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A Food-Grade System for Production of Pediocin PA-1 in Nisin-Producing and Non–Nisin-Producing *Lactococcus lactis* Strains: Application To Inhibit *Listeria* Growth in a Cheese Model System

C. REVIRIEGO, L. FERNÁNDEZ, AND J. M. RODRÍGUEZ*

Departamento de Nutrición, Bromatología y Tecnología de los Alimentos, Facultad de Veterinaria, Universidad Complutense de Madrid, 28040 Madrid, Spain

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ABSTRACT

Food-grade heterologous production of pediocin PA-1 in nisin-producing and non–nisin-producing *Lactococcus lactis* strains, previously selected because of their technological properties for cheese making, was achieved. Plasmid pGA1, which contains the complete pediocin operon under the control of the strong P32 promoter and is devoid of any antibiotic marker, was introduced into *L. lactis* ESI 153 and *L. lactis* ESI 515 (Nis$^+$). Transformation of *L. lactis* ESI 515 with pGA1 did not affect its ability to produce nisin. The antimicrobial activity of the pediocin-producing transformants on the survival of *Listeria innocua* SA1 during cheese ripening was also investigated. Cheeses were manufactured from milk inoculated with 1% of the lactic culture and with or without approximately 4 log CFU/ml of the *Listeria* strain. *L. lactis* ESI 153, *L. lactis* ESI 515, and their transformants (*L. lactis* GA1 and GA2, respectively) were used as starter cultures. At the end of the ripening period, counts of *L. innocua* in cheeses made with the bacteriocin-producing lactococcal strains were below 50 CFU/g in the *L. lactis* GA1 cheeses and below 25 CFU/g in the *L. lactis* GA2 ones, compared with 3.7 million CFU/g for the controls without nisin or pediocin production.

Listeria monocytogenes represents a major biological hazard in the dairy industry *(19)* and, among the strategies suggested to avoid or reduce its presence in cheeses, the in situ production of the anti-*Listeria* bacteriocin pediocin PA-1 by wild strains of dairy origin constitutes an attractive research target *(30).* The potential of bacteriocin-producing wild lactic acid bacteria strains to control *Listeria* growth in different cheese varieties has been demonstrated previously. Nisin-producing lactococcal starters inhibited *L. monocytogenes* in Camembert *(17)* and *Listeria innocua* in a semihard cheese *(26),* and similar results have been achieved with bacteriocinogenic starter cultures producing lacticin 3147 *(18),* lacticin 481 *(27),* or enterococcal bacteriocins *(22).*

Direct application of pediocin PA-1–producing pediococci as an anti-*Listeria* tool is constrained by their inability to ferment lactose rapidly, which makes them metabolically and technologically unsuited for dairy fermentations *(2).* However, the fact that one of the main characteristic features of pediocin PA-1 is their strong activity against *Listeria* species *(21)* has led to the development of different strategies for the heterologous production of this bacteriocin in lactococci *(1, 3, 12–14, 23).* Although such attempts have been useful for research purposes, the resulting pediocin-producing lactococci could not be transferred to industries because, often, the selected lactococcal hosts consisted of laboratory strains that lacked the properties required for food fermentations or because the genetic determinants for pediocin PA-1 biosynthesis were carried in non–food-grade vectors that harbored one or more genes conferring antibiotic resistance.

In a recent study, we found that *Lactococcus lactis* ESI 153 and ESI 515 could be successfully used as hosts for the heterologous production of pediocin PA-1 through the introduction of a plasmid conferring resistance to chloramphenicol *(23).* In addition, the pediocin-producing recombinant strains led to a significant inhibition of *L. monocytogenes* growth in cheeses without exerting adverse effects on their sensorial properties *(25).* These two strains were originally isolated from artisanal raw milk cheeses and, later, selected because of their technological properties for cheese making *(4),* and have been used in several trials as adjuncts and starter cultures in cheese manufacture *(9, 25, 26).* The achievement of pediocin production in such wild lactococcal hosts cannot be considered a trivial result, because many difficulties are usually found when transferring technology from laboratorial lactococci to industrial strains *(15).*

In this context, the objective of this study has been the development of a food-grade vector for the heterologous production of pediocin PA-1, its introduction in *L. lactis* ESI 153 and ESI 515, and an evaluation of their usefulness to control the growth of an *L. innocua* strain in experimental cheeses. Because *L. lactis* ESI 515 naturally produces nisin and the combination of pediocin PA-1 and nisin seems

* Author for correspondence. Tel: +34-91-3943837; Fax: +34-91-3943743; E-mail: jmrodrig@vet.ucm.es.

