Isolation and Selection of Lactic Acid Bacteria as Biocontrol Agents for Nonacidified, Refrigerated Pickles

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ABSTRACT: A nonacidified, deli-type pickle product was used as a model system to study the potential use of biocontrol as a means to prevent the growth of pathogens in minimally processed fruits and vegetables (MPFV). Fresh pickling cucumbers were blanched and brined with sterile spices and garlic oil. The product was stored at 5 °C for 3 wk and then transferred to various abuse temperatures (16 °C, 25 °C, 30 °C). Lactic acid bacteria (LAB) were isolated and characterized as potential biocontrol agents, and the isolates were tested for bacteriocin-like activity. A total of 118 LAB isolates were obtained. Among the LAB identified were species of *Lactococcus*, *Leuconostoc*, *Lactobacillus*, *Weissella*, and *Enterococcus*. Three isolates showed transient bacteriocin activity against—*Listeria monocytogenes*, and 7 isolates (*Lactococcus*) had bacteriocin-like activity against other LAB. Although it did not produce a bacteriocin, a *Lactobacillus curvatus* isolate (LR55) was found to have desirable characteristics for use as a biocontrol (competitive exclusion) culture to enhance the safety of nonacidified deli-type pickles.

Keywords: lactic acid bacteria, minimally processed vegetables, bacteriocin, biocontrol

Introduction

Refrigerated cucumber pickles have gained in popularity over the past few decades (currently estimated at about 25% of all pickle sales) because they retain crispness and other quality characteristics compared with shelf-stable, heat-processed, fermented pickles. To manufacture refrigerated pickles, the cucumbers are washed and packed in brine with appropriate spices. Equilibrated NaCl concentrations range from 1% to 3%. Unlike shelf-stable pickles, they are usually not heat-processed. The shelf life of refrigerated pickles depends on the refrigeration temperature and whether vinegar (acetic acid) and preservatives (for example, sodium benzoate) are added to the brine. Characteristics of the products at the end of their shelf life can include an increase in turbidity and acidity of the brine, gas formation, and a change in color of the cucumbers from green to olive. The shelf life of refrigerated pickles varies and is about 3 wk when vinegar and sodium benzoate are not added. The shelf life is extended to about 3 mo with these ingredients if the temperature is maintained at 2 °C to 5 °C. The acidified type of product typically contains 0.2% to 0.4% acetic acid and 0.1% sodium benzoate, with a brine pH well below 4.6 and typically 4.2 or less. Nonacidified refrigerated pickles, which are the subject of this study, have an initial pH of about 5.5.

Etchells and Bell (1976) reviewed a wide range of refrigerated pickle products, including both nonacidified and acidified types. They cited the popularity of the nonacidified type, especially in metropolitan areas such as New York City where they are identified by names such as "overnight," "half-sour," "Kosher new dills," and "half-sour new pickles." The nonacidified products may undergo fermentation either intentionally or unintentionally. When fermentation is intended, the brined product is held out of refrigeration for a few days to allow slight fermentation before packing into wholesale or retail containers and placing them under refrigeration. When brine turbidity becomes obvious in refrigerated pickles, lactic acid bacteria (LAB) are primarily responsible for the turbidity (Reina 2003). Because the product is not acidified or heat-treated, has a relatively high pH (about 5.5), and contains fresh vegetable ingredients, the safety of this type of product has been a concern, although there are no known instances of food-borne illness by such products. The number of LAB in or on fresh cucumber fruit is variable, depending on harvest season, fruit origin, and fruit size (Etchells and Jones 1943). *Listeria monocytogenes* has been shown to grow when inoculated into product with low numbers of LAB under laboratory conditions (Romick 1994). If these products were inoculated with a LAB culture, it may be possible to prevent the growth or survival of pathogenic bacteria by competitive exclusion or biocontrol (Breidt and Fleming 1997).

