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Research Paper

Survey of Foodborne Pathogens, Aerobic Plate Counts, Total Coliform Counts, and *Escherichia coli* Counts in Leafy Greens, Sprouts, and Melons Marketed in the United States

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ABSTRACT

The objective of this research was to assess the microbiological status of leafy greens, sprouts, and melons from U.S. markets. A total of 14,183 samples of leafy greens, 2,652 samples of sprouts, and 3,411 samples of melons were collected throughout the United States from 2009 to 2014. The samples were analyzed for aerobic plate counts, total coliform counts, *Escherichia coli* counts, and the presence and levels of *Salmonella, Shigella, Listeria monocytogenes,* and Shiga toxin–producing *E. coli* (STEC), depending on the year and type of produce. Among the leafy greens, no *E. coli* O157:H7 or non-O157 STEC were detected from iceberg lettuce samples. The overall prevalences of *Salmonella, E. coli* O157:H7, non-O157 STEC, and *L. monocytogenes* in the 14,183 samples of leafy greens were 0.05, 0.01, 0.07, and 0.11%, respectively. Among sprout samples, no *Salmonella* or *E. coli* O157:H7 was detected, and the overall prevalences of non-O157 STEC and *L. monocytogenes* were 0.04 and 0.11%, respectively. Among melon samples, no *Salmonella* was detected from cucumbers, no *L. monocytogenes* was detected from cantaloupes, and the overall prevalences of *Salmonella* and *L. monocytogenes* were 0.12 and 0.23%, respectively. *L. monocytogenes* levels were 0.4 to 1,470 most probable number (MPN)/g in leafy greens, 0.36 to 1,100 MPN/g in sprouts, and <0.03 to 150 MPN/g in melons, and most positive samples had low levels of these pathogens. The isolates from these foods were very diverse genetically. Foodborne pathogens, including *Salmonella,* STEC, and *L. monocytogenes,* had relatively low prevalences in the produce surveyed. Because these foods are usually consumed raw, measures should be taken to significantly minimize the presence and levels of human pathogens.

Key words: Aerobic plate count; *Escherichia coli* O157; *Listeria*; *Salmonella*; Shiga toxin–producing *Escherichia coli*; *Shigella*

Fresh produce is popular because it is perceived to be a healthy component of a well-balanced diet. However, produce has also emerged as a food safety concern because of the potential presence of enteric pathogens. Cantaloupes, packaged leafy green salads, stone fruits, caramel apples, coleslaw, and sprouts have been associated with listeriosis outbreaks, including multistate U.S. and international outbreaks *(9, 10, 15, 22, 25, 38)*. Cantaloupes, mangoes, peppers, tomatoes, papayas, cucumbers, and sprouts have been associated with both multistate U.S. and international *Salmonella* infection outbreaks *(3, 9, 28)*. Similarly, sprouts, spinach, lettuce, and cucumbers have been associated with Shiga toxin–producing *Escherichia coli* (STEC) infection outbreaks in many countries. Recalls of produce commodities due to contamination by foodborne pathogens have been reported frequently in recent years *(7, 9, 24)*.

Microbiological surveys of fresh produce have been conducted in various countries and regions owing to the relatively high risk of foodborne pathogens associated with fresh produce. In a survey of leafy greens marketed in Italy for the presence of *Salmonella, Listeria monocytogenes, E. coli* O157:H7, *Campylobacter, Yersinia enterocolitica,* and norovirus, 3.7% of fresh leafy green vegetables and 1.8% of fresh-cut or ready-to-eat (RTE) vegetables were contaminated with one or more foodborne pathogens *(20)*. In a survey of over 3,000 samples of raw produce and minimally processed packaged salad in The Netherlands, *E. coli* O157:H7, *L. monocytogenes,* and *Salmonella* were found in 0.11, 0.11, and 0.38% of samples, respectively, and 2.7% of the cucumber samples contained *Salmonella (39)*. In another survey of fresh and minimally processed fruits and vegetables and sprouts conducted in several retail establishments in Catalonia, Spain, from 2005 to 2006, *E. coli* O157:H7, *L. monocytogenes,* and *Salmonella* were found in 0, 0.7, and 1.3% of the samples, respectively *(1)*. In a survey of sprout products in Mumbai, India, a high prevalence of *Salmonella* and no *L. monocytogenes* or *E. coli* O157 were found *(29)*. In a survey of sprout products in Korea, no *Salmonella* or *E. coli* O157 were found *(16)*. Contaminated water is considered one of the major sources of contamination for leafy greens. In a survey of the watershed sites in

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a leafy green growing region on the California central coast, STEC, *E. coli* O157:H7, *Salmonella,* and *L. monocytogenes* were found in 11, 8, 65, and 43% of environmental samples, respectively *(11)*. In a recent interagency *Listeria monocytogenes* market basket survey, the proportions of RTE produce samples positive for *L. monocytogenes* were 0.11% in sprouts, 0.37% in low-acid cut fruit, and 1.07% in raw cut vegetables *(21)*.

The objective of the present study was to conduct a baseline survey of the prevalence of non-O157 STEC, *E. coli* O157:H7, *Salmonella, L. monocytogenes,* and *Shigella* in leafy greens (spinach, iceberg lettuce, and romaine lettuce), sprouts, cantaloupes, mangos, and cucumbers marketed in the United States. For *L. monocytogenes,* contamination levels were also determined for the samples that tested positive. This independent scientific research project was not associated with any national food safety program.

MATERIALS AND METHODS

Sample collection: leafy greens. From October 2009 to September 2010 (fiscal year 2010), a total of 8,068 iceberg lettuce, romaine lettuce, and spinach samples, both organic and conventional, were collected in various states around the United States: Arizona, Arkansas, California, Colorado, Florida, Illinois, Indiana, Iowa, Kentucky, Maryland, Michigan, Minnesota, New Jersey, New Mexico, North Carolina, Ohio, Oregon, Texas, Washington, and Wisconsin. Approximately two-thirds (64%) of the samples came from producers located in California. Detailed information about the samples is listed in Tables 1 through 3.

The fiscal year 2010 samples of spinach, iceberg lettuce, and romaine lettuce were grouped as either RTE, i.e., samples that have been washed and processed in some manner (e.g., chopped or trimmed) and bagged for retail sale, or unprocessed, i.e., samples that did not undergo further handling after harvest and before retail sale.

From October 2010 to November 2011 (fiscal year 2011 with an extension), 1,113 samples of bagged leafy greens were collected from grocery stores. From January 2012 to December 2012 (fiscal year 2012 with an extension), 5,002 samples of bagged leafy greens were collected from grocery stores but were not designated as RTE or unprocessed.

All samples were packaged with frozen gel packs to maintain refrigeration during transportation and were sent by FedEx Priority overnight express (early morning arrival) to the laboratory either on the day of sample collection or no later than the following day.

Sample collection: sprouts. Retail sprout samples were collected from July 2012 to August 2014 from both supermarket chains and independent stores located in the Centers for Disease Control and Prevention (CDC) Foodborne Diseases Active Surveillance Network (FoodNet) in California, Connecticut, Georgia, and Maryland. Samples of both organic and conventional products were collected: (i) alfalfa sprouts; (ii) bean sprouts (soybean or mung); and (iii) other sprouts, including radish, snow pea, daikou, clover, broccoli, dill, sunflower, green pea, adzuki, lentil, and mixed. Cooked sprouts and sprouts mixed with other food items were not collected. Each sample weighed at least 340 g. When multiple containers were purchased to form one sample from a single lot, the containers were placed in a single Ziploc bag. All samples were packaged with frozen gel packs to maintain refrigeration during transportation and were sent by FedEx Priority overnight express (early morning arrival) to the laboratory either on the day of sample collection or no later than the following day. Detailed information about the samples is listed in Table 2.