TABLE 1. *Lactococcal strains used in this study*

Host and strain	Plasmid	Reference or source
ESI 153		*4*
CL1	pFI2160 (Cm^R, pTG262 derivative, contains the hybrid *L-pedA* gene preceded by the lactococcin A promoters, *lcnC* and *lcnD*)	*23*
MC1	pMC117 (Ery^R, pMG36e derivative, contains the pediocin operon preceded by the P32 promoter)	*24*
RK1	pRK119 (Cm^R, pMG36c derivative, contains the pediocin operon preceded by the P32 promoter)	*24*
CNC1	pCNC16 (Cm^R, pTG262 derivative, contains the pediocin operon preceded by the P32 promoter)	*24*
GA1	pGA1 (pCNC16 derivative, contains the pediocin operon preceded by the P32 promoter; devoid of antibiotic-resistant genes)	This study
ESI 515 (Nis^+)		*4*
CL2	pFI2160	*23*
MC2	pMC117	*24*
RK2	pRK119	*24*
CNC2	pCNC16	*24*
GA2	pGA1	This study

to inhibit the growth of *L. monocytogenes* and other gram-positive bacteria more efficiently than either of the bacteriocins alone *(10, 20),* the food-grade coproduction of both bacteriocins by an *L. lactis* ESI 515–derived strain was considered a particularly attractive target in this study.

MATERIALS AND METHODS

Microorganisms and culture conditions. Lactococcal strains and plasmids used in this study are listed in Table 1. The lactococcal host strains *L. lactis* ESI 153 and *L. lactis* ESI 515 (Nis^+) were isolated from artisanal raw milk cheeses *(4).* The lactic acid bacteria used in this study were routinely grown in deMan Rogosa Sharpe agar (Oxoid, Basingstoke, UK) at 32°C without agitation. Agar plates were made by the addition of 1.5% (wt/vol) of agar to broth media. When appropriate, chloramphenicol (Sigma-Aldrich, St. Louis, Mo.) was added (5 μg/ml). *L. innocua* SA1 (our own collection) was grown in brain heart infusion broth (Oxoid) and PALCAM agar plates (bioMérieux, Marcy l'Etoile, France) at 32°C. Lactic acid bacteria strains were stored at −80°C in deMan Rogosa Sharpe broth containing 15% glycerol (vol/vol).

Construction of pGA1 and transformation of *L. lactis* ESI 153 and ESI 515. In a previous study, a DNA fragment containing the pediocin PA-1 operon (*pedABCD*) under the control of the P32 promoter (P32-*pedABCD*) was cloned into the *Bam*HI site of the lactococcal–*E. coli* shuttle vector pTG262 *(13),* generating pCNC16 *(24).* In this study, the P32-*pedABCD* fragment (3,658 bp) was amplified from plasmid pCNC16 with primers operonF (5′-CGAAGATCTGATATGATAAGATTAATAGTT-3′) and R003 (5′-GCGGATCCCTATTCTTGATTATGAATTAAC-3′), which include *Bgl*II and *Bam*HI sites (underlined), respectively, with the Platinum *Taq* DNA polymerase (Invitrogen, Paisley, UK). PCR conditions were as follows: first, one cycle of 95°C for 5 min; then, 35 cycles of 95°C for 45 s, 50°C for 30 s, and 72°C for a time corresponding to 1 min/kb; and finally, an extension step of 72°C for 7 min. Parallel, pCNC16 also served as the template for amplification of a 1,234-bp fragment containing the lactococcal origin of replication and the gene encoding the replication protein RepA with primers F002 (5′-GCGGATCCAATTTGGAATTTG GAAAAAAATGGG-3′) and rep*Bgl*II (5′-CGAAGATCTAAAT AACTCTTTTTCTTTGTTTGTCA-3′), which included *Bam*HI and *Bgl*II sites (underlined), respectively. Both PCR fragments were purified with the Qiaquick Rapid Purification kit (Qiagen, Hilden, Germany), submitted to a double digestion with *Bgl*II and *Bam*HI (New England Biolabs, Ipswich, Mass.) and, finally, ligated with T4 DNA ligase (New England Biolabs) to generate plasmid pGA1 (4,870 bp) (Fig. 1).

