APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1996, p. 2610–2614 Vol. 62, No. 7
0099-2240/96/$04.00+0

Control of Beef Spoilage by a Sulfide-Producing *Lactobacillus sake* Strain with Bacteriocinogenic *Leuconostoc gelidum* UAL187 during Anaerobic Storage at 2°C

JØRGEN J. LEISNER,[1] G. GORDON GREER,[2] AND MICHAEL E. STILES[1]*

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5,[1] and Lacombe Research Centre, Agriculture and Agri-Food Canada, Lacombe, Alberta, Canada T0C 1S0[2]

Received 6 February 1995/Accepted 16 April 1996

Chill-stored, vacuum-packaged beef inoculated with sulfide-producing *Lactobacillus sake* 1218 developed a distinct sulfide odor within 3 weeks of storage at 2°C, at which time the bacteria had reached maximum numbers of 10^6 CFU cm^{-2}. Coinoculation of the meat with the wild-type, bacteriocinogenic (Bac^+) strain of *Leuconostoc gelidum* UAL187 delayed the spoilage by *L. sake* 1218 for up to 8 weeks of storage. Coinoculation of meat samples with an isogenic, slowly growing Bac^+ variant, UAL187-22, or with the Bac^- variant UAL187-13 did not delay the onset of spoilage by *L. sake* 1218. The study showed that spoilage of chill-stored, vacuum-packaged beef by a susceptible target organism could be dramatically delayed by the Bac^+ wild-type strain of *L. gelidum* UAL187. Inoculation with *L. sake* 1218 can be used as a model system to determine the efficacy of biopreservation of vacuum-packaged meats.

Bacteriocinogenic strains of lactic acid bacteria (LAB) have been isolated from raw meat packaged under vacuum or in modified atmosphere, and considerable research has been done on their ability to inhibit growth of pathogenic microorganisms (1, 16, 21, 38). The potential of these bacteria to control growth of spoilage microorganisms, including the indigenous lactic microflora, has not been examined to the same extent; however, bacteriocinogenic LAB cultures have been successfully used to control the natural LAB microflora in Spanish-style green olive fermentations with *Lactobacillus plantarum* producing plantaricins S and T (28) and in sausage fermentations with *Lactobacillus curvatus* producing curvacin A (36). In contrast to the potential benefits of biopreservation, LAB can be significant spoilage agents of normal (pH 5.6), vacuum-packaged meat (11, 34). It has also been shown that they can cause spoilage of meat stored under aerobic conditions (20). Spoilage by LAB cannot be controlled by traditional preservation methods such as low temperature, anaerobic conditions, or addition of organic acids because the spoilage LAB of meats are psychrotrophic, facultatively anaerobic, and acid tolerant. Hence, their growth might best be controlled with bacteriocinogenic, nonspoiling LAB.

To be successful in biopreservation, a bacteriocinogenic LAB culture must compete with the relatively high indigenous microbial loads of raw meat, actively inhibit pathogenic and spoilage bacteria, and not cause undesirable organoleptic changes. We previously studied the production of leucocin A by *Leuconostoc gelidum* UAL187 that we isolated from vacuum-packaged meat in our laboratory (16). Leucocin A is bacteriostatic and active against a broad spectrum of LAB, and strains of *Listeria monocytogenes* and *Enterococcus* spp., but it is not active against *Brochothrix* spp., *Staphylococcus* spp., or gram-negative bacteria. Leucocin A has been well characterized (15, 35). It is a heat-stable, class II bacteriocin (19) with a molecular mass of 3.93 kDa. It is detected early in the growth cycle of the producer organism (16). Its production in meat during chilled storage was demonstrated (29), indicating that it is active and stable in the meat environment despite the fact that it is inactivated by common proteolytic enzymes of the intestinal tract (16). The production of leucocin A is plasmid mediated (15, 35). During the study of the genetic control of leucocin production, two variants of the wild-type strain were produced: UAL187-22, which is missing plasmid pLG5.0 but is bacteriocin positive (Bac^+); and UAL187-13, which is missing two plasmids, pLG5.0 and pLG7.2, and is bacteriocin negative (Bac^-).

We previously studied the effects of four bacteriocinogenic meat isolates of LAB, including *L. gelidum* UAL187-22 but not the wild-type strain UAL187, on the anaerobic and aerobic storage life of chilled beef (20). *L. gelidum* UAL187-22 was the strain of choice for use as an antagonistic competitive strain because it grows on beef at low storage temperatures without the development of undesirable changes in odor or appearance. It remains to be shown whether this relatively slowly growing organism competes efficiently with spoilage bacteria in meats. In this study, the biopreservative capabilities of bacteriocinogenic (Bac^+) and bacteriocin-negative (Bac^-) variants of *L. gelidum* UAL187 were studied by determining the abilities of these variants to control spoilage caused by a sulfide-producing strain of *Lactobacillus sake* inoculated onto vacuum-packaged beef.