Despite efforts to develop protective cultures of LAB as biocontrol agents in minimally processed fruits and vegetables (MPFV) in recent years (Bennik and others 1999; Leroy and others 2002; Palmai and Buchanan 2002), few cultures have been identified for use in commercial food products. Satisfactory performance under optimal laboratory conditions is no guarantee for success when potential biocontrol microorganisms are applied to foods under typical processing conditions. The ecological adaptation and growth characteristics of cultures in food products will determine their effectiveness as biocontrol cultures. Bacterial cultures isolated from the same type of vegetable or product in which they will be subsequently used as biocontrol agents may have the greatest chance of success in controlling pathogens (Vescovo and others 1996; Breidt and Fleming 1997). The objectives of this research were to select, isolate, and characterize naturally occurring LAB from nonacidified brined cucumbers suitable for use as biocontrol agents in nonacidified refrigerated pickle products.

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Materials and Methods

Deli-type pickle product

Cucumbers (3.5- to 3.8-cm dia) were washed and blanched for 15 s at 80 °C (Breidt and others 2000) and immediately placed in 46-oz glass jars containing 680 mL cold (5 °C), sterile brine prepared in an autoclave (4% NaCl, to equilibrate at 2% NaCl) with 1 g of sterile garlic oil (Aquaresin garlic, Kalsec, Kalamazoo, Mich., U.S.A.) and 5 g of sterile, irradiated pickling spices (Ba-Tempte, Brooklyn, N.Y., U.S.A.). We have previously found that the Aquaresin garlic oil contains no detectable microbial flora (data not shown). This procedure significantly reduces the total microflora and increases shelf life, but does not eliminate the LAB and other microflora typically present in nonacidified refrigerated pickle products (Breidt and others 2000). Spices were irradiated with a dose of 13 kGy in plastic containers of 1-L capacity by using Co^{60} (Nuclear Engineering Dept., NCSU, Raleigh, N.C., U.S.A.).

Jars were refrigerated at 5 °C for 3 wk and then transferred to various abuse temperatures (16 °C, 25 °C, 30 °C). Brine samples were appropriately diluted in sterile saline solution (0.85% NaCl) and plated on De Man, Rogose, and Sharpe (MRS) agar (Difco, Detroit, Mich., U.S.A.) supplemented with 0.02% sodium azide (modified MRS or MMRS) to select for LAB. Bacteria were enumerated by automated plating using a spiral plating machine (Autoplate 4000, Spiral Biotech, Bethesda, Md., U.S.A.) and an automated plate reader (QCount, Spiral Biotech). Isolated colonies with different morphology were re-streaked on MRS agar.

Samples of blanched and unblanched cucumbers were also taken to enumerate the initial load of LAB. Cucumbers (200 g) were blended with sterile saline solution (0.85% NaCl) for 2 min at high speed. Blended samples were mixed in a stomacher for 1 min on the "high" setting (Stomacher 400, Spiral Biotech); the cell suspension was removed from the filter side of the stomacher bags (Spiral Biotech) and plated. For enumerating low (<100 colony-forming units [CFU]/g) numbers of LAB, 3 different methods of plating were used: spread plating (0.1 mL) using a glass spreader, membrane-filtration using bottle filters (100 mL samples, Millipore Corp., Bedford, Mass., U.S.A.), and pour plating (1 mL samples) on MMRS agar.

Bacterial cultures and identification methods

Bacteria used in this study, in addition to the isolates described subsequently, are listed in Table 1. LAB were grown overnight (15 h) at 30 °C in MRS broth. *Escherichia coli* K12 and *Pseudomonas fluorescens* were grown overnight at 37 °C in Tryptic Soy Glucose (TSG) broth. LAB isolates were grown on MRS agar and tested for gas production from glucose in MRS broth containing 1% added glucose with Durham tubes. Fermentative metabolism (homofermentor or heterofermentor) was determined with Heterolactic-Homolactic Differentiation (HHD) medium (McDonald and others 1987). Catalase reaction was determined by the 3% H_2O_2 method (Paludan-Muller and others 1999) and cytochrome oxidase by Dry Slide Oxidase strips (Difco). Presumptive LAB isolates (catalase and oxidase negative Gram-positive bacteria from the MRS agar plates) were further characterized by a polymerase chain reaction (PCR) method (Breidt and Fleming 1996) using the 16S-23S intergenic transcribed spacer (ITS) region, followed by restriction with *Rsa*I (ITS-PCR). Primers for the PCR were G1-16S (5′ GAAGTCGTAACAAGG 3′) and L2-23S (5′ GGGTTTCCCCATTCGGA 3′). PCR was performed using a Robocycler Gradient 96 (Stratagene, La Joya, Calif., U.S.A.). Representative isolates from each group were further characterized by sequencing variable regions in the 1st 350 bases from the 5′ end the 16S rRNA gene (Barrangou and others 2002) using primers RU-16S (5' AGAGTTTGATCCTGGCTCAG 3′) and RD-16S (5′ GTCTCAGTCCCAATGTGGCC 3′). All primers were obtained from Sigma-Genosys (The Woodlands, Tex., U.S.A.). PCR products were purified before sequencing using a Wizard SV PCR purification kit (Promega, Madison, Wis.), and sequencing was done by Davis Sequencing (Davis, Calif., U.S.A.). Biochemical identification was done by a microplate method (AN Anaerobic Microplate, Biolog, Hayward, Calif., U.S.A.) or the API 50 CHL kit (Biomerieux, Hazelwood, Mich., U.S.A.) in accordance with the manufacturer's instructions.