Lactococcal strains ESI 153 and ESI 515 were transformed with the recombinant plasmid following the procedure described by Wells et al. *(32).* Because both strains are naturally resistant to pediocin PA-1 and pGA1 is devoid of marker genes, the selection of recombinant colonies was based on their ability to produce the bacteriocin. For this purpose, electroporation mixtures were seeded on deMan Rogosa Sharpe agar plates, and once the colonies were clearly visible (approximately 24 h), replica plates were performed. Subsequently, 5 ml of soft (0.8%) agar containing approximately 6 log CFU/ml of the indicator strain, *Enterococcus faecalis* TAB 28 *(16),* was added to one set of replica plates. Plates containing colonies of *L. lactis* ESI 153 and *Pediococcus acidilactici* 347 were used as negative and positive controls, respectively. For routine PCR screening of potential recombinant clones, primers Fintped (5′-TGGGGTAAGGCTACCACTTG-3′) and Rintped (5′-GGCGTTTGCTGGTTATGTTT-3′) were used to amplify a 282-bp fragment comprising the end of *pedA* and the beginning of *pedB* with Biotools *Taq* DNA polymerase (Biotools, Madrid, Spain) and the PCR program described above (annealing at 59°C). The new strains were called *L. lactis* GA1 (ESI 153 and pGA1) and GA2 (ESI 515 and pGA1).

Bacteriocin assays. Pediocin PA-1 and nisin antimicrobial activities in cultures were assayed by the agar diffusion bioassay described by Dodd et al. *(5)* with *E. faecalis* TAB28 (pediocin sensitive, nisin resistant) and *L. lactis* MG1614 (pediocin resistant, nisin sensitive *(8)*) as indicator organisms. *L. lactis* BB24 *(28)* and *P. acidilactici* 347 *(29)* were used as positive controls of nisin and pediocin PA-1 production, respectively. The lactococcal strains CL1, MC1, RK1, CNC1, CL2 MC2, RK2, and CNC2, which are other pediocin-producing ESI 153– or ESI 515–derived strains constructed in previous studies *(23, 24),* were also included for comparison. All the supernatants were obtained from cultures with a bacterial concentration of approximately 9 log CFU/ml.

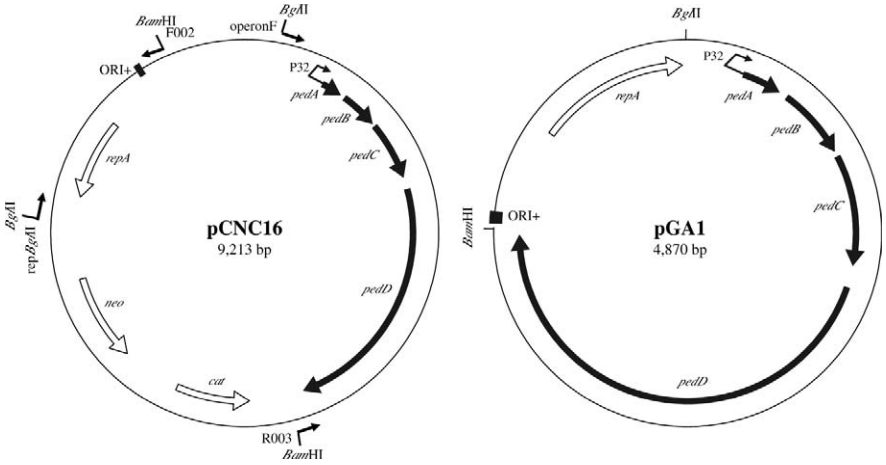

FIGURE 1. *Construction of plasmid pGA1 from pCNC16.*

The microtiter plate assay system developed by Holo et al. *(11)* was employed to quantify the pediocin PA-1 activities present in the culture supernatants of all the strains investigated. One bacteriocin unit was defined as the reciprocal of the highest dilution causing 50% growth inhibition of the indicator organism *E. faecalis* TAB 28, measured as the reduction in culture density (A_{620}). The assays were performed in quadruplicate, and the standard deviation values were calculated. The pediocin PA-1 activity corresponding to the supernatants of the wild-type pediocin-producing strain *P. acidilactici* 347 was considered the 100% value.

