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Enhanced Control of *Listeria monocytogenes* by In Situ-Produced Pediocin during Dry Fermented Sausage Production†

P. M. FOEGEDING,* A. B. THOMAS, D. H. PILKINGTON, AND T. R. KLAENHAMMER

Department of Food Science, North Carolina State University, Raleigh, North Carolina 27695-7624

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To determine whether pediocin is produced and has effective antilisterial activity during food fermentation, six sausage fermentation trials were conducted with antibiotic-resistant, pediocin-producing (Bac$^+$) *Pediococcus acidilactici* PAC 1.0 (Str^r Rif^r) and an isogenic pediocin-negative (Bac$^-$) derivative used as a control. Meat was inoculated (ca. 10^5 CFU/g) with a composite of five *Listeria monocytogenes* strains, each electrotransformed with pGK12 (Cm^r Em^r). *P. acidilactici* and *L. monocytogenes* populations were selectively enumerated by plating on media with antibiotics. This study indicated that the dry sausage fermentation process can reduce *L. monocytogenes* populations. Effective inactivation of *L. monocytogenes* was observed when the pH at the end of the fermentation portion of the process was <4.9. Pediocin was responsible for part of the antilisterial activity during the fermentation in each of the six trials. Furthermore, inhibition of *L. monocytogenes* during drying was enhanced in the presence of pediocin in the three trials in which *L. monocytogenes* could be detected throughout the drying process. Thus, pediocin production contributed to an increase in safety during both the fermentation and drying portions of sausage manufacturing.

Food-borne listeriosis outbreaks have been associated with the consumption of cole slaw (30), milk (8), and cheese products (2). Two cases of listeriosis have been linked to consumption of poultry products (3, 19). Although a major outbreak of listeriosis related to meat consumption has not been identified, the association of *Listeria monocytogenes* with meat, including cured and fermented products, is well documented (6, 7, 25, 26, 32). Recent studies have shown that *L. monocytogenes* may survive the combination of low pH, low water activity, sodium chloride, and sodium nitrite associated with the fermentation and drying of meat (4, 9, 16, 17).

Inhibition of *L. monocytogenes* by bacteriocins produced by *Pediococcus acidilactici* may provide added control against this pathogen in fermented meat products. *P. acidilactici* PAC 1.0 produces a bacteriocin designated pediocin PA-1 (11) and has inhibitory activity directed against *L. monocytogenes* (12, 28). Although pediocins are known to inhibit *L. monocytogenes* (5, 12, 14, 28, 33), no one has clearly indicated that bacteriocin production in food provides added safety above and beyond that provided by other fermentation end products. One problem is the lack of a method for enumerating mixed populations, particularly when low target populations are encountered and enrichment protocols are required. A second problem is the need for a proper control fermentation where all variables except the possible bacteriocin production and activity are held constant. To address these issues, we employed a streptomycin- and rifampin-resistant bacteriocin-producing *P. acidilactici* strain and its isogenic derivative, which had been cured of the plasmid encoding bacteriocin production, to ferment meat inoculated with *L. monocytogenes*. The *L. monocytogenes* cells were genetically marked by electrotransformation with plasmid pGK12, which encodes resistance to chloramphenicol and erythromycin (20). Thus, both pediococcal and listerial populations were readily enumerated on antibiotic-containing media. This study indicated that meat fermentation in and of itself was effective in controlling *L. monocytogenes*, especially if the pH at the end of fermentation was less than 4.9. When the pH was not lowered sufficiently, *L. monocytogenes* survived the fermentation and bacteriocin contributed to inactivation of *L. monocytogenes* during the drying process.

MATERIALS AND METHODS

Bacterial cultures and media. *L. monocytogenes* cultures (Scott A [clinical isolate, serotype 4b, originally obtained from C. Donnelly, University of Vermont], F5069 [raw milk isolate, serotype 4b, originally obtained from C. Donnelly], ATCC 19115 [clinical isolate, serotype 4b, from the American Type Culture Collection, Rockville, Md.], and NCF-U2K3 and NCF-F1KK4 [liquid whole egg isolates from our laboratory]) were grown in brain heart infusion (BHI) broth (Difco, Detroit, Mich.) at 37°C. Bacteriocin-producing (Bac$^+$) *P. acidilactici* PAC 1.0 strains (obtained from D. Hoover, University of Delaware) and bacteriocin-negative (Bac$^-$) *P. acidilactici* PAC 1.0 (11) strains were grown in MRS broth (Difco) at 37°C. Cultures were stored at −20°C in double-strength broth with 20% glycerin (Fisher Scientific Co., Pittsburg, Pa.). Before experimental use, cells were subcultured twice (1% inoculum for 24 h). Solid medium was prepared by adding 1.5% granulated agar (Difco) to the broth. The overlays were prepared with 0.75% agar. Growth and selection of antibiotic-resistant organisms used, unless otherwise noted, 10 μg of chloramphenicol per ml and 5 μg of erythromycin per ml in BHI or 500 μg of streptomycin per ml and 50 μg of rifampin per ml in MRS. In all meat fermentation trials, 5 μg of chloramphenicol per ml and 2 μg of erythromycin per ml in BHI were used for the recovery of *L. monocytogenes*. A specific resuscitation step was not needed to selectively recover heated listeria cells (data not shown). The antibiotics were from Sigma Chemical Co. (St. Louis, Mo.).