MATERIALS AND METHODS

Bacterial cultures and identification of *L. sake* 1218. The LAB used in this study are listed in Table 1. *L. sake* 1218 is a sulfide-producing LAB isolated from modified-atmosphere-packaged pork stored at −1°C (21). The strain was initially identified by McMullen and Stiles (21) by standard techniques (24, 31). Its identity was confirmed in the present study by the following biochemical and cultural tests: no production of slime from sucrose, ability to grow on acetate agar (6), reduction of tetrazolium (37), final pH in La broth (27, 32), presence or absence of *meso*-diaminopimelic acid (18), sugar fermentation pattern (33), and lactic acid isomer determination by an enzymatic UV method (5). *L. sake* 1218 was tested for bacteriocinogenic activity against all of the *L. gelidum* strains by direct and deferred inhibition tests (1, 2).

Inhibition of *L. sake* 1218 by *L. gelidum* strains in APT broth. Growth rates of *L. gelidum* UAL187 and its variants were determined in pure culture at 2 and 25°C in APT broth (Difco Laboratories, Inc., Detroit, Mich.) containing 2% glucose or in modified APT broth (mAPT) made according to Difco's instructions (10) but containing 0.05 or 0.1% glucose inoculated at 4.2 to 4.3 log CFU ml^{-1}. The initial pH of APT broth was adjusted to 5.6 or 6.5. Competitive growth

* Corresponding author. Phone: (403) 492-2386. Fax: (403) 492-8914. Electronic mail address: mstiles@afns.ualberta.ca.

TABLE 1. Bacterial strains used in this study

Strain	Relevant phenotype and/or description[a]	Source or reference(s)
L. gelidum		
UAL187	Bac^+ wild-type strain with plasmids pLG5.0, pLG7.6, and pLG9.2	16, 35
UAL187-22	Bac^+ strain with plasmids pLG7.6 and pLG9.2	16
UAL187-13	Bac^- strain with plasmid pLG9.2	16
L. sake 1218	Sulfide-producing spoilage organism	McMullen[b]
C. divergens LV13	Leucocin A-sensitive indicator strain	NCFB 2855[c]

[a] Bac^+, bacteriocin producing; Bac^-, nonbacteriocinogenic.
[b] L. McMullen, University of Alberta.
[c] NCFB, National Collection of Food Bacteria, Institute for Food Research, Reading, United Kingdom.

studies of *L. gelidum* UAL187, UAL187-22, or UAL187-13 with *L. sake* 1218 were done in mAPT containing 0.1% glucose with its initial pH adjusted to 5.6. pH was determined for all samples during growth.

Inocula for all experiments were grown in APT broth at 25°C for 18 h. Cells were washed three times by centrifugation at 16,000 × *g*; washed with sterile, 0.1% peptone water; and resuspended in peptone water at the desired cell density. Samples for bacterial enumeration were diluted in 0.1% peptone water and surface streaked onto M5 agar, consisting of (in grams per liter) tryptone, 10; yeast extract, 5; fructose, 2.5; KH_2PO_4, 2.5; L-cysteine–HCl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; $MnSO_4 \cdot H_2O$, 0.05; calcium pantothenate, 0.01; and agar, 20; and (in milliliters per liter) Tween 80, 1; and bromocresol green (0.1 g in 30 ml of 0.01 N NaOH), 20 (40). This medium differentiated the heterofermentative *L. gelidum* colonies (white) from homofermentative *L. sake* 1218 colonies (blue). The two species could also be distinguished by their colony morphology on M5 agar. Representative colonies were checked by their phenotypic characteristics to determine the reliability of the differentiation (see below). MRS (Difco)-sorbic acid agar (3) was used for selective enumeration of *L. sake* 1218. Plates were incubated at 25°C for 3 days. Identical counts for *L. sake* 1218 were obtained on M5 and MRS-sorbic acid agars, whereas colonies of *L. gelidum* were observed only on M5 agar. Antimicrobial activity of leucocin A in the supernatant was assayed by the spot-on-lawn method (1, 2) with *Carnobacterium divergens* LV13 as the indicator strain. All experiments were done in duplicate.

Inoculation of beef samples. Sterile, lean slices of beef (surface area, 20 cm^2) were excised aseptically from normal-pH longissimus dorsi muscle as described by Greer and Jones (13). Beef slices were suspended from sterile clips, immersed for 15 s in a bacterial suspension containing 10^5 CFU of *L. gelidum* ml^{-1} and 10^3 CFU of *L. sake* ml^{-1}, and allowed to air dry at 25°C for 15 min. This produced an inoculum of approximately 10^4 CFU of *L. gelidum* cm^{-2} and 10^2 CFU of *L. sake* cm^{-2}. An equal number of beef slices was immersed in sterile, 0.1% peptone water for use as controls.

Beef storage. Three inoculated beef slices from each sample were placed in sterile stomacher bags (Seward Medical, London, United Kingdom), enclosed in gas-impermeable foil laminate bags (Printpac-UEB, Auckland, New Zealand), and vacuum packaged with a Captron II Packaging System (RMF Inc., Grandview, Mo.). Vacuum-packaged beef samples were stored at 2°C for 8 weeks, and samples were removed for analysis after 0, 1, 2, 3, 4, 4.5, 5, 6, and 8 weeks of storage. Three or four independent trials were done for microbiological content and sensory analysis of each combination of bacterial inocula, except for meat inoculated with pure cultures of *L. gelidum* UAL187, UAL187-22, and UAL187-13, for which only one trial was done.

