Food Control 25 (2012) 740–744

Contents lists available at SciVerse ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Increasing the microbial inactivation of *Staphylococcus aureus* in sauces by a combination of enterocin AS-48 and 2-nitropropanol, and mild heat treatments

Maria José Grande Burgos, Hikmate Abriouel, Rosario Lucas, Antonio Gálvez*

Área de Microbiología, Departamento de Ciencias de la Salud, Facultad de Ciencias Experimentales, Universidad de Jaén, 23071-Jaén, Spain

ARTICLE INFO

Article history:
Received 29 July 2011
Received in revised form
8 December 2011
Accepted 14 December 2011

Keywords:
Staphylococcus
2-Nitropropanol
Bacteriocin
Biopreservation

ABSTRACT

Staphylococcus aureus was challenged with the cyclic bacteriocin AS-48 in four commercial sauces singly and in combination with sublethal heat treatments (50–60 °C, for 5 min) and with 2-nitro-1-propanol (2NPOH). Heating at 60 °C in combination with AS-48 (25 μg/ml) reduced initial counts (4.1–4.5 log CFU/ml) below detection limit (1.0 log CFU/ml), while a combined treatment at 55 °C inhibited proliferation of staphylococci in sauces for 24 h at 22 °C. In BHI broth, enterocin AS-48 (0–15 μg/ml) acted synergistically with 2NPOH (0–220 mM), reducing the concentrations of both antimicrobials required for inactivation of *S. aureus*. In sauces, enterocin AS-48 (25 μg/ml) plus 2NPOH (25 mM) reduced initial cell concentrations (4.1–5.0 log CFU/ml) below detectable levels within 8–24 h, depending on the sauce. The combinations of enterocin AS-48 and 2NPOH or sublethal heat are interesting for testing as part of hurdle technology aimed at reducing the risks for proliferation of *S. aureus* in sauces.

1. Introduction

Staphylococcal food poisoning is among the most common causes of reported foodborne diseases (EFSA, 2010; Le Loir, Baron, & Gautier, 2003; Tirado & Schimdt, 2001; WHO, 2002), requiring hospital attention by up to 19.5% of the affected individuals (EFSA, 2010). *Staphylococcus aureus* is found in the nostrils as well as on the skin and hair of warm-blooded animals, and up to 30–50% of human population are carriers (Le Loir et al., 2003). *S. aureus* has been isolated from several foods including meat and meat products, chicken, milk and dairy products, fermented food items, salads, vegetables, fish products, etc. (Jørgensen et al., 2005; Seo & Bohach, 2007; Tamarapu, McKillip, & Drake, 2001). Most strains are capable of producing one or more heat stable enterotoxins (Balaban & Rasooly, 2000; Ortega, Abriouel, Lucas, & Gálvez, 2010) which are the cause of the gastrointestinal symptoms observed during intoxications (Tamarapu et al., 2001).

One of the approaches proposed for the control of *S. aureus* in foods is application of bacteriocins either singly or in combination with other antimicrobials (Gálvez, Lucas-López, Abriouel, Valdivia, & Ben Omar, 2008). Among them, the cyclic peptide bacteriocin enterocin AS-48 has been thoroughly investigated for its antimicrobial activity against foodborne pathogens both in liquid cultures and in food systems (Abriouel, Lucas, Ben Omar, Valdivia, & Gálvez, 2010). In a previous study, we showed that inactivation of *S. aureus* in sauces required addition of high concentrations of enterocin AS-48 (up to 80 μg/ml) and prolonged incubation compared to culture media, and also that bactericidal activity was greatly influenced by the type of sauce (Ananou, Valdivia, Martínez-Bueno, Gálvez, & Maqueda, 2004; Grande et al., 2007). Addition of phenolic compounds significantly improved microbial inactivation of *S. aureus* in sauces. Among them, hydrocinnamic acid and carvacrol showed the best results in combination with enterocin AS-48 (Grande et al., 2007). The purpose of the present study was to investigate alternative approaches based on moderate heat treatment and addition of 2-1-nitro-propanol (2NPOH), in order to reduce the effective concentration of enterocin AS-48 required for complete inactivation of *S. aureus* in sauces.