Table 1—Bacterial strains used in this study

Strain	Source, strain identification
Lactobacillus plantarum LA70	ATCC,[a] 14917
Lactobacillus plantarum BI7	FFL[b]
Pediococcus dextrinicus LA77	ATCC, 33087
Pediococcus pentosaceus LA61	ATCC, 432001
Leuconostoc mesenteroides LA81	ATCC, 8293
Listeria monocytogenes B164 serotype 4b	FFL
Listeria monocytogenes B181 serotype 4b	SK[c]
Listeria monocytogenes B182 serotype 4b	SK
Listeria monocytogenes B183 serotype 1/2a	SK
Listeria monocytogenes B184 serotype 1/2b	SK
Escherichia coli B41	FFL
Pseudomonas fluorescens B14	FFL
Lactococcus lactis LA218	FFL

[a]American Type Culture Collection.
[b]USDA Food Fermentation Laboratory Culture Collection, Raleigh, N.C.
[c]Dr. S. Kathariou, NCSU, Raleigh, N.C.

Bacteriocin assay

Screening for bacteriocin production was carried out using an agar overlay spot test method (Fleming and others 1975). Cultures were grown overnight (15 h) in MRS broth at 30 °C and harvested by centrifugation (13000 × *g*, 10 min). The supernatant was adjusted to pH 7 with NaOH, boiled for 1 min at 100 °C to select for thermostable bacteriocins, and filter sterilized. For overlay plates, 10 μL of the cell free supernatant was spotted onto MRS agar or TSG (trypticase soy agar plus 1% glucose) agar plates and allowed to dry. The plates were then overlaid with 3 mL of soft agar (0.75% agar) containing approximately 10^6 CFU/mL cells from a turbid overnight culture of the indicated target organisms (discussed subsequently). The plates were incubated 24 h at 30 °C or 37 °C and checked for inhibition zones around the spot area after 24 h. A positive control supernatant spot from a known nisin producer, *Lactococcus lactis* LA218, was included in each test. In addition to 30 °C incubation, plates with *Listeria* as the indicator organism were incubated at 5 °C for 15 d.

Growth rate determinations and biocontrol experiments

Growth rates of LAB and *L. monocytogenes* B164 (serotype 4b) were determined using an automated microplate reader (Model EL_x808_{IU} Bio-Tek Microplate reader with Kineticalc V2.0 Software, Bio-Tek, Winooski, Vt.) at A_{600} nm optical density (OD). Rates were calculated from OD data using a Gompertz model with a custom nonlinear curve fitting software package (F. Breidt, unpublished). Experiments were done in a final volume of 200 μL with cells from an overnight culture inoculated to an initial A_{600} of 0.05 (approx. 1×10^7 CFU/mL) in cucumber juice medium containing 2% or 4% salt at 5 °C, 10 °C, 15 °C, and 21 °C. Cucumber juice medium (Breidt and others 2004) consisting of 60% cucumber juice and 40% water was filter-sterilized and stored at 5 °C until use. For growth curves and biocontrol experiments, cells were incubated in 15-mL

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Table 2—Bacteriological attributes and putative identification of lactic acid bacteria (LAB) isolates from brined, refrigerated cucumbers[a]