Bacterial sampling and determination of antimicrobial activity on meat. At each sampling time, three beef slices from one package were homogenized separately in a Colworth Stomacher 400 (Canlab Division, Baxter Diagnostics Corp., Edmonton, Alberta, Canada) in 90 ml of sterile 0.1% peptone water. Samples were diluted and surface plated onto M5 and MRS-sorbic acid agar and incubated at 25°C for 3 days. The reliability of detection of the *Leuconostoc* strain was checked by the ability to produce slime on APT agar containing 2% sucrose. An average of eight colonies of each of the *L. gelidum* variants was picked from M5 agar plates from meat samples analyzed after 3 or 8 weeks of storage. These colonies were grown in APT broth for determination of their carbohydrate fermentation patterns (33), and some were also examined for plasmid profiles (2) and for bacteriocin production by overlayering with the indicator strain. After enumeration, M5 plates were overlayered with soft APT agar (0.75% agar) containing 1% of an overnight culture of *C. divergens* LV13 or *L. sake* 1218 to determine antimicrobial activity by the deferred inhibition test.

Production of bacteriocin during growth of the producer strain on beef was determined by a modification of the procedure described by Ruiz-Barba et al. (28). One beef slice was homogenized in 90 ml of 0.1% peptone water, heated in a boiling water bath for 15 min, cooled rapidly on ice, and centrifuged at 8,000 × *g* for 15 min. Ammonium sulfate (Fisher Scientific, Fair Lawn, N.J.) was added to 70% saturation, and the mixture was stirred at 4°C overnight and centrifuged at 20,000 × *g* for 1 h at 0.5°C. The precipitate was resuspended in 1.5 ml of sodium phosphate buffer (50 mM; pH 7.0), and activity was determined by the spot-on-lawn method (1, 2) using *C. divergens* LV13 as the indicator. The presence of bacteriocin was confirmed by adding 10 μl of pronase E (1 mg ml^{-1}; Sigma Chemical Co., St. Louis, Mo.) to appropriate samples of supernatant.

Sensitivity of *L. sake* 1218 to bacteriocin produced by *L. gelidum* UAL187. After 8 weeks of storage under vacuum at 2°C, one of the beef slices from each inoculum type was homogenized in 90 ml of sterile, 0.1% peptone water. Seventy-five microliters of liquid was withdrawn from each sample, mixed with 7.5 ml of soft MRS-sorbic acid agar (0.75% agar), and plated on MRS-sorbic acid agar (1.5% agar) for selective growth of *L. sake* 1218. Supernatants of APT broth cultures of *L. gelidum* UAL187 or UAL187-13 grown at 25°C for 18 h were adjusted to pH 6.5 with 1 N NaOH and heated at 65°C for 30 min. From these preparations, 20 μl of appropriate twofold dilutions was spotted onto the *L. sake* 1218 indicator lawns to be tested for sensitivity to the inhibitory substance. Plates were incubated anaerobically at 25°C overnight and observed for zones of inhibition.

Sensory assessment of beef samples. Qualitative analysis of odor acceptability, based on detection of sulfide odor in vacuum-packed beef samples, was done as described by McMullen and Stiles (22). An experienced five-member panel was used. Each packaged sample containing three slices of beef was filled with 200 ml of helium, and 5 ml of headspace gas was withdrawn for sensory analysis through a "sticky nickel" (Mocon Modern Controls, Inc., Minneapolis, Minn.) sampling port with a gastight syringe (SGE; Mandel Scientific, Guelph, Ontario, Canada) equipped with a button lock. Acceptability was judged by the absence or presence of sulfide odor. A sample was deemed spoiled if 50% or more of the panelists rejected the sample because of a sulfide odor.

RESULTS

Characterization and identification of *L. sake* 1218. The gram-positive, rod-shaped, catalase- and oxidase-negative strain 1218 was classified as *L. sake* on the basis of the following characteristics: no gas from glucose, no slime from sucrose, growth on acetate agar, degradation of arginine, tetrazolium not reduced, absence of *meso*-diaminopimelic acid in the cell wall, production of D- and L-lactic acid isomers, and final pH of <4.15 in La broth. This classification was also based on the following carbohydrate fermentation pattern: negative for amygdalin, cellobiose, inulin, inositol, lactose, maltose, mannitol, melezitose, raffinose, and salicin and positive for arabinose, fructose, glucose, mannose, melibiose, ribose, and sucrose. No acid was produced from glycerol or pyruvate. The organism grew in the presence of 6.5% NaCl but not at 45°C. Preliminary experiments showed that *L. sake* 1218 produced a strong sulfide odor when inoculated onto vacuum-packaged beef but not when inoculated onto beef stored under aerobic conditions. *L. sake* 1218 was not found to be bacteriocinogenic against any of the *L. gelidum* variants when tested by deferred and spot-on-lawn techniques. Colony color on M5 agar did not give reliable differentiation between the test strains; however, colony types were readily differentiated by their morphology. Counts of *L. sake* 1218 obtained by differential counting based on morphological differentiation on M5 agar and counts on MRS-sorbic acid agar were identical.