2. Materials and methods

2.1. Bacterial strains and cultivation conditions

The toxicogenic strain *S. aureus* CECT 976 was from the Spanish Type Culture Collection (CECT). *Enterococcus faecalis* A-48-32 (Martínez-Bueno, Gálvez, Valdivia, & Maqueda, 1990) was used to

* Corresponding author. Present address: Área de Microbiología, Departamento de Ciencias de la Salud, Facultad de Ciencias Experimentales, Edif. B3, Universidad de Jaén, Campus Las Lagunillas s/n, 23071-Jaén, Spain. Tel.: +34 953 212160; fax: +34 953 212943.
E-mail address: agalvez@ujaen.es (A. Gálvez).

0956-7135/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodcont.2011.12.018

produce enterocin AS-48, and *E. faecalis* S-47 was used as an indicator strain for determination of bacteriocin activity. Bacterial strains were grown on brain heart infusion broth (BHI; Scharlab, Barcelona, Spain) at 37 °C and maintained routinely on BHI agar slants at 4 °C.

2.2. Bacteriocin preparation

Enterocin AS-48 was obtained as described elsewhere (Abriouel, Valdivia, Martínez-Bueno, Maqueda, & Gálvez, 2003). Briefly, the producer strain *E. faecalis* A-48-32 was incubated for 8 h at 37 °C in a modified complex medium (MCM broth) (Abriouel et al., 2003). Bacteriocin was recovered by cation exchange chromatography on CM-25 Sephadex by elution with 1.5 M NaCl as described by Abriouel et al. (2003). Bacteriocin concentrates (containing ca. 1 mg bacteriocin/ml) were desalted through 2000 mw cut-off dialysis tubing (Sigma) and filtered through 0.22 μm pore size low protein binding filters (Millex GV; Millipore Corp., Belford, MA, USA) under sterile conditions.

2.3. Treatments combining enterocin AS-48 and 2-nitro-1-propanol or heat

Commercial sauces were purchased from local supermarkets: carbonara sauce (containing cheese, cream, bacon, onion, egg white and oil as main ingredients; pH 6.03; Knorr), napoletana sauce (tomato, onion, carrots, vegetable oil; pH 4.33; Buitoni, Barcelona), pesto (basil, cheese, powdered milk whey, pine nuts, garlic, xantan gum, olive oil; pH 4.77; Gallo), and green sauce for fish (vegetables, peas, onion, garlic, parsley, starch, fish flour, olive oil; pH 5.45; Knorr, Barcelona). Sauces (20 g) were inoculated (0.2%, vol/vol) with a 100-fold dilution (in sterile saline solution) of an overnight culture of *S. aureus* CECT 976 grown in BHI broth at 37 °C for 15 h.

In order to test the combined effect of enterocin AS-48 and 2-nitro-1-propanol (2NPOH; Fluka, Madrid, Spain), the inoculated sauces (viable counts of 4.1–4.5 log CFU/ml at time 0) were supplemented or not with enterocin AS-48 (25 μg/ml, final concentration) and 2NPOH (25 mM, final concentration) singly or in combination, thoroughly mixed and distributed in sterile capped plastic test tubes before they were stored at 22 °C. At desired intervals of incubation, aliquots (1 ml) of control and treated sauces were serially diluted in sterile saline solution (0.85% NaCl) and plated in triplicate on Vogel and Jonson agar (VJ agar, Scharlab). After 48 h incubation at 37 °C, the average number of colonies on the plates was used to calculate the viable cell concentration of samples, expressed as colony forming units (CFU) per ml.

In order to test the combined effect of heat treatments and enterocin AS-48, sauce samples prepared as above in sterile capped plastic test tubes (viable counts of 4.1–5.0 log CFU/ml at time 0), supplemented or not with enterocin AS-48 (25 μg/ml), were heated by immersion in water baths (Memmert, Schwabach, Germany) at desired temperatures (50, 55 and 60 °C) for 5 min. After treatments, samples were cooled under running tap water for 5 min and processed for viable counts or stored at 22 °C for 24 h in order to determine the growth of survivors with or without bacteriocin being present. Aliquots (1 ml) of control and treated sauces (taken at 0 and 24 h) were serially diluted in sterile saline solution and plated in triplicate on VJ agar for viable cell determinations. All experiments were carried out in duplicate.