Microscopic morphology	HHD[b]	Temperature (°C)[c]	ITS pattern	AN plate[d]	API 50 CHL[d,e]	16S rRNA sequencing[f]
Cocci	–	25	*Enterococcus*	No ID	No ID	*E. casseliflavus* (98)
Cocci	–	25	*Enterococcus*	No ID	No ID	*E. durans* (98)
Cocci	–	25	*Enterococcus*	No ID	No ID	*E. mundtii* (98)
Cocci, chains	–	16, 18, 25	*Lactococcus*	*L. lactis*	*L. lactis*	*L. lactis lactis* (98)
Coccobacilli	+	16, 25	*Leuconostoc*	*L. citreum*	ND	*L. citreum* (97.5)
Coccobacilli	+	5, 18	*Leuconostoc*	*L. mesenteroides*	*L. mesenteroides*	*L. mesenteroides* (98)
Short rods	–	5	*Lactobacillus*	ND	*L. curvatus*	*L. curvatus* (98)
Short rods, chains	–	18	*Lactobacillus*	ND	*L. plantarum*	*L. plantarum* (98)
Cocci, pairs	–	5	*Pediococcus*	ND	ND	*P. pentosaceus* (96.5)
Coccobacilli (96.5)	+	18, 25	*Weissella*	*W. confusa*	*L. coprophilus*	*W. confusa/kimchi*

[a]AN = Anaerobic Microplate (Biolog); HHD = Heterolactic-Homolactic Differentiation; ITS = intergenic transcribed spacer.
[b]Fermentative metabolism on the HHD medium: – = homofermentative; + = heterofermentative.
[c]Temperature (°C) at which cultures were isolated.
[d]No ID = no identification; ND = not determined.
[e]Two isolates were identified as *Lactococcus (L). lactis raffinolactis* by API50 CHL kit.
[f]Numbers in parentheses indicates the % of similarity of the 300 bp of the 16S (PCR) product.

plastic screw cap test tubes with similarly prepared cells (as discussed previously) in a 10-mL final volume. Tubes were placed in a heating-cooling waterbath (Neslab, RTE-211, Neslab Instruments, Inc., Newington, N.H., U.S.A.). For temperature shift experiments, bath temperature was adjusted as indicated. Cells were enumerated by plating at appropriate intervals on MRS (LAB) or TSG agar (*Listeria*). All assays were conducted with 2 or more independent replications.

Statistical analysis

The analysis of variance was computed by the General Linear Models Procedure of SAS version 8.0 (SAS Inc., Cary, N.C., U.S.A.).

Results and Discussion

Microbiology of deli-type pickles

The total aerobic plate count on the cucumbers were between 10^3 and 10^4 CFU/g after blanching. LAB dominated the microflora of the deli-style pickles as fermentation occurred, even though they represented only about 1% of the total microbial count (10 to 100 CFU/g) in the cucumbers after blanching. Turbidity of the brine for samples at 5 °C typically occurred at 5 wk or longer after brining. In some cases, no turbidity was apparent after several months.

Bacterial isolates

A total of 118 LAB isolates were obtained from the brine of the product stored at 5 °C, 16 °C, 25 °C, and 30 °C. Of the Gram-positive isolates tentatively identified as LAB by biochemical tests, we found that 45.8% were homofermentors and 54.2% were heterofermentors using both HHD medium and Durham tube methods. Bacteria with different ITS PCR-RFLP molecular fingerprinting patterns were chosen for identification by sequencing the 5' end of the 16S rRNA gene (containing 2 variable regions). Genotypic characterization of the isolates correlated well with their biochemical profile as determined by API or BIOLOG tests, with the exception of *Weissella* and *Enterococcus* (Table 2). In the case of *Enterococcus*, no identification could be found in the BIOLOG or the bioMerieux (API) databases. In the case of *Weissella* spp., 16 isolates were identified as *Lactobacillus coprophillus* by API 50 CHL. Some *Lactobacillus* and *Leuconostoc* spp. have been reclassified as *Weissella*, and *L. coprohillus* is now known as *Weissella confusa* (Paludan-Muller and others 1999). The bacteria identified included *Enterococcus*, *Lactococcus*, *Leuconostoc*, *Lactobacillus*, *Pediococcus*, and *Weissella* species. Temperature appeared to be an important factor influencing the type of microflora isolated from the nonacidified pickle product: *Lactobacillus* species were isolated at 5 °C; *Leuconostoc* species at 5 °C and 16 °C; *Enterococcus* at 16 °C, 25 °C, and 30 °C; and *Lactococcus* and *Weissella* at 16 °C, 18 °C, and 25 °C (Table 2).

