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Genomic Analysis of *Pediococcus* Starter Cultures Used To Control *Listeria monocytogenes* in Turkey Summer Sausage

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The pulsed-field technique of clamped homogeneous electric field electrophoresis was employed to characterize and size genomic DNA of three pediocin-producing (Ped$^+$) and two non-pediocin-producing (Ped$^-$) strains of *Pediococcus acidilactici*. Comparison of genomic fingerprints obtained by digestion with the low-frequency-cleavage endonuclease *Asc*I revealed identical restriction profiles for four of the five strains analyzed. Summation of results for 10 individually sized *Asc*I fragments estimated the genome length to be 1,861 kb for the four strains (H, PAC1.0, PO2, and JBL1350) with identical fingerprints. Genomic analysis of the pediocin-sensitive, plasmid-free strain *P. acidilactici* LB42 with the unique fingerprint revealed nine *Asc*I fragments and a genome length of about 2,133 kb. Ped$^-$ (JBL1350) and Ped$^+$ (JBL1095) starter cultures (one each) were used to separately prepare turkey summer sausage coinoculated with a four-strain *Listeria monocytogenes* mixture (ca. 10^5 CFU/g). The starter cultures produced equivalent amounts of acid during fermentation, but counts of *L. monocytogenes* were reduced to a greater extent in the presence of the Ped$^+$ starter culture (3.4 $\log_{10}$ unit decrease) than in the presence of the Ped$^-$ starter culture (0.9 $\log_{10}$ unit decrease). Although no listeriae were recovered from sausages following the cook/shower, appreciable pediocin activity was recovered from sausages prepared with the Ped$^+$ strain for at least 60 days during storage at 4°C. The results of this study revealed genomic similarities among pediococcal starter cultures and established that pediocins produced during fermentation provide an additional measure of safety against listerial proliferation in turkey summer sausage.

In recent years, there have been several episodes of food-borne illness involving *Listeria monocytogenes* (11). Surveillance studies have shown that the pathogen is common in agricultural ecosystems (39) and prevalent on various foods at the slaughterhouse and retail levels, most notably on poultry (2, 13, 14, 23, 40). With the exception of sporadic listeriosis linked to consumption of undercooked chicken and nonreheated turkey frankfurters (1, 36), there have been no major outbreaks of listeriosis associated with poultry. However, conventional preservation methods, including refrigeration, may not preclude survival or growth of *L. monocytogenes* in foods, including turkey products. For example, the organism grew on vacuum-packaged refrigerated turkey loaf (24) and sliced turkey (16). Thus, the development of innovative and complementary methods for controlling this pathogen in poultry and other foods has become an active area of research.

During the past decade, considerable research has focused on the application of molecular technologies to study lactic acid bacteria, an economically significant group of organisms used to prepare and preserve foods (27). Regarding this group of organisms, relatively little information has accumulated concerning genetic characterization of pediococcal starter cultures used in meat fermentation. There have been a few reports describing pediococci that harbor plasmids, including plasmids encoding pediocins (9, 18, 19, 21, 33), but thus far efforts have not been made to characterize the chromosome of *Pediococcus* spp. For several other gram-positive bacteria (for examples, see references 6–8, 22, and 30), including other lactic acid bacteria (25, 38), the technique of pulsed-field gel electrophoresis (PFGE) has been used to extensively characterize and size genomic DNA. In a similar fashion, genomic analyses of pediococci by PFGE would expand our knowledge of these genetically ill-defined organisms and provide considerable information on genetic relatedness among starter cultures.

In addition to acid and flavor compounds, many *Pediococcus* spp. also produce pediocins that exhibit antilisterial activity. The efficacy of biopreservatives (i.e., pediocins and pediococci) for controlling *L. monocytogenes* has been demonstrated with fresh (29), processed (3, 10a, 42), and fermented meats (4, 12, 15, 34) as well as refrigerated dairy-based products (32). However, there have been no reports on the use of biopreservation systems to control *L. monocytogenes* associated with turkey products. In the present study, we compared the efficacy of pediocin-producing (Ped$^+$) and non-pediocin-producing (Ped$^-$) pediococcal starter cultures for controlling *L. monocytogenes* in turkey summer sausage. In addition, we utilized pulsed-field analysis (i.e., genomic fingerprinting) to establish genetic relationships among *Pediococcus* starter cultures.