2.4. Determination of synergistic activity between enterocin AS-48 and 2-nitro-1-propanol

The concentration-dependent effect of enterocin AS-48 and 2NPOH was determined by the checkerboard method using 96-well, flat-bottom microtiter plates (Becton Dickinson Labware, Franklin Lakes, NJ). An overnight culture of *S. aureus* CECT 976 was diluted 100-fold in sterile saline solution and inoculated (0.2%, vol/vol) in duplicate on BHI broth (ca. 5.0 log CFU/ml) supplemented with different concentrations of enterocin AS-48 (0–15 μg/ml) and 2NPOH (0–220 mM), and incubated at 22 °C for 24 h. After this, the concentrations of viable cells were determined by serial dilution and plating on tryptic soy agar plates (TSA, Scharlab). The resulting isobolograms were interpreted according to Williamson (2001). The minimum bactericidal concentrations (MBC) of enterocin AS-48 and 2NPOH was established as the lowest concentration of antimicrobial that reduced the population of *S. aureus* below detectable levels within 24 h incubation.

2.5. Statistical analysis

All experiments were carried out in duplicate, and the average data ± standard deviations were determined with Excel programme (Microsoft Corp., USA). A *t*-test was performed at the 95% confidence interval with Statgraphics Plus version 5.1 (Statistical Graphics Corp, USA), in order to determine the statistical significance of data.

3. Results

3.1. Potentiation of bacteriocin activity in sauces in combination with heat treatments

At the final concentration tested (25 μg/ml) enterocin AS-48 alone had no significant inhibitory effect on the viability of *S. aureus* in all four sauces tested. In contrast, after 5 min heat treatments, reductions of viable counts increased in the samples heated with enterocin AS-48 compared to samples heated without bacteriocin, in proportion to the intensity of the heat treatment (Fig. 1A). Microbial inactivation was significantly higher ($P < 0.05$) for combined treatments at 55 °C and 60 °C compared to the heat treatments applied singly, with increased log reductions of ca. 1.2 and 2 log units, respectively. After the 60 °C treatment, no viable staphylococci were detected in the samples heated with bacteriocin, while an average of 2 log CFU/ml viable cells were still detected in sauces heated without bacteriocin.

Samples treated at a sublethal temperature of 55 °C were further incubated for 24 h at 22 °C in order to determine the fate of survivors during storage (Fig. 1B). Viable counts of controls heated without bacteriocin increased during storage especially for the sauces with higher pH values (carbonara and green sauce for fish), but not so much in the samples heated with bacteriocin. Viable counts after 24 h were always significantly lower ($P < 0.05$) than controls heated without bacteriocin. Samples heated at 60 °C with bacteriocin did not yield any viable counts after 24 h incubation at 22 °C.

3.2. Synergism with 2-nitro-1-propanol

The interaction of enterocin AS-48 and 2NPOH against *S. aureus* CECT 976 was investigated in BHI broth by using a range of concentrations of bacteriocin and 2NPOH (Fig. 2A). The minimum bactericidal concentration (MBC) of enterocin AS-48 that reduced the population of *S. aureus* below detectable levels within 24 h incubation at 22 °C was 15 μg/ml. In contrast, a concentration of 220 mM 2NPOH was required to achieve the same bactericidal effect (Fig. 2A), although this compound was bacteriostatic at much lower concentrations (55 mM). The combinations of enterocin AS-48 and 2NPOH were far more effective in killing *S. aureus* than the single treatments. The concave isobole obtained for the MBC of

