Prevalence of *Listeria monocytogenes* during Production and Postharvest Processing of Cabbage

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MS 01-308: Received 21 August 2001/Accepted 6 March 2002

**ABSTRACT**

From November 1999 to May 2000, analyses of 425 cabbage, 205 water, and 225 environmental sponge samples from four cabbage farms with packing sheds and from two packing sheds in the Rio Grande Valley and Uvalde, Tex., were conducted to determine whether *Listeria monocytogenes* was present. Samples were tested by the Food and Drug Administration method for the isolation of *Listeria* spp., and confirmed isolates were DNA fingerprinted by repetitive-element sequence-based polymerase chain reaction (rep-PCR). *L. monocytogenes* was isolated from 3% (26 of 855) of the samples. Twenty of these isolates were obtained from cabbage (7 isolates from farms and 13 from packing sheds). Three isolates were from water samples (two from farms and one from a packing shed), and three were from environmental sponge samples of packing shed surfaces. Rep-PCR–generated fingerprints of 21 of the isolates revealed 18 distinctive banding patterns. Four isolates from environmental sponge samples of conveyor belts and from cabbage samples shared identical banding patterns, suggesting common sources of contamination. These identical environmental isolates suggest that contact with packing shed surfaces may be a source of contamination of cabbage. However, the cabbage samples could have arrived contaminated, since they were not washed.

*L. monocytogenes*, a ubiquitous foodborne pathogen, causes an illness (listeriosis) that can manifest itself in forms ranging from gastroenteritis to meningitis, septicemia, encephalitis, and spontaneous abortion (7, 9, 13, 17, 22, 28). The ability of *L. monocytogenes* to grow at temperature extremes from $-0.4$ to 50°C (16, 29) and to survive for long periods in far less than optimal conditions (3, 6, 24) gives rise to a threat to the safety of a variety of foods, including fresh fruits and vegetables. In addition, *L. monocytogenes* is a ubiquitous organism, so it is not surprising that it is present naturally on fresh produce (23, 27).

A well-documented outbreak of listeriosis caused by contaminated cabbage occurred in 1981 in the Maritime Provinces of Canada (25). In this instance, a regional processor who had obtained cabbage from a farm on which there were known cases of ovine listeriosis prepared the coleslaw that resulted in the outbreak. The cabbage farmer involved had used both raw and composted sheep manure to fertilize his fields. The cabbage was harvested and stored on the farm for shipment during the following winter and spring. The extended cold storage probably allowed the growth of *L. monocytogenes*. In all, the outbreak comprised 7 adult cases and 34 perinatal cases of listeriosis, resulting in 18 deaths.

Cabbage is one of many fresh fruits and vegetables that is not washed prior to its arrival at the point of sale (e.g., a retail supermarket). A few studies have evaluated fresh vegetables, including cabbage, for the presence of *L. monocytogenes* just prior to the point at which these vegetables reach the consumer (2, 3, 11). Other researchers have evaluated the ability of this microorganism to attach to various surfaces, in addition to evaluating the effectiveness of washing methods and chemical sprays in removing the pathogen from fresh produce (4, 5, 8, 12, 18, 19, 20, 30). In spite of the information provided by these studies, the contamination of cabbage with *L. monocytogenes* from the field to the packing shed and to the point of shipment for retail sale has not been evaluated.

Fresh vegetable producers and packing shed managers would benefit from a thorough understanding of the prevalence of *L. monocytogenes* in their growing and storage environments. If contamination with this pathogen was found to exist, measures could be taken to prevent it from reaching the consumer.

An assortment of characterization assays are currently used to fingerprint species and/or strains of microorganisms that are of interest in foods. These procedures potentially aid researchers in linking strains of pathogens from various sources to each other. A technique growing in popularity because of its speed, lower cost, and ease of use is repetitive-element sequence-based polymerase chain reaction (rep-PCR) (27). Rep-PCR provides ample DNA fingerprints that assist in discriminating between bacterial isolates.