MATERIALS AND METHODS

Bacteria. All strains used in this study are listed in Table 1. *Pediococcus acidilactici* H (wild type of JBL1095 [5]), PAC1.0 (also called JBL1096), PO2 (also called JBL1097), and JBL1350 were isolated from commercial starter culture preparations or from fermented sausages prepared with such cultures. *P. acidilactici* LB42 (also called JBL1146), kindly provided by Bibek Ray (University of Wyoming, Laramie),

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TABLE 1. Designations, characteristics, and origins of bacterial strains

Organism	Other designation	Relevant characteristics[a]	Reference or source
P. acidilactici			
JBL1095		Pediocin AcH producer, Suc^+ *str-10 rif-10* (pSMB74)	42
JBL1096[b]	PAC1.0	Pediocin PA-1 producer, Suc^+ (pSRQ10, pSRQ11)	18
JBL1097	PO2	Ped^r Ped^+ ccc^+ Suc^+	21
JBL1146	LB42	Ped^s Ped^- ccc^-	33
JBL1350		Ped^r Ped^- ccc^+	Commercial starter culture
L. monocytogenes			
JBL1002	101M	Meat isolate, serotype 4b	15
JBL1003	103M	Meat isolate, serotype 1/2c	15
JBL1006		Hyperhemolytic derivative of NCTC 5105; serotype 3a	Lammerding[c]
JBL1012	8732		35
JBL1226	AP63	Turkey frankfurter isolate, serotype 1/2a	Bailey[d]

[a] Ped^r, pediocin resistant; Ped^s, pediocin sensitive; ccc^+, contains plasmid (5); ccc^-, plasmid free; *str-10*, streptomycin resistant (1,000 μg/ml); *rif-10*, rifamycin resistant (100 μg/ml); Suc^+, ferments sucrose.
[b] Isolated from LACTACEL 110 (Microlife Technics, Inc., Sarasota, Fla.).
[c] A. M. Lammerding, Health of Animals Laboratory, Guelph, Ontario, Canada.
[d] J. S. Bailey, Russell Research Center, Athens, Ga.

and all *L. monocytogenes* strains used in this study are pediocin sensitive (10, 10a, 33, 42). Pediococci and listeriae were maintained as previously described (10a, 42).

Plasmid isolation. Covalently closed circular DNA was extracted from pediococci by the method described by Muriana and Klaenhammer (28), but in addition to lysozyme, 10 μl of mutanolysin (1 mg/ml in H_2O; Sigma Chemical Company, St. Louis, Mo.) was added to facilitate cell lysis. Plasmid DNA was further purified by ethidium bromide density gradient ultracentrifugation (26). After fractionation by agarose gel electrophoresis (3 h at 70 V using electrophoresis-grade agarose [0.7%]), gels were stained in ethidium bromide (<1 mg/ml; Sigma) for about 15 min and then photographed after visualization on a shortwave UV transilluminator.

Purification, digestion, and resolution of high-molecular-weight DNA fragments. Pediococci were passed twice in brain heart infusion (Difco Laboratories, Detroit, Mich.) broth at 37°C, harvested, washed, and suspended in agarose plugs essentially as described previously (22). Intact high-molecular-weight genomic DNA was prepared from pediococcus cells embedded in agarose plugs by using the method of Carriere et al. (8), but 20 μl of RNase (10 mg/ml in H_2O; Sigma) was included during the lysozyme step, and plugs were gently shaken while immersed in this solution. Prior to digestion, inserts were washed once with 10 mM Tris–0.1 mM EDTA (pH 7.5) (TE buffer) plus 1 mM phenylmethylsulfonyl fluoride and twice in TE buffer only, as described previously (8). Each washing step was performed at room temperature for 1 h.

Next, each plug was cut into about five inserts, and each insert was placed into a sterile microcentrifuge tube for digestion of embedded DNA with approximately 2 U of the "rare cutting" restriction endonuclease *Asc*I as described by the manufacturer (Promega Corporation, Madison, Wis.). Following digestion for at least 16 h at 37°C with gentle shaking, 20 μl of 0.5 M EDTA (pH 8) was added to stop the reaction. Inserts could then be stored at 4°C for 1 to 2 days before fractionation by PFGE.

A clamped homogeneous electric field (CHEF) CHEF-DR II (Bio-Rad Laboratories, Richmond, Calif.) pulsed-field system and electrophoresis-grade (GIBCO-Bethesda Research Laboratories, Life Technologies, Inc., Gaithersburg, Md.) agarose were used to resolve high-molecular-weight restriction fragments. Running buffer (0.5× Tris-borate-EDTA) was cooled to 4°C prior to electrophoresis and maintained at 14°C for the entire run by using an Isotemp refrigerated circulator (Fisher Scientific, Pittsburgh, Pa.). Electrophoresis was performed for 18 h at 200 V by using electrophoresis-grade agarose (1%) and pulse times ramped from 5 to 30 s. Gels were stained and photographed as described above for plasmid DNA.

