fig. 1. The acid treatment at pH 4·5 for 2 h and chemical stress with TSP (20 mmol l^{-1}) and EDTA (20 mmol l^{-1}) for 30 min did not affect the viability of *Salmonella* at the time periods evaluated ($P > 0{\cdot}05$). Significant decimal reductions ($P < 0{\cdot}05$) of 3 and 2 log cycles were observed with the treatments of 55°C for 10 min and −26°C for 2 h, respectively.

Action of bacteriocins on previously stressed *Salmonella* Typhimurium cells

The viability of non-stressed cells in presence of nisin did not differ statistically ($P > 0{\cdot}05$) from those in the absence of bacteriocin during the first hour of treatment (Fig. 2a, Table S1). However, after 6 h of treatment with the bacteriocin, the non-stressed cells showed significant decimal reductions of approximately 1·5 log cycles (Fig. 2a, Table S1).

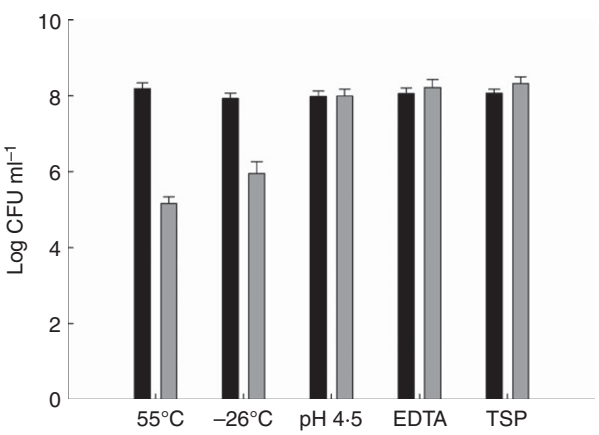

Figure 1 Viability of *Salmonella* Typhimurium treated under different stress conditions. Approximately 10^8 CFU ml^{-1} *Salmonella* cells was inoculated in BHI broth and subjected to 55 ± 1°C for 10 min, −26 ± 1°C for 2 h, pH 4·5 for 2 h, or 20 mmol l^{-1} ethylenediaminetetracetate or 20 mmol l^{-1} trisodium phosphate for 30 min. The black and grey bars represent viability before and after treatment, respectively. Error bars indicate standard deviations ($n = 3$).

Similar results were observed when *Salm.* Typhimurium was previously stressed with acid (pH 4·5) and chemical stresses with TSP (20 mmol l^{-1}) and EDTA (20 mmol l^{-1}). The presence of nisin did not influence statistically ($P > 0{\cdot}05$) the number of viable cells of *Salm.* Typhimurium during the first hour of treatment (Fig. 2b, Table S1). After 6 h of treatment, cells previously stressed at pH 4·5 and in the presence of TSP and EDTA presented decimal reductions of approximately 2·9, 4·1 and 4·0 log cycles, respectively (Fig. 2b, Table S1). It is noteworthy that during treatment with bacteriocins, the cells were under nutritional stress because they were resuspended in 0·85% saline plus glucose (22 mmol l^{-1}).

The bactericidal effect of nisin was higher on cells previously subjected to heat and cold stresses. After 20 min of treatment, it was possible to verify a significant reduction ($P < 0{\cdot}05$) compared to the stressed cells in absence of bacteriocin (Fig. 3). After 1 h of treatment, decimal reductions of 4 log cycles were observed in the population of cells pre-stressed at 55°C, and 3·5 log cycles in the cells pre-stressed at −26°C (Fig. 3, Table S1). After 6 h, the counts were below the detection limit of the technique, which is 10 CFU ml^{-1} (Fig. 3).