Cheese model. Manufacture of cheese was carried essentially as described by Rodríguez et al. *(25).* Cheeses were made in three trials carried out on different days from pasteurized milk. In each trial, milk at 32°C with 0.01% $CaCl_2$ was distributed into 11 vats (1.5 liters per vat). Five vats were independently inoculated with 1% of a milk culture (~9 log CFU/ml) of *L. lactis* ESI 153, *L. lactis* ESI 515, *L. lactis* GA1, *L. lactis* GA2, or *P. acidilactici* 347. Parallel, other five vats were coinoculated with each of the lactic acid bacteria strains as described above and also with *L. innocua* SA1 (final concentration, 4 log CFU/ml). Finally, the remaining vat was inoculated only with *L. innocua* SA1 and served as a control. This strain was selected as indicator to evaluate the anti-*Listeria* activity of the lactic acid bacteria strains because it is nonpathogenic and because preliminary assays showed that its sensitivity to pediocin PA-1 is very similar to that of some pathogenic *L. monocytogenes* strains, such as Scott A (data not shown). Rennet (Naturen Powder, Chr. Hansen, Hørsholm, Denmark) was added as recommended to milk 20 min after the inoculation of cultures. The curds were cut 40 min after the rennet addition and heated at 38°C for 20 min. Whey was drained off, and curds were distributed into plastic cylindrical molds. One cheese (~190 g) was obtained from each vat. Cheeses were pressed for 4 h at room temperature, salted in 15% brine for 45 min, and kept at 20°C for 16 h. Then, cheeses were cut into five pieces, which were individually vacuum packed in Cryovac plastic bags and ripened at 12°C for 4 weeks.

Microbiological analysis. Initially, two samples (2 g) of curd were obtained from each vat before pressing. Later, cheeses were sampled at days 1 (prior vacuum packaging), 7, 14, 21, and 28. *L. innocua* counts were determined in duplicate on PALCAM agar plates (bioMérieux) incubated at 32°C for 48 h. At the end of the ripening period, 50 colonies of *L. innocua* SA1 were recovered from cheeses coinoculated with pediocin-producing lactococci and tested for their sensitivity to pediocin PA-1 with the supernatant of *P. acidilactici* 347 in the agar diffusion test described previously. A random amplified polymorphic DNA assay with primer Arg Dei (5′-ACCYTRGAAGGYGGYGATGTB-3′) was performed as described by Veyrat et al. *(31)* to confirm the identity of these 50 colonies grown in PALCAM agar plates. Parallel, 25 colonies of *L. lactis* GA1 and 25 of strain GA2 were recovered from the same cheeses and tested for pediocin PA-1 production. Cheese pH was measured in duplicate on the same sampling days with a Crison pH meter (GPL 22, Crison Instruments, Alella, Spain) provided with a Crison electrode (model 52-3,2, Crison). To detect nisin and pediocin PA-1 activities, the cheese extracts were prepared following the procedure of Rodríguez et al. *(25).* Then, bacteriocin assays were carried as described previously.

Statistical analysis. Microbiological data, recorded as CFU per gram, were transformed to logarithmic values before statistical analysis. Analysis of variance and the Duncan multiple range test were performed to determine significant differences in the logarithm of *Listeria* population densities at each time point by Statgraphics Plus 5.0 software (Manugistics, Inc., Rockville, Md.). Analysis was conducted with a significance level of $P < 0.01$.