Preparation of bacteria for inoculation of sausage. (i). Pediococci. *P. acidilactici* starter cultures JBL1095 and JBL1350 were passed twice in MRS (Difco) broth at 30°C and screened for pediocin production (described below) prior to use. To prepare turkey inocula, starter cultures were separately grown in 500 ml of MRS broth for 16 h at 30°C. Cells were harvested by centrifugation (2,000 × *g*, 20 min, 4°C), suspended in 150 ml of 0.1% peptone water (ca. 10^9 CFU/ml), and added directly to sausage batter as described below.

(ii). Listeriae. *L. monocytogenes* JBL1002, JBL1003, JBL1012, and JBL1226 were grown individually in 10 ml of tryptose phosphate broth (Difco) at 37°C for 16 h. The cells from each strain were harvested by centrifugation and suspended in a nominal volume of 0.1% peptone. Next, all four cell suspensions were combined and the volume was adjusted to 75 ml with 0.1% peptone (ca. 5×10^7 CFU/ml) before the four-strain mixture was added to sausage batter as described below.

Manufacture of turkey summer sausage. Turkey summer sausage was prepared from 90% hand-deboned turkey thigh meat, 5% turkey hearts, and 5% turkey fat by using a procedure modified from that of Wilson (41). Turkey components were ground first through a 9.5-mm plate by using a Hobart laboratory grinder (model 84142; Hobart Manufacturing Co., Troy, Ohio). Commercial turkey summer sausage seasoning (0.5%, formulated to provide glucose [1%] and NaCl [2.5%]; Milwaukee Seasonings, Inc., Germantown, Wis.) and $NaNO_2$ (0.015%) were added and mixed with 15-kg quantities of turkey meat for 3 min at 4°C in a Hobart mixer (model 1661682). Three samples (ca. 100 g each) were removed for analyses of uninoculated batter.

Sausage batter was inoculated with 150 ml of pediococcal starter culture (JBL1095 or JBL1350; ca. 10^7 CFU/g of batter) and mixed for 2 min at 4°C. A 7.5-kg portion of batter was removed, ground through a 6.3-mm plate, stuffed (150 g per chub) by using a hand stuffer (Koch Supplies, Inc., Kansas City, Mo.) into mahogany fibrous casings (75 by 152 mm; Vista International Packaging, Inc., Kenosha, Wis.), and hand tied. To the remaining 7.5 kg of batter was added 75 ml of the four-strain *L. monocytogenes* mixture (ca. 5×10^5 CFU/g of batter), and the batter was mixed, reground, and stuffed as described above. Sausages were fermented at 37°C (85% relative humidity) in a Vortron smokehouse (model 1000; Vortron, Inc., Beloit, Wis.) to pH 5 (ca. 12 h), cooked to an internal temperature of 66.5°C for 45 min, and cold-showered to an internal temperature of 55°C. After sausages were processed, individual chubs were vacuum-packaged in gas-impermeable Curlon bags (nylon-Saran-polyethylene; O_2 transmission of 0.8 to 1.0 cm^3/645 cm^2/24 h at 22.8°C, CO_2 transmission of 2.5 to 3.0 cm^3/645 cm^2/24 h at 22.8°C, H_2O transmission of 0.5 g/645 cm^2/24 h at 37.8°C, and 90% relative humidity; Curwood, Inc., New London, Wis.) by using a Multivac AGW vacuum-packaging unit (Sepp Haggemuller KG, Wolfertschwenden, Germany) and then stored at 4°C (for up to 60 days) or 25°C (for up to 7 days).

Enumerating listeriae and pediococci from sausages and determining pH and TA. Three sausage chubs from each treatment (JBL1095 only, JBL1095 plus *L. monocytogenes*, JBL1350 only, and JBL1350 plus *L. monocytogenes*) at each sampling time were tested for (i) *L. monocytogenes* count by direct plating and by enrichment (at the end of cook/shower and subsequent sampling times), (ii) *Pediococcus* count by direct plating, (iii) pH, and (iv) titratable acidity (TA) as percent lactic acid. Sausage samples were taken immediately after stuffing (time 0), during fermentation (e.g., at 4 and 8 h after the start and at the end), at the end of the cook/shower, and during storage at 4°C (at 1, 7, and 60 days) or 25°C (at 1, 3, 5, and 7 days). In addition, uninoculated sausage batter samples were analyzed to determine the presence of indigenous *L. monocytogenes* and lactic acid bacteria.