The heat and cold stresses were chosen for treatments with bovicin HC5, due to the greater bactericidal effect observed in the treatments with nisin. Bovicin HC5 clearly acted more rapidly than nisin. The number of viable cells of *Salm.* Typhimurium subjected to heat and cold stresses and exposed to bovicin HC5 showed counts below the detection limit of the technique (10 CFU ml^{-1}) after only 20 min of treatment, whereas similar results with nisin only were observed after 6 h of treatment (Fig. 3).

Salmonella cells stressed with different methods (acid, chemical, heat and cold stresses) did not presented significant reduction in the number of viable cells in absence of bacteriocins. Surprisingly, it was observed a considerable increase in the number of viable cells (Figs 2 and 3).

Discussion

The antimicrobial activity of the lantibiotic nisin and bovicin HC5 is related to their interaction with lipid II, an important precursor of cell wall synthesis in bacteria (Hasper *et al.* 2006; Paiva *et al.* 2011). The access of lantibiotics to the cellular membrane of Gram-negative bacteria is limited due to the presence of the outer membrane. Therefore, in this study, stress factors were used to destabilise the outer membrane of *Salm.* Typhimurium, allowing the lantibiotics to interact with the inner membrane.

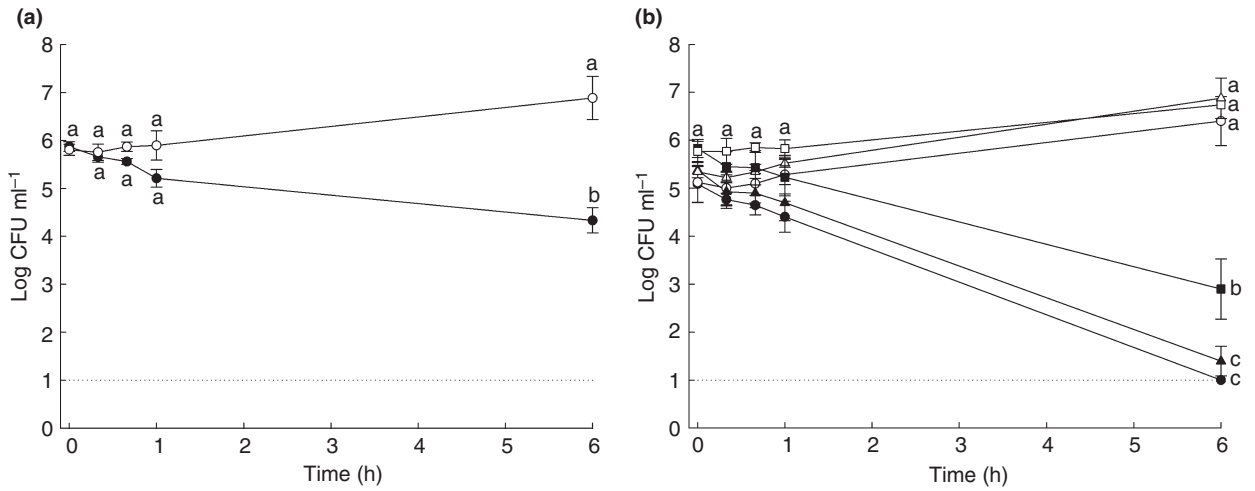

Figure 2 Viability of *Salmonella* Typhimurium non-stressed or stressed with acid and chemical stresses and treated with nisin (500 AU ml^{-1}). (a) Control cells (non-stressed cells). Caption: (○) non-stressed cells in absence of nisin, (●) non-stressed cells in presence of nisin. (b) Cells subjected to acid and chemical stresses (trisodium phosphate (TSP) and ethylenediaminetetracetate (EDTA)) and then treated with nisin. Caption: (●) cells stressed with TSP in presence of nisin, (○) cells stressed with TSP in absence of nisin, (▲) cells stressed with EDTA in presence of nisin, (Δ) cells stressed with EDTA in absence of nisin, (■) cells stressed at acidic pH (4·5) in presence of nisin and (□) cells stressed at acidic pH (4·5) in absence of nisin. Detection limit of the technique (. . . .). Error bars indicate standard deviations ($n = 2$). [a–c]Counts with a same letter in each time intervals do not differ between themselves by Tukey's test ($P < 0{\cdot}05$).