RESULTS AND DISCUSSION

Transformation of *L. lactis* ESI 153 and ESI 515 with plasmid pGA1 generated the lactococcal strains GA1 and GA2, respectively. Inhibition of the pediocin-sensitive indicator strain *E. faecalis* TAB 28 by culture supernatants of *L. lactis* GA1 and GA2 was detected by plate diffusion bioassays and compared with that displayed by supernatants of strains CL1, MC1, RK1, CNC1, CL2 MC2, RK2, and CNC2, other pediocin-producing ESI 153– or ESI 515–derived strains constructed in previous studies (Fig. 2). The smallest inhibition zones corresponded to CL1 and CL2 supernatants, whereas those obtained by RK1, RK2, CNC1, and CNC2 displayed the largest halos (Fig. 2). Supernatants from strains MC1, MC2, GA1, and GA2 produced intermediate inhibition zones (Fig. 2). Similar results were obtained with supernatants of strains CL1, MC1, RK1, CNC1, CL2 MC2, RK2, and CNC2 in a previous study *(24).*

The pediocin activity in the culture supernatants of the different lactococcal strains was also determined by microtiter plate assays (Table 2), and the results were in agreement with those obtained by agar diffusion assays (Fig. 2). Interestingly, the antimicrobial activities of the ESI 153 derivative strains CNC1 (pCNC16) and GA1 (pGA1) supernatants were 165 and 17%, respectively, that found in the

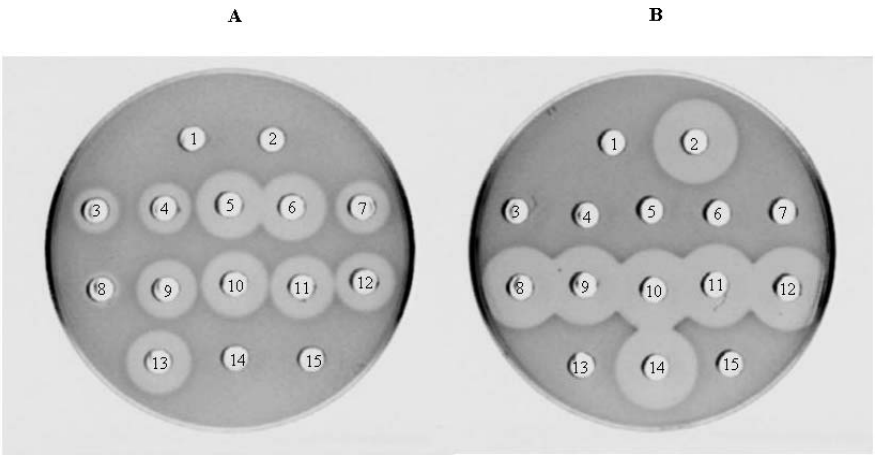

FIGURE 2. *Agar diffusion bioassay for detection of pediocin PA-1 activity against* Enterococcus faecalis *TAB 28 (A) and of nisin activity against* L. lactis *MG 1614 (B). Samples: 1,* L. lactis *ESI 153; 2,* L. lactis *ESI 515; 3,* L. lactis *CL1; 4,* L. lactis *MC1; 5,* L. lactis *RK1; 6,* L. lactis *CNC1; 7,* L. lactis *GA1; 8,* L. lactis *CL2; 9,* L. lactis *MC2; 10,* L. lactis *RK2; 11,* L. lactis *CNC2; 12,* L. lactis *GA2; 13,* P. acidilactici *347; 14,* L. lactis *BB24; 15,* L. lactis *MG 1614.*

supernatants of *P. acidilactici* 347. Similarly, those corresponding to the supernatants of the ESI 515–derivative strains CNC2 (pCNC16) and GA2 (pGA1) supernatants were 95 and 35%, respectively, when compared with the pediococcal strain. The new plasmid pGA1 was designed to exclusively contain pTG262 lactococcal sequences and was generated from pCNC16 by ligation of two PCR-derived fragments, which provided the minimal genetic requirements for replication and pediocin production. Therefore, the removal of nonlactococcal sequences from pCNC16 led to an important reduction in pediocin PA-1 production. However, the generation of pGA1 can have practical consequences because, in contrast to pCNC16, it can be considered a food-grade plasmid. In addition, previous studies have shown that the heterologous production of pediocin PA-1 at a level of 10 to 30% that of *P. acidilactici* 347 can notably increase the anti-*Listeria* activity of nisin-producing lactococci *(12, 14).* In this context, transformation of ESI 515 with pGA1 did not alter the nisin-producing property of the host (Fig. 2), a finding that has been previously observed after introduction of the genes required for the heterologous production of pediocin PA-1 in lactococci producing this lantibiotic *(12, 14, 23).* Anti-*Listeria* additive or synergistic effects resulting from the combination of pediocin PA-1 and nisin have been reported previously *(10, 20).*