To obtain sausage samples, casings were wiped with 70% ethanol and cut with a sterile scalpel to facilitate removal of casings. For each sample, three 25-g portions were aseptically removed to separate stomacher bags for direct plating, enrichment, and pH and TA determinations. For determining bacterium numbers, each 25-g portion was macerated with 225 ml of 0.1% peptone water for 2 min by using a stomacher (model 400; Tekmar Co., Cincinnati, Ohio) and serially diluted (1:10) for direct plate counts. As described previously (10a, 42), pediococci were enumerated on MRS agar (JBL1350; not genetically marked) or MRS agar plus 1,000 μg of streptomycin per ml and 100 μg of rifamycin per ml (JBL1095; Str^r Rif^r) and incubated for 24 to 48 h at 30°C. *L. monocytogenes* organisms were enumerated on a selective medium (modified Oxford agar) and confirmed essentially as described previously (17).

The pH and TA were determined by a procedure modified from that of Sebranek (37). In brief, a 25-g portion of sausage was macerated in a stomacher with 225 ml of hot (ca. 70°C) distilled, deionized water for 2 min. The homogenate was poured into a 250-ml Erlenmeyer flask and cooled to room temperature, and the fat layer was removed with the aid of a pipet. The pH of the mixture was measured with a combination electrode and a Corning model 140 pH meter (Corning Glass Works, Corning, N.Y.). The homogenate was filtered through Whatman no. 1 filter paper, and the filtrate (100 ml) was titrated with 0.1 N NaOH to pH 8.1. The TA was expressed as the percent lactic acid.

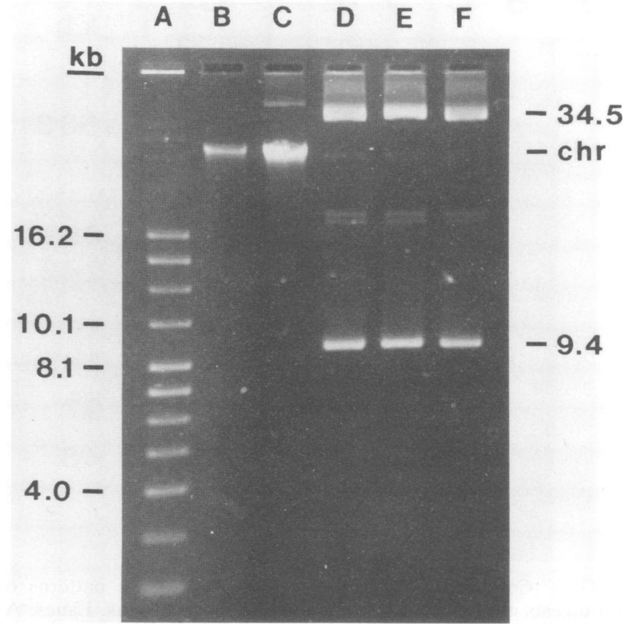

FIG. 1. Comparison of plasmid profiles of *Pediococcus* strains. Lanes: A, supercoiled ladder molecular weight size standard (Bio-Rad); B, LB42; C, JBL1350; D, JBL1095; E, PAC1.0; F, PO2. The plasmid profiles shown are identical to profiles of each strain obtained in at least nine replicate gels. chr, chromosomal DNA.

Monitoring pediocin AcH activity in sausages. At appropriate time intervals, 10-g samples of sausage were removed from chubs as described above, combined with 90 ml of sterile H_2O, and blended to homogeneity (ca. 2 min) by using the highest setting on a commercial blender (model 32BL97; Waring Products Division, New Hartford, Conn.). Next, the sausage-H_2O blend was centrifuged (9,820 × *g*, 5 min, 4°C), and the resulting supernatant (ca. 80 ml) was transferred to a fresh tube and heated (100°C, 10 min) to eliminate residual bacteria. Pediocin activity was then concentrated (80 ml reduced to ca. 5 ml) by lyophilization. Antilisterial activity was determined by the spot-on-lawn method and expressed as arbitrary units per gram of sausage essentially as described previously (10a).

RESULTS

Preliminary characterization of pediococcal starter cultures. For application to this study, experiments were conducted to confirm production of pediocin AcH by JBL1095 and to determine whether JBL1350 produced or was sensitive to already characterized pediocins. Strain JBL1095, a derivative of *P. acidilactici* H (33), and the associated pediocin AcH (5) have been at least partially characterized, but essentially no information is available for JBL1350. In this study, JBL1350 did not exhibit antimicrobial activity related to the production of a pediocin (data not shown). On the basis of these results, JBL1095 and JBL1350 were employed as Ped^+ and Ped^- starter cultures, respectively, to manufacture turkey summer sausage.