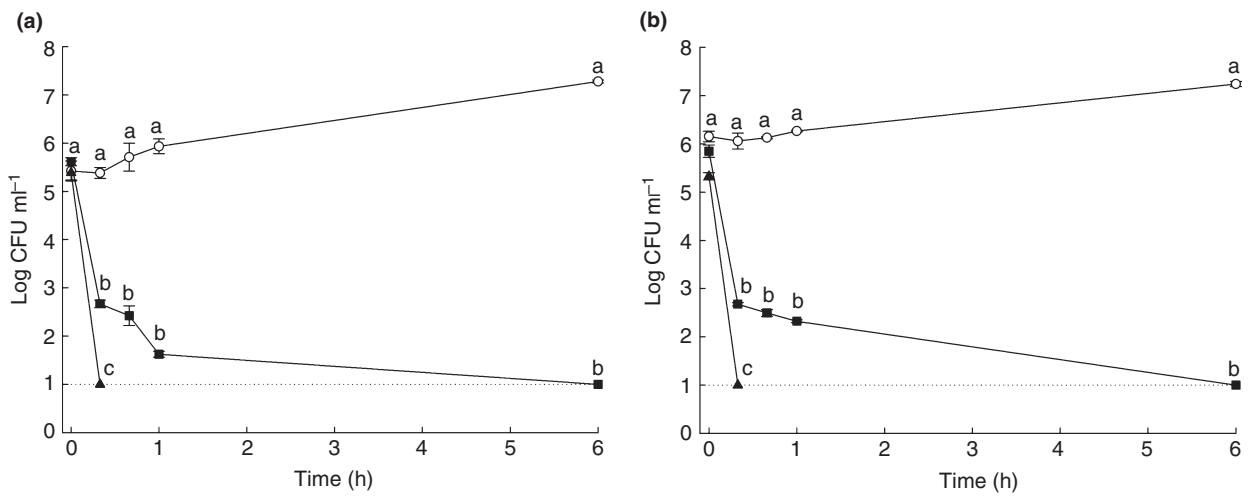

Figure 3 Viability of *Salmonella* Typhimurium subjected to stress and then treated with nisin or bovicin HC5 (500 AU ml^{-1}). (a) Cells subjected to heat stress (55°C). (b) Cells subjected to cold stress (−26°C). Caption: (○) cells stressed in absence of bacteriocins, (■) cells stressed in presence of nisin, (▲) cells stressed in presence of bovicin HC5. Detection limit of the technique (. . . .). Error bars indicate standard deviations ($n = 2$). [a–c]Counts with a same letter in each time intervals do not differ between themselves by Tukey's test ($P < 0{\cdot}05$).

The loss of viability observed in the presence of bacteriocins was due to the bactericidal activity of the peptide, whereas there was an increase in the number of viable cells under all conditions in the absence of the bacteriocin. The order of stress conditions that best sensitized the cells to treatment with nisin (500 AU ml^{-1}, pH 6·5) was the treatment at 55°C and freezing at −26°C, chemical treatment with TSP and EDTA, and acid stress at pH 4·5.

Additionally, the non-stressed cells incubated in saline plus glucose and, consequently, in a state of nutritional stress during the treatment with nisin, became sensitive to the bacteriocin along the course of the experiment (Fig. 2a). Changes in any of the parameters for optimal microbial growth, such as abundant nutrients and temperature and pH optimum will disturb the maximum growth rate, representing a stress to the cell (Spector and

Kenyon 2012). Responses to nutritional stress result in an overall reprogramming of cellular metabolism, including the production of enzymes for the 'cannibalisation' or turnover of unnecessary cellular components, enzymes that modify the components of the inner membrane, peptidoglycan and outer membrane (Spector and Kenyon 2012). Although there are no studies correlating nutritional stress to the sensitivity of Gram-negative to bacteriocins, the cell death observed can be due to the large number of physiological changes resulting from an attempt by the bacteria to maintain homoeostasis.