Characterization of isolates

The growth rates of selected LAB were compared with growth rate of *L. monocytogenes* B164 at 20 °C and 5 °C in cucumber juice medium at different salt concentrations (0%, 2%, and 4% NaCl). At 20 °C, *L. mesenteroides* LR50, *Lactobacillus curvatus* LR55, isolates from this study, as well as *L. lactis* LA218 (a nisin-producing strain isolated originally from sauerkraut; Harris and others 1992) were found to grow faster than *L. monocytogenes* B164 at all salt concentrations tested (Figure 1), although at 4% NaCl, the growth rate of the *L. lactis* strain was similar to the *L. monocytogenes* culture. At

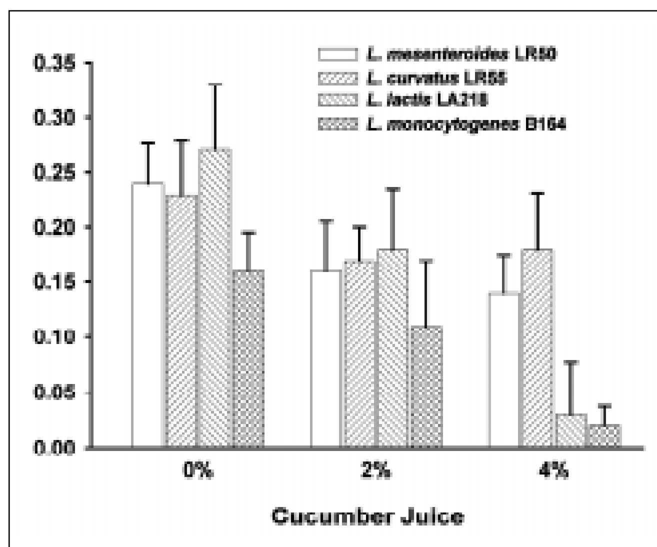

Figure 1—Growth rates of lactic acid bacteria and *Listeria monocytogenes* in cucumber juice at 20 °C with 0%, 2%, and 4% NaCl. Open bars = *Lactobacillus mesenteroides* LR50; upward diagonal = *Lactobacillus curvatus* LR55; downward diagonal = *Lactococcus lactis* LA218; crosshatch = *Listeria monocytogenes* B164.

5 °C, *L. monocytogenes* grew faster than all of the LAB isolates, reaching maximum cell density in 5 to 7 d (data not shown).

Because of its homofermentative metabolism and growth characteristics, *L. curvatus* LR55 was chosen for further testing as a putative biocontrol organism. We found that *L. curvatus* LR55 did not produce a bacteriocin (as observed by the agar plate spot method) and grew slower than *L. monocytogenes* at 5 °C. However, an abuse temperature of 21 °C could inhibit *L. monocytogenes*. In a temperature shift experiment (from 5 °C to 21 °C) carried out in cucumber juice medium with 4% NaCl, *L. monocytogenes* counts were quickly reduced after the shift up in temperature when *L. curvatus* LR55 was present, compared with a pure culture of *L. monocytogenes* (Figure 2).

Bacteriocin production by isolates

Potential bacteriocin producing LAB from brine samples were identified on MRS agar plates by the inhibition of growth of neighboring colonies in the spiral patterns obtained by plating brine samples. The spectrum of antimicrobial activity analyzed by the zone of inhibition test against the panel of strains shown in Table 1. Three of these putative bacteriocin-producing isolates showed heat-stable antimicrobial activity of culture supernatants when the brine supernatants were tested. These isolates belonged to the genera *Lactococcus* (LR80), *Enterococcus* (LR110), and *Leuconostoc* (LR50), and showed bacteriocin-like activity against 3 *L. monocytogenes* serotype 4b strains (B181, B182, B184). Interestingly no inhibition zone was apparent when the brine supernatants were tested against *L. monocytogenes* strains B183 (serotype 1/2a) and B184 (serotype 1/2b). To our knowledge, this type of strain selectivity of bacteriocin activity has not been previously reported. However, after the cultures were transferred in the laboratory and grown for frozen storage, the anti-listerial bacteriocin activity was apparently lost. Similar results with class II bacteriocin producers were reported by other authors (Vaughan and others 2001).