Plasmid DNA extraction and visualization. Plasmid pGK12

* Corresponding author.

† Paper FS91-28 of the Journal Series of the Department of Food Science, North Carolina State University, Raleigh, NC 27695-7624.

(20), used in the electroporation experiments, was isolated from *Escherichia coli* JM110 (21). Plasmids for molecular weight markers were isolated from *E. coli* V157 (22). Large-scale extraction of plasmid DNA was by the alkali procedure of Rodriguez and Tait (29). Plasmid DNA was purified through cesium chloride-ethidium bromide equilibrium density gradients (23). Small-scale extraction of plasmid DNA was as detailed by Anderson and McKay (1), except that lysozyme was increased to 15 mg/ml with 30 min of incubation at 37°C and phenol was removed by two extractions with 300 μl of chloroform-isoamyl alcohol (24:1).

Electroporation. *L. monocytogenes* was electroporated as detailed by Luchansky et al. (21) with 2.5× PEB electroporation buffer at 6,250 V cm^{-1} and 25 μF in 0.2-cm cuvettes. Transformants of strains Scott A, ATCC 19115, and F5069 were selected first on BHI agar with chloramphenicol (7.5 μg/ml) and then plated on BHI agar with erythromycin (3.0 μg/ml). Other transformants were selected on BHI agar with each antibiotic at 3 μg/ml.

Plasmid stability studies. Six successive 1% transfers of *L. monocytogenes* containing pGK12 in BHI broth were incubated at 37°C for 24 h. At each transfer interval, samples were diluted and plated on BHI agar; 100 isolated colonies were transferred to BHI agar with chloramphenicol. The percent loss of pGK12 during growth in BHI broth was determined from the percentage of colonies that did not grow on the BHI agar with chloramphenicol.

A five-strain composite consisting of equal aliquots of transformed *L. monocytogenes* cultures was used to inoculate autoclaved (121°C, 15 min) ground pork. The inoculated pork was incubated for 24 h at 37°C. The pork culture was mixed (ca. 5% inoculum) into autoclaved pork, and this culture was also incubated for 24 h at 37°C. After each 24-h interval, portions of the pork cultures were diluted in peptone-water and plated on BHI agar. The percent loss of plasmids was determined as detailed above.

Bacteriocin detection and assays. *P. acidilactici* strains were examined for bacteriocin production by direct (34) and deferred (18) methods. A modification of the critical dilution assay (24) was used to determine the bacteriocin titer. Serial twofold dilutions of sterile culture supernatant were spotted (10 μl) onto indicator lawns of *Lactobacillus* sp. strain LA51 (a low-pH- and acid-resistant organism from H. Fleming, U.S. Department of Agriculture, Raleigh, N.C.) or *L. monocytogenes* strains and incubated for 16 to 18 h at 37°C. Indicator lawns were prepared by adding 0.1 ml of an overnight culture (18 h, 37°C) to 4 ml of overlay agar. The titer was defined as the reciprocal of the highest dilution exhibiting complete inhibition of the indicator lawn and was expressed in activity units per milliliter. Bacteriocin assays with samples neutralized to pH 7.0 were included to eliminate inhibition due to acid or low pH.

Mutant isolation and screening. Spontaneous mutants of *P. acidilactici* PAC 1.0 with resistance to streptomycin (2,000 μg/ml) and rifampin (200 μg/ml) were isolated by sequential selection on MRS agar containing increasing antibiotic concentrations. Bac^- derivatives were isolated from antibiotic-resistant cells by repeated culturing in broth containing 20 μg acriflavin per ml at 47°C for 18 h. After three transfers, appropriate dilutions were plated on MRS agar with streptomycin and rifampin and incubated for 24 h at 37°C. Colonies were replica plated to test for bacteriocin production against *Lactobacillus* sp. strain LA51. Suspected Bac^- colonies were picked from the original plate and grown in MRS at 37°C for 24 h. Loss of bacteriocin production was confirmed by direct and deferred antagonism and by plasmid visualization. Plasmids were extracted by the modified small-scale extraction method detailed above.