Among the stresses used, acid was less effective in sensitizing cells to bacteriocins. Acidic conditions may affect the growth of micro-organisms by interfering with the synthesis of cellular components and inducing cell death as a result of damage to the outer membrane, the denaturation of proteins at the cell surface, the disruption of cytoplasmic pH homoeostasis and subsequent damage to DNA and enzymes (Brown *et al.* 1997; Cheroutre-Vialette *et al.* 1998). Thongbai *et al.* (2006) verified that nisin caused a 5·36 log cycles reduction and extensive morphological changes in the cell envelope of *Salm.* Typhimurium S36 cells pre-stressed at pH 4·5 and treated with cetylpyridinium chloride (CPC). However, in this case, the CPC possibly enhanced the activity of the bacteriocin, which is different from what was found in this study, whereby acid stress most likely did not cause sufficient damage to the outer membrane to sensitize the cells to the action of nisin (Fig. 2b).

This study also demonstrated that the use of EDTA, a chelating agent widely used to destabilise the structure of the outer membrane, facilitates the inactivation of the cells by nisin, promoted a reduction of 4·0 log cycles (Fig. 2b). This result agrees with that obtained by Phillips and Duggan (2001), who observed reductions of 4·4 log cycles in the number of viable *Arcobacter butzleri* cells treated with EDTA (20 mmol l^{-1}) and then with nisin (500 AU ml^{-1}) for 30 min. It is known that EDTA promotes the disintegration, at least in part, of the LPS layer, possibly due to the binding of this compound to calcium and magnesium ions, which are essential in establishing cross-links with the KDO residues and phosphate radicals to reinforce the structure of the outer membrane of Gram-negative bacteria (Alakomi *et al.* 2003). In contrast, 30-min treatments with EDTA alone (20 mmol l^{-1}) do not inactivate several serovars of *Salmonella* (Stevens *et al.* 1991), similar to our results (Fig. 1).

The use of TSP alone did not affect the viability of *Salm.* Typhimurium, though it did stress the cells, sensitizing them to treatment with nisin (Figs 1 and 2b). The antimicrobial action of this compound involves a combination of ionic strength and the effect of a detergent, resulting in the disruption of the cytoplasmic membrane (Mendonça *et al.* 1994; Capita *et al.* 2002; Del Rio *et al.* 2006). Capita *et al.* (2002) have documented that the antimicrobial effect of TSP is more effective against Gram-negative bacteria than Gram-positive bacteria. Studies also suggest that TSP has a mode of action that involves the chelation of divalent cations in the outer membrane (Sampathkumar *et al.* 2003). Phillips and Duggan (2001) reported that treatment with TSP (20 mmol l^{-1}) followed by nisin (500 AU ml^{-1}) for 30 min caused a reduction of 5·2 cycles in a population of *A. butzleri*. TSP is approved as an antimicrobial agent at levels 80–120 mg ml^{-1} for raw and chilled bird carcasses (Federal Register 1994), and these concentrations are considerably higher compared with those used in the present work: 20 mmol l^{-1} or 3·28 mg ml^{-1}.