Six *L. lactis* isolates (strains LR1 to LR7) showed stable bacteriocin-like activity when *Pediococcus dextrinicus* was used as the indicator organism (Figure 3). The putative bacteriocin activity of these isolates was also found to inhibit *Lactobacillus plantarum*, *L. mesenteroides*, and *L. monocytogenes* 4b, which is similar to the expected pattern for the bacteriocin nisin. Interestingly, 3 of these isolates, LR5, LR6, and LR7, showed no detectable activity against *L. monocytogenes* 1/2a and 1/2b strains. Further characterization of this bacteriocin activity will be the subject of future work.

Discussion

The microflora of fruits and vegetables are related to agronomic practices, including irrigation water, fertilizers, and soil type in the field (Taormina and Beuchat 2002). The ecology of the resulting fresh vegetable products depends on the processing conditions and characteristics of the fruits and vegetables being processed. For fruits with low pH, such as tomatoes, lactobacilli typically predominate during storage, although the initial microflora include yeasts, molds, and Gram-negative bacteria (Drosinos and others 2000). Spoilage of leafy vegetables, naturally low in sugar content, is mainly due to Gram-negative bacteria, but in sugar-rich produce such as cucumbers and cabbage, spoilage or fermentative microflora typically consists of LAB and yeasts (Jacxsens and others 2003).

In brined vegetable products, the decline in pH, salt concentration, and temperature influence the type of microorganisms carrying out the fermentation (Fleming and others 1995). Four stages can be differentiated in vegetable fermentations, each characterized by different groups of microorganisms: initiation (various Gram-positive and Gram-negative bacteria), primary fermentation (LAB and yeasts), secondary fermentation (yeasts), and post-fermentation (surface growth of oxidative yeasts, molds, and bacteria). In vegetable fermentations where low salt and cold temperatures are common, such as cabbage fermentations (2% NaCl and 18 °C), the LAB populations are dominated by heterofermentors such as *Leuconostoc* or *Weisella* spp. at the start of the fermentation, followed by a variety of homofermentors (Plengvidhya 2003). In fermentations with higher initial salt concentrations and warmer temperatures

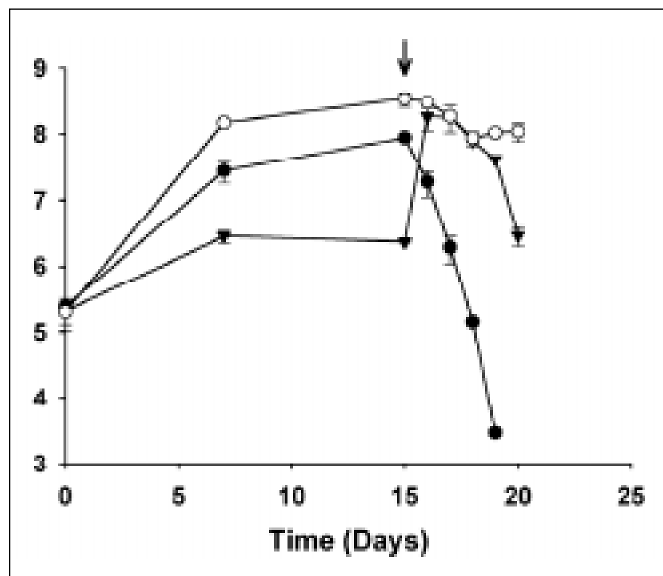

Figure 2—Growth of *Listeria monocytogenes* B164 in pure and mixed cultures with *Lactobacillus curvatus* LR55 in cucumber juice with 4% NaCl. The arrow indicates a temperature shift from 5 °C to 21 °C. Open circles = *L. monocytogenes* B164 in pure culture; filled circles = *L. monocytogenes* B164 in mixed culture; triangles = *L. curvatus* LR55 in mixed culture.