TABLE 2. *Pediocin PA-1 activity in the culture supernatants of the strains used in this study*

Strain	Pediocin PA-1 activity (BU)[a]	%
P. acidilactici 347	1,500	100
L. lactis ESI 153	ND[b]	—
L. lactis CL1	106	7
L. lactis MC1	225	15
L. lactis RK1	2,447	163
L. lactis CNC1	2,478	165
L. lactis GA1	255	17
L. lactis ESI 515	ND	—
L. lactis CL2	33	2
L. lactis MC2	751	50
L. lactis RK2	1,503	100
L. lactis CNC2	1,427	95
L. lactis GA2	525	35

[a] Pediocin PA-1 activity as determined by a microtiter plate assay. One bacteriocin unit (BU) is the reciprocal of the highest dilution causing 50% growth inhibition of the indicator organism *Enterococcus faecalis* TAB 28.

[b] ND, not detected.

Subsequently, the anti-*Listeria* activity of *L. lactis* ESI 153, ESI 515, GA1, and GA2 was tested in cheeses inoculated with *L. innocua* SA1. Values of pH were between 5.08 and 5.69 on day 1 and between 4.51 and 4.67 at the end of the ripening period. Counts of *L. innocua* SA1 in inoculated milk were approximately 4 log CFU/ml. In control cheeses, counts were 7.60 log CFU/g after 1 day and decreased during ripening to 6.57 log CFU/g after 28 days (Table 3). Nonbacteriocinogenic *L. lactis* ESI 153 and nisin-producing *L. lactis* ESI 515 reduced *L. innocua* counts, which, after 28 days, were 4.06 and 4.22 log units lower, respectively, than in control cheese (Table 3). The application of the food-grade pediocin-producing derivatives of *L. lactis* resulted in significantly ($P < 0.01$) higher rates of inactivation of *L. innocua.* The *L. innocua* numbers in cheese made with *L. lactis* GA1 (pediocin producer) and *L. lactis* GA2 (nisin and pediocin producer) were 4.87 and 5.17 log units lower than control cheese at the end of the ripening period. Interestingly, the *Listeria* reduction by strain GA1, which produces only a moderate amount of pediocin PA-1 (17% that of *P. acidilactici* 347), was higher than that achieved by strain ESI 515, a natural nisin-producing strain. This fact reflects the strong anti-*Listeria* activity of pediocin PA-1 *(21).* Bacteriocin activity in cheeses made with the different strains is shown in Table 4. Nisin activity could be detected throughout ripening in those cheeses inoculated with ESI 515 or GA2, whereas pediocin PA-1 activity was detected only in the extracts obtained from the cheeses inoculated with *L. lactis* GA1 and GA2. The ability of different lactic acid bacteria strains to produce bacteriocins during cheese ripening has been described previously *(1, 7, 22, 25).*

At the end of the ripening period, 50 *L. innocua* colonies were tested for sensitivity to supernatants of *P. acidilactici* 347, and all of them remained sensitive to pediocin PA-1; their identity to *L. innocua* SA1 was confirmed by the random amplified polymorphic DNA assay (data not

TABLE 3. *Survival of* Listeria innocua *SA1 in cheeses made from milk coinoculated with* L. innocua *SA1 and each of five pediocin-producing or nisin-producing lactic acid bacteria (LAB)*

LAB strain	*L. innocua* (log CFU/g)[a]				
	1 day	7 days	14 days	21 days	28 days
P. acidilactici 347	4.02 ± 0.12 A[b]	3.65 ± 0.07 A	2.69 ± 0.13 A	1.87 ± 0.09 A	1.87 ± 0.05 A
L. lactis ESI 153	4.09 ± 0.11 A	3.78 ± 0.06 AB	3.60 ± 0.04 B	3.54 ± 0.14 B	2.51 ± 0.12 B
L. lactis GA1	4.03 ± 0.07 A	3.30 ± 0.06 C	2.70 ± 0.12 A	1.70 ± 0.05 A	1.70 ± 0.05 A
L. lactis ESI 515	4.17 ± 0.15 A	3.93 ± 0.09 B	3.51 ± 0.06 B	3.14 ± 0.10 C	2.35 ± 0.08 B
L. lactis GA2	3.65 ± 0.12 B	3.32 ± 0.08 C	2.63 ± 0.05 A	1.40 ± 0.05 D	1.40 ± 0.05 C
None	7.60 ± 0.13 C	7.27 ± 0.10 D	7.25 ± 0.14 C	7.04 ± 0.07 E	6.57 ± 0.06 D