Preparation of bacterial inocula for sausage fermentation. A five-strain composite of the transformed *L. monocytogenes* cultures was used for the inoculated meat studies. Strains were grown individually in BHI at 37°C for 18 h. Equal portions of each culture were mixed to yield ca. 10^5 CFU/g when added to the meat.

P. acidilactici were subcultured twice in MRS broth containing 4.5% NaCl at 37°C for 18 h, concentrated by centrifugation (4,000 × *g*, 15 min), and resuspended in sterile 0.1% Difco peptone-water for an inoculum of ca. 10^8 CFU/g when added to the meat.

Sausage manufacture. Six independent sausage trials were conducted, each consisting of two simultaneous fermentations with either the Str^r Rif^r Bac^+ *P. acidilactici* strain or its isogenic Bac^- derivative. Sausage was made from fresh pork shoulders. The shoulders were deboned, and the subcutaneous fat layer was removed. The defatted pork was vacuum packaged and stored at −20°C for ≤2 weeks. The meat was tempered at 4°C and then ground through a plate with 1.2-mm holes and then a plate with 0.3-mm holes. A cure mixture, consisting of glucose (0.75%), salt (3.3%), and $NaNO_2$ (156 ppm), was premixed in a bag, added to the ground pork, and mixed in a Keebler Engineering Co. (Chicago, Ill.) mixer for 1.75 min. The appropriate starter culture and the five-strain *L. monocytogenes* composite culture were mixed and immediately added to the pork and cure mixture, and mixing was continued for an additional 1.75 min. For any given trial (consisting of a Bac^+ strain and a control Bac^- strain), the batter was prepared first with the Bac^- culture and then with the Bac^+ culture to avoid carryover of the Bac^+ culture into batter intended to contain the Bac^- culture. The inoculated sausage batter was stored overnight at 4°C before sausages were stuffed.

Two sausage trials each were conducted with casings 99, 77, and 52 mm in diameter (Fibrous Securex; Teepak, Chicago, Ill.). Casings were hydrated in warm water, stuffed by using a KOCH KVF80 vacuum stuffer (Koch Supplies, Inc., Kansas City, Mo.), and double clipped on both ends. The sausages were approximately the same length; the weights were about 1.82, 1.10, and 0.52 kg, respectively, for sausages made in 99-, 77-, and 52-mm-diameter casings. For each trial the Bac^- batter was stuffed first.

After stuffing, the casings were washed three times in a 200-ppm hypochlorite solution, labeled, weighed, hung on stainless steel smokesticks, and placed in a smokehouse (Drying Systems Co., Morton Grove, Ill.) for fermentation. The fermentation chamber was 38°C at 85 to 90% relative humidity to achieve an internal sausage temperature of ca. 32°C during a 48-h fermentation. This is referred to as the fermentation portion of the process. After fermentation, the internal temperature of the sausage was raised to 49°C and the chamber relative humidity was raised to 100%. Sausages were showered with warm and then cooler water until the internal temperature was 38°C. Sausages were hung in the drying room at 13°C and 65% relative humidity for 44 days. This is referred to as the drying portion of the process.

Sausage sampling and evaluation. Sausage was sampled at selected times to determine *L. monocytogenes* and *P. acidilactici* populations by aseptically removing 11 g from the geometric center. Samples were mixed with 0.1% peptone–water and placed in a Stomacher Lab-blender (model 400; Cooke Laboratory Products, Alexandria, Va.) for 2 min. *L. monocytogenes* was enumerated after incubating for 48 h at 37°C on BHI agar pour plates containing chloramphenicol

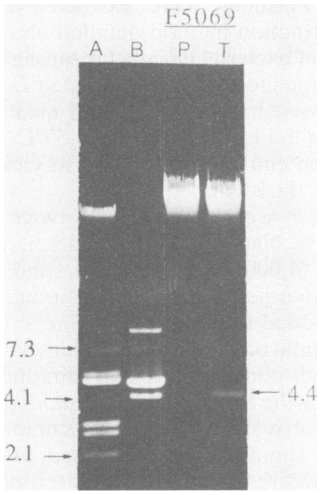

FIG. 1. Agarose gel electrophoresis of plasmid DNA isolated from parental and pGK12-transformed *L. monocytogenes* F5069. Lanes: A, *E. coli* V157 reference plasmids (sizes in kilobases indicated by arrows); B, pGK12 plasmid DNA isolated from *E. coli* JM110; P and T, parental and transformed *L. monocytogenes* cultures, respectively.