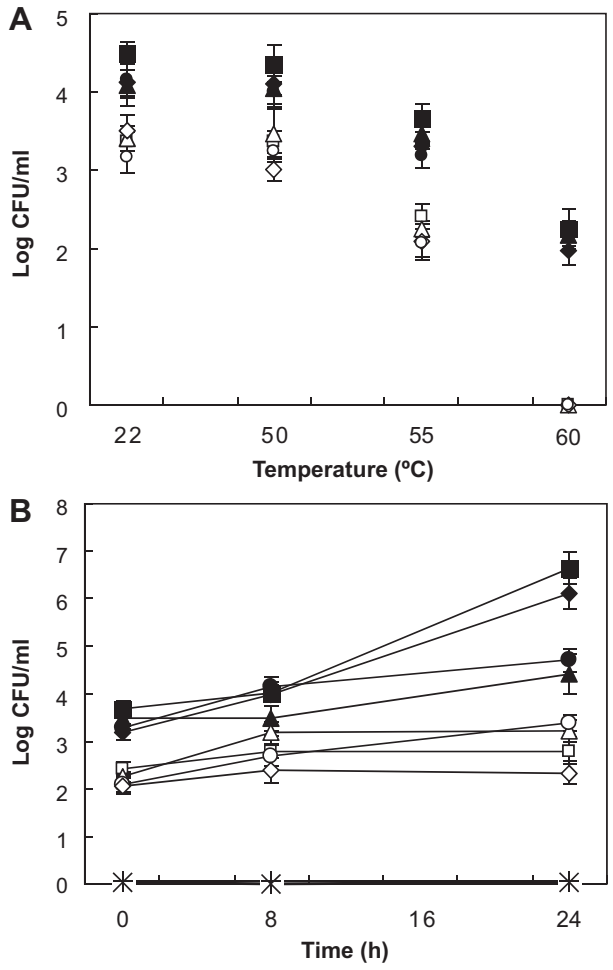

Fig. 1. Effect of heating temperature applied singly (closed symbols) or of a combination of heating and enterocin AS-48 (25 μg/ml; open symbols) on survival (A) and proliferation during storage (B) of *S. aureus* CECT 976 inoculated in sauces: carbonara (■), napoletana (◆), pesto (▲) and sauce for fish (●). In (A), survival was determined right after the 5-min heat treatments. In (B), viable counts were determined during 24 h incubation at 22 °C of controls and bacteriocin-treated samples heated at 55 °C. Samples heated at 60 °C with AS-48 did not yield any viable counts as shown in the graph for carbonara (+) and napoletana (*) sauces.

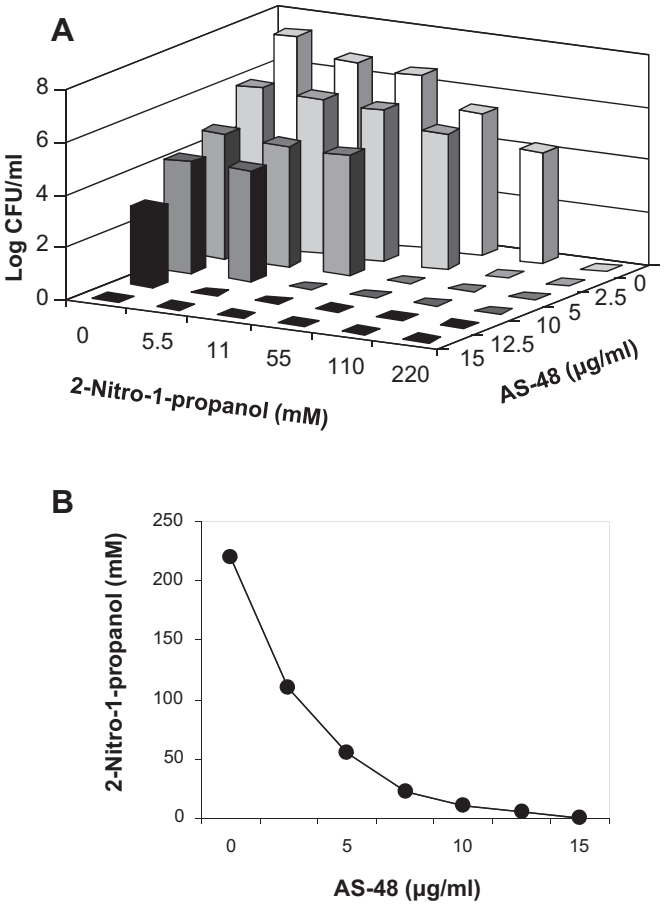

Fig. 2. Effect of different concentrations of enterocin AS-48 and 2-nitro-1-propanol on survival of *S. aureus* CECT 976 in BHI broth (A). In (B), the different combinations of enterocin AS-48 and 2-nitro-1-propanol that caused complete inactivation of *S. aureus* are shown. Samples were incubated at 22 °C for 24 h.