Although it has been demonstrated that bovicin HC5 alone, at concentrations up to 200 AU ml^{-1}, does not exert an bactericidal effect on *Salm.* Typhimurium (Prudêncio *et al.* 2014, 2015b), the present results provide further evidence that it is necessary to destabilize the outer membrane to reduce the resistance of this micro-organism to the bacteriocin. Furthermore, bovicin HC5 at the same concentration of nisin (500 AU ml^{-1}) showed the highest bactericidal effect on *Salm.* Typhimurium cells pre-stressed at 55 and −26°C, being able to reduce the number of viable cells to undetectable levels in a shorter period of time (Fig. 3). These results corroborate those presented by Paiva *et al.* (2011), who showed that bovicin HC5 was five times more effective than nisin in inhibiting the growth of Gram-positive *Staphylococcus warneri*, which is resistant to nisin. However, the treatment conditions can influence the bacteriocin activity, so that the efficiency of each bacteriocin is variable according to environmental conditions, such as temperature and pH (Balciunas *et al.* 2013; Prudêncio *et al.* 2015b).

The present sequential combination of heat or cold stress and bacteriocin treatment resulted in significant synergistic decreases in the viability of *Salmonella*, suggesting that these stresses caused a sublethal injury to the outer membrane and allowed the access of the bacteriocin. In particular, a variety of morphological and structural changes can occur in the outer membrane due to heating or freezing, including the formation of vesicles and bullae on the cell surface, the release of LPS and damage or conformational changes in proteins and LPS. These changes may alter the permeability of the barrier, causing an efflux of periplasmic enzymes and sensitivity to dyes, surfactants and hydrophobic compounds, such as bacteriocins (Kempler and Ray 1978; Tsuchido *et al.* 1985; Ueckert *et al.* 1998; Cao-Haong *et al.* 2008). Boziaris and Adams (2001) have noted an increased in the hydrophobicity of the cell surface of Gram-negative bac-

teria after heating and freezing, which appears to be related to LPS release.

It may be concluded from the present findings that heating and freezing stresses reduced the number of viable cells of *Salm.* Typhimurium and allowed a greater action of nisin and bovicin HC5, which promoted the rapid loss of cell viability, with bovicin HC5 being more effective than nisin. These observations show that nisin and bovicin HC5 exhibit bactericidal activity against *Salm.* Typhimurium when stress conditions are used to disrupt the LPS layer of the outer membrane.

Acknowledgements

The authors thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for funding this project.

Conflict of interest

No conflict of interest declared.

References

Alakomi, H.L., Skytta, E., Saarela, M., Mattila-Sandholm, T., Latva-Kala, K. and Helander, I.M. (2000) Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. *Appl Environ Microbiol* **66**, 2001–2005.

Alakomi, H., Saarela, M. and Helander, J. (2003) Effect of EDTA on *Salmonella enterica* serovar *Typhimurium* involves a component not assignable to lipopolysaccharide release. *Microbiology* **149**, 2015–2021.

Balciunas, E.M., Martinez, F.A.C., Todorov, S.D., Franco, B.D.G.M., Converti, A. and Oliveira, R.P.S. (2013) Novel biotechnological applications of bacteriocins: a review. *Food Control* **32**, 134–142.

Boziaris, I.S. and Adams, M.R. (1999) Effect of chelators and nisin produced *in situ* on inhibition and inactivation of gram negatives. *Int J Food Microbiol* **53**, 105–113.

Boziaris, I.S. and Adams, M.R. (2001) Temperature shock, injury and transient sensitivity to nisin in Gram-negatives. *J Appl Microbiol* **91**, 715–724.

Breukink, E., Van Heusden, H.E., Vollmerhaus, P.J., Swiezewska, E., Brunner, L., Walker, S., Heck, A.J. and de Kruijff, B. (2003) Lipid II is an intrinsic component of the pore induced by nisin in bacterial membranes. *J Biol Chem* **278**, 19898–19903.

Brown, J.L., Ross, T., Mcmeekin, T.A. and Nichols, P.D. (1997) Acid habituation of *Escherichia coli* and the potential role of cyclopropane fatty acids in low pH tolerance. *Int J Food Microbiol* **37**, 163–173.

Cao-Haong, L., Marechal, P.A., Le-Thanh, M. and Gervais, P. (2008) Synergistic action of rapid chilling and nisin on the inactivation of *Escherichia coli*. *Appl Microbiol Biotechnol* **79**, 105–109.