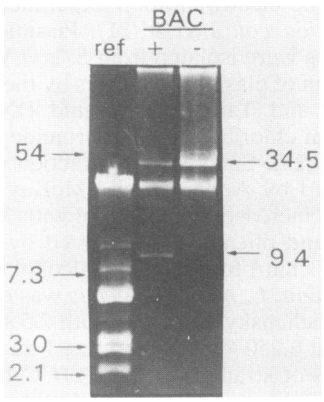

FIG. 2. Agarose gel electrophoresis of plasmid DNA from *P. acidilactici* PAC 1.0 Str^r Rif^r Bac$^+$ and Str^r Rif^r Bac$^-$ derivative strains. Lanes: ref, *E. coli* V157 reference plasmids; +, parental strain PAC 1.0 (Str^r Rif^r Suc$^+$ Bac$^+$); −, PAC 1.0 derivative cured of the 9.4-kb plasmid (Str^r Rif^r Suc$^+$ Bac$^-$). The 34.5- and 9.4-kb plasmids are indicated to the right.

and erythromycin. *P. acidilactici* was enumerated on MRS pour plates containing streptomycin and rifampin after 72 h of incubation at 37°C.

The weights of five sausages were recorded before and periodically during fermentation and drying and used to calculate the percent weight loss during fermentation and drying.

The sausage pH and titratable acidity, expressed as the percent lactic acid, were determined as detailed by Sebranek (31). pH was monitored by using a combination electrode (Fisher Scientific Co., Pittsburg, Pa.) and an Accumet 910 pH meter (Fisher). Titrations were to an endpoint pH of 8.3.

RESULTS

Electroporation of *L. monocytogenes*. Plasmid profiles of parental and electrotransformed *L. monocytogenes* F5069 are shown in Fig. 1. All five transformed strains contained the 4.4-kb plasmid (data not shown). The acquisition of plasmid pGK12 was confirmed phenotypically by resistance to 10 μg each of chloramphenicol and erythromycin per ml. The growth rates in BHI of the transformants were 0.7 to 1.2 times those of the parent cultures.

Plasmid stability. The stability of pGK12 in each of the five *L. monocytogenes* grown in BHI was 99 to 100% after six successive transfers without selective pressure to retain the plasmid. Plasmid stability in autoclaved ground pork with the five strains as a composite culture ranged from 74 to 99% after two successive transfers.

***P. acidilactici* mutant isolation and screening.** A Str^r Rif^r chromosomal mutant of *P. acidilactici* PAC 1.0 was selected to permit monitoring the starter culture population during the fermentation process. The *P. acidilactici* PAC 1.0 (Str^r Rif^r Bac$^+$) strain was compared with the parental *P. acidilactici* PAC 1.0 strains for growth characteristics and acid production. The growth and acid production rates of both cultures were virtually identical (data not shown), indicating that chromosomal marking of the PAC 1.0 strain did not alter these properties, which are important to sausage fermentation.

The Bac$^-$ control starter culture was isolated by curing the antibiotic-resistant Bac$^+$ culture of the 9.4-kb plasmid that encodes pediocin production. The plasmid-cured (Bac$^-$) derivative was missing the 9.4-kb plasmid (Fig. 2). The inability to produce bacteriocin was verified by direct and deferred antagonism assays against *Lactobacillus* sp. strain LA51 (data not shown) and *L. monocytogenes* (Table 1).

Pediocin antagonism against *L. monocytogenes*. Deferred and direct antagonism of *P. acidilactici* against parental and transformed *L. monocytogenes* strains was measured (Table 1). The parental strains and transformed *L. monocytogenes* derivatives were similarly sensitive to pediocin. Therefore, the presence of pGK12 in *L. monocytogenes* had no substantial effect on its sensitivity to pediocin. Unusual differences in the sensitivity or resistance to pediocin were not

TABLE 1. Antagonism of *L. monocytogenes* strains bearing pGK12 by *P. acidilactici*[a]

L. monocytogenes strain	Deferred antagonism (zone size in mm)			Direct antagonism (activity units/ml)		
	Bac$^+$ (PAC 1.0 parent)	Str^r Rif^r Bac$^+$	Str^r Rif^r Bac$^-$	Bac$^+$ (PAC 1.0 parent)	Str^r Rif^r Bac$^+$	Str^r Rif^r Bac$^-$
Scott A	1.0	1.0	0	800	1,600	0
ATCC 19115	4.0	1.0	0	400	1,600	0
F5069	5.0	2.0	0	800	1,600	0
NCF-U2K3	5.0	2.0	0	800	1,600	0
NCF-F1KK4	6.0	2.0	0	800	1,600	0

[a] Phenotypes given in column subheadings are those of the *P. acidilactici* strains used for antagonism assays.