the different combinations of enterocin AS-48 and 2NPOH clearly indicated that the two antimicrobials act synergistically (Fig. 2B). Remarkably, the effective concentration of 2NPOH in the combination was reduced below 55 mM when the bacteriocin concentration increased above 5 μg/ml (Fig. 2B).

3.3. Combined effect of enterocin AS-48 and 2-nitro-1-propanol in sauces

Because of the lower bactericidal effect demonstrated in previous work for enterocin AS-48 in sauces compared to culture media, a higher concentration of bacteriocin (25 μg/ml) was tested in combination with 25 mM 2NOPH in sauces stored at 22 °C (Fig. 3). The single application of both antimicrobials did not have significant effects on the viability of *S. aureus*. Growth inhibition was clearly observed in carbonara (Fig. 3A) and in sauce for fish (Fig. 3D), in which the population of staphylococci increased in the absence of added antimicrobials. In contrast, significant bactericidal effects ($P < 0.05$) were observed in all sauces for the combined treatments of enterocin AS-48 plus 2NPOH, reducing the viable cell counts of staphylococci below detectable levels after 8 h incubation in napoletana (Fig. 3B) and pesto (Fig. 3C) sauces and after 24 h in carbonara (Fig. 3A) and sauce for fish (Fig. 3D).

4. Discussion

Vegetable sauces that become contaminated during handling may be considered as a potential risk and also as vehicles for transmission of staphylococci to other foods. Therefore, the use of additional hurdles such as bacteriocins to decrease the risks for transmission and proliferation of this pathogen should be recommended. In the present study we have shown that the antimicrobial activity of enterocin AS-48 against *S. aureus* in sauces can be enhanced by sublethal heat and by the antimicrobial compound 2NPOH. In a previous study, Ananou et al. (2004) showed that sublethal heat treatment (65 °C, 5 min) increased the sensitivity of *S. aureus* CECT 976 to enterocin AS-48 in BHI broth at low bacteriocin concentrations of 7 or 10 μg/ml. The present study is the first report where the combined treatment has been tested in a food system. While bacteriocin concentrations up to 80 μg/ml may be required for complete inactivation of *S. aureus* in carbonara sauce (Grande et al., 2007), a much lower bacteriocin concentration (25 μg/ml) in combination with sublethal heat (60 °C) reduced viable counts below detectable levels within 5 min treatment in all sauces tested. In combined treatments at 55 °C, the bacteriocin also inhibited proliferation of survivors during storage of carbonara sauce and green sauce for fish. These results suggest that enterocin