Inhibition of *L. sake* 1218 by *L. gelidum* strains in APT broth. At 25°C the three isogenic variants of *L. gelidum* UAL187 had identical doubling times of 1.64 h when grown as pure cultures or in combination with *L. sake* 1218. mAPT with an initial glucose concentration of 0.05, 0.1, or 2% or with the initial pH adjusted to 5.6 or 6.5 had no effect on the growth rate of the *L. gelidum* variants. At 2°C the initial doubling times for *L. gelidum* UAL187, UAL187-13, and UAL187-22 were similar, averaging 1.75 days, but after 4 to 8 days of incubation the doubling time of *L. gelidum* UAL187-22 increased to 3.15 days. This change in growth rate could not be attributed to glucose concentration or pH of the growth medium or to whether *L. gelidum* UAL187-22 was grown as a pure culture or together with *L. sake* 1218.

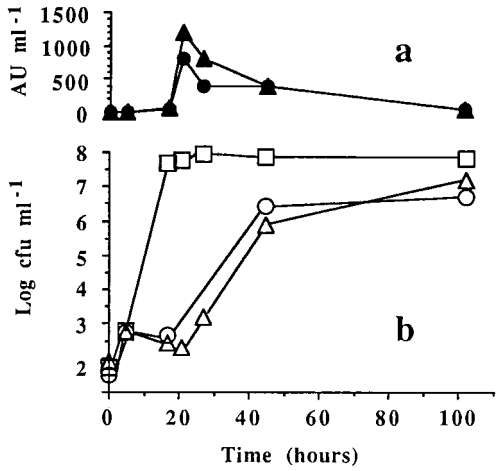

FIG. 1. Bacteriocin activity and growth of *L. sake* 1218 in mixed culture with variants of *L. gelidum* at 25°C in mAPT with 0.1% glucose and the initial pH adjusted to 5.6. (a) Bacteriocin activity in arbitrary units (AU) per milliliter of supernatant for mixed cultures of *L. sake* 1218 and *L. gelidum* UAL187 (▲) and mixed cultures of *L. sake* 1218 and *L. gelidum* UAL187-22 (●). (b) Growth of *L. sake* 1218 with *L. gelidum* UAL187 (△), *L. gelidum* UAL187-22 (○), and *L. gelidum* UAL187-13 (□).

L. sake 1218 grown in APT broth in mixed culture with *L. gelidum* UAL187 or UAL187-22 at 25°C was inhibited at the time (17 h) at which antimicrobial activity was detected in the supernatant (Fig. 1). Growth of *L. sake* 1218 resumed after 21 h, coinciding with a decrease in antimicrobial activity, and reached a population of approximately 10^7 CFU ml^{-1} after extended incubation for 100 h at 25°C (Fig. 1). *L. sake* 1218 grew rapidly in pure culture (results not shown) or in mixed culture with *L. gelidum* UAL187-13 (Fig. 1). Antimicrobial activity was not detected in these cultures.

Growth of *L. sake* 1218 in APT broth at 2°C was inhibited in mixed culture with *L. gelidum* UAL187 after 8 days of incubation, coinciding with the time at which antimicrobial activity was first detected in the supernatant (Fig. 2). The cell density of *L. sake* 1218 decreased to the minimum detection limit after

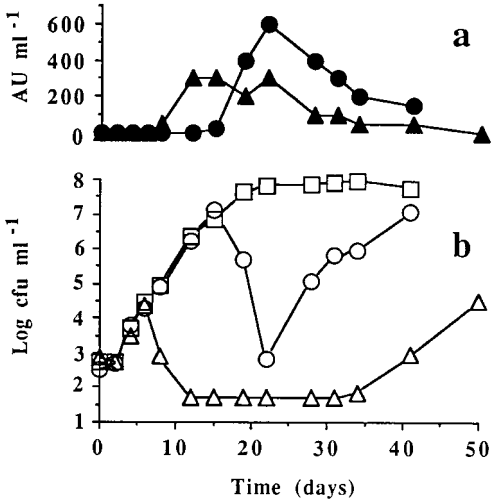

FIG. 2. Bacteriocin activity and growth of *L. sake* 1218 in mixed culture with variants of *L. gelidum* at 2°C in mAPT with 0.1% glucose and the initial pH adjusted to 5.6. See the legend to Fig. 1 for definitions of symbols.

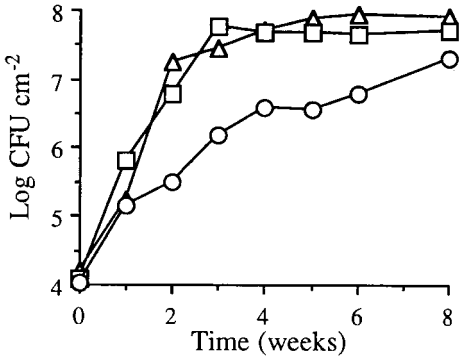

FIG. 3. Log_{10} CFU of variants of *L. gelidum* grown in mixed culture with *L. sake* 1218 per square centimeter of vacuum-packaged beef stored at 2°C. △, *L. gelidum* UAL187; □, UAL187-13; ○, UAL187-22. The data represent the means for three trials.