Sample collection: melons. Cantaloupe, cucumber, and mango samples were acquired at retail locations from FoodNet-specified sites in Colorado, Connecticut, Georgia, Maryland, and Minnesota. Other locations in California, Texas, and Washington were added to provide more comprehensive geographic diversity. Samples were collected from January to October 2014. Melons and cucumbers were purchased from large chain supermarkets, smaller regional chain supermarkets, and ethnic markets to maximize brand and source variability.

Sample sizes were two whole cantaloupes, two whole mangos, and two to six whole cucumbers. Individual samples came from a single unique traceable source and were collected in a manner that minimized any opportunities for cross-contamination by the sample collector and/or the retail outlet. Only items offered in sealed packages were acceptable, such as unopened bagged cartons or other unopened bulk packaging. Samples were collected by turning a new plastic bag inside out, grasping the produce item, and transferring it to a Ziploc bag. When multiple produce items were purchased to form one sample from a single lot, the items were placed in a single Ziploc bag. Samples were transferred in coolers at ambient temperature to the analytical laboratory by FedEx Priority overnight express (early morning arrival). Coolers contained about one pack of blue ice per every four samples to prevent extreme temperature fluctuations during shipment. The blue ice was wrapped in bubble wrap to prevent direct contact with samples and localized cooling. Detailed information about the samples is listed in Table 2.

Microbiological assays: leafy greens. Samples of leafy greens from fiscal year 2010 were analyzed for aerobic plate counts (APCs), total coliform counts (TCCs), *E. coli* counts (ECCs), *Salmonella, L. monocytogenes, E. coli* O157:H7, non-O157 STEC, and *Shigella*. Samples of leafy greens from fiscal years 2011 and 2012 were analyzed for *Salmonella, L. monocytogenes, E. coli* O157:H7, and non-O157 STEC.

APCs were conducted according to AOAC official method 990.12 *(5)*. TCCs and ECCs were conducted according to AOAC method 991.14 *(4)*. The analytical sample size was 50 g for each assay.

For *Salmonella,* 375-g samples were analyzed using the U.S. Department of Agriculture (USDA) method with the BAX PCR *(31)*.

For *L. monocytogenes,* 250-g samples were analyzed using AOAC method 2003.12 (BAX screening) *(6)*. In positive samples, the *L. monocytogenes* level was determined following the U.S. Food and Drug Administration (FDA) *Bacteriological Analytical Manual* (BAM) method *(34)*.

For *E. coli* O157:H7 and non-O157 STEC, 200-g samples were analyzed using the BAM method for diarrheagenic *E. coli* *(35)*.

For *Shigella,* 225-g samples were placed in sterile plastic bags, and 225 mL of Butterfield's phosphate buffer solution was added. The sample was shaken at 100 rpm for 5 min using a shaker, and 125 mL of the sample rinse solution was transferred to a sterile 500-mL Erlenmeyer flasks containing 125 mL of 2× *Shigella* broth with novobiocin (1.0 μg/mL) and incubated at 40 ± 2°C for 24 h under aerobic conditions with shaking at 150 rpm. Two 10-mL portions from novobiocin sample were transferred into 15-mL centrifuge tubes and centrifuged at 8,000 × *g* for 5 min. The supernatants were decanted, and the pellets from each tube were

TABLE 1. *APCs, TCCs, and ECCs for iceberg lettuce, romaine lettuce, and spinach samples collected in fiscal year 2010*[a]

Sample group	Mean ± SD (log CFU/g) (no. of samples)[b]		
	APC	TCC	ECC
Iceberg lettuce			
All unprocessed	A 6.04 ± 1.01 (1,145)	A 0.53 ± 1.14 (1,222)	A 0.00 ± 0.03 (1,223)
Unprocessed organic	6.28 ± 0.83 (160) A	0.51 ± 1.16 (164) A	0.00 ± 0.00 (164) A
Unprocessed conventional	6.01 ± 1.06 (985) B	0.54 ± 1.15 (1,058) A	0.00 ± 0.03 (1,059) A
All RTE	B 6.59 ± 0.83 (818)	B 0.78 ± 1.43 (912)	A 0.00 ± 0.00 (914)
RTE organic	6.64 ± 0.78 (16) A	0.22 ± 0.87 (16) A	0.00 ± 0.00 (16) A
RTE conventional	6.59 ± 0.87 (802) A	0.78 ± 1.44 (896) A	0.00 ± 0.00 (898) A
All iceberg	6.27 ± 0.98 (1,988)	0.64 ± 1.28 (2,160)	0.00 ± 0.02 (2,164)
All organic	6.31 ± 0.82 (178) A	0.48 ± 1.13 (182) A	0.00 ± 0.00 (182) A
All conventional	6.27 ± 1.02 (1,810) A	0.65 ± 1.29 (1,978) A	0.00 ± 0.02 (1,982) A
Romaine lettuce			
All unprocessed	A 6.29 ± 0.77 (1,886)	A 0.84 ± 1.35 (1,957)	A 0.00 ± 0.11 (1,956)
Unprocessed organic	6.49 ± 0.64 (587) A	0.94 ± 1.43 (611) A	0.00 ± 0.07 (612) A
Unprocessed conventional	6.19 ± 0.86 (1,299) B	0.81 ± 1.31 (1,346) B	0.00 ± 0.12 (1,334) A
All RTE	B 6.37 ± 0.89 (1,488)	A 0.80 ± 1.38 (1,558)	A 0.01 ± 0.12 (1,560)
RTE organic	6.41 ± 0.83 (292) A	0.89 ± 1.42 (304) A	0.00 ± 0.06 (304) A
RTE conventional	6.37 ± 0.91 (1,196) A	0.78 ± 1.37 (1,254) A	0.00 ± 0.14 (1,256) A
All romaine	6.32 ± 0.83 (3,439)	0.83 ± 1.36 (3,581)	0.01 ± 0.11 (3,582)
All organic	6.45 ± 0.75 (905) A	0.93 ± 1.43 (940) A	0.00 ± 0.07 (941) A
All conventional	6.28 ± 0.88 (2,534) B	0.80 ± 1.34 (2,641) B	0.01 ± 0.13 (2,641) A
Spinach			
All unprocessed	A 6.92 ± 0.65 (779)	A 0.97 ± 1.55 (854)	A 0.01 ± 0.22 (854)
Unprocessed organic	6.98 ± 0.59 (110) A	1.12 ± 1.65 (119) A	0.00 ± 0.00 (119) A
Unprocessed conventional	6.92 ± 0.62 (669) A	0.94 ± 1.53 (735) A	0.01 ± 0.24 (735) A
All RTE	A 6.95 ± 0.63 (1,252)	B 0.70 ± 1.33 (1,416)	A 0.00 ± 0.03 (1,418)
RTE organic	6.93 ± 0.55 (337) A	0.70 ± 1.33 (372) A	0.00 ± 0.05 (373) A
RTE conventional	6.96 ± 0.65 (915) A	0.71 ± 1.33 (1,044) A	0.00 ± 0.00 (1,045) A
All spinach	6.93 ± 0.64 (2,065)	0.80 ± 1.42 (2,305)	0.00 ± 0.05 (2,306)
All organic	6.95 ± 0.56 (457) A	0.79 ± 1.42 (501) A	0.00 ± 0.04 (502) A
All conventional	6.94 ± 0.64 (1,608) A	0.80 ± 1.42 (1,804) A	0.01 ± 0.16 (1,804) A
Total	6.46 ± 0.91 (7,492)	0.77 ± 1.36 (8,046)	0.00 ± 0.11 (8,052)
Total organic	6.58 ± 0.75 (1,540) A	0.83 ± 1.40 (1,623) A	0.00 ± 0.06 (1,625) A
Total conventional	6.44 ± 0.86 (5,952) B	0.76 ± 1.35 (6,423) A	0.00 ± 0.12 (6,427) A
Total unprocessed	A 6.33 ± 0.93 (3,810)	A 0.77 ± 1.35 (4,033)	A 0.01 ± 0.13 (4,031)
Total RTE	B 6.62 ± 0.86 (3,558)	A 0.76 ± 1.37 (3,886)	A 0.00 ± 0.08 (3,892)