Capita, R., Alonso-Calleja, C., García-Fernández, M.C. and Moreno, B. (2002) Review: trisodium phosphate (TSP) treatment for decontamination of poultry. *Food Sci Technol Int* **8**, 11–24.

Carneiro De Melo, A.M.S., Cassar, C.A. and Miles, R.J. (1998) Trisodium phosphate increases sensitivity of Gram-negative bacteria to lysozyme and nisin. *J Food Prot* **61**, 839–844.

Cheroutre-Vialette, M., Lebert, I., Hebraud, M., Labadie, J.C. and Lebert, A. (1998) Effects of pH or a_w stress on growth of *Listeria monocytogenes*. *Int J Microbiol* **42**, 71–77.

Cotter, P.D., Hill, C. and Ross, R.P. (2005) Bacteriocins: developing innate immunity for food. *Nat Rev Microbiol* **3**, 777–788.

De Man, J.C., Rogosa, M. and Sharpe, M.E. (1960) A medium for the cultivation of *lactobacilli*. *J Appl Bacteriol* **23**, 130–135.

Del Rio, E., Capita, R., Prieto, M. and Alonso-Calleja, C. (2006) Comparison of pathogenic and spoilage bacterial levels on refrigerated poultry parts following treatment with trisodium phosphate. *Food Microbiol* **23**, 195–198.

Delcour, A.H. (2009) Outer membrane permeability and antibiotic resistance. *Biochim Biophys Acta* **1794**, 808–816.

Delves-Broughton, J., Blackburn, P., Evans, R.J. and Hugenholtz, J. (1993) Applications of the bacteriocin nisin. *Antonie Van Leeuwenhoek* **69**, 193–202.

Dischinger, J., Chipalu, S.B. and Bierbaum, G. (2014) Lantibiotics: promising candidates for future applications in health care. *Int J Med Microbiol* **304**, 51–62.

Federal Register (1988) Nisin preparation: affirmation of GRAS status as a direct human food ingredient. *Fed Reg* **54**, 11247–11251.

Federal Register (1994) Use of trisodium phosphate on raw, chilled poutry carcasses. *Fed Reg* **59**, 551–554.

Gálvez, A., Abriouel, H., López, R.L. and Omar, N.B. (2007) Bacteriocin-based strategies for food preservation. *Int J Food Microbiol* **120**, 51–70.

García, P., Rodríguez, L., Rodríguez, A. and Martínez, B. (2010) Food biopreservation: promising strategies using bacteriocins, bacteriophages and endolysins. *Food Sci Technol Int* **21**, 373–382.

Hasper, H.E., Kramer, N.E., Smith, J.L., Hillman, J.D., Zachariah, C., Kuipers, O.P., De Kruijff, B. and Breukink, E. (2006) An alternative bactericidal mechanism of action for lantibiotic peptides that target lipid II. *Science* **313**, 1636–1637.

Hoover, D.G. and Harlander, S.K. (1993) Screening methods for detecting bacteriocin activity. In *Bacteriocins of Lactic Acid Bacteria* ed. Hoover, D.G. and Steenson, L.R. pp. 23–29. San Diego, CA: Academic Press.

Kempler, G. and Ray, B. (1978) Nature of freezing damage on the lipopolysaccharide molecule of *Escherichia coli* B. *Cryobiology* **15**, 578–583.

Mantovani, H.C. and Russell, J.B. (2001) Nisin resistance of *Streptococcus bovis*. *Appl Environ Microbiol* **67**, 808–813.

Mantovani, H.C. and Russell, J.B. (2003) Inhibition of *Listeria monocytogenes* by bovicin HC5, a bacteriocin produced by *Streptococcus bovis* HC5. *Int J Food Microbiol* **89**, 77–83.