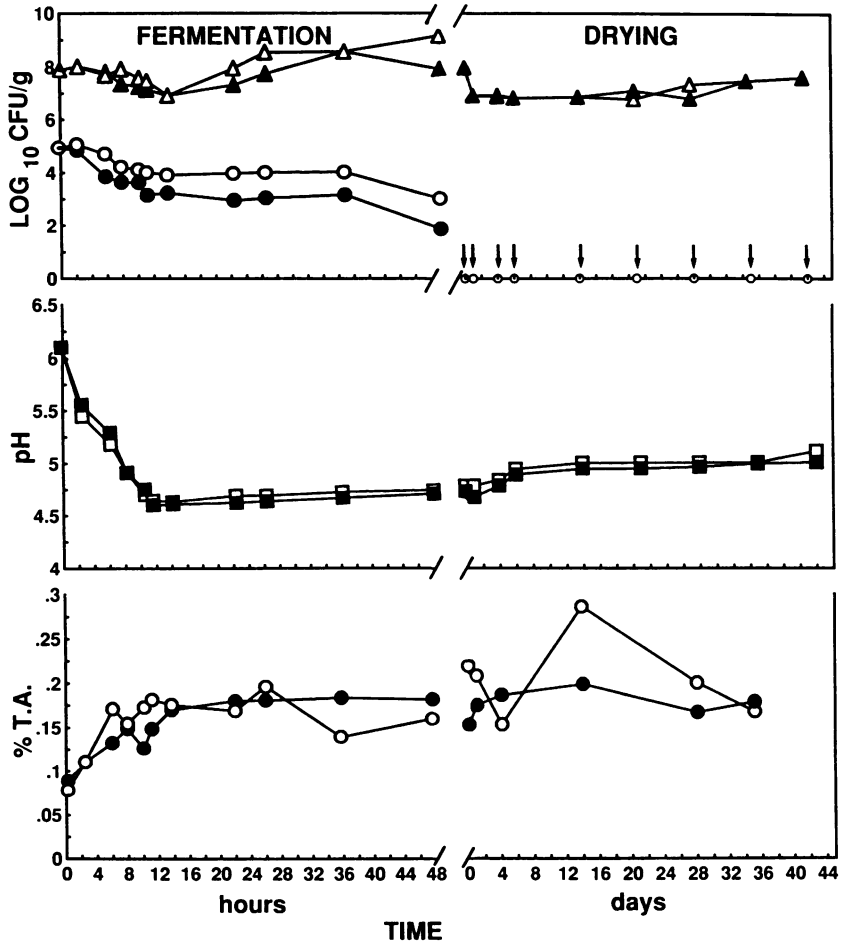

FIG. 3. Sausage fermentation in 77-mm-diameter casings, trial 1. Symbols: ○ and ●, *P. acidilactici* PAC 1.0 Str^r Rif^r; △ and ▲, *L. monocytogenes* (pGK12) five-strain composite; □ and ■, pH; ○ and ●, titratable acidity (T.A.); ▲, ■, and ●, Bac$^+$ fermentation; △, □, and ○, Bac$^-$ fermentation. Arrows indicate populations of <1 CFU/g.

apparent in any of the *L. monocytogenes* transformants. The PAC 1.0 parent and the antibiotic-resistant mutant were similarly bacteriocinogenic (Table 1).

Sausage fermentation and drying. Populations of *P. acidilactici* and *L. monocytogenes*, pH, and titratable acidity are shown in Fig. 3 (trial 1) and 4 (trial 2) for selected sausage fermentation and drying trials (in 77-mm-diameter casings). The weight loss was faster and more extensive in trial 1 than trial 2 (data not shown). At the end of the fermentation periods, average weight losses were 16.7% (trial 1) and 9.8% (trial 2) for fermentations when either the Bac$^+$ or Bac$^-$ pediococci were used. Weight loss was not affected by use of the Bac$^+$ or Bac$^-$ starter culture. The corresponding pH values at the end of the fermentation periods were 4.8 and 4.9, respectively. During the drying periods, the average weight loss percents after 7 days with either the Bac$^+$ or Bac$^-$ starter culture were 37% (trial 1) and 26% (trial 2). During drying, the pH of the sausage in each trial increased slightly. The moisture, fat, and protein contents of the test and control sausages did not differ significantly (data not shown) and were not unlike those of sausage produced industrially, except that all sausages were dried for 44 days, resulting in sausages that were dryer than is typical, especially for the medium and small casings.