[a] APC, aerobic plate count; TCC, total coliform count; ECC, *E. coli* count. Numbers may not be consistent across different tests for the same sample type.

[b] For unprocessed and RTE samples in the same category, means preceded by different letters are significantly different ($P \leq 0.05$, *t* test). For organic and conventional samples in the same category, means followed by different letters are significantly different ($P \leq 0.05$, *t* test).

suspended in l mL of phosphate-buffered saline and combined into a single tube. This solution (100 μL) was spread plated onto two *Shigella* rainbow agar plates. One loopful (10 μL) was streaked onto *Shigella* rainbow, Hektoen enteric, and xylose lysine desoxycholate agar plates and incubated for 16 to 24 h at 37 ± 2°C. Typical *Shigella* colonies from these plates were confirmed using a PCR protocol.

A small portion of a presumptive *Shigella* colony was added to 150 μL of distilled water, boiled in a water bath for 5 min, cooled on ice, and centrifuged at 16,000 × *g* for 3 min for use as the DNA template for the PCR. PCR primers were ipaHF (5′-GTT CCT TGA CCG CCT TTC CGA TAC CGT C-3′) and ipaHR (5′-GCC GGT CAG CCA CCC TCT GAG AGT AC-3′). Each PCR mixture included 6.5 μL of distilled water, 12.5 μL of HotStart *Taq* Master Mix (Qiagen, Valencia, CA), 2.5 μL of primer ipaHF (10 pmole/μL stock), 2.5 μL of primer ipaHR (10 pmole/μL stock), and 1 μL of DNA template. PCR conditions were 95°C for 15 min and then 30 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min. The PCR products were kept at 4°C until gel analysis. After amplification, 10 μL of the PCR products was transferred to a new microcentrifuge tube containing 2 μL of tracking dye and loaded onto a 1% agarose gel. A 620-bp product was expected from a positive sample.

Microbiological assays: sprouts. Sprout samples were analyzed for the presence of *Salmonella, L. monocytogenes, E. coli* O157:H7, and non-O157 STEC. Samples that were positive for these pathogens were further evaluated to determine pathogen population levels.

TABLE 2. *Sample characteristics and pathogen prevalence for leafy greens, sprouts, and melons*

				No. (%, 95% CI)[a]			
Produce	Conventional	Organic	Total no. of samples tested	Samples positive for *Salmonella*	Samples positive for *E. coli* O157:H7	Samples positive for non-O157 STEC	Samples positive for *L. monocytogenes*[b]
Leafy greens							
2010							
Iceberg lettuce	1,985	182	2,167	1 (0.05, <0.01–0.26)	0 (0, 0–0.17)	0 (0, 0–0.17)	
Romaine lettuce	2,647	942	3,589	2 (0.06, <0.01–0.20)	1 (0.03, <0.01–0.16)	3 (0.08, 0.02–0.24)	
Spinach	1,810	502	2,312	2 (0.09, 0.01–0.31)	1 (0.04, <0.01–0.24)	2 (0.09, 0.01–0.31)	
Total	6,442	1,626	8,068	5 (0.06, 0.02–0.14)	2 (0.02, <0.01–0.09)	5 (0.06, 0.02–0.14)	
2011							
Iceberg lettuce	215	0	215	0 (0, 0–1.70)	0 (0, 0–1.70)	0 (0, 0–1.70)	0 (0, 0–1.70)
Romaine lettuce	344	33	377	0 (0, 0–0.97)	0 (0, 0–0.97)	0 (0, 0–0.97)	2 (0.53, 0.06–1.90)
Spinach	397	124	521	0 (0, 0–0.71)	0 (0, 0–0.71)	0 (0, 0–0.71)	5 (0.96, 0.31–2.23)
Total	956	157	1113	0 (0, 0–0.33)	0 (0, 0–0.33)	0 (0, 0–0.33)	7 (0.63, 0.25–1.29)
2012							
Iceberg lettuce	928	0	928	0 (0, 0–0.40)	0 (0, 0–0.40)	0 (0, 0–0.40)	2 (0.22, 0.03–0.78)
Romaine lettuce	1,363	219	1,582	1 (0.06, <0.01–0.35)	0 (0, 0–0.23)	2 (0.13, 0.02–0.46)	6 (0.38, 0.14–0.82)
Spinach	1,749	743	2,492	1 (0.04, <0.01–0.22)	0 (0, 0–0.15)	3 (0.12, 0.02–0.35)	0 (0, 0–0.15)
Total	4,040	962	5,002	2 (0.04, <0.01–0.14)	0 (0, 0–0.07)	5 (0.10, 0.03–0.23)	8 (0.16, 0.07–0.31)
3 yr combined							
Iceberg lettuce	3,128	182	3,310	1 (0.03, <0.01–0.17)	0 (0, 0–0.11)	0 (0, 0–0.11)	2 (0.06, <0.01–0.22)
Romaine lettuce	4,354	1,194	5,548	3 (0.05, 0.01–0.16)	1 (0.02, <0.01–0.10)	5 (0.09, 0.03–0.21)	8 (0.14, 0.06–0.28)
Spinach	3,956	1,369	5,325	3 (0.06, 0.01–0.16)	1 (0.02, <0.01–0.10)	5 (0.09, 0.03–0.22)	5 (0.09, 0.03–0.22)
Total	11,438	2,745	14,183	7 (0.05, 0.02–0.10)	2 (0.01, 0–0.05)	10 (0.07, 0.03–0.13)	15 (0.11, 0.06–0.17)
Sprouts							
Alfalfa	240	251	491	0 (0, 0–0.75)	0 (0, 0–0.75)	0 (0, 0–0.75)	2 (0.41, 0.05–1.46)
Bean	658	148	806	0 (0, 0–0.46)	0 (0, 0–0.46)	1 (0.12, <0.01–0.69)	0 (0, 0–0.46)
Other	946	409	1,355	0 (0, 0–0.27)	0 (0, 0–0.27)	0 (0, 0–0.27)	1 (0.07, <0.01–0.41)
Total	1,844	808	2,652	0 (0, 0–0.14)	0 (0, 0–0.14)	1 (0.04, <0.01–0.21)	3 (0.11, 0.02–0.33)
Melons							
Cantaloupe	993	82	1,075	2 (0.19, 0.02–0.67)			0 (0, 0–0.34)
Cucumber	994	166	1,160	0 (0, 0–0.32)			5 (0.43, 0.14–1.00)
Mango	1,076	100	1,176	2 (0.17, 0.02–0.61)			3 (0.26, 0.05–0.74)
Total	3,063	348	3,411	4 (0.12, 0.03–0.30)			8 (0.23, 0.10–0.46)

[a] CI, Clopper-Pearson exact 95% confidence interval.
[b] Because *L. monocytogenes* was not analyzed for 2010 samples, values for the 3 years combined are from only 2011 and 2012.