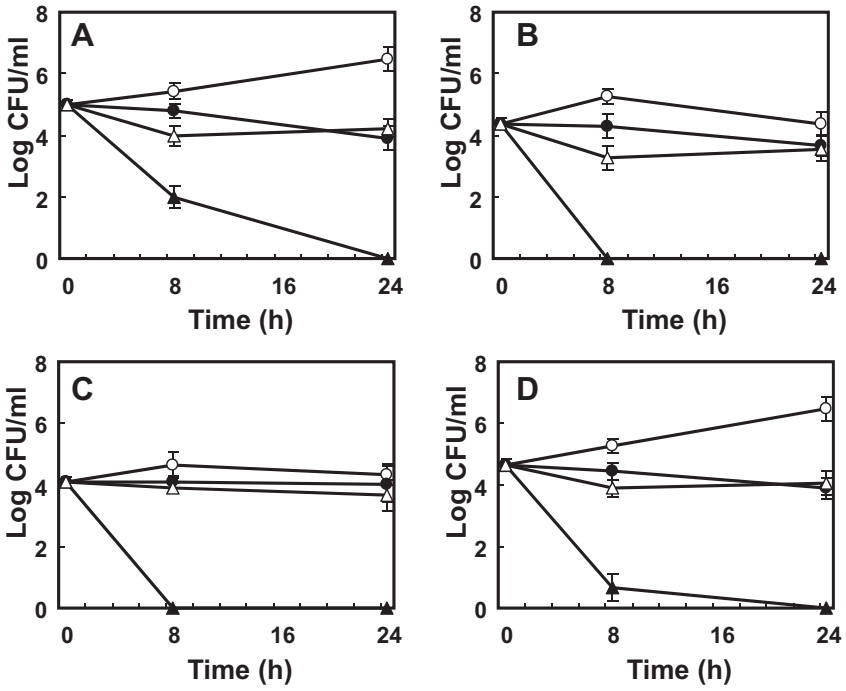

Fig. 3. Effect of enterocin AS-48 (25 μg/ml) in combination with 2-nitro-1-propanol (25 mM) on survival of *S. aureus* CECT 976 in carbonara (A), napoletana (B), pesto (C) and sauce for fish (D) stored at 22 °C. Symbols: *S. aureus* control cultures (○). Cultures treated with 2NPOH (△), enterocin AS-48 (●) or enterocin AS-48 plus 2NPOH (▲).

AS-48 could be applied in combination with mild heat treatments in sauces as a hurdle for inactivation or growth inhibition of *S. aureus*.

In addition to classical food preservatives, new chemical compounds with antimicrobial activity may find a place for applications in the food industry sector and decrease the problems of resistance to common preservatives. One candidate is 2NPOH. This compound exhibits broad-spectrum antimicrobial activity, inhibiting the growth of *Salmonella enterica* serovar Typhimurium, *Escherichia coli* O157:H7, *Campylobacter* sp., *Listeria monocytogenes* Scott A and *E. faecalis* in vitro and reducing gut concentrations of *Salmonella* Typhimurium in broilers (Dimitrijevic et al., 2006; Jung, Anderson, Callaway et al., 2004, Jung, Anderson, Edrington et al., 2004). Due to its low toxicity (Jung, Anderson, Edrington et al., 2006; Majak & Clark, 1980) and its antimicrobial activity against foodborne pathogens, this compound may find potential applications in preharvest safety programs, as a feed additive, or in the control *Listeria* in natural and man-made environments (Anderson et al., 2006; Dimitrijevic et al., 2006; Jung, Anderson, Callaway et al., 2004, Jung, Anderson, Edrington et al., 2006). A recent study indicated that 2NPOH had an MBC value greater than 200 mM against *S. aureus* CECT 976 in corn flour dough stored at 37 °C (Ortega, Abriouel, Lucas-López, Ben Omar, & Gálvez, 2010).2NPOH increases the bactericidal effect of enterocin AS-48 against *L. monocytogenes* and *S. enterica* cells in deli-type salads (Cobo Molinos, Abriouel et al., 2009, Cobo-Molinos, Lucas et al., 2009). In chocolate syrup, the combination of enterocin AS-48 (50 μg/ml) and 2NPOH (55 mM) increased the microbial inactivation of *S. aureus* (Martínez-Viedma, Abriouel, Ben Omar, Lucas, & Gálvez, 2009). In the present study, by using a range of concentrations of enterocin and 2NPOH, a synergistic interaction between the two compounds was demonstrated, resulting in greater reduction of viable counts than the single application of the compounds. This could lead to a reduction in the concentrations of both compounds required for complete inactivation of *S. aureus* in food systems, as shown in the results obtained in the four types of sauces tested. The combined treatment allowed a significant reduction of the effective concentrations of 2NPOH (from 220 mM to 25 mM) and enterocin AS-48 from 80 μg/ml (as described in a previous work by Grande et al., 2007) to 25 μg/ml in the present study. Like many other bacteriocins, the efficacy of enterocin AS-48 in food systems depends greatly on the degree of interaction of bacteriocin molecules with food components (Gálvez, Abriouel, Lucas-López, & Ben Omar, 2007). According to results of the present study, suitable combinations of enterocin AS-48 and 2NPOH could find application in foods where higher bacteriocin concentrations are required. Possible synergistic effects of 2NPOH and other bacteriocins should also be investigated. Bacteriocins can be approved as food preservatives (as exemplified by Nisin, E234) based on their lack of toxicity to eukaryotic cells and long history of safe consumption along with foods, but they can also be incorporated in foods under other categories such as “cultured dairy solids”, “ingredients”, “shelf life extenders” or “processing aids” (Gálvez, Abriouel, Ben Omar, & Lucas, 2011). Because of its broad antimicrobial spectrum and lack of toxicity to human eukaryotic cells (Abriouel et al., 2010), enterocin AS-48 is likely a candidate for commercial use in food preservation under at least some of the categories indicated above.