The purpose of this study was to evaluate the contamination of cabbage with *L. monocytogenes* during production and postharvest handling in Texas. Specifically, the objectives were (i) to determine the effect of the water source (river versus well) and the time of irrigation (before or after final irrigation) on surface contamination with *L. monocytogenes*, (ii) to examine packing shed surfaces (conveyor belts, cooler floors, walls, drains, and the hands of workers) for the presence of *L. monocytogenes*, (iii) to determine if...
growing seasons have an effect on the contamination of cabbage, and (iv) to carry out DNA fingerprint analysis of isolates to determine common contamination sources.

**MATERIALS AND METHODS**

**Overall experimental design.** Cabbage, water, and environmental samples were obtained from six farms and/or packing sheds in Texas. Five of the sampling sites were located in the Rio Grande River Valley, and one was located in Uvalde. Two of the farms with packing sheds (farm–packing sheds A and B) in the Rio Grande Valley were irrigated from the Rio Grande River, while the other two farms with packing sheds (farm–packing sheds C and D) were irrigated with well water. At an additional two packing sheds (packing sheds E and F), produce was received from growers, packed, and distributed to the market. Samples were collected in fall, winter, and spring. These samples were evaluated for numbers of *Escherichia coli* biotype 1 and for the presence of *L. monocytogenes*.

**Sample collection.** Cabbage samples were obtained at the following points during production and processing: (i) just prior to the final irrigation, (ii) after the final irrigation, (iii) immediately on arrival at the packing shed (before packing), and (iv) during storage in boxes in the packing shed cooler. Twenty-five heads of cabbage were sampled at each point. Samples were collected with latex gloves by removing three to four outer cabbage leaves from each head (ca. 100 g). These samples were placed in individual Whirl-pak bags (Nasco, Ft. Atkinson, Wis.), packed in ice chests containing frozen refrigerants, and sent by overnight courier to Kleberg Center at Texas A&M University (College Station, Tex.) for microbial analysis. Water samples were collected aseptically at the following points: (i) from each irrigation water source (river water or well water), (ii) before water filtration, (iii) after water filtration, (iv) from delivery pipes into the field, and (v) from water flowing in the field. In addition to these samples, wash and spray waters and packing ice used to wash and cool vegetables other than cabbage were collected. Although these water samples were not used on cabbage, they were a part of the shed environment and could indicate sources of contamination. Five replicate samples were obtained each time from each of these locations. Sterile polypropylene 4-oz (120-ml) cups (Oxford Labware, St. Louis, Mo.) were used to collect approximately 90 ml of water for each sample. Samples were packed in ice chests containing frozen refrigerants and sent by overnight courier to Kleberg Center at Texas A&M University for microbial analysis.

Environmental samples were obtained from various packing shed surfaces, including (i) conveyer belts, (ii) the hands of workers, (iii) cooler floors, (iv) walls, and (v) drains. To collect these samples, a sponge-and-glove sampling system (International Bioproducts, Muncie, Ind.) was used. This system consists of an 18-oz (532-ml) sample bag that contains a dry, sterile biocide-free sponge (3.81 by 7.62 cm) in the top compartment and a pair of sterile polyethylene gloves in the bottom compartment. Sponges were rehydrated with 25 ml of Mega-Reg 25 sterile buffered peptone water (International Bioproducts). The USDA Template 100 (International Bioproducts), a sterile disposable plastic template (10 by 10 cm), was used to designate each environmental sample area, with the exception of samples from the hands of the workers. For these samples, each worker was asked to take a rehydrated sponge into his or her hands, squeeze it and rub it (as if washing one’s hands with a bar of soap), and then place it back into the sample bag. Samples were packed in ice chests containing frozen refrigerants and sent by overnight courier to Kleberg Center at Texas A&M University for microbial analysis.

**Microbial evaluation methods:** *E. coli* biotype 1. *E. coli* biotype 1 cells were enumerated with Petrifilm *E. coli*logiform count plates (3M, St. Paul, Minn.). Serial dilutions of up to 10⁻⁴ were made for each homogenized sample with 0.1% buffered peptone water (Difco Laboratories, Detroit, Mich.), and 1 ml of solution was added to each plate. Plates were incubated for 24 h at 35°C, and colonies were counted on a Quebec colony counter (Leica, Inc., Buffalo, N.Y.).