12 days of incubation, but growth resumed after approximately 30 to 35 days of storage (Fig. 2). *L. sake* 1218 grew rapidly at 2°C in pure culture (results not shown) and in mixed culture with *L. gelidum* UAL187-13 (Fig. 2). Antimicrobial activity was not detected in these cultures. *L. sake* 1218 in mixed culture with *L. gelidum* UAL187-22 grew actively for the first 15 days of incubation, after which a rapid decline in cell counts of *L. sake* 1218, coinciding with the detection of antimicrobial activity, was observed (Fig. 2). After 22 days of incubation there was a loss of antimicrobial activity and *L. sake* 1218 resumed its growth. The pH did not change by more than 0.2 units from the initial value in any of the experiments done with mAPT.

Growth of bacteria and detection of bacteriocin on vacuum-packaged beef. The data shown in Fig. 3 illustrate the growth of the three isogenic strains of *L. gelidum* UAL187 coinoculated with *L. sake* 1218 on beef stored under vacuum at 2°C. *L. gelidum* UAL187 and UAL187-13 again exhibited identical growth rates, while *L. gelidum* UAL187-22 grew at a considerably slower rate. The same result was obtained with *L. gelidum* strains inoculated onto meat as pure cultures. Growth and survival of *L. sake* 1218 alone or in mixed culture with the isogenic variants of *L. gelidum* are shown in Fig. 4. *L. sake* 1218 grew rapidly as a pure culture on vacuum-packaged beef, pro-

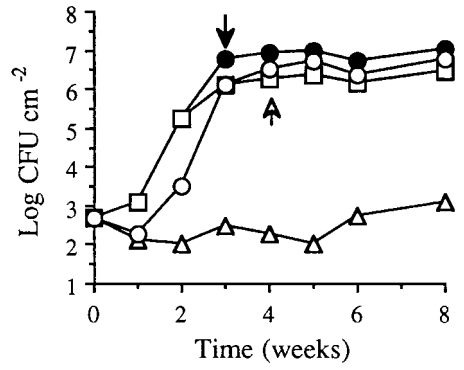

FIG. 4. Log_{10} CFU of *L. sake* 1218 showing growth and survival in mixed culture with variants of *L. gelidum* per square centimeter of vacuum-packaged beef stored at 2°C. ●, *L. sake* 1218 alone; △, *L. sake* with *L. gelidum* UAL187; □, *L. sake* with UAL187-13; ○, *L. sake* with UAL187-22. The solid arrow indicates the sampling time at which a sulfide odor was first detected in samples inoculated with *L. sake* 1218; the open arrow indicates the sampling time at which a sulfide odor was first detected in samples inoculated with *L. sake* 1218 and *L. gelidum* UAL187-13 or UAL187-22. The data represent the means for three trials.

ducing a sulfide odor within three weeks at 2°C. Marked inhibition of *L. sake* 1218 was observed in three of four trials in which *L. sake* 1218 was coinoculated with *L. gelidum* UAL187 onto meat. There was a delay of growth for 5 weeks with a 4-log-lower count of *L. sake* 1218 after 8 weeks of incubation. In a fourth trial, there was a delay of 2 weeks before initiation of growth of *L. sake* 1218, and a relatively low maximum count of 10^5 to 10^6 log CFU cm^{-2} was observed. These data were not included in the means calculated for Fig. 4. Similar growth of *L. sake* 1218 but with a maximum count approximately 1 log lower than that in pure culture was observed when *L. sake* 1218 was coinoculated with *L. gelidum* UAL187-13. A slight delay in initiation of growth and a reduction of 0.5 to 1 log unit in the maximum count were observed when *L. sake* 1218 was coinoculated with *L. gelidum* UAL187-22. Comparison with pure culture studies indicated that growth of *L. gelidum* UAL187 and its isogenic variants was not affected by the presence of *L. sake* 1218 in any trial. The identity of each variant of *L. gelidum* was confirmed by comparison of plasmid profiles, carbohydrate fermentation patterns, and production of slime by colonies isolated after 8 weeks of storage from each experiment.

The possibility that *L. sake* 1218 developed resistance to the inhibitory substance during the trial with extended growth in the presence of *L. gelidum* UAL187 was tested. Spot-on-lawn tests of isolates of *L. sake* 1218 were done after 8 weeks of storage. Results showed that *L. sake* 1218 was sensitive to 800 arbitrary units ml^{-1} in heat-treated supernatant of *L. gelidum* UAL187 grown in APT broth. The same sensitivity was observed for isolates of *L. sake* 1218 grown as pure cultures or in mixed culture with *L. gelidum* UAL187-22 or UAL187-13. Growth of *L. sake* 1218 with extended incubation was apparently due to loss of activity of the inhibitory substance rather than development of resistant strains of *L. sake* 1218.