Mantovani, H.C., Hu, H., Worobo, R.W. and Russell, J.B. (2002) Bovicin HC5, a bacteriocin from *Streptococcus bovis* HC5. *Microbiology* **148**, 3347–3352.

Mendonça, A.F., Amoroso, T.L. and Knabel, S.J. (1994) Destruction of Gram-negative food-borne pathogens by high pH involves disruption of the cytoplasmic membrane. *Appl Environ Microbiol* **60**, 4009–4014.

Molinos, A.C., Abriouel, H., Omar, N.B., López, R.L., Valdivia, E., Omar, N.B. and Gálvez, A. (2008) Combined physico-chemical treatments based on enterocin AS-48 for inactivation of Gram-negative bacteria in soybean sprouts. *Food Chem Toxicol* **48**, 2912–2921.

Morton, R.D. (2001) Aerobic plate count. In *Compendium of Methods for the Microbial Examination of Foods* ed. Downes, F.P. and Ito, K. pp. 63–67. Washington, DC: American Public Health Association.

Paiva, A.D., Breukink, E. and Mantovani, H.C. (2011) The role of Lipid II and membrane thickness in the mechanism of action of the lantibiotic bovicin HC5. *Antimicrob Agents Chemother* **55**, 5284–5293.

Phillips, C.A. and Duggan, J. (2001) The effect of EDTA and trisodium phosphate, alone and in combination with nisin, on the growth of *Arcobacter butzleri* in culture. *Food Microbiol* **18**, 547–554.

Prudêncio, C.V., Mantovani, H.C. and Vanetti, M.C.D. (2014) Inhibition of *Salmonella* Typhimurium by bovicin HC5 associated with chelating agents and surfactants. *Afr J Microbiol Res* **8**, 12–18.

Prudêncio, C.V., Vanetti, M.C.D. and Prieto, M. (2015a) Tolerance of *Salmonella enterica* serovar Typhimurium to nisin combined with EDTA is accompanied by changes in cellular composition. *Food Res Int* **69**, 281–288.

Prudêncio, C.V., Mantovani, H.C. and Vanetti, M.C.D. (2015b) Differences in the antibacterial activity of nisin and bovicin HC5 against *Salmonella* Typhimurium under different temperature and pH conditions. *J Appl Microbiol* **118**, 18–26.

Sampathkumar, B., Khachatourians, G.G. and Korber, D.R. (2003) High pH during trisodium phosphate treatment causes membrane damage and destruction of *Salmonella enterica* serovar Enteritidis. *Appl Environ Microbiol* **69**, 122–129.

Spector, M.P. and Kenyon, W.J. (2012) Resistance and survival strategies of *Salmonella enterica* to environmental stresses. *Food Res Int* **45**, 455–481.

Stevens, K.A., Sheldon, B.W., Klapes, N.A. and Klaenhammer, T.R. (1991) Nisin treatment for inactivation of *Salmonella* species and other gram-negative bacteria. *Appl Environ Microbiol* **57**, 3613–3615.

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

Thongbai, B., Gasaluck, P. and Waites, W.M. (2006) Morphological changes of temperature- and pH-stressed *Salmonella* following exposure to cetylpyridinium chloride and nisin. *LWT-Food Sci Technol* **39**, 1180–1188.

Tsuchido, T., Katsui, N., Takeuchi, A., Takano, M. and Shibasaki, I. (1985) Destruction of the outer membrane permeability barrier of *Escherichia coli* by heat treatment. *Appl Environ Microbiol* **50**, 298–303.

Ueckert, J.E., ter Steeg, P.F. and Coote, P.J. (1998) Synergistic antibacterial action of heat in combination with nisin and magainin II amide. *J Appl Microbiol* **85**, 487–494.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Viability (log CFU ml^{-1}) of *S.* Typhimurium subjected to stress and then treated with nisin (500 AU ml^{-1}) or not treated (0 AU ml^{-1}) and controls (cells not stressed).