TABLE 3. *Prevalence of pathogens in conventional versus organic produce*

Produce	No. of samples tested	No. (%, 95% CI)[a] Samples positive for *Salmonella*	Samples positive for *E. coli* O157:H7	Samples positive for non-O157 STEC	Samples positive for *L. monocytogenes*[b]
Leafy greens					
2010					
Organic	1,626	2 (0.12, 0.01–0.44)	0 (0, 0–0.23)	2 (0.12, 0.01–0.44)	
Conventional	6,442	3 (0.05, <0.01–0.14)	2 (0.03, <0.01–0.11)	3 (0.05, <0.01–0.14)	
2011					
Organic	157	0 (0, 0–2.32)	0 (0, 0–2.32)	0 (0, 0–2.32)	2 (1.27, 0.15–4.53)
Conventional	956	0 (0, 0–0.39)	0 (0, 0–0.39)	0 (0, 0–0.39)	5 (0.52, 0.17–1.22)
2012					
Organic	962	0 (0, 0–0.38)	0 (0, 0–0.38)	3 (0.31, 0.06–0.91)	0 (0, 0–0.38)
Conventional	4,040	2 (0.05, <0.01–0.18)	0 (0, 0–0.09)	2 (0.05, <0.01–0.18)	8 (0.20, 0.09–0.39)
3 yr combined					
Organic	2,745	2 (0.07, <0.01–0.26)	0 (0, 0–0.13)	5 (0.18, 0.06–0.42)	2 (0.07, <0.01–0.26)
Conventional	11,438	5 (0.04, 0.01–0.10)	2 (0.02, 0–0.06)	5 (0.05, 0.01–0.10)	13 (0.11, 0.06–0.19)
Sprouts					
Organic	808	0 (0, 0–0.46)	0 (0, 0–0.46)	0 (0, 0–0.46)	2 (0.25, 0.03–0.89)
Conventional	1,844	0 (0, 0–0.20)	0 (0, 0–0.20)	1 (0.05, <0.01–0.30)	1 (0.05, <0.01–0.30)
Melons					
Organic	348	0 (0, 0–1.05)			2 (0.57, 0.07–2.06)
Conventional	3,063	4 (0.13, 0.04–0.33)			6 (0.20, 0.07–0.43)

[a] CI, Clopper-Pearson exact 95% confidence interval.
[b] Because *L. monocytogenes* was not analyzed for 2010 samples, values for the 3 years combined are from only 2011 and 2012.

For *Salmonella,* 50-g samples was analyzed using the USDA method with the BAX PCR *(31)* and the USDA DNA extraction method *(32)*. For positive samples, *Salmonella* was enumerated by analyzing triplicate samples of weights 10, 1, 0.1, and 0.01 g according to BAM instructions for *Salmonella (36)* and instructions for most-probable-number (MPN) determinations *(33)*.

For *L. monocytogenes,* 25-g samples were analyzed using AOAC method 2003.12 *(6)*. For positive samples, pathogen levels were determined using a three-tube four-level MPN method. The first, second, third, and fourth sets of the MPN tubes represent 10, 1.0, 0.1, and 0.01 g of the original sample, respectively. Samples were analyzed according to the BAM method for *L. monocytogenes (34)*.

For *E. coli* O157:H7 and non-O157 STEC, 50-g samples were analyzed according to the BAM instructions for diarrheagenic *E. coli (35)*. For positive samples, the pathogen level was determined with triplicate samples of 10, 1, 0.1, and 0.01 g per according to the BAM methods *(33, 35)*.

Microbiological assays: melons. Melon samples were analyzed for *Salmonella* and *L. monocytogenes*. For *Salmonella,* samples were analyzed using the BAM method *(36)*. The sample sizes for analysis were one cantaloupe, 450 g of mangoes, and 450 g of cucumbers. The sample-to-broth ratios (w/w) were 1:1.5 for cantaloupe and 1:1 for mangos and cucumbers. The preenrichment broths used for cantaloupes, mangoes, and cucumbers were universal preenrichment broth, buffered peptone water, and lactose broth, respectively.

For *L. monocytogenes,* the sample sizes for analysis were one cantaloupe, 450 g of mangoes, and 450 g of cucumbers. For cantaloupes, peels were cut and analyzed. For mangos and cucumbers, entire samples were analyzed. Cantaloupe peels, mangoes (seeds removed), and cucumbers were mixed with enrichment broths at 1:1 (w/w) and thoroughly blended, and then 50 g of the blended mix was added to 200 mL of preenrichment broth. Buffered *Listeria* enrichment broth *(34)* was used for enrichment. The rest of the blended mix was retained under refrigerated conditions. For positive samples triplicates of 20, 2, 0.2, and 0.02 g of the retained blended mix were added to enrichment broth at 1:4 (w/w) for pathogen enumeration. The rest of the procedure was according the BAM instructions *(33, 34)*.

Serotyping of *Salmonella*. *Salmonella* isolates from produce were serotyped using Luminex xMAP *Salmonella* serotyping assay (https://www.luminexcorp.com/research/applied-markets/salmonella-serotyping-assay/). Isolates untypeable by the Luminex assay were serotyped using the conventional Kauffman-White antigenic formulae scheme *(13, 36)*. Other pathogens were not serotyped.

PFGE. Pulsed-field gel electrophoresis (PFGE) was conducted according to the CDC PulseNet protocols *(8)*. For *Salmonella, Xba*I was the primary restriction enzyme and *Bln*I was the secondary restriction enzyme. For *L. monocytogenes, Asc*I was the primary restriction enzyme, and *Apa*I was the secondary restriction enzyme. The PFGE profile data were analyzed using Bionumerics v.7.6 (Applied Maths, Austin, TX). PFGE data were not collected for the other pathogens.

Genome sequencing. Isolates were grown in tryptic soy broth (Difco, BD, Franklin Lakes, NJ) overnight at 37°C, and genomic DNA was extracted using the DNeasy Blood & Tissue kit

TABLE 4. *Leafy green samples containing pathogens during fiscal year 2010*

Sample code	NCBI accession no.[a]	Collection date	Produce type	Condition	Non-O157 STEC	*E. coli* O157:H7	*Salmonella*	Serotype	*Shigella*
80959-06	SAMN00754301	22 Mar.	Iceberg	Unprocessed	ND[b]	ND	Pos[c]	Montevideo	ND
81038-01	SAMN00710599	7 Apr.	Romaine, organic	RTE	ND	ND	Pos	Montevideo	ND
81255-34	NA[d]	5 May	Romaine	RTE	ND	ND	Pos	Anatum	ND
81275-32	NA	10 May	Spinach, organic	RTE	ND	ND	Pos	Duisburg	ND
81593-41	NA	23 June	Spinach	Unprocessed	ND	ND	Pos	Mbandaka	ND
81655-26	NA	28 June	Spinach	RTE	ND	Pos	ND		ND
81701-38	NA	5 July	Romaine	RTE	ND	Pos	ND		ND
81780-19	SAMN04273144	18 July	Romaine, organic	Unprocessed	Pos	ND	ND	Not typed	ND
81849-61	SAMN04273145	29 July	Spinach	RTE	Pos	ND	ND	Not typed	ND
81869-25	SAMN04273146	03 Aug.	Romaine, organic	Unprocessed	Pos	ND	ND	Not typed	ND
81990-62	NA	30 Aug.	Spinach	RTE	Pos	ND	ND	Not typed	ND
82012-16	NA	30 Aug.	Romaine	Unprocessed	Pos	ND	ND	Not typed	ND

[a] Isolate identification number assigned for the National Center for Biotechnology Information (NCBI) database.
[b] ND, not detected.
[c] Pos, positive.
[d] NA, not available. Neither an NCBI number nor sequencing data were available.