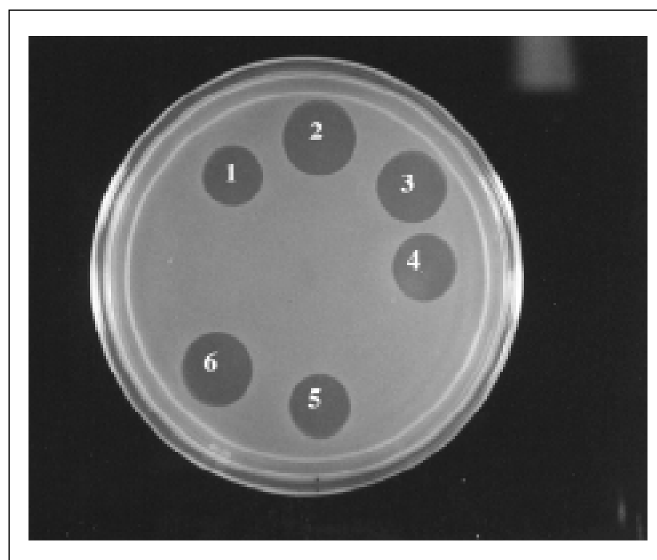

Figure 3—Zones of inhibition from bacteriocin-producing *Lactococcus lactis* isolates on De Man, Rogosa, Sharpe (MRS) agar seeded with a lawn of *Pediococcus dextrinicus*. Indicated spots contain supernatant from (1) LR1; (2) LR2; (3) LR4; (4) LR5; (5) LR6; (6) LR7.

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typical of cucumber and olive fermentations (6% or greater NaCl, 25 °C), homofermentative LAB such as *L. plantarum* predominate the fermentations (Fleming and others 1995). Refrigerated deli-style pickle products contain a low-salt brine typically about 2% NaCl.

The ideal biocontrol agent for nonacidified deli style pickles could be described as follows: (1) belonging to the LAB group; (2) a homofermentor to avoid gas production; (3) able to survive the initial harsh environment of up to 4% salt and low temperatures of storage; (4) able to grow slowly but faster than pathogenic bacteria during refrigerated storage, but without greatly shortening the shelf life of the product; and (5) able to grow faster than pathogens and to produce inhibitory substances (bacteriocins) against the pathogen at abuse temperatures. Ideally, candidate biocontrol organisms would be able to utilize available biochemical resources, and therefore may be preferably isolated from the product for which they are intended to be used (Breidt and Fleming 1997). The terminal pH should be about 4 to preserve the fresh cucumber flavor and color in deli-style pickles. Consumers of this type of product do not like the sharp acidic flavor of pickles that results from high concentrations of lactic acid. The spontaneous fermentation of the nonacidified refrigerated pickles at abuse or refrigerated temperatures was due mainly to LAB. This suggests that the product can be considered safe for human consumption after temperature abuse.

Two LAB isolates identified in this study, *L. mensenteroides* LR50 and *L. curvatus* LR55, were able to grow faster than *L. monocytogenes* in the presence of 4% salt in cucumber juice (Figure 1), whereas a nisin-producing—*L. lactis* strain from sauerkraut (typically fermented at 2% NaCl) was apparently less salt-tolerant. The mechanism used by the LAB isolates (LR50 and LR55) to inhibit *Listeria* at abuse temperatures remains to be characterized, but did not appear to involve heat-stable bacteriocins. Other factors are likely involved, such as the production of acids and accompanying lowering of pH, and production of other metabolites. Similar conclusions have been drawn with *Salmonella enteritidis*, which could grow in tomatoes at room temperature, but not at refrigeration temperatures due to the growth of LAB that produced lactic, acetic, propionic, and formic acids, thus resulting in a decrease in pH (Drosinos and others 2000). *Lactobacillus curvatus* LR55, isolated from nonacidified pickles, meets several, but not all, of the criteria for an ideal biocontrol agent against *L. monocytogenes* in minimally processed pickle products, including homofermentative metabolism (which is desirable so gas production does not occur in jars) survival at low temperatures, and growth characteristics over a range of salt conditions. As an isolate from minimally processed pickles, it is presumably well adapted for growth in the presence of the garlic, salt, and spices present in these products. Trials of minimally processed pickle products co-inoculated with *L. monocytogenes* and *L. curvatus* or similar cultures producing bacteriocins active against *L. monocytogenes* (which *L. curvatus* LR55 does not do) will be the subject of future investigations.

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