Acknowledgements

This work was supported by the Spanish Ministry of Education (research project AGL2005-07665-C02-02/ALI; CeiA3). We also acknowledge the Research Programme of the University of Jaén, and the Research Plan of the Junta de Andalucía (research group AGR230).

References

Abriouel, H., Lucas, R., Ben Omar, N., Valdivia, E., & Gálvez, A. (2010). Potential applications of the cyclic peptide enterocin AS-48 in the preservation of vegetable foods and beverages. *Probiotics and Antimicrobial Proteins, 2*, 77–89.

Abriouel, H., Valdivia, E., Martínez-Bueno, M., Maqueda, M., & Gálvez, A. (2003). 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., Valdivia, E., Martínez-Bueno, M., Gálvez, A., & Maqueda, M. (2004). Effect of combined physico-chemical preservatives on enterocin AS-48 activity against the enterotoxigenic *Staphylococcus aureus* CECT 976 strain. *Journal of Applied Microbiology, 97*, 48–56.
Anderson, R. C., Carstens, G. E., Miller, R. K., Callaway, T. R., Schultz, C. L., Edrington, T. S., et al. (2006). Effect of oral nitroethane and 2-nitropropanol administration on methane-producing activity and volatile fatty acid production in the ovine rumen. *Bioresource Technology, 97*, 2421–2426.
Balaban, N., & Rasooly, A. (2000). Staphylococcal enterotoxins. *International Journal of Food Microbiology, 61*, 1–10.
Cobo Molinos, A., Abriouel, H., Ben Omar, N., Lucas, R., Valdivia, E., & Gálvez, A. (2009). Enhanced bactericidal activity of enterocin AS-48 in combination with essential oils, natural bioactive compounds, and chemical preservatives against *Listeria monocytogenes* in ready-to-eat salads. *Food and Chemical Toxicology., 47*, 2216–2223.
Cobo-Molinos, A., Lucas, R., Abriouel, H., Ben Omar, N., Valdivia, E., & Gálvez, A. (2009). Inhibition of *Salmonella enterica* cells in deli-type salad by enterocin AS-48 in combination with other antimicrobials. *Probiotics and Antimicrobials Proteins, 1*, 85–90.
Dimitrijevic, M., Anderson, R. C., Callaway, T. R., Jung, Y. S., Harvey, R. B., Ricke, S. C., et al. (2006). Inhibitory effect of select nitrocompounds on growth and survivability of *Listeria monocytogenes* in vitro. *Journal of Food Protection, 69*, 1061–1065.
EFSA. (2010). The community summary report on trends and sources of zoonoses and zoonotic agents and food-borne outbreaks in the European Union in 2008. *The EFSA Journal*, 1946.
Gálvez, A., Abriouel, H., Ben Omar, N., & Lucas, R. (2011). Food applications and regulations. In D. Drider, & S. Rebuffat (Eds.), *Prokaryotic antimicrobial peptides: From genes to applications* (pp. 353–390). New York: Springer.
Gálvez, A., Abriouel, H., Lucas-López, R., & Ben Omar, N. (2007). Bacteriocin-based strategies for food biopreservation. *International Journal of Food Microbiology, 120*, 51–70.
Gálvez, A., Lucas-López, R., Abriouel, H., Valdivia, E., & Ben Omar, N. (2008). Application of bacteriocins in the control of foodborne pathogenic and spoilage bacteria. *Critical Reviews in Biotechnology, 28*, 125–152.