Antimicrobial activity that was sensitive to pronase E was demonstrated for extracts prepared from beef samples coinoculated with *L. gelidum* UAL187 and *L. sake* 1218. The antibacterial activity on the meat persisted from 2 to 8 weeks of storage, but the level of activity was near the lowest detectable limit and activity could not be detected on all samples that were tested. At least half of the trials were positive at each sampling time. Antimicrobial activity was also observed on beef coinoculated with *L. gelidum* UAL187-22 and *L. sake* 1218 after 6 weeks of storage. No activity was observed for beef coinoculated with *L. gelidum* UAL187-13 and *L. sake* 1218. *L. gelidum* UAL187 and UAL187-22 were found to have retained their bacteriocinogenic potential when tested at each storage interval for antagonistic activity by the deferred inhibition test.

Detection of spoilage of beef samples. *L. gelidum* UAL187 completely inhibited sulfide-mediated spoilage of beef by *L. sake* 1218 for up to 8 weeks, except in two of four trials, in which spoilage was detected in samples taken at 4.5 weeks but not at 6 and 8 weeks of storage at 2°C. Spoilage produced by *L. sake* 1218 in the presence or absence of *L. gelidum* UAL187-22 or UAL187-13 was detected within 3 to 4.5 weeks of storage and is illustrated by arrows in Fig. 4. No spoilage was detected in beef samples inoculated with pure cultures of *L. gelidum* UAL187, UAL187-22, or UAL187-13 and stored for up to 8 weeks under vacuum at 2°C (data not shown).

DISCUSSION

In our previous study (20) we demonstrated that inoculation of beef with the bacteriocinogenic LAB *L. gelidum* UAL187-22 did not spoil beef stored under aerobic or anaerobic conditions for extended periods. This might have been the result of biphasic growth of UAL187-22 that resulted in slower overall growth of this strain on meat stored at 2°C. The reason for biphasic and ultimately slow growth of UAL187-22 at this low temperature could not be determined; however, a transformed variant of *L. gelidum* UAL187-13 containing an 8-kb insert with the active gene for leucocin A (data not shown) also exhibited the reduced growth rate at 2°C. Both *L. gelidum* UAL187-22 and the transformed variant of UAL187-13 lacked a small plasmid with a molecular mass of 5.0 MDa which was present in *L. gelidum* UAL187 (16). This small plasmid might account in some way for the difference in growth rate between the wild-type strain UAL187 and the partially cured variant UAL187-22.

L. sake 1218 can be used as a model organism for testing the efficacy of added bacteriocins or bacteriocinogenic LAB in preventing spoilage of vacuum-packaged meats. On vacuum-packaged beef it produced a readily detectable sulfide odor within 3 weeks when it was inoculated at 10^3 CFU cm^{-2} and stored at 2°C. This occurred well in advance of spoilage by the normal adventitious lactic microflora of meats (8, 11). Development of a sulfide odor was reported as a form of spoilage by some adventitious LAB on meat (12, 34). Inhibition of growth and sulfide-mediated spoilage by *L. sake* 1218 in vacuum-packaged beef by *L. gelidum* UAL187 can be attributed to the rapid growth of and early production of bacteriocin by this strain. This was confirmed by the rapid growth and spoilage observed for *L. sake* 1218 when coinoculated with the fast-growing isogenic Bac^- variant *L. gelidum* UAL187-13. Slow growth of and delayed production of bacteriocin by *L. gelidum* UAL187-22 are considered to be the reason why this variant failed to inhibit development of the sulfide odor by *L. sake* 1218. The possibility exists that a mixed culture of the rapidly and slowly growing strains of *L. gelidum* UAL187 and UAL187-22 could further delay the growth of *L. sake* 1218 because of the potential for sustained inhibitory concentrations of the inhibitory substance in the meat.

Nisin cannot be used as a preservative in meats because it is inactivated at the pH of meat (17, 25) and has limited activity on beef (7). *Lactococcus lactis* is a mesophile, and so production of nisin in situ in chilled meat is not possible. Although bacteriocins produced by psychrotrophic LAB of meat origin have a greater potential to exert an inhibitory effect on spoilage LAB in meats, the effect of the meat environment on their activity remains to be studied. Bacteriocins might be sensitive to proteases of meat or microbial origin, as suggested by the observed loss of activity of sakacin A produced by *L. sake* Lb706 in minced meat at 8°C (30). In the present study, the inhibitory substance was produced and remained active in the meat throughout the 8-week storage period at 2°C. In contrast, the bacteriocin was partially inactivated during prolonged storage in APT broth. In beef stored at 2°C, resistance of *L. sake* 1218 cells to the inhibitory substance was not detected after 8 weeks of storage. Development of resistance of target organisms such as *L. monocytogenes* to bacteriocins has been noted for sakacin A (30); nisin (9, 14, 23); and mesenterocin 52, curvaticin 13, and plantaricin C19 (26).

Yousef et al. (39) showed that pediocin AcH (PA-1) was less effective against *L. monocytogenes* in wiener exudates stored at 25°C than it was in those stored at 4°C. A similar effect was noted for bacteriocinogenic *Pediococcus acidilactici* against *L. monocytogenes* in frankfurters (4). The efficacy of leucocin A produced by *L. gelidum* UAL187 at abusive storage temperatures remains to be determined. The results obtained in the present study with mixed cultures in APT broth incubated at 25°C indicated that leucocin might be active for shorter periods at abusive storage temperatures.