L. monocytogenes populations were reduced during the fermentation period when the Bac$^+$ or Bac$^-$ starter culture was used. The Bac$^+$ starter culture resulted in a 10-fold (trial 1) or $10^{1.2}$-fold (trial 2) greater decrease in *L. monocytogenes* populations at the end of the fermentation period. After fermentation and heating (to 49°C [internal temperature]), listerial populations were reduced to <1 CFU/g in trial 1, in which the pH at the end of the fermentation period was 4.8 (Fig. 3). In trial 2, in which the pH at the end of fermentation was 4.9, *L. monocytogenes* survived the fermentation period, heating, and extended drying. One advantage of using the Bac$^+$ starter culture was that pediocin contributed to a reduction in the listerial populations during the fermentation period in both trials and throughout the drying process in trial 2. Upon completion of drying in trial 2, a further reduction of 2.4 log cycles in *L. monocytogenes* populations was observed only with the Bac$^+$ starter culture.

The precise results with the three casing sizes differed. However, the trends observed with the 77-mm-diameter casing also were observed with 99- and 52-mm-diameter casings (data not shown). Table 2 summarizes the results of the six sausage fermentation trials. In each trial, at the end of the fermentation period the *L. monocytogenes* population was reduced to a greater extent when the Bac$^+$ starter culture was used. In four of the six trials, the additional reduction was at least 10-fold.

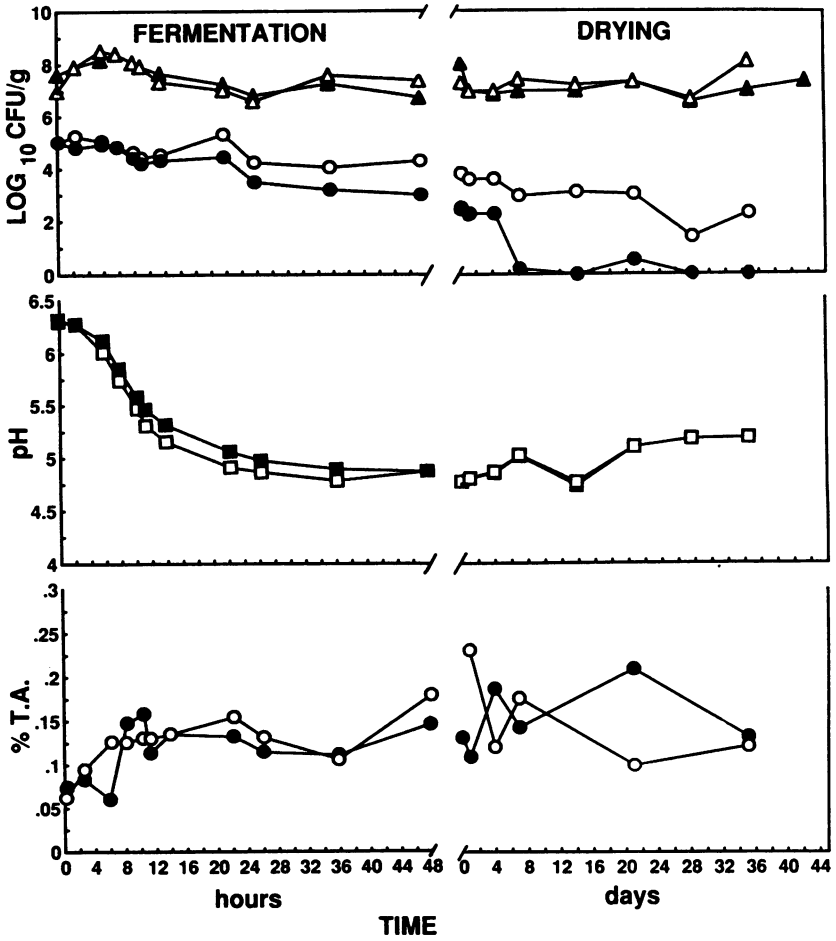

FIG. 4. Sausage fermentation in 77-mm-diameter casings, trial 2. Symbols are as defined in the legend to Fig. 3.

Detectable *L. monocytogenes* remained after the 48-h fermentation period for all trials. The inhibitory effect of bacteriocin could not be evaluated during drying for three trials in which the pH was generally 4.8 at the end of the fermentation period; *L. monocytogenes* populations were <1 CFU/g initially and throughout drying in these trials. In the three trials in which *L. monocytogenes* was detectable after fermentation and mild heating, the pH was ≥4.9 at the end of the fermentation period. In these trials, the *L. monocytogenes* population was reduced to a greater extent during drying when the Bac^+ starter was used. For two of the three trials, the additional reduction was greater than 100-fold.