(Qiagen). DNA concentrations were measured with a Qubit fluorometer (Life Technologies, Invitrogen, CA), standardized to 0.2 ng/μL, and stored at –20°C until library preparation. Libraries were prepared with the Nextera XT DNA sample preparation kit (Illumina, San Diego, CA) according to the manufacturer's instructions. Genomic libraries were paired-end sequenced using the MiSeq sequencing technology (Illumina) with 500 (2 × 250 bp) cycles to a coverage depth of 30 to 90× at the FDA Center for Food Safety and Applied Nutrition (CFSAN) genomics laboratory. All genomes were submitted as assembled reads to the National Center for Biotechnology Information (NCBI) *(2)*.

Statistical analyses. Standard statistical tests were employed in the analysis using SAS 9.4 (SAS Institute, Cary, NC).

RESULTS

APCs, TCCs, and ECCs of leafy greens. APCs, TCCs, and ECCs were collected only in fiscal year 2010. The mean (±standard deviation) APCs among different types of leafy greens, i.e., unprocessed versus RTE, organic versus conventional, and lettuces versus spinach, were similar, from 6.01 ± 1.06 log CFU/g (unprocessed conventional iceberg lettuce) to 6.98 ± 0.59 log CFU/g (unprocessed organic spinach). The mean APCs for iceberg and romaine lettuces were similar, 6.27 ± 0.98 and 6.32 ± 0.83 log CFU/g, respectively. Spinach had a slightly higher APC of 6.93 ± 0.64 log CFU/g, although this value was not significantly different from those of lettuces ($P > 0.05$). The overall mean APC for the combined leafy greens (iceberg lettuce, romaine lettuce, and spinach samples) was 6.46 ± 0.91 log CFU/g (Table 1).

TCCs for different types and categories of leafy greens were all <1 log CFU/g, except for the mean for unprocessed organic spinach samples (1.12 ± 1.65 log CFU/g). Significant differences in TCCs ($P \leq 0.05$) were found between unprocessed organic and unprocessed conventional romaine lettuce samples and between all organic and all conventional romaine lettuce samples. All other organic and conventional comparisons among leafy greens were not significantly ($P > 0.05$). Significant differences in TCCs ($P \leq 0.05$) were found between unprocessed and RTE iceberg lettuce and spinach samples but not for the romaine lettuce and combined leafy greens (Table 1).

ECCs for different categories of leafy greens were all very low, at ≤0.01 log CFU/g. No significant differences ($P > 0.05$) were observed between organic and conventional or between unprocessed and RTE samples regardless of produce type (iceberg lettuce, romaine lettuce, or spinach) (Table 1).

Prevalence of pathogens in leafy greens. For fiscal year 2010, *Shigella* was not detected among the 8,068 iceberg lettuce, romaine lettuce, and spinach samples tested (Table 4). Only 1 (0.05%) of the 2,167 iceberg lettuce samples was positive for *Salmonella*; no *E. coli* O157:H7 or non-O157 STEC were detected in these samples. *Salmonella, E. coli* O157:H7, and non-O157 STEC were detected in romaine lettuce and spinach samples at prevalences of 0.03 to 0.09% (Table 2). For fiscal year 2011, no *Salmonella, E. coli* O157:H7, or non-O157 STEC were detected in the 1,113 lettuce and spinach samples tested. All 215 iceberg lettuce samples were also negative for *L. monocytogenes*. *L. monocytogenes* was detected in 0.53 and 0.96% of romaine lettuce and spinach samples, respectively (Table 2). For fiscal year 2012, no *Salmonella, E. coli* O157:H7, or non-O157 STEC were detected in 928 iceberg lettuce samples; however, 0.22% of the samples were positive for *L. monocytogenes*. *E. coli* O157:H7 was not detected in romaine lettuce and spinach samples, but these samples were positive for *Salmonella* and non-O157 STEC at prevalences of 0.04 to 0.13%. The prevalence of *L. monocytogenes* in iceberg lettuce, romaine lettuce, and spinach samples was 0.22, 0.38, and 0%, respectively (Table 2).

When combining 3 years of data (2010, 2011, and 2012) for the 14,183 leafy green samples, no *E. coli* O157:H7 or non-O157 STEC were detected in iceberg

TABLE 5. *Leafy green samples containing foodborne pathogens during fiscal years 2011 and 2012*

Sample code	NCBI accession no.[a]	Collection date	Produce type	Non-O157 STEC	*E. coli* O157:H7	*Salmonella*	*Listeria monocytogenes* (MPN/g) (95% CI)[b]
300635-38	SAMN01939115	6 June 2011	Spinach	ND[c]	ND	ND	Pos[d]
300673-29	SAMN01816207	26 July 2011	Spinach, organic	ND	ND	ND	42.70 (10.40–175)
300711-24	SAMN01816208	19 Sep. 2011	Romaine	ND	ND	ND	9.20 (2.30–36.80)
300722-07	SAMN01939120	19 Sep. 2011	Spinach	ND	ND	ND	Pos
300716-05	SAMN01939127	10 Oct. 2011	Spinach	ND	ND	ND	462 (104–2050)
300717-02	SAMN01816214	18 Oct. 2011	Spinach, organic	ND	ND	ND	0.40 (0.10–2.50)
300719-14	SAMN01939123	31 Oct. 2011	Romaine	ND	ND	ND	Pos
300753-08	SAMN03218212	7 May 2012	Romaine, organic	Pos	ND	ND	ND
300754-23	SAMN03760076	7 May 2012	Iceberg	ND	ND	ND	1.47 (0.41–5.21)
300755-38	SAMN02211847	8 May 2012	Iceberg	ND	ND	ND	2.10 (0.61–7.30)
300757-03	SAMN03760077	14 May 2012	Romaine	ND	ND	ND	1,100 (260–4,700)
300766-09	NA[e]	30 May 2012	Spinach	ND	ND	Pos	ND
300776-26	SAMN02211849	18 June 2012	Romaine	ND	ND	ND	149 (41.60–536)
300808-44	SAMN01939126	15 Aug. 2012	Romaine	ND	ND	ND	149 (41.60–536)
300823-15	SAMN01939125	12 Sep. 2012	Romaine	ND	ND	ND	1,470 (413–5,220)
300825-04	SAMN01939116	17 Sep. 2012	Romaine	ND	ND	ND	1.43 (0.40–5.11)
300829-16	SAMN04273140	24 Sep. 2012	Spinach, organic	Pos	ND	ND	ND
300833-46	SAMN04273141	1 Oct. 2012	Spinach, organic	Pos	ND	ND	ND
300833-51[f]	NA	1 Oct. 2012	Romaine	ND	ND	Pos	ND
300858-10	SAMN04273142	19 Nov. 2012	Romaine	Pos	ND	ND	ND
300866-41	SAMN02569957	3 Dec. 2012	Romaine	ND	ND	ND	42.70 (10.40–175)
300868-48	SAMN04273143	5 Dec. 2012	Spinach	Pos	ND	ND	ND