Grande, M. J., Lucas-López, L., Abriouel, H., Valdivia, E., Ben Omar, N., Maqueda, M., et al. (2007). Treatment of vegetable sauces with enterocin AS-48 alone or in combination with phenolic compounds to inhibit proliferation of *Staphylococcus aureus*. *Journal of Food Protection, 70*, 405–411.
Jørgensen, H. J., Maticen, T., Løvseth, A., Oboe, K., Qvale, K. S., & Loncarevic, S. (2005). An outbreak of staphylococcal food poisoning caused by enterotoxin H in mashed potato made with raw milk. *FEMS Microbiology Letters, 252*, 267–272.
Jung, Y. S., Anderson, R. C., Callaway, T. R., Edrington, T. S., Genovese, K. J., Harvey, R. B., et al. (2004). Inhibitory activity of 2-nitropropanol against select food-borne pathogens in vitro. *Letters in Applied Microbiology, 39*, 471–476.
Jung, Y. S., Anderson, R. C., Edrington, T. S., Genovese, K. J., Byrd, J. A., Callaway, T. R., et al. (2004). Experimental use of 2-nitropropanol for reduction of *Salmonella typhimurium* in the ceca of broiler chicks. *Journal of Food Protection, 67*, 1945–1947.
Le Loir, Y., Baron, F., & Gautier, M. (2003). *Staphylococcus aureus* and food poisoning. *Genetics and Molecular Research, 2*, 63–76.
Majak, W., & Clark, L. J. (1980). Metabolism of aliphatic nitro compounds in bovine rumen fluid. *Canadian Journal of Animal Science, 60*, 319–325.
Martínez-Bueno, M., Gálvez, A., Valdivia, E., & Maqueda, M. (1990). A transferable plasmid associated with AS-48 production in *Enterococcus faecalis*. *Journal of Bacteriology, 172*, 2817–2818.
Martínez-Viedma, P., Abriouel, H., Ben Omar, N., Lucas, R., & Gálvez, A. (2009). Anti-staphylococcal effect of enterocin AS-48 in bakery ingredients of vegetable origin, alone and in combination with selected antimicrobials. *Journal of Food Science, 74*, M384–M389.
Ortega, E., Abriouel, H., Lucas, R., & Gálvez, A. (2010). Multiple roles of *Staphylococcus aureus* enterotoxins: pathogenicity, superantigenic activity, and correlation to antibiotic resistance. *Toxins, 2*, 2117–2131.
Ortega, E., Abriouel, H., Lucas-López, R., Ben Omar, N., & Gálvez, A. (2010). Antibacterial activity of carvacrol and 2-nitro-1-propanol against single and mixed populations of foodborne pathogenic bacteria in corn flour dough. *Food Microbiology, 27*, 274–279.
Seo, K. S., & Bohach, G. A. (2007). *Staphylococcus aureus*. In M. P. Doyle, & L. R. Beuchat (Eds.), *Food microbiology: Fundamentals and frontiers* (3rd ed). (pp. 493–518). Washington, DC: ASM Press.
Tamarapu, S., McKillip, J. L., & Drake, M. (2001). Development of a multiplex polymerase chain reaction assay for detection and differentiation of *Staphylococcus aureus* in dairy products. *Journal of Food Protection, 64*, 664–668.
Tirado, C., & Schimdt, K. (2001). WHO surveillance programme for control of foodborne infections and intoxications: preliminary results and trends across greater Europe. *Journal of Infection, 43*, 80–84.
WHO, (2002). Statistical information on food-borne disease in Europe. Microbiological and chemical hazards. In: *FAO/WHO Pan-European conference on food quality and safety, Budapest*.
Williamson, E. M. (2001). Synergy and other interactions in phytomedicines. *Phytomedicine, 8*, 401–409.