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Antimicrobial activity of pediocin-producing *Lactococcus lactis* on *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli* O157:H7 in cheese

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Abstract

The antimicrobial activity of two pediocin-producing transformants obtained from wild strains of *Lactococcus lactis* on the survival of *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli* O157:H7 during cheese ripening was investigated. Cheeses were manufactured from milk inoculated with the three pathogens, each at approximately 6 log cfu mL^{-1}. *Pediococcus acidilactici* 347 (Ped^+), *Lc. lactis* ESI 153, *Lc. lactis* ESI 515 (Nis^+) and their respective pediocin-producing transformants *Lc. lactis* CL1 (Ped^+) and *Lc. lactis* CL2 (Nis^+, Ped^+) were added at 1% as adjuncts to the starter culture. After 30 d, *L. monocytogenes*, *S. aureus* and *E. coli* O157:H7 counts were 5.30, 5.16 and 4.14 log cfu g^{-1} in control cheese made without adjunct culture. On day 30, pediocin-producing derivatives *Lc. lactis* CL1 and *Lc. lactis* CL2 lowered *L. monocytogenes* counts by 2.97 and 1.64 log units, *S. aureus* by 0.98 and 0.40 log units, and *E. coli* O157:H7 by 0.84 and 1.69 log units with respect to control cheese. All cheeses made with nisin-producing LAB exhibited bacteriocin activity throughout ripening. Pediocin activity was only detected throughout the whole ripening period in cheese with *Lc. lactis* CL1. Because of the antimicrobial activity of pediocin PA-1, its production in situ by strains of LAB growing efficiently in milk would extend the application of this bacteriocin in cheese manufacture.

Keywords: *L. monocytogenes*; *S. aureus*; *E. coli* O157:H7; Bacteriocin; Pediocin; Cheese

1. Introduction

Listeria monocytogenes, *Staphylococcus aureus* and *Escherichia coli* O157:H7 are pathogens of major concern for the dairy industry. Their survival in different cheese varieties has been well documented (Ibrahim, Baldock, Radford, & Ireland, 1981; Reitsma & Henning, 1996; Nuñez, Rodríguez, García, Gaya, & Medina, 1997).

The potential of bacteriocin-producing lactic acid bacteria (LAB) to control undesirable microorganisms in cheese has been demonstrated. Nisin-producing starters inhibited *L. monocytogenes* in Camembert (Maisnier-Patin, Deschamps, Tatini, & Richard, 1992) and *L. innocua* in a semi-hard cheese (Rodríguez, Gaya, Nuñez, & Medina, 1998). Other bacteriocinogenic cultures as lacticin 3147- and lacticin 481- producing strains of *Lactococcus lactis* (McAuliffe, Hill, & Ross, 1999; Rodríguez, Arqués, Gaya, Nuñez, & Medina, 2001), and different enterocin-producing strains of enterococci (Nuñez et al., 1997; Giraffa & Carminati, 1997; Farias et al., 1999) have shown antilisterial activity in cheese. Less-known is the efficacy of bacteriocins to control *S. aureus* in cheese. *S. aureus* counts were reduced in a semi-hard cheese made with a nisin-producing starter (Rodríguez, Arqués, Gaya, Nuñez, & Medina, 2000) and, more efficiently, in cheese spreads from Cheddar cheese manufactured with nisin-producing lactococci (Zottola, Yezzi, Ajao, & Roberts, 1994).

Bacteriocins of LAB are generally inactive against Gram-negative bacteria due to the resistance conferred by the outer membrane. However, inhibitory effects of nisin (Cutter & Siragusa, 1995) and some enterocins

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(Gálvez, Maqueda, Martínez-Bueno, & Valdivia, 1989; Simonetta, Moragues de Velasco, & Frison, 1997) on Gram-negative bacteria have been described. The potential application of bacteriocins against Gram-negative bacteria through their synergistic effects with other antimicrobials has gained increased interest (Helander, Wright, & Mattila-Sandholm, 1997).

Pediocin PA-1 is a class II bacteriocin with strong antilisterial activity produced by some strains of pediococci, generally of meat origin (Bhunia, Johnson, & Ray, 1988; Rodríguez, Cintas, Casaus, Suárez, & Hernández, 1997). The application of pediococci in milk fermentations is restricted by their inability to ferment lactose rapidly, which results in slow growth in milk and dairy products (Caldwell, McMahon, Oberg, & Broadbent, 1996). A rapid decrease in counts of *L. monocytogenes* was reported by Pucci, Vedamuthu, Sunka, and Vanderbergh (1988) when culture supernatant from pediocin-producing *Pediococcus acidilactici* PAC1.0 was added to cottage cheese. Cell suspensions of pediocin-producing *Lactobacillus plantarum* WHE92 sprayed on the surface of Munster cheeses at the beginning of the ripening period eliminated *L. monocytogenes* (Ennahar, Assobhei, & Hasselmann, 1998). The inhibition of this pathogen in Cheddar cheese by pediocin PA-1 produced in situ by a lactococcal starter culture containing a plasmid coding the pediocin PA-1 operon has also been demonstrated (Buyong, Kok, & Luchansky, 1998).