[a] *L. innocua* SA1 on PALCAM agar. Values are means ± standard deviations ($n = 3$).
[b] Means in the same column followed by different letters are significantly different ($P < 0.01$).

shown). In addition, 25 colonies of strain GA1 and 25 of GA2 were isolated at the end of ripening, and all of them kept their pediocin production ability. Therefore, resistance to this bacteriocin and instability of the bacteriocinogenicity cannot explain the presence of the surviving *Listeria* in the lactic acid bacteria–inoculated cheeses. Probably, the *L. innocua* inoculum was too high, and there were not enough bacteriocin molecules available to completely inactivate the pathogen. Previously, Ennahar et al. *(6)* observed that the amount of pediocin PA-1 produced by *Lactobacillus plantarum* WHE92 in Munster cheese crust was insufficient to eliminate high numbers of *L. monocytogenes,* but at levels below 2 log CFU/g, the bacteriocin inactivated completely the pathogen.

In the past, pediocin PA-1 production by *L. lactis* strains has been generally achieved with well-characterized laboratory strains as hosts *(3, 12–14)*; however, such lactococcal hosts are not suited for application in the dairy industry, because they are proteinase negative, lactose negative, or unable for adequate growth and acid production in milk. Although genetic manipulation of wild dairy lactococci is more difficult than that of laboratory strains, Buyong et al. *(1)* transformed *L. lactis* MM210, a starter strain used in Cheddar cheese manufacture, with pMC117, a plasmid containing the pediocin operon, under the control of the lactococcal promoter P32. These authors tested the anti-*Listeria* activity of the recombinant strain in Cheddar cheese and showed a 3-log reduction in the counts of a mixture of three *L. monocytogenes* strains within 3 months of ripening. More recently, heterologous pediocin PA-1 production was achieved in *L. lactis* ESI 153 and ESI 515, two strains previously selected because of their technological properties for cheese making *(4),* through a strategy based on exchange of the leader and transporter system of the bacteriocin lactococcin A *(23).* Later, their antimicrobial activity against *L. monocytogenes* was confirmed in experimental cheeses *(25).* However, such recombinant strains cannot be considered safe for food production because they carry plasmids with undesirable features, such as the presence of genes conferring antibiotic resistances. In contrast, *L. lactis* GA1 and GA2, the two new strains developed in this study, harbor a plasmid that exclusively contains lactococcal sequences and that is devoid of antibiotic resistance markers. Obviously, these strains are genetically modified organisms, and their industrial application should follow the corresponding regulatory requirements. Research is in progress to evaluate the potential of the culture supernatants of the recombinant strains for use as fermentates for protection against *Listeria* in ready-to-eat foods.

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TABLE 4. *Bacteriocin activity in cheese manufactured with different lactococci*

Strain	Bacteriocin	Bacteriocin activity (mm)[a]							
		L. lactis MG 1614				*E. faecalis* TAB 28			
		7 days	14 days	21 days	28 days	7 days	14 days	21 days	28 days
L. lactis ESI 153	—	ND[b]	ND	ND	ND	ND	ND	ND	ND
L. lactis GA1	Pediocin	ND	ND	ND	ND	7	9	9	8
L. lactis ESI 515	Nisin	13	14	15	14	ND	ND	ND	ND
L. lactis GA2	Nisin, pediocin	15	17	18	17	ND	6	7	7

[a] Bacteriocin activity (halo diameter in millimeters) as determined by an agar diffusion bioassay with *Lactococcus lactis* MG1614 (nisin sensitive) and *Enterococcus faecalis* TAB 28 (pediocin sensitive) as the indicator microorganisms.
[b] ND, not detected.

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