Plasmid analysis. Plasmid DNA was extracted from Ped^+ (JBL1095) and Ped^- (JBL1350) starter cultures and from three other (PAC1.0, PO2, and LB42) strains of *P. acidilac-*

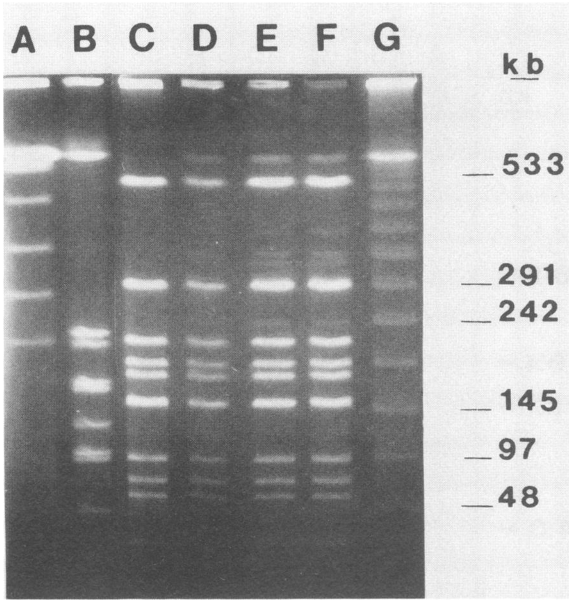

FIG. 2. Comparison of CHEF gel electrophoresis patterns of *Asc*I digests of genomic DNA from *Pediococcus* strains. Lanes: A, *Saccharomyces cerevisiae* chromosome size standard (Bio-Rad); B, LB42; C, JBL1350; D, JBL1095; E, PAC1.0; F, PO2; G, lambda ladder size standard (Bio-Rad). The genomic fingerprints shown are identical to restriction profiles of each strain obtained in at least five replicate gels.

tici (Fig. 1). Heretofore, the plasmid profiles of these strains have not been directly compared (i.e., on the same gel). Plasmid DNA was not observed in the Ped$^-$, plasmid-free control strain, LB42 (lane B), but plasmids were discernible in all other strains screened (lanes C to F). In addition to at least one large (>30 kb) plasmid, *P. acidilactici* JBL1095, PAC1.0, and PO2 contained small plasmids (one each) of similar size (ca. 9.4 kb). Strain JBL1350 did not contain this small (ca. 9.4-kb) plasmid, but at least one high-molecular-weight band was evident (Fig. 1). These results established that strains JBL1095, PAC1.0, and PO2 possess highly similar plasmid profiles. Further work is required to determine whether the resident plasmids in these strains are identical.

Genomic fingerprinting of pediococci by using CHEF. Since *Pediococcus* spp. have relatively low G+C contents (ca. 37 to 44%), and on the basis of encouraging results with *L. monocytogenes* (ca. 36 to 38% G+C [22]), we used the low-frequency-cleavage endonuclease *Asc*I (GGCGCGCC) for CHEF analyses of pediococci (Fig. 2). Digestion of genomic DNA from LB42 produced nine *Asc*I fragments ranging in length from about 84 to 950 kb. CHEF analyses revealed identical restriction profiles (10 *Asc*I fragments; ca. 62 to 525 kb) for *P. acidilactici* JBL1095, PAC1.0, PO2, and JBL1350. The sizes and numbers of fragments generated by digestion of intact pediococcal DNA with *Asc*I are listed in Table 2. Depending on the strain, summation of results with *Asc*I fragments estimated the genome sizes to be 1,861 kb (JBL1095, PAC1.0, PO2, and JBL1350) and 2,133 kb (LB42).

Control of *L. monocytogenes* during turkey summer sausage fermentation. Sausages prepared with a Ped$^-$ or Ped$^+$ starter culture were compared for the ability to support the growth of *L. monocytogenes* during manufacture and storage of sausages (Fig. 3). Levels of pediococcal starter cultures

TABLE 2. Restriction analysis of *Pediococcus* genomic DNA

Fragment	Size (kb) of *Asc*I fragments from strains[a]	
	JBL1146	PAC1.0, PO2, JBL1095, and JBL1350
1	950.5 ± 29.5	525 ± 35
2	212.5 ± 2.5	288 ± 3
3	200 ± 5	207.5 ± 2.5
4	178.5 ± 21.5	180 ± 5
5	170 ± 20	171.5 ± 1.5
6	127 ± 2	147.5 ± 2.5
7	107.5 ± 2.5	105 ± 0
8	102.5 ± 2.5	91 ± 3
9	84 ± 10	83 ± 3
10		62.5 ± 2.5
Total	2,133 ± 10.6	1,861 ± 5.8

[a] Individual and total fragment sizes were calculated as an average from at least three different gels for fragments of <550 kb and one gel for fragments of >600 kb.

decreased by the end of fermentation; pediococci were not recovered from properly cooked sausages (data not shown). In sausages fermented with a Ped$^-$ strain, counts of *L. monocytogenes* decreased about 0.9 $\log_{10}$ units during the 12-h fermentation period. In contrast, counts of the pathogen were reduced by 3.4 $\log_{10}$ units during fermentation of sausages prepared with a Ped$^+$ pediococcal starter culture. Regardless of the choice of starter culture, no listeriae were recovered from sausages following the cook/shower process by either direct plating or the enrichment procedure.