**Microbial evaluation methods:** *L. monocytogenes*. *L. monocytogenes* was detected qualitatively by enrichment in listeria enrichment broth (Difco). For cabbage samples, 25 g of leaves was aseptically added to 225 ml of listeria enrichment broth in a 500-ml Nalgene bottle (Nalge Nunc Int., Rochester, N.Y.). The bottle was shaken 20 times in 7 s in a 1-ft (30.5-cm) arc. Shaken bottles were incubated at 35°C for 24 h. For environmental sponge samples, 25 ml of listeria enrichment broth was added to the sample bag. Sponges were massaged for approximately 20 s and were incubated at 35°C for 24 h. For water samples, 3 ml of sample water was added to 27 ml of listeria enrichment broth in 50-ml Nalgene bottles (Nalge Nunc Int.). Bottles were shaken and incubated by the same method used for cabbage samples.

Following incubation, a loopful of the enriched sample was plated onto LiCl-phenylethanol-moxalacam agar (Difco) and modified Oxford agar (Difco). LiCl-phenylethanol-moxalacam plates were incubated for 24 to 48 h at 30°C, and modified Oxford agar plates were incubated for the same time at 35°C. Plates were observed at 24-h intervals for characteristic colonies. Potential positive colonies from LiCl-phenylethanol-moxalacam agar and/or modified Oxford agar plates were streaked onto tryptic soy agar (Difco) plates and incubated for 24 h at 35°C. A commercially available enzyme-linked immunosorbent assay kit (Tecra, International Pty. Ltd., New South Wales, Australia) was used to screen the initial samples. Confirmation was carried out with both traditional and commercially available biochemical tests as follows. Gram staining was performed according to the method described in the *Bacteriological Analytical Manual* (BAM). Motility was observed on minimal medium agar (MMA, Difco). Fermentation of dextrose, maltose, mannitol, rhamnose, and xylose was determined by using Wedett's rapid fermentation tablets (Key Scientitic Products Co., Round Rock, Tex.). Esculin hydrolysis was determined by plating on modified Oxford agar (Difco). The catalase test was performed by streaking each isolate onto a microscope slide, adding a drop of 3% hydrogen peroxide (Fisher Scientific, Fair Lawn, N.J.), and observing the development of gas bubbles.

**Fingerprinting of isolates by rep-PCR.** Rep-PCR DNA fingerprinting was carried out with a Uprime-Dt rep<sub>900</sub> kit (Bacterial Barcodes, Inc., Houston, Tex.). This kit contains high-performance liquid chromatography-grade water (100%); 5× buffer; a mixture of nucleotides, or dNTPs (dATP, dTTP, dCTP, dGTP) (each 10 mM); bovine serum albumin (20 mg/ml); dimethyl sulfoxide (100%); positive control DNA consisting of genomic DNA from a *L. monocytogenes* isolate (100 ng/µl); a 1-kb lambda ladder, or molecular weight marker (0.25 µg/µl); 3× loading buffer; and Uprime-Dt (0.3 µg/µl). The repetitive-element sequence-based primer sequences (Uprime-Dt) used were REPIR-Dt (5'-HNCNCNGCATTGCGG-3' and REP-2-Dt (5'-NCGCTTATCNGGGTAC-3') (N = A, C, G, and T; I = inosine).

DNA from each *L. monocytogenes* isolate was extracted with the High Pure PCR template kit (catalog no. 1 796 828, Roche
Diagnósticos Corporation, Indianapolis, Ind.). Extracted DNA was resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]) at a concentration of approximately 100 ng/µl and was stored at −20°C. The protocol provided with the Uprima-Dt repPCR kit was used as follows: PCR tubes were placed in a thermal cycler (Perkin-Elmer Gene Amp PCR System 2400, Norwalk, Conn.), and rep-PCR was carried out with 31 cycles of 95°C for 3 s, 92°C for 2 min, 94°C for 3 s, 92°C for 30 s, 40°C for 1 min, and 65°C for 8 min, followed by a final extension at 65°C for 8 min. When the reaction was complete, 2 µl of the loading buffer was added to a fresh PCR tube. Ten microliters of each amplified reaction was added to its corresponding tube. A 20-cm 1.5% agarose (Bio-Rad) gel was produced with 1× Tris-acetate-EDTA buffer (Sigma Chemical Co., St. Louis, Mo.) and 3 µg of ethidium bromide per ml (Bio-Rad, Hercules, Calif.). Generally, 10 µl of amplified reaction with dye per lane was added. Electrophoresis of DNA was carried out in a horizontal gel rig (Bio-Rad) in 1× Tris-acetate-EDTA buffer (Sigma) at 120 V for 2 to 3 h. Gels were viewed with a UV transilluminator (UVP, San Gabriel, Calif.) and photographed with a Polaroid MP-4 camera (Polaroid Corporation, Cambridge, Mass.) with Polaroid 665 film.