DISCUSSION

Studies have shown that the addition of pediocin to cottage cheese, half-and-half cream, cheese sauce (28), and meat (27) inhibits or kills *L. monocytogenes*. In this study we investigated the inactivation of *L. monocytogenes* due to bacteriocin production in situ during a meat fermentation. This study was innovative in the direct comparison of fermentations with Bac^+ and Bac^- derivatives to determine whether pediocin production during food fermentation enhances the destruction of *L. monocytogenes* and thereby improves safety.

A five-strain *L. monocytogenes* composite culture was used in meat challenge studies to eliminate the chances of selecting one strain that was unusually sensitive or resistant to pediocin or other fermentation conditions. Transformation with pGK12 permitted direct quantitation of *L. monocytogenes* from a food system bearing a mixed microflora. Since the transformed *L. monocytogenes* strains were resistant to two antibiotics, the probability of indigenous microflora having the same resistance was small and below the detection limits of this study (<1 CFU/g in the raw meat; data not shown). To our knowledge, this is the first example in which electroporation of plasmid DNA encoding antibiotic resistance was used to monitor cultures during food fermentation. The slight retardation in the growth of *L. monocytogenes* from maintenance of the plasmid was not substantial enough to interfere with the use of the transformants in the meat system. The genetic stability of pGK12 in the five *L. monocytogenes* strains was also acceptable for these studies. Without selective pressure, pGK12 was essentially 100% stable in BHI broth, but stability was lower (74 to 99% retention) in autoclaved ground pork. Thus, the actual *L. monocytogenes* populations theoretically could be 1.4 times higher than those recovered with antibiotic selection. However, the relative difference in *L. monocytogenes* population would not be expected to differ for test (Bac^+) and control (Bac^-) fermentation processes within each trial. Reasons for the lower stability in pork were not determined. Johnson et al. (16) suggested that meat lacks a nutrient

TABLE 2. Summary of pilot plant fermentations

Casing diameter (mm)	Trial no.	Bac phenotype	*L. monocytogenes* population[a]					pH at end of fermentation	Summary of effect[c] of using Bac$^+$ or Bac$^-$ strain during:	
			Initial log CFU/g	End of fermentation		End of drying			Fermentation	Drying
				Log CFU/g	Log decrease[b]	Log CFU/g	Log decrease			
99	1	Bac$^+$	5.00 (0.11)	2.80 (0.02)	2.20	<0	>5.00	4.8		
		Bac$^-$	5.01 (0.09)	3.00 (0.06)	2.01	<0	>5.01	4.9	(+)	NA
99	2	Bac$^+$	4.96 (0.03)	3.04 (0.03)	1.92	<0	>4.96	4.8		
		Bac$^-$	4.48 (0.01)	3.98 (0.01)	0.50	<0	>4.48	4.8	+	NA
77	1	Bac$^+$	5.00 (0.07)	1.90 (0.01)	3.10	<0	>5.00	4.8		
		Bac$^-$	5.04 (0.02)	2.94 (0.01)	2.10	<0	>5.04	4.8	+	NA
77	2	Bac$^+$	5.11 (0.03)	2.91 (0.04)	2.20	<0	>5.01	4.9		
		Bac$^-$	5.00 (0.06)	3.96 (0.02)	1.04	2.36 (0.02)	2.64	4.9	+	+
52	1	Bac$^+$	4.85 (0.02)	3.52 (0.05)	1.33	2.12 (0.02)	2.73	5.2		
		Bac$^-$	3.84 (0.02)	2.95 (0.04)	0.89	1.93 (0.02)	1.91	5.1	(+)	(+)
52	2	Bac$^+$	4.91 (0.01)	3.29 (0.10)	1.62	0.74 (0.05)	4.17	5.0		
		Bac$^-$	4.78 (0.02)	4.76 (0.03)	0.02	2.66 (0.07)	2.12	5.0	+	+

[a] Values reported are means of triplicate determinations. Values within parentheses represent standard errors.
[b] Decreases in *L. monocytogenes* populations were determined by subtracting the log population at the end of either fermentation or drying from the log initial population. Thus, these decreases do not reflect the overall population differences during the process.
[c] +, advantage (≥1 log unit difference); (+), questionable advantage (difference of <1 log unit); NA, not applicable (inconclusive data).

required for growth of *L. monocytogenes*; this may limit metabolic energy generation and hinder plasmid retention under nonselective conditions. In these studies, the autoclaved ground pork did not contain added carbohydrate. With the addition of glucose to the sausage formulation, higher plasmid stability may have occurred. This was not evaluated.