Production of acid and pediocin during fermentation of turkey summer sausage. The use of a Ped$^+$ starter culture rather than a Ped$^-$ strain resulted in a greater reduction in counts of *L. monocytogenes* during fermentation. The pH, TA, and pediocin activity of sausages prepared with JBL1095 or JBL1350 were monitored to determine whether antilisterial activity was due to acid and/or pediocin production. The data revealed that the pH was reduced to similar levels (reduced from an average pH of 6.3 to 5.0) at equivalent rates (ca. 12 h) in sausage prepared with either starter culture (Table 3). Likewise, the TA (expressed as percent lactic acid) increased to the same extent in sausage prepared with either pediococcal starter culture. More important, as the fermentation proceeded, counts of JBL1095 decreased by about 1.1 $\log_{10}$ unit while the titer of pediocin activity increased dramatically (from 0 to 5,000 arbitrary units per gram of sausage in 12 h) in sausage prepared with JBL1095 (Fig. 4). Moreover, after the cook/shower, pediococci were not detectable ($<10^2$ CFU/g) whereas pediocin activity was maintained at high levels (ca. 5,000 arbitrary units per gram of sausage) for 60 days at 4°C. No pediocin activity was recovered from sausages prepared with JBL1350. These data confirm that additional antilisterial activity (i.e., pediocin AcH) was available in sausage prepared with a Ped$^+$ starter culture and that pediocin AcH was not affected by fermentation, cook/shower, or storage of turkey summer sausage.

DISCUSSION

L. monocytogenes has been implicated in food-related listeriosis outbreaks involving dairy products and coleslaw, and the pathogen is common in meat and poultry products. The frequent association of *L. monocytogenes* with fresh, frozen, and ready-to-eat poultry (up to 60% [2, 13, 14, 23,

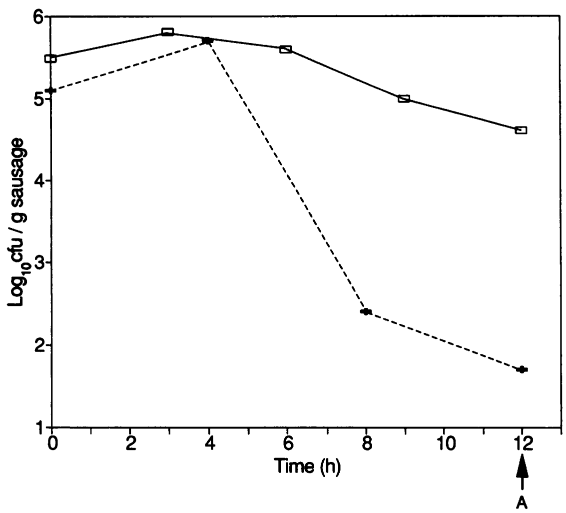

FIG. 3. Behavior of *L. monocytogenes* in the presence of pediococci during fermentation of turkey summer sausage (trial 2). Open rectangles represent the four-strain *L. monocytogenes* mixture in the presence of *P. acidilactici* JBL1350; thick crosses represent the four-strain *L. monocytogenes* mixture in the presence of *P. acidilactici* JBL1095. "A" signals the end of fermentation.

31]) and the perceived risk from poultry-related listeric illness prompted us to evaluate the use of pediocin-producing pediococci as an additional method to ensure product safety. Also, published information intimating similarities among various pediocins, pediocinogenic plasmids, and pediococci prompted us to investigate genomic relationships among characterized starter cultures.

Prior to the use of JBL1095 and JBL1350 in production of turkey summer sausage, pediocin activity, plasmid content, and genomic fingerprints of these strains were compared with each other and with those of characterized *P. acidilactici* strains. Previous studies (18, 21, 33) revealed that strains H, PAC1.0, and PO2 contained a small plasmid necessary for pediocin production and an additional plasmid of about 34.5 kb. Our data do not confirm the presence of a third plasmid as previously reported for strains PO2 (127 MDa [21]) and H (40 MDa [33]). Aside from the initial observation of them, there have been no reports substantiating the presence or function of these very large plasmids in strain PO2 or H. Moreover, it is possible that the ca. 40-MDa plasmid reported for strain H represents the dimeric form of the 23-MDa resident plasmid. Although further work is warranted to unravel relationships among strains and plasmids, Hoover et al. (20) reported that the plasmid profiles of strains PAC1.0 and PO2 were similar and that the corresponding pediocins possessed similar activities and physical properties. Our results established that the large (ca. 34.5-kb) and small (ca. 9.4-kb) plasmids present in strains PAC1.0 and PO2 are equivalent in size to plasmids in strain JBL1095 (Fig. 1). It was also of interest that the Ped$^-$ commercial starter culture JBL1350 contained a high-molecular-weight (>35 kb) plasmid band but was missing the small pediocin plasmid common among the other strains. The inability to produce pediocin (i.e., absence of pediocin plasmid) was in large measure the reason that JBL1350 was less effective than JBL1095 in controlling *L. monocytogenes* during fermentation.