[a] Isolate identification number assigned for the National Center for Biotechnology Information (NCBI) database.
[b] MPN, most probable number; CI, Clopper-Pearson exact 95% confidence interval.
[c] ND, not detected.
[d] Pos, positive. For samples recorded as positive for *L. monocytogenes,* the MPN was not determined.
[e] NA, not available. Neither an NCBI number nor sequencing data were available.
[f] Isolate from sample 300833-51 was identified as *Salmonella enterica* subsp. *arizonae*. No serotype information was available for other isolates.

lettuce samples (3,310), and only 1 (0.03%) iceberg lettuce sample was positive for *Salmonella*. Prevalences of *Salmonella, E. coli* O157:H7, and non-O157 STEC were 0.02 to 0.09% for romaine lettuce and spinach. The prevalence of *L. monocytogenes* in iceberg lettuce, romaine lettuce, and spinach samples (fiscal years 2011 and 2012) was 0.06, 0.14, and 0.09%, respectively. Fiscal year 2010 samples were not tested for *L. monocytogenes*. The overall prevalences of *Salmonella, E. coli* O157:H7, non-O157 STEC, and *L. monocytogenes* for the 14,183 leafy green samples were 0.05, 0.01, 0.07, and 0.11%, respectively (Table 2).

Prevalence of pathogens in sprouts. Sprout samples were analyzed for *Salmonella, E. coli* O157:H7, non-O157 STEC, and *L. monocytogenes*. No *Salmonella* or *E. coli* O157:H7 were detected from the 2,652 tested samples. Non-O157 STEC was detected in one (0.12%) mung bean sprout sample. *L. monocytogenes* was detected in two (0.41%) alfalfa sprout samples and one broccoli sprout sample (0.07%). The overall prevalences of non-O157 STEC and *L. monocytogenes* in tested sprout samples were 0.04 and 0.11%, respectively (Table 2). Some information related to *L. monocytogenes* and sprouts was reported by Luchansky et al. *(21)*.

Prevalences of *Salmonella* and *L. monocytogenes* in melons. Melon samples were analyzed for *Salmonella* and *L. monocytogenes*. No *Salmonella* was detected in 1,160 cucumber samples. *Salmonella* prevalences in cantaloupes and mangos were 0.19 and 0.17%, respectively. No *L. monocytogenes* was detected in 1,075 cantaloupe samples, but *L. monocytogenes* prevalence in cucumbers and mangos was 0.43 and 0.26%, respectively. The overall prevalences of *Salmonella* and *L. monocytogenes* in 3,411 melon samples tested were 0.12 and 0.23%, respectively (Table 2).

Prevalence of *Salmonella, E. coli* O157:H7, non-O157 STEC, and *L. monocytogenes* in organic versus conventional produce. The comparison of prevalences of pathogens in organic and conventional produce is presented in Table 3. The majority of prevalences of *Salmonella, L. monocytogenes, E. coli* O157:H7, and non-O157 STEC in these organic and conventional samples were 0 to 0.31%, with most on the lower end of the range. Two exceptions were *L. monocytogenes* prevalence of 0.57 and 1.27% in organic melons and leafy greens (fiscal year 2011), respectively. These two produce groups also had the smallest numbers of samples analyzed.

Contamination levels of *L. monocytogenes* in produce. Samples positive for *L. monocytogenes* were analyzed

TABLE 6. *Sprout and melon samples containing foodborne pathogens*

Sample code	NCBI accession no.[a]	Collection date	Produce type	Non-O157 STEC	*E. coli* O157:H7	*Salmonella*	*L. monocytogenes* (MPN/g) (95% CI)[b]
Sprouts							
300856-1	SAMN01939121	14 Nov. 2012	Alfalfa, organic	ND[c]	ND	ND	Pos,[d] <0.036
300879-14	NA[e]	30 Jan. 2013	Mung bean	Pos	ND	ND	ND
1101236-33	SAMN02850684	9 Jan. 2014	Broccoli, organic	ND	ND	ND	0.36 (0.05–2.50)
1101236-38	SAMN02850685	9 Jan. 2014	Alfalfa	ND	ND	ND	1100 (100–2,100)
Melons							
1101422-16	NA	5 Feb. 2014	Cucumber			ND	Pos, not enumerated
1102342-13	NA	10 July 2014	Cucumber			ND	<0.03
1102410-08	NA	24 July 2014	Cucumber			ND	Pos, not enumerated
1102350-01	NA	11 July 2014	Cucumber			ND	1.1 (0.3–4.2)
1102550-30	SAMN04325075	14 Aug. 2014	Mango			ND	Pos, not enumerated
1102606-31	SAMN04325057	21 Aug. 2014	Mango			ND	<0.03
1101923-19	SAMN02850694	24 Apr. 2014	Mango, organic			ND	<0.03
1102273-09	SAMN02943415	25 June 2014	Cucumber, organic			ND	150 (42–540)
1102018-13	SAMN02800603	14 May 2014	Mango			Pos	ND
1103041-06[f]	SAMN03285119	21 Oct. 2014	Cantaloupe			Pos	ND
1103041-06	SAMN03285120	21 Oct. 2014	Cantaloupe			Pos	ND
1102204-15	NA	13 June 2014	Cantaloupe			Pos	ND
1102550-03	NA	14 Aug. 2014	Mango			Pos	ND

[a] Isolate identification number assigned for the National Center for Biotechnology Information (NCBI) database.
[b] MPN, most probable number; CI, Clopper-Pearson exact 95% confidence interval.
[c] ND, not detected.
[d] Pos, positive.
[e] NA, not available. Neither an NCBI number nor sequencing data were available.
[f] Two strains of *Salmonella* were isolated from this sample: *Salmonella* Minnesota (SAMN03285119) and *Salmonella* 6,7:m,t:– (SAMN03285120). No serotype information was available for other isolates.

to determine the contamination levels (Tables 5 and 6), which ranged from 0.4 to 1,470 MPN/g for leafy green samples; two conventional romaine lettuce samples had >1,000 MPN/g. Among the three sprouts samples that were positive for *L. monocytogenes,* the level in the organic alfalfa sprout sample was not enumerated, and the organic broccoli and conventional alfalfa sprout samples had 0.36 and 1,100 MPN/g, respectively. Among the eight melon samples positive for *L. monocytogenes,* the levels in three samples (two conventional cucumbers and one conventional mango) were not determined due to lack of material, two conventional cucumber samples had 0.03 and 1.1 MPN/g, an organic cucumber sample had 150 MPN/g, and two mango samples (one organic and one conventional) both had <0.03 MPN/g.

Diversity of foodborne pathogens isolated from produce samples. Among the eight serotyped *Salmonella* isolates obtained from these produce samples, seven serotypes were found (Tables 4 through 6). PFGE profiles of these isolates indicated a similar level of diversity (Fig. 1). For *L. monocytogenes,* PFGE profiles by both restriction enzymes *Asc*I and *Apa*I were very diverse (Fig. 2).