In this study, even the quite extensive growth of *L. sake* 1218

in the presence of *L. gelidum* UAL187 in one of four trials did not result in spoilage of the beef. This result indicated that addition of bacteriocinogenic *L. gelidum* UAL187 as a protective culture to meat could delay spoilage by an overt spoilage organism, such as *L. sake* 1218. This study illustrated the potential for *L. gelidum* UAL187 to act as a microbial antagonist on meat against the adventitious spoilage microflora of LAB. Current work is aimed at expanding the application of *L. gelidum* UAL187 to preserve other meat products and to study its activity against other spoilage and pathogenic microorganisms on meat, such as *Brochothrix thermosphacta* and *L. monocytogenes.*

ACKNOWLEDGMENTS

We gratefully acknowledge Lynn McMullen for the partial identification of *L. sake* 1218 and Brian Dilts (Lacombe Research Centre) and Alison Poon for their valued assistance.

Financial support for this study was received from the Cargill Research Fund, the Alberta Cattle Commission, and the Alberta Agricultural Research Institute.

REFERENCES

1. **Ahn, C., and M. E. Stiles.** 1990. Antibacterial activity of lactic acid bacteria isolated from vacuum packaged meats. J. Appl. Bacteriol. **60:**302–310.
2. **Ahn, C., and M. E. Stiles.** 1990. Plasmid-associated bacteriocin production by a strain of *Carnobacterium piscicola* from meat. Appl. Environ. Microbiol. **56:**2503–2510.
3. **Anonymous.** 1987. de Man, Rogosa and Sharpe agar with sorbic acid (MRS-S agar). Int. J. Food Microbiol. **5:**230–232.
4. **Berry, E. D., R. W. Hutkins, and R. W. Mandigo.** 1991. The use of bacteriocin-producing *Pediococcus acidilactici* to control postprocessing *Listeria monocytogenes* contamination of frankfurters. J. Food Prot. **54:**681–686.
5. **Boehringer Mannheim Biochemicals.** 1987. Biochemica information, p. 45. Boehringer Mannheim Biochemicals, Indianapolis, Ind.
6. **Cavett, J. J.** 1963. A diagnostic key for identifying the lactic acid bacteria of vacuum packed bacon. J. Appl. Bacteriol. **26:**453–470.
7. **Chung, K.-T., J. S. Dickson, and J. D. Crouse.** 1989. Effects of nisin on growth of bacteria attached to meat. Appl. Environ. Microbiol. **55:**1329–1333.
8. **Dainty, R. H., and B. M. Mackey.** 1992. The relationship between the phenotypic properties of bacteria from chill-stored meat and spoilage processes. J. Appl. Bacteriol. Symp. Suppl. **73:**103S–114S.
9. **Davies, E. A., and M. R. Adams.** 1994. Resistance of *Listeria monocytogenes* to the bacteriocin nisin. Int. J. Food Microbiol. **21:**341–347.
10. **Difco Laboratories, Inc.** 1984. Difco manual, 10th ed., p. 1071. Difco Laboratories, Inc., Detroit.
11. **Egan, A. F.** 1983. Lactic acid bacteria of meat and meat products. Antonie Leeuwenhoek **49:**327–336.
12. **Egan, A. F., B. J. Shay, and P. J. Rogers.** 1989. Factors affecting the production of hydrogen sulphide by *Lactobacillus sake* L13 growing on vacuum packaged beef. J. Appl. Bacteriol. **67:**255–262.
13. **Greer, G. G., and S. D. M. Jones.** 1991. Effects of lactic acid and vacuum packaging on beef processed in a research abattoir. Can. Inst. Food Sci. Technol. J. **24:**161–168.
14. **Harris, L. J., H. P. Fleming, and T. R. Klaenhammer.** 1991. Sensitivity and resistance of *Listeria monocytogenes* ATCC 19115, Scott A and UAL500 to nisin. J. Food Prot. **54:**836–840.
15. **Hastings, J. W., M. Sailer, K. Johnson, K. L. Roy, J. C. Vederas, and M. E. Stiles.** 1991. Characterization of leucocin A-UAL 187 and cloning of the bacteriocin gene from *Leuconostoc gelidum*. J. Bacteriol. **173:**7491–7500.
16. **Hastings, J. W., and M. E. Stiles.** 1991. Antibiosis of *Leuconostoc gelidum* isolated from meat. J. Appl. Bacteriol. **70:**127–134.
17. **Hurst, A.** 1981. Nisin. Adv. Appl. Microbiol. **27:**85–123.
18. **Kandler, O., and N. Weiss.** 1986. Regular, nonsporing gram-positive rods, p. 1208–1260. *In* P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. Williams and Wilkins, Baltimore.
19. **Klaenhammer, T. R.** 1993. Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiol. Rev. **12:**39–86.