Thus far, the technique of PFGE-CHEF has not been employed to size or discriminate genomic DNA from *Pediococcus* spp. CHEF analysis of pediococci using the enzyme *Asc*I revealed identical genomic fingerprints for the Ped$^+$ strains JBL1095, PAC1.0, and PO2. Moreover, the genomic fingerprint of a Ped$^-$ pediocin-resistant commercial starter culture (JBL1350) was identical to the *Asc*I profiles for strains JBL1095, PAC1.0, and PO2. The plasmid-free Ped$^-$ pediocin-sensitive strain LB42 displayed an *Asc*I migration profile significantly different from those of the other strains tested. Summation of results for individually sized *Asc*I fragments from strains JBL1095, PAC1.0, PO2, and JBL1350 and strain LB42 estimated the genome lengths to be 1,861 and 2,133 kb, respectively. These values are in agreement with pulsed-field analysis determinations of the genome sizes of other lactic acid bacteria (range of 1,700 to 2,700 kb [25, 38]). Future efforts will be directed to analyze more strains, evaluate additional rare cutting endonucleases, and optimize conditions for enhanced resolution of high-molecular-weight pediococcal DNA for molecular tracking of strains and construction of macrorestriction maps.

In the last few years, there have been several reports on the use of pediococci and pediocins to control *L. monocytogenes* in foods (3, 4, 10a, 12, 29, 32, 34, 42). Previous studies established that pediocin AcH (added directly or produced in situ by JBL1095) was a potent inhibitor of *L. monocytogenes* associated with all-beef wieners (10a, 42). Degnan et al. (10a) implemented genetically marked Ped$^+$ and genetically related Ped$^-$ pediococci to compare the antilisterial activities of organic acids and pediocins in thermally processed meat. These investigators demonstrated that *L. monocytogenes* survived but did not grow in temperature-abused vacuum-packaged wieners stored at 25°C for 8 days in the presence of a Ped$^-$ strain, whereas counts of the pathogen were reduced by an average of 2.7 $\log_{10}$ units in the presence of a Ped$^+$ (JBL1095) strain. More recently, a similar approach was used to confirm the antilisterial role of pediocin production during dry fermented sausage production (12). The results of the present work reveal the added safeguard of bacteriocin production by pediococcal starter cultures for reducing the potential for listerial proliferation in poultry. More specifically, the production of acid by a Ped$^-$ starter culture produced primarily a listeriostatic effect in turkey summer sausage, but a listericidal response was associated with production of pediocin AcH by a Ped$^+$ starter culture.

In summary, the results of this study, as well as other work cited herein, suggest that some *P. acidilactici* starter cultures and pediocin-encoding plasmids are genetically related. However, the pediocin immunity genes are chromosomal in strain PAC1.0 (18) and presumably in PO2 and are plasmid borne in strain H (parental strain of JBL1095 [33]). Strain JBL1350 (Ped$^-$) was phenotypically similar to plasmid-cured derivatives (Ped$^-$) of PAC1.0 and PO2 because it remained resistant to pediocin despite the absence of the small pediocin plasmid; the location of the JBL1350 immunity/resistance gene(s) is presently unknown. Our data expanded upon previous reports and revealed that *P. acidilactici* strains JBL1095, PAC1.0, and PO2, as well as JBL1350, have identical *Asc*I genomic fingerprints. However, neither the amino acid sequences of the various pediocins nor the nucleotide sequences of the corresponding genes have been compared. These data argue strongly for additional studies to substantiate common parentage among strains. Our results also established that not all commercially available starter cultures encode pediocins or are equally effective in controlling *L. monocytogenes*. The fermentation of turkey sausage with a Ped$^+$ starter culture resulted in a dramatic

TABLE 3. Changes in pH and TA during manufacture and storage of turkey summer sausage inoculated with *L. monocytogenes* and/or *P. acidilactici* JBL1350 or JBL1095[a]