Lc. lactis ESI 153 and *Lc. lactis* ESI 515 (Nis^+) were isolated from artisanal raw milk cheeses (Cogan et al., 1997), selected by their technological and/or antimicrobial properties, and used as starter cultures in cheese manufacture (Rodríguez et al., 1998; Gómez, Rodríguez, Gaya, Nuñez, & Medina, 1999). Both strains were transformed by Reviriego et al. (2004) to produce pediocin PA-1 heterologously. The objective of the present work was to evaluate the antimicrobial ability of the transformants *Lc. lactis* CL1 (Ped^+) and *Lc. lactis* CL2 (Nis^+, Ped^+) against *L. monocytogenes*, *S. aureus* and *E. coli* O157:H7 in cheese.

2. Materials and methods

2.1. Microorganisms and culture conditions

L. monocytogenes Ohio serotype 4b (from R.G. Crawford, Food and Drug Administration, Cincinnati, OH, USA), *S. aureus* CECT 4013 and *E. coli* O157:H7 ATCC 43894, were propagated in tryptic soy broth (TSB; Biolife, Milano, Italy) at 37°C for 18 h and subcultured twice in sterile reconstituted skim milk supplemented with 0.3% yeast extract before use in cheesemaking.

P. acidilactici 347 (Ped^+) (Rodríguez et al., 1997), *Lc. lactis* ESI 153, *Lc. lactis* ESI 515 (Nis^+) and their respective pediocin-producing transformants *Lc. lactis* CL1 (Ped^+) and *Lc. lactis* CL2 (Nis^+, Ped^+) (Reviriego et al., 2004) were used as adjuncts to the starter culture in cheesemaking. They were grown in MRS broth (Biolife) at 30°C for 18 h and subcultured twice in sterile reconstituted skim milk supplemented with 0.3% yeast extract before use in cheesemaking.

Lc. lactis MG 1614 and *Enterococcus faecalis* TAB 28 were used as indicator organisms to evaluate nisin and pediocin activity, respectively. They were propagated in MRS broth at 30°C for 18 h.

All strains were maintained as frozen stocks in milk supplemented with 15% glycerol at −80°C.

2.2. Manufacture of cheese

Cheeses were made in two trials carried out on different days from pasteurized (75°C/15 s) milk. In each trial, milk at 32°C with 0.02% $CaCl_2$ was distributed in six 2 L vats and inoculated with the three pathogens, each at approximately 10^6 cfu mL^{-1}. Commercial mesophilic lactic culture (CLC) MA 016 (Rhodia, Dangé Saint-Romain, France) was added at 1% to the six vats. Vat 1 served as control. The other five vats were individually inoculated with 1% of a culture of *P. acidilactici* 347, *Lc. lactis* ESI 153, *Lc. lactis* ESI 515, *Lc. lactis* CL1, or *Lc. lactis* CL2, respectively. Rennet (Maxiren 150, Gist-brocades, Delft, The Netherlands) was added to milk 20 min after inoculation of cultures. The curds were cut 40 min after rennet addition and heated at 37°C for 25 min. Whey was drained off and curds were distributed into plastic cylindrical moulds. One cheese (~240 g weight) was obtained from each vat. Cheeses were pressed for 4 h at room temperature, salted in 20% brine for 30 min, kept at 20°C for 16 h, vacuum packed in Cryovac plastic bags and ripened at 12°C for 30 d.

2.3. Microbiological analysis

Cheeses were sampled at days 4, 15 and 30. Two 5 g samples from two different sectors were pooled and homogenized with 90 mL of sterile sodium citrate solution, and decimal dilutions in sterile 0.1% peptone water were prepared (Nuñez, Gaya, & Medina, 1985). *L. monocytogenes* counts were determined on duplicate plates of PALCAM Listeria agar (Merck, Darmstadt, Germany) with PALCAM Listeria selective supplement (Merck) incubated at 37°C for 48 h, *S. aureus* on duplicate plates of Baird–Parker (Oxoid) with RPF Supplement II (Biolife) incubated at 37°C for 48 h and *E. coli* O157:H7 counts on duplicate plates of McConkey sorbitol agar (Scharlau Chemie, Barcelona, Spain) with selective Cefixime–Tellurite supplement (Oxoid, Basingstoke, United Kingdom) incubated at 37°C for 24 h.