DISCUSSION

APCs, TCCs, and ECCs can reflect the actual sanitary conditions of produce. Zhang and Lampel *(43)* reported that mean APCs of parsley, cilantro, spinach, and lettuce ranged from 7.06 to 7.51 log CFU/g. Korir et al. *(17)* estimated that the mean APCs of basil, cilantro, lettuce, scallion, spinach, and parsley were 7.49 to 8.06 log CFU/g, and mean TCCs and ECCs were <1 log CFU/g. Vital et al. *(37)* investigated a few produce samples from both open air markets and supermarkets and found *E. coli* levels of 0.30 to 4.15 log CFU/g. Wood et al. *(40)* reported that green, red, and romaine lettuce had mean APCs of 6.1 to 6.7 log CFU/g, and their median TCCs and ECCs were 1.9 and 0.7 log CFU/g, respectively. Apple, mango, and orange samples were estimated to have aerobic psychrotrophic bacterial levels of 1.5, 0.2, and 1.5 log CFU/g, respectively, aerobic mesophilic counts of 3.4, 4.0, and 3.4 log CFU/g, respectively, and coliforms counts of 0.1, 1.1, and 0.9 log CFU/g, respectively *(40)*. Aerobic psychrotrophic bacterial counts, aerobic mesophilic counts, and coliform counts in bean sprouts were estimated to be 1.6, 8.0, and 5.7 log CFU/g, respectively *(30)*. Cucumbers were reported to have thermotolerant coliform levels of 2.75 to 9.04 log CFU per fruit *(23)*. The estimated APCs, TCCs, and ECCs for produce sampled in our present survey were within the ranges reported previously, although our results tended to be slightly lower in some cases (Table 1). The difference between the results from the present research and previous publications might be due to the sample sizes. Our study had much larger sample sizes than did most other surveys, making the estimated values statistically closer to the actual values.

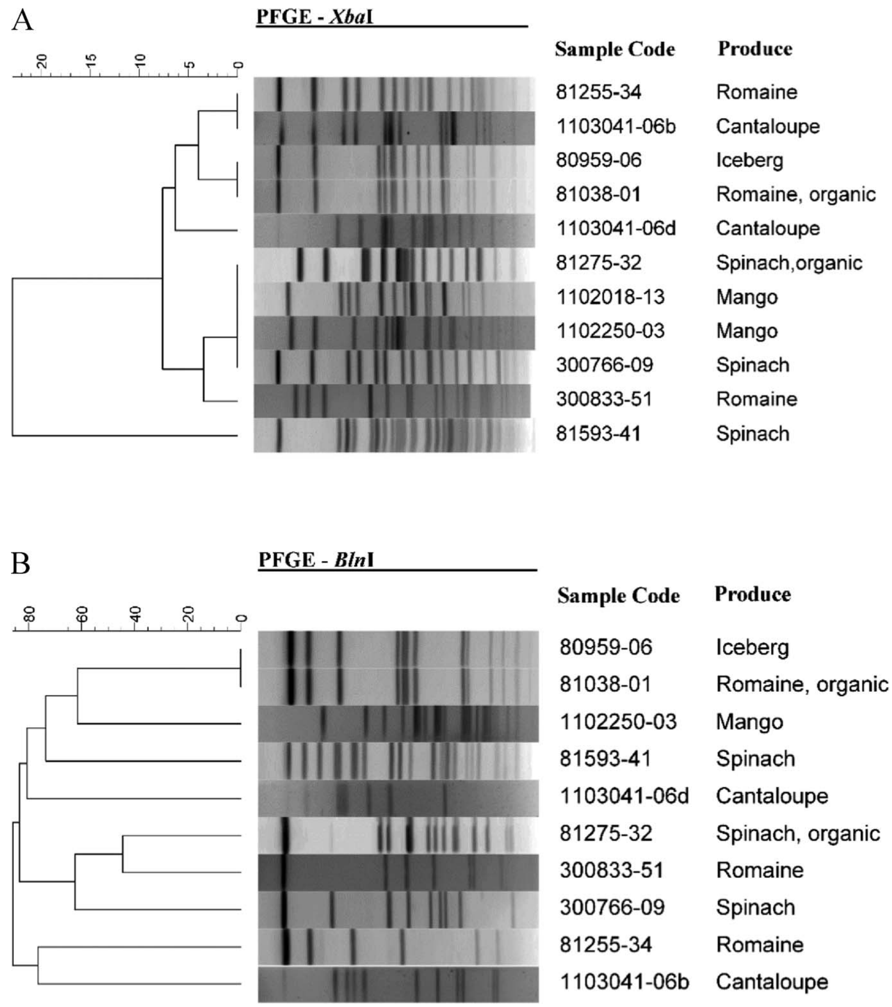

FIGURE 1. *PFGE profiles of* Salmonella *isolates from produce samples determined with restriction enzymes* Xba*I (A) and* Bln*I (B).*

Prevalences of common foodborne pathogens, such as *Salmonella, L. monocytogenes,* and STEC, in fresh produce is one of the main inputs used in risk assessment of these commodities. In a recent Canadian survey *(12),* among leafy vegetables (leafy lettuce, head lettuce, mixed greens, spinach, etc.), no *E. coli* O157:H7, *E. coli* O157:NM, *Campylobacter* spp., or *Shigella* spp. were detected, but *L. monocytogenes* and *Salmonella* prevalences were 0.32 and 0.02%, respectively. Among the leafy herbs (parsley, cilantro, basil, dill, mint, etc.) tested, no *E. coli* O157:H7, *E. coli* O157:NM, *Campylobacter* spp., or *Shigella* spp. were detected, but the *Salmonella* prevalence was at 0.08%. Among cantaloupe samples, no *E. coli* O157:H7, *E. coli* O157:NM, *Campylobacter* spp., or *Shigella* spp. were detected, but *Salmonella* and *L. monocytogenes* prevalences were 0.06 and 1.43%, respectively. In a recent U.S. survey of many different types of produce (including fresh fruits, vegetables, sprouts, etc.) collected from 2002 to 2012, *Salmonella* prevalence was 0 to 0.34%, and cilantro was the most contaminated produce type *(27). E. coli* O157:H7 and *Salmonella* were not detected in a small-scale survey of fresh fruits and vegetables sold in Singapore *(30). Salmonella* was found in 0.8% of fresh produce samples (pepper, tomato, parsley, iceberg lettuce, and green leaf lettuce) in a 2012 survey in Turkey *(14)*. In a 2011 survey in Japan, no *E. coli* O157:H7, *Salmonella,* or *L. monocytogenes* were found in iceberg lettuce *(18)*. Among 17 vegetable types collected and analyzed in Mexico from 2004 to 2005, 98 (5.8%) of 1,700 samples were positive for *Salmonella (26)*. In comparison, in our study prevalences were 0 to 0.23% for *Salmonella, E. coli* O157:H7, non-O157 STEC, *L. monocytogenes,* and *Shigella* among leafy greens, melons, and sprouts (Table 2). These findings are similar to previous North American survey results, although our results tend to be a little bit lower possibly because of the large number of samples we analyzed. Our prevalences also were slightly lower than those reported in some other countries, possibly because of more stringent U.S. regulations and enforcement of food and produce safety rule. In our surveyed vegetables and fruits, *L. monocytogenes* seemed to be more prevalent than *Salmonella, E. coli* O157:H7, and non-O157 STEC in the (Table 2). *L. monocytogenes* can grow under refrigerated conditions. This characteristic in combination with higher prevalence and less research on *L. monocytogenes* than on *Salmonella* and STEC in the past decades suggest that more attention and research should be focused on survival, growth, and control of *L. monocytogenes* in produce.