Time	Result for sausage inoculated with:															
	JBL1350 + *L. monocytogenes*				JBL1350				JBL1095 + *L. monocytogenes*				JBL1095			
	Trial 1		Trial 2		Trial 1		Trial 2		Trial 1		Trial 2		Trial 1		Trial 2	
	pH	TA	pH	TA	pH	TA	pH	TA	pH	TA	pH	TA	pH	TA	pH	TA
0[b]	6.14	0.46	6.44	0.43	6.14	0.46	6.44	0.43	6.31	0.48	6.41	0.44	6.31	0.48	6.41	0.44
During manufacture (h)																
0	6.14	0.47	6.48	0.43	6.11	0.47	6.49	0.43	6.39	0.42	6.41	0.41	6.39	0.41	6.35	0.44
4	5.72	0.57	6.22	0.48	5.82	0.55	6.21	0.46	6.17	0.48	5.99	0.51	6.19	0.51	6.00	0.52
8	4.94	0.81	5.75	0.58	4.93	0.80	5.70	0.58	5.53	0.60	5.41	0.60	5.48	0.59	5.44	0.60
12	—[c]	—	4.94	0.82	—	—	4.99	0.79	5.14	0.68	4.98	0.78	5.07	0.73	5.02	0.77
After shower	4.91	0.80	5.07	0.81	4.86	0.80	5.08	0.81	5.17	0.75	4.97	0.76	5.13	0.75	5.02	0.77
During storage (days)																
At 4°C																
1	4.94	0.78	5.07	0.85	4.97	0.77	5.13	0.84	5.18	0.76	4.96	0.79	5.13	0.78	5.02	0.77
7	4.97	0.94	5.13	0.88	4.96	0.93	5.14	0.89	5.17	0.77	5.04	0.90	5.11	0.79	5.20	0.78
60	5.05	0.88	5.20	0.94	5.02	0.90	5.21	0.96	—	—	5.12	0.95	5.25	0.82	5.17	0.91
At 25°C																
1	4.99	0.81	5.07	0.84	4.97	0.78	5.10	0.82	5.20	0.80	4.98	0.79	5.14	0.80	5.02	0.78
3	5.10	0.79	—	—	4.94	0.92	—	—	5.18	0.77	—	—	5.14	0.76	—	—
5	5.02	0.85	—	—	4.97	0.87	—	—	5.11	0.88	—	—	5.06	0.90	—	—
7	4.94	0.92	5.13	0.95	4.94	0.94	5.19	0.94	5.12	0.91	5.07	0.86	5.01	0.92	5.20	0.84

[a] Prior to stuffing, sausage batter was inoculated with a four-strain mixture of *L. monocytogenes* and/or JBL1350 (Ped$^-$) or JBL1095 (Ped$^+$).
[b] Values at time zero are initial values.
[c] —, not determined.

reduction in counts of *L. monocytogenes* during fermentation. More important, the recovery of pediocin AcH activity after 60 days of storage at 4°C established another hurdle for *L. monocytogenes* in the event of insufficient processing and/or postprocess contamination. Future efforts to construct and genetically characterize bacteriocinogenic pediococci for use as starter cultures will lead to strains with enhanced capabilities for ensuring the safety and extending the shelf life of foods.

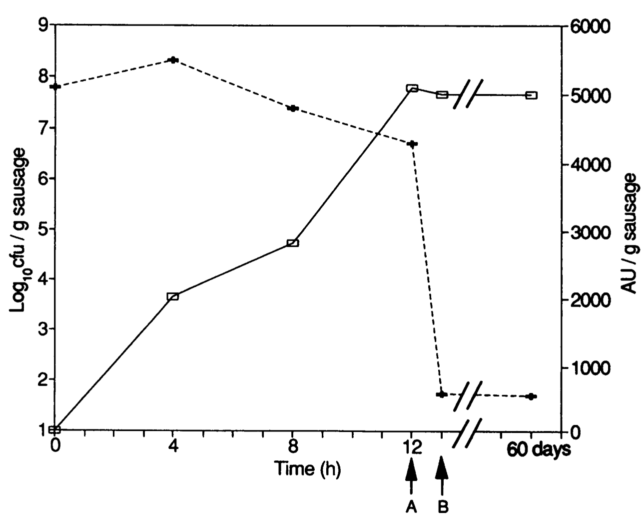

FIG. 4. Behavior of Ped$^+$ pediococci (average of two trials) and pediocin activity (duplicate sampling of trial 1) in turkey summer sausage. Open rectangles represent pediocin AcH produced during fermentation of sausage with JBL1095; thick crosses represent JBL1095 during fermentation of sausage. "A" signals the end of fermentation, and "B" signals the end of the cooking step.

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