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Identification of *Salmonella* Serotypes Isolated from Cantaloupe and Chile Pepper Production Systems in Mexico by PCR–Restriction Fragment Length Polymorphism

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ABSTRACT

A study was conducted in 2006 to determine the prevalence of *Salmonella* on three cantaloupe farms in Matamoros, Coahuila, Mexico, and on one farm that cultivates chile peppers var. Bell in Culiacán, Sinaloa, Mexico. Samples from cantaloupe farms consisted of cantaloupe rinses, irrigation water, water from furrows in the field, and workers' hands. Samples from the chile pepper farm consisted of rinses of chile peppers obtained at the field, pepper rinses obtained at the packing house, and irrigation water from the field. A total of 55 samples were obtained from both production systems. Twelve and 10 samples from the cantaloupe and chile pepper production systems, respectively, tested positive for *Salmonella* according to a traditional culture method. The difference between the proportion of *Salmonella*-positive samples from the cantaloupe production system (12 of 28 = 0.43) and the chile pepper production system (10 of 27 = 0.37) was not statistically significant ($P > 0.05$). A PCR–restriction fragment length polymorphism (RFLP) method based on the *fliC* gene was used to determine the serotype of the isolates. *Salmonella* Typhimurium was the only serotype found associated with the cantaloupe production system, whereas both *Salmonella* Typhimurium and Enteritidis serotypes were found associated with the chile pepper production system. Results showed that 91% (20 of 22) and 9% (2 of 22) of the isolates from both agricultural systems matched with the *Salmonella* Typhimurium and *Salmonella* Enteritidis reference strain restriction profiles, respectively. This study demonstrates the utility of the PCR-RFLP technique for determining the serotypes of *Salmonella* isolates obtained from cantaloupe and chile pepper production systems.

The genus *Salmonella* comprises over 2,700 serotypes that are found in different hosts and environments and that can cause human illness, including enteric fever, gastroenteritis, and septicemia. *Salmonella* infection has been associated with the consumption of contaminated fresh fruits and vegetables, raw and undercooked poultry, or other red meat and poultry products *(1, 13, 24).* Most studies in Mexico on *Salmonella* tend to corroborate the presence or absence of *Salmonella* in food and water and as a cause of outbreaks; however, only a few studies have been performed investigating the prevalence of different *Salmonella* serotypes in Mexico *(6, 14).* An important phenotypic characteristic of the genus is the antigenic diversity in the flagellar antigens, which is also observed at the genetic level *(10).*

The procedures used to identify *Salmonella* are laborious, time-consuming, and require a number of biochemical and serological tests to confirm presumptive isolates *(25).* On the other hand, tests using molecular tools have been useful in reducing the steps and the time needed for the detection, identification, and characterization of specific pathogens. The *fliC* gene, encoding for the flagellin protein, has been used as a target gene in assays to test the genetic diversity in *Salmonella (10).* The *fliC* gene has a conserved terminal region and a variable central region, which determines the antigenic specificity *(4, 15).* The goals of this study were to determine the prevalence and sources of *Salmonella* in cantaloupe and chile pepper production systems in Mexico and to apply a PCR–restriction fragment length polymorphism (RFLP) method based on the *fliC* gene to identify the serotypes of the *Salmonella* isolates.

MATERIALS AND METHODS

Samples from cantaloupe and chile pepper farms. Samples from cantaloupe production systems were obtained at three farms (fields) in Matamoros, Coahuila, Mexico, during June 2006. The samples consisted of cantaloupe rinses (five composite samples per farm, 125 ml per composite sample), water obtained from an irrigation channel that irrigated the three farms (four samples, 25 ml per sample), water from furrows in fields (one sample per farm, 25 ml per sample), and samples from workers' hands (six samples from one farm, 1 cotton swab per worker). Samples from the chile pepper var. Bell production system were obtained from only one farm located in Culiacan, Sinaloa, Mexico, during April

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2006 and consisted of rinses of chile fruits obtained at the field (five composite samples, 125 ml per composite sample), fruit rinses obtained at the packing house (eight samples, 25 ml per sample), and water from the irrigation channel (14 samples, 25 ml per sample). A total of 55 samples were obtained from both production systems. Cantaloupe and chile pepper rinses were obtained at both production systems from five representative points at each farm, and at each point, five fruits were taken randomly within a radius of 3 m. Each fruit was washed individually by placing the fruit into a sterile Whirl-Pak bag (Nasco, Modesto, Calif.) containing 25 ml of 0.1% buffered peptone water (Becton Dickinson, Sparks, Md.) with a different sterile pair of gloves for each fruit. The fruit was washed by shaking and mixing the bag for at least 2 min. The washings from the five cantaloupes obtained from the same point were combined into a single glass bottle (125 ml per composite sample). Samples from water sources were placed directly into sterile glass bottles (25 ml per sample). Samples from hands of workers in the field were obtained by rubbing the hands with a sterile cotton swab (Dequinsa, D.F. México) moistened in 0.85% saline solution and then placing the cotton swab into a tube with 5 ml of saline solution. Fruit rinses obtained at the packing house were obtained in a similar way as the rinses in the field, except each fruit constituted an individual sample. The samples were immediately transported in a cooler containing ice to the laboratory and processed within 24 h. Each sample was mixed by shaking; 1.0 ml was removed and added to 9.0 ml of buffered peptone water and then incubated at 35°C for 24 h. Enrichment and microbiological analyses were performed according to the method described in the U.S. Department of Agriculture–Food Safety and Inspection Service *Microbiology Laboratory Guidebook (23).* Briefly, 0.5 ± 0.05 ml from the buffered peptone water preenrichment was transferred into 10 ml of tetrathionate broth and 0.1 ± 0.02 ml into 10 ml of modified Rappaport-Vassiliadis broth, which were then incubated at 42 ± 0.5°C for 22 to 24 h. Next, both enrichments were streaked onto brilliant green sulfa and double-modified lysine iron agar plates and incubated at 35 ± 2°C for 18 to 24 h. Plates were then examined for the presence of colonies meeting the description of suspect *Salmonella* colonies.

***Salmonella* strains.** The