Antibiotic-resistant mutants of *P. acidilactici* PAC 1.0 were selected to permit enumeration of the starter culture during sausage manufacture. The *P. acidilactici* PAC 1.0 Str^r Rif^r Bac$^+$ strain grew slightly slower in BHI than did the parent culture, but acid production and pediocin production were comparable to those of the parental *P. acidilactici*. These data suggested that this mutant would perform acceptably (grow and produce acid and bacteriocin at adequate rates and in appropriate amounts) in the sausage fermentation experiments.

Harris et al. (13) used chromosomally encoded antibiotic resistance to quantitiate populations of a mixed starter culture of *Lactococcus lactis* and *Leuconostoc mesenteroides* during a model sauerkraut fermentation. The approach used by Harris and coworkers was similar to that used to monitor *P. acidilactici* in this study. These two methods to genetically mark organisms of interest, with either plasmid- or chromosome-encoded determinants, permit selection of cultures from a mixed background microflora and provide models to monitor minority populations of interest in other food fermentation systems or other microbiologically complex systems. In this research, Cm^r and Em^r encoded by plasmid determinants permitted enumeration of *L. monocytogenes* when it represented as little as 10^{-8} of the population.

P. acidilactici PAC 1.0 had previously been reported to contain two plasmids of 34.5 and 9.4 kb, designated as pSRQ10 and pSRQ11, respectively (10). The presence of the 34.5-kb plasmid (pSRQ10) correlates with a sucrose-fermenting phenotype (Suc$^+$), and the presence of the 9.4-kb plasmid (pSRQ11) correlates with bacteriocin production (Bac$^+$). The ability to cure bacteriocin-producing plasmids in *P. acidilactici*, resulting in a bacteriocin-negative phenotype (Bac$^-$), was demonstrated by Gonzalez and Kunka (11) and Hoover et al. (14). The Bac$^-$ control starter culture was isolated by curing the antibiotic-resistant Bac$^+$ culture of the 9.4-kb plasmid; the 34.5-kb plasmid and sucrose fermenting ability were retained by the culture. This was important, since it resulted in identical genomes in Bac$^+$ and Bac$^-$ strains except for the 9.4-kb plasmid, providing a control differing from the bacteriocin-producing test culture only in its inability to produce bacteriocin.

To account for different conditions that may occur during fermentation and their possible effects on bacteriocin production and inhibition, three sausage casing sizes were used. The casing diameter affects the rate of heat penetration and consequently the rates of metabolism, including acid production, moisture loss, water activity reduction, and other factors that contribute to terminating the fermentation.

The survival of *L. monocytogenes* during fermentation and drying observed in this study was consistent with data reported by others (4, 9, 15–17). This study is unique, however, because it clearly demonstrates that antagonism of *L. monocytogenes* may be enhanced by bacteriocin production in situ and bacteriocin activity throughout the fermentation process. Berry et al. (4) reported that a bacteriocin-producing starter culture effectively inhibited *L. monocytogenes* during sausage fermentation. Their study was not designed to detect the contribution of bacteriocin per se and compared fermentations with a bacteriocin-producing *P. acidilactici* strain with those with a non-bacteriocin-producing *Pediococcus pentosaceous* strain. Our study differs because it clearly shows that bacteriocin production, in combination with other fermentation end products, increases the inhibition of *L. monocytogenes* during fermentation and drying.

This research indicates that meat fermentation, minimal heating after fermentation, and drying of sausage reduced *L. monocytogenes* populations by 5 log units or more, regardless of whether bacteriocin production occurred. Effective reduction in *L. monocytogenes* populations correlated with an adequate pH drop (below 4.9) during the fermentation portion of the process. The pH affects the inhibitory role of

other fermentation preservation factors, such as heating, acid, and drying. In situ bacteriocin production enhanced the inhibition of *L. monocytogenes* during both fermentation and drying. This added inhibition would be especially important if a sufficiently low pH were not achieved during the fermentation.

Where acid production may be insufficient, bacteriocin production would facilitate the reduction of any remaining *L. monocytogenes*. Starter cultures, such as *P. acidilactici* PAC 1.0, that produce bacteriocins inhibitory to *L. monocytogenes* could be utilized in meat fermentations to provide added inhibition against this pathogen. Thus, utilization of bacteriocin-producing starter cultures is recommended to help control *L. monocytogenes* in meat fermentations. Also, using bacteriocin-producing cultures in other food fermentations may prove to be beneficial in controlling this or other food-borne pathogens. For application, it should be recognized that bacteriocin-resistant strains of *L. monocytogenes* may arise due to prolonged or repeated exposure to bacteriocins in a laboratory or food production system.

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