20. **Leisner, J. J., G. G. Greer, B. D. Dilts, and M. E. Stiles.** 1995. Effect of growth of selected lactic acid bacteria on storage life of beef stored under vacuum and in air. Int. J. Food Microbiol. **26:**231–243.
21. **McMullen, L. M., and M. E. Stiles.** 1993. Microbial ecology of fresh pork stored under modified atmosphere at −1, 4.4 and 10°C. Int. J. Food Microbiol. **18:**1–14.
22. **McMullen, L. M., and M. E. Stiles.** 1994. Quality of fresh retail pork cuts stored in modified atmosphere under temperature conditions simulating export to distant markets. Meat Sci. **38:**163–177.
23. **Ming, X., and M. A. Daeschel.** 1993. Nisin resistance of foodborne bacteria and the specific resistance responses of *Listeria monocytogenes* Scott A. J. Food Prot. **56:**944–948.
24. **Montel, M.-C., R. Talon, J. Fournaud, and M.-C. Champomier.** 1991. A simplified key for identifying homofermentative *Lactobacillus* and *Carnobacterium* spp. from meat. J. Appl. Bacteriol. **70:**469–472.
25. **Ramseier, H. R.** 1960. Die Wirkung von Nisin auf *Clostridium butyricum* Prazm. Arch. Mikrobiol. **37:**57–94.
26. **Rekhif, N., A. Atrih, and G. Lefebvre.** 1994. Selection and properties of spontaneous mutants of *Listeria monocytogenes* ATCC 15313 resistant to different bacteriocins produced by lactic acid bacteria strains. Curr. Microbiol. **28:**237–241.
27. **Reuter, G.** 1970. Laktobazillen und eng verwandte Mikroorganismen in Fleisch und Fleischerzeugnissen. 2. Mitteilung: die Charakterisierung der isolierten Laktobazillenstämme. Fleischwirtsch. **50:**954–962.
28. **Ruiz-Barba, J. L., D. P. Cathcart, P. J. Warner, and R. Jiménez-Díaz.** 1994. Use of *Lactobacillus plantarum* LPCO10, a bacteriocin producer, as a starter culture in Spanish-style green olive fermentations. Appl. Environ. Microbiol. **60:**2059–2064.
29. **Saucier, L., L. McMullen, and M. E. Stiles.** Unpublished data.
30. **Schillinger, U., M. Kaya, and F.-K. Lücke.** 1991. Behaviour of *Listeria monocytogenes* in meat and its control by a bacteriocin-producing strain of *Lactobacillus sake*. J. Appl. Bacteriol. **70:**473–478.
31. **Schillinger, U., and F.-K. Lücke.** 1987. Identification of lactobacilli from meat and meat products. Food Microbiol. **4:**199–208.
32. **Shaw, B. G., and C. D. Harding.** 1984. A numerical taxonomic study of lactic acid bacteria from vacuum packed beef, pork, lamb and bacon. J. Appl. Bacteriol. **56:**25–40.
33. **Shaw, B. G., and C. D. Harding.** 1985. Atypical lactobacilli from vacuum packaged meats: comparison by DNA hybridization, cell composition and biochemical tests with a description of *Lactobacillus carnis* sp. nov. Syst. Appl. Microbiol. **6:**291–297.
34. **Shay, B. J., and A. F. Egan.** 1981. Hydrogen sulphide production and spoilage of vacuum-packed beef by a *Lactobacillus*, p. 241–251. *In* T. A. Roberts, G. Hobbs, J. H. B. Christian, and N. Skovgaard (ed.), Psychrotrophic microorganisms in spoilage and pathogenicity. Academic Press, Inc., New York.
35. **van Belkum, M. J., and M. E. Stiles.** 1995. Molecular characterization of genes involved in the production of the bacteriocin leucocin A from *Leuconostoc gelidum*. Appl. Environ. Microbiol. **61:**3573–3579.
36. **Vogel, R. F., B. S. Pohle, P. S. Tichaczek, and W. P. Hammes.** 1993. The competitive advantage of *Lactobacillus curvatus* LTH 1174 in sausage fermentations is caused by formation of curvacin A. Syst. Appl. Microbiol. **16:**457–462.
37. **Wilkinson, B. J., and D. Jones.** 1977. A numerical taxonomic survey of *Listeria* and related bacteria. J. Gen. Microbiol. **98:**399–421.
38. **Winkowski, K., A. D. Crandall, and T. J. Montville.** 1993. Inhibition of *Listeria monocytogenes* by *Lactobacillus bavaricus* MN in beef systems at refrigeration temperatures. Appl. Environ. Microbiol. **59:**2552–2557.
39. **Yousef, A. E., J. B. Luchansky, A. J. Degnan, and M. P. Doyle.** 1991. Behavior of *Listeria monocytogenes* in wiener exudates in the presence of *Pediococcus acidilactici* H or pediocin AcH during storage at 4 or 25°C. Appl. Environ. Microbiol. **57:**1461–1467.
40. **Zúñiga, M., I. Pardo, and S. Ferrer.** 1993. An improved medium for distinguishing between homofermentative and heterofermentative lactic acid bacteria. Int. J. Food Microbiol. **18:**37–42.