Banding patterns were visually inspected for similarity to determine differences in the DNA fingerprints of the isolates. If at least two different bands were present or absent in one of the patterns, two fingerprints were considered different. Deviations in band intensity were not considered differences. If bands were too faint to be read, they were not considered.

### RESULTS AND DISCUSSION

Overall, 26 isolates (3%) of *L. monocytogenes* and 73 isolates (8.5%) of *E. coli* biotype 1 were identified from 855 cabbage, water, and environmental samples (Table 1). Nine of the *L. monocytogenes* isolates (38.5%) were found on farms (Tables 2 and 3). Two of these isolates were obtained from water samples taken from irrigation water flowing in the furrows (Table 2), and seven were obtained from cabbage in the field (Table 3). One of the irrigation water isolates came from a farm that irrigated with well water, while the other came from a farm that irrigated with river water. However, *L. monocytogenes* was not found directly in either the river or the well water. Thus, the isolates could have contaminated the water in the furrow through contact with birds, insects, or the soil itself. It should be noted that soil samples taken in the field next to cabbage heads tested positive for *E. coli* biotype 1 (EC) and *L. monocytogenes* (LM) in samples obtained from various vegetable farms and packing sheds in Texas

### TABLE 3. Overall contamination of cabbage with E. coli biotype 1 (EC) and *L. monocytogenes* (LM) in samples obtained from various vegetable farms and packing sheds in Texas

<table>
<thead>
<tr>
<th>Sample location</th>
<th>No. of samples</th>
<th>EC</th>
<th>LM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before FI</td>
<td>100</td>
<td>1 (1)</td>
<td>A</td>
</tr>
<tr>
<td>After FI</td>
<td>50</td>
<td>0 (0) A</td>
<td>4 (8) A</td>
</tr>
<tr>
<td>Upon arrival</td>
<td>125</td>
<td>9 (7.2) B</td>
<td>6 (4.8) A</td>
</tr>
<tr>
<td>In cooler</td>
<td>150</td>
<td>0 (0) A</td>
<td>7 (4.7) A</td>
</tr>
<tr>
<td>Total</td>
<td>425</td>
<td>10 (2.4) A</td>
<td>20 (4.7) A</td>
</tr>
</tbody>
</table>

* a Values with different letters in the same column are significantly different ($P < 0.05$).

#### TABLE 4. Environmental contamination with E. coli biotype 1 (EC) and *L. monocytogenes* (LM) in samples from various vegetable farms and packing sheds in Texas

<table>
<thead>
<tr>
<th>Sample location</th>
<th>No. of samples</th>
<th>EC</th>
<th>LM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil (next to cabbage heads)</td>
<td>5</td>
<td>0 (40) A</td>
<td>0 (0) A</td>
</tr>
<tr>
<td>Transport trailers</td>
<td>35</td>
<td>0 (0) B</td>
<td>0 (0) A</td>
</tr>
<tr>
<td>Transport bins</td>
<td>10</td>
<td>1 (10) B</td>
<td>0 (0) A</td>
</tr>
<tr>
<td>Conveyor belts</td>
<td>50</td>
<td>2 (4) B</td>
<td>2 (4) A</td>
</tr>
<tr>
<td>Cooler surfaces</td>
<td>85</td>
<td>1 (1.2) B</td>
<td>1 (1.2) A</td>
</tr>
<tr>
<td>Workers’ hands</td>
<td>40</td>
<td>0 (0) B</td>
<td>0 (0) A</td>
</tr>
<tr>
<td>Total</td>
<td>225</td>
<td>6 (2.7) A</td>
<td>3 (1.3) A</td>
</tr>
</tbody>
</table>

* a Values with different letters in the same column are significantly different ($P < 0.05$).
positive for *E. coli* biotype 1 but not for *L. monocytogenes* (Table 4). Another possible explanation is that the organism may be present at such low numbers in water that our procedure was not able to detect it.

*E. coli* biotype 1 was detected in 19.2% of the water samples collected on farms, with the highest incidence found in samples taken prior to filtering (Table 2). Only samples taken prior to filtering were significantly different from samples taken at the other points on the farms. The largest number of positive samples was taken from the source of the water, where 20% of samples were found to be positive for *E. coli* biotype 1 in both the river and the well water. *E. coli* biotype 1 is commonly used as a fecal indicator for water because of its period of survival (14), so it is not surprising to find it in source water samples. Under conditions similar to those found in water distribution systems, *E. coli* would be expected to survive for about 30 days. Grabow et al. (10) reported that *E. coli* survived for 55 days in dialysis bags suspended in river water at 9 to 16°C.

Sixteen of the 26 (62%) *L. monocytogenes* isolates were identified from samples taken from packing sheds (Tables 3, 4, and 5). One sample was from nonchlorinated wash water that was used to wash produce other than cabbage (Table 5). Environmental contamination with the pathogen was detected on the conveyor belts and in the cooler (Table 4). The isolate from the cooler was found on a damp wooden pallet. This finding supports the notion that *L. monocytogenes* is an environmental contaminant and may have been brought into the packing shed by workers, equipment, or produce. In addition, conveyor belts and cooler surfaces are not likely to be disinfected or cleaned often, allowing for an increase in the bacterial load.

Packing sheds process many kinds of vegetables in addition to cabbage. *L. monocytogenes* has been isolated from several other fresh vegetables, including cucumbers, potatoes, radishes (11), asparagus, broccoli, and cauliflower (1). It is possible that contamination in Texas packing sheds results from the variety of vegetables processed. Further investigation is necessary to assess the effect that other vegetables have on *L. monocytogenes* contamination of the packing shed environment.

*E. coli* biotype 1 was isolated from conveyor belts, coolers, and transport bins (Table 4). *E. coli* biotype 1 was not found in water samples collected from the packing sheds. There was a highly significant difference between *E. coli* biotype 1 contamination of water samples from farms and that of samples from the sheds. These data suggest that water used at the packing shed is not a likely source of contamination involving fecal material.

Twenty-four nonpathogenic *Listeria* isolates were also identified in samples from throughout the farms and packing sheds (Table 6). Eighty-three percent (20 of 24) of these isolates were found in the packing shed environment. These findings indicate that the packing shed provides a suitable environment for *Listeria* spp. and that there is a need to

<table>
<thead>
<tr>
<th>Sample location</th>
<th>No. of samples</th>
<th>No. (%) positive for:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reservoir tanks</td>
<td>15</td>
<td>0 (0) A</td>
</tr>
<tr>
<td>Cooler fan water</td>
<td>10</td>
<td>0 (0) A</td>
</tr>
<tr>
<td>Wash water</td>
<td>40</td>
<td>0 (0) A</td>
</tr>
<tr>
<td>Spray water</td>
<td>10</td>
<td>0 (0) A</td>
</tr>
<tr>
<td>Packing ice</td>
<td>5</td>
<td>0 (0) A</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>0 (0) A</td>
</tr>
</tbody>
</table>

* Values with different letters in the same column are significantly different (*P* < 0.05).

b This water was used to clean and cool produce other than cabbage.

**TABLE 6.** Other *Listeria* species isolated in the farms and packing sheds investigated

<table>
<thead>
<tr>
<th>Species</th>
<th>Source of isolate(s) (no. of isolates)</th>
<th>Farm or shed</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. grayi</em></td>
<td>Water (1) Environmental sponge of conveyor (1)</td>
<td>Farm</td>
</tr>
<tr>
<td><em>L. innocua</em></td>
<td>Environmental sponge of conveyor (1) Environmental sponge of cooler (1) Cabbage on arrival at shed (1) Cabbage in cooler (1)</td>
<td>Shed</td>
</tr>
<tr>
<td><em>L. ivanovii</em></td>
<td>Environmental sponge of conveyor (1) Water (2)</td>
<td>Farm</td>
</tr>
<tr>
<td><em>L. welshimeri</em></td>
<td>Cabbage on arrival at shed (2) Cabbage in cooler (2) Cabbage before final irrigation (1)</td>
<td>Shed</td>
</tr>
</tbody>
</table>

**FIGURE 1.** Percentages of *E. coli* biotype 1 and *L. monocytogenes* isolated from farms and packing sheds during three sampling seasons. Error bars indicate 95% confidence intervals. *n* = 273 samples; **n** = 330 samples; ***n*** = 250 samples.
FIGURE 2. DNA fingerprint analysis of L. monocytogenes isolates identified from samples taken in Uvalde and the Rio Grande River Valley in Texas. Agarose gel electrophoresis of rep-PCR reactions was carried out with the Uprime-Dt primer. DNA was extracted with the High Pure PCR template kit (Roche). (A) Isolates from samples taken in the Rio Grande Valley only. Lanes 1 and 2, L. monocytogenes isolated from a sample of river water during delivery in the field by furrow irrigation (duplicate); lanes 3 and 4, L. monocytogenes isolated from a cabbage sample on arrival at the packing shed (duplicate); lanes 5 and 6, L. monocytogenes isolated from an environmental sponge sample of a conveyor belt in a packing shed (duplicate); lane 7, L. monocytogenes isolated from an environmental sponge sample of a conveyor belt at a different packing shed; lanes 8 and 9, L. monocytogenes isolated from a cabbage sample on arrival at a packing shed (duplicate); lanes 10 and 11, L. monocytogenes isolated from the same packing shed’s cooler (duplicate); lanes 12 and 13, L. monocytogenes isolated from a cabbage sample in a packing shed cooler (two views of the same isolate); lane 3, L. monocytogenes isolated from a cabbage sample in the field before final irrigation with well water on a farm (duplicate). (B) Isolates from samples taken in the Rio Grande Valley only. Lanes 1 and 2, L. monocytogenes isolated from a cabbage sample in a packing shed cooler (two views of the same isolate); lane 3, L. monocytogenes isolated from a cabbage sample in the field before final irrigation with well water; lane 4, L. monocytogenes isolated from a nonchlorinated sample of water used to wash produce other than cabbage in a packing shed; lanes 6 and 7, L. monocytogenes isolated from a cabbage sample in the cooler at a packing shed; lane 8, L. monocytogenes isolated from a cabbage sample in a box in a packing shed cooler; lane 9, L. monocytogenes isolated from a cabbage sample on arrival at a packing shed.
study the ecology of the packing shed environment in depth. Overall, *Listeria* spp. were isolated from 5.9% of the samples, with 52% (26 of 50) of these isolates being *L. monocytogenes* isolates.

*L. monocytogenes* was isolated from 4.7% of the cabbage samples at the four stages of production and processing (before final irrigation, after final irrigation, on arrival at the shed, and during storage in the cooler) (Table 3). Statistical analysis revealed no significant difference between the contamination of cabbage before final irrigation and that after final irrigation. These data suggest that the irrigation of cabbage does not have an effect on contamination with *L. monocytogenes*.

*E. coli* biotype 1 was found less frequently in cabbage, with only 2.4% of the samples being contaminated (Table 3). Such contamination was found most often on cabbage on arrival at the shed, suggesting that cabbage itself may be a likely source of the *E. coli* biotype 1 contamination in the packing shed environment. In a survey of 116 samples of 11 different fresh vegetables (spinach, coriander, marjoram, radishes, lettuce, carrots, beets, cabbage, tomatoes, cucumbers, and capsicum) from a local market in Mumbai, India, the most-probable-number (MPN) index per gram for coliforms ranged from $<3$ to $>1,100$ (21). While cabbage samples were not positive for fecal coliforms, coriander, marjoram leaves, radishes, and lettuce were.

Isolates were identified from samples taken during fall, winter, and spring. The majority of *L. monocytogenes* isolates were found during the winter growing season (Fig. 1). In the Canadian outbreak of 1981 (25), contaminated cabbage had been stored for extended periods, allowing *L. monocytogenes* to proliferate. The increased contamination observed in Texas during the winter could similarly be due to a delayed harvest or a long storage period. *L. monocytogenes* is a robust microorganism even under less-than-optimal conditions. Beuchat et al. (3) reported that this pathogen survives and multiplies in cabbage extracts at 4°C for long periods at fairly low pHs and high salt concentrations. This psychrotrophic pathogen may favor the conditions of the Texas winter season; however, more research into the effects of seasonal conditions and variations due to seasonality is required.

Rep-PCR of genomic DNA from the *L. monocytogenes* isolates generated numerous DNA fragments with sizes ranging between approximately 100 and 6,500 bp (Fig. 2). A common band at about 506 bp was present in all of the isolates, all but one isolate shared a band at 2,036 bp, and the majority of isolates also shared a band at about 3,054 bp. Four isolates from the Rio Grande River Valley had identical banding patterns (Fig. 2). Two of these isolates were from environmental sponge samples of conveyor belts at different packing sheds (farm--packing shed C and packing shed E), while the other two isolates were from cabbage samples from the same packing shed (packing shed F). The fact that these samples came from the same packing shed suggests that contact with packing shed surfaces may be a source of contamination of cabbage there. However, the cabbage isolates could have arrived contaminated, as cabbage is not washed at packing shed F.

Identical banding patterns were not observed among other isolates (Fig. 2). Visual comparison of banding patterns revealed 18 distinct REP profiles for the 21 isolates fingerprinted. Other researchers have reported wide diversity in fingerprints for *L. monocytogenes* isolated from food samples (15), pointing to the ubiquitous nature of this organism in a variety of environments.

**CONCLUSIONS AND RECOMMENDATIONS**

*L. monocytogenes* contamination of cabbage from Texas farms and packing sheds was not due to a single source. Because each shed has different contamination problems, it is necessary to identify the particular sources of *L. monocytogenes* within individual packing sheds and to find means to reduce environmental contamination. Removal of the outer leaves of the cabbage prior to its entry into the packing shed environment could reduce the potential for the introduction of this pathogen into the packing shed. Because *L. monocytogenes* is such a ubiquitous pathogen, its complete elimination from the packing shed environment may not be possible.

Postharvest washing of cabbage could be beneficial in that it may reduce or eliminate contamination of the product with environmental contaminants like *Listeria*. Chlorine is commonly added to water that is used to wash whole fruits and vegetables, but it does not eliminate pathogenic bacteria (4). Thus, studies of the rinsing and/or washing of cabbage with other commercially available chemicals are desirable. The implementation of washing or rinsing steps in postharvest processing of cabbage at packing sheds could reduce contamination, but investigations must take into account the effect of such treatments on the quality of the product.

Presently, the best way to maintain the safety of fresh vegetables is to ensure that good agricultural practices are used during the growth of crops and that good hygienic practices are employed during harvesting and postharvest processing. The U.S. Food and Drug Administration (26) publishes an industry guide that is a good source of information for farm managers and packing shed managers in this respect.

Future research is needed to determine if the growing season affects cabbage contamination. Perhaps a correlation between certain environmental conditions could be used to determine an optimal harvesting time when contamination is at its lowest.

Rep-PCR was a rapid, easy-to-use technique that provided quality fingerprints that were visually distinguishable. The collection and fingerprinting of more *L. monocytogenes* isolates from Texas vegetable farms and/or packing sheds could produce a database of banding patterns common to these areas. This database could be useful should cases of listeriosis need to be traced to this particular production area.

**ACKNOWLEDGMENTS**

We gratefully acknowledge the technical assistance of Jennifer Hudnall, Lisa Lucia, and others for their help in processing the many samples.
that were collected. This research was made possible through grants from the USDA CSREES and the Texas Advanced Technology Program.

REFERENCES