The difference in contamination levels of organic versus conventional produce has been debated for many years and remains a topic of interest. In both 2010 and 2012, a higher prevalence of non-O157 STEC was observed in leafy green

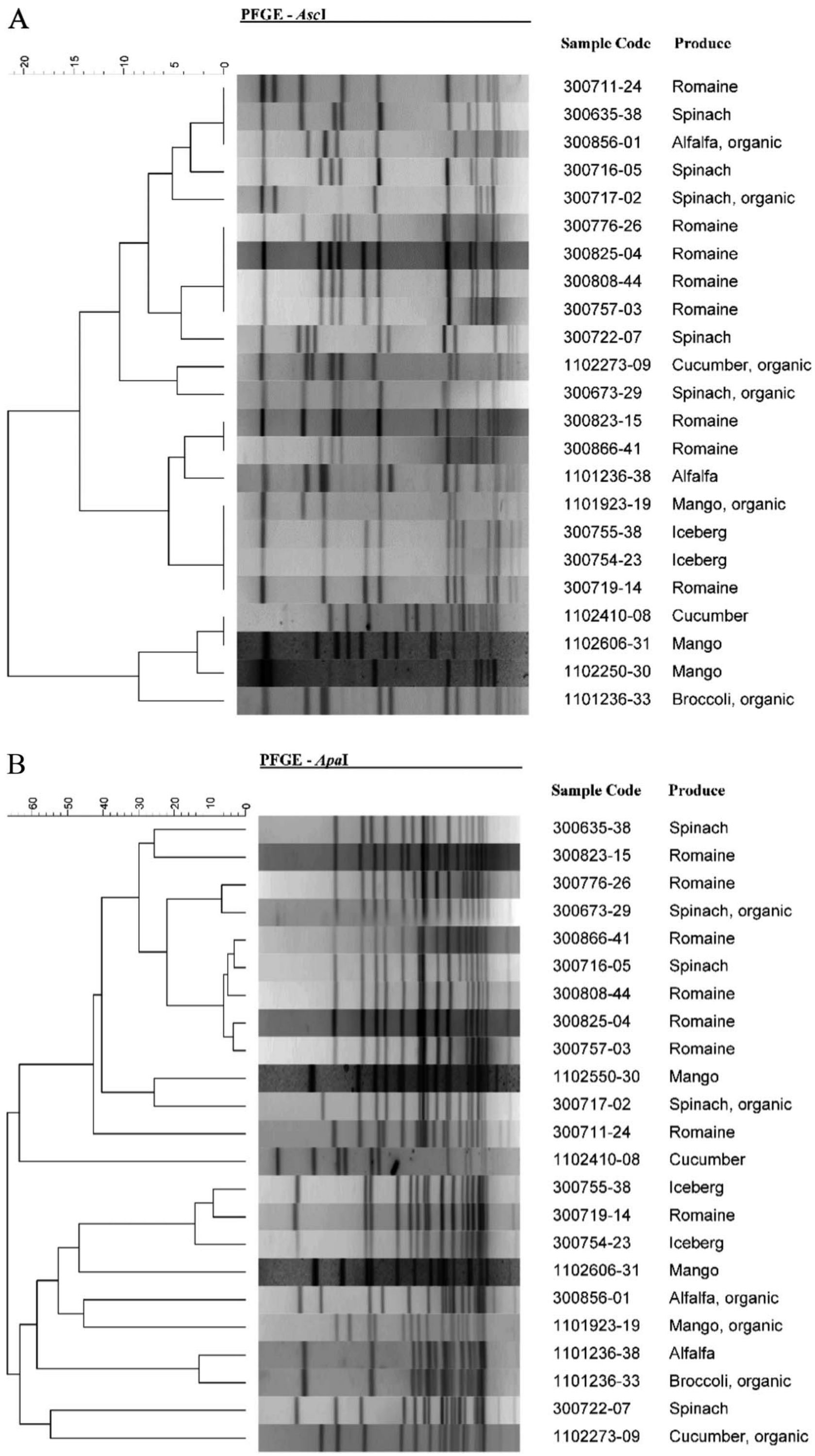

FIGURE 2. *PFGE profiles of* L. monocytogenes *isolates from produce samples determined with restriction enzymes* AscI *(A) and* ApaI *(B).*

samples. However, none of the sprout and melon samples were positive for non-O157 STEC (Table 3). Among all the samples tested in this project, none of the organic samples but four conventional samples were positive for *E. coli* O157:H7 (Table 3). *Salmonella* prevalence was higher in organic leafy greens (0.07%) than in conventional samples (0.04%). No organic sprout and melon samples were positive for *Salmonella,* but four conventional melon samples were positive (Table 3). A higher *L. monocytogenes* prevalence was found in for organic sprouts, melons, and 2011 leafy greens samples than in conventional samples. However, among the 2012 leafy greens samples, no organic samples but eight conventional samples were positive for *L. monocytogenes* (Table 3). The survey created a mixed picture of contamination of organic versus conventional produce. Further research is warranted to determine the difference between organic and conventional produce in their rates of contamination with foodborne pathogens.

Both serotype and PFGE data (Tables 4 through 6 and Figs. 1 and 2) indicated that foodborne pathogens isolated from the surveyed produce were genetically diverse. Recent national FDA surveys of tree nuts (cashews, hazelnuts, macadamia nuts, pecans, pine nuts, and walnuts) and spices (basil leaf, black pepper, coriander seed, cumin seed, curry powder, dehydrated garlic, oregano leaf, paprika, red pepper, sesame seed, and white pepper) produced similar results *(41, 42)*. These findings are logical because the samples analyzed were collected nationwide and a large portion of them were imported from other countries. Globalization of food supplies has made fresh produce available all year round. However, this globalization may also have contributed to more diverse foodborne pathogen populations associated with these foods.

In our present survey, the levels of *L. monocytogenes* in positive samples ranged from <0.03 to 1,470 MPN/g, with three samples at $>1,000$ MPN/g (Tables 3, 5, and 6). In a survey of RTE minimally processed and refrigerated vegetables from Osijek, Croatia, one red cabbage sample contained *L. monocytogenes* at 60 CFU/g *(19)*. In a survey of *Listeria* spp. in RTE foods containing fresh salad vegetables and fresh produce, the maximum levels of *Listeria* spp. were 150, 33, 87, and 2 CFU/g in purple cabbage, green cabbage, lettuce, and carrot, respectively *(44)*. *L. monocytogenes* was found in 53.3% of 105 fresh whole stone fruit samples linked to an outbreak, and the levels ranged from 0.04 to 22 CFU/g *(10)*.

In conclusion, this study has highlighted the potential public health risk associated with the consumption of certain types of fresh leafy greens, sprouts, and melons contaminated with *Salmonella, L. monocytogenes, E. coli* O157:H7, and non-O157 STEC. The pathogens isolated from these fresh produce types were genetically diverse, although the overall pathogen prevalence was relatively low. Regardless of how low the pathogen prevalence is, pathogen-positive produce can cause illness in consumers, especially because most produce is eaten raw. Some of the samples in this survey had high levels of *L. monocytogenes* (as high as 1,470 MPN/g). Therefore, to minimize these human pathogens in fresh produce, good agricultural practices and good hygiene measures should be implemented from farm to fork during production, harvesting, processing, packaging, transporting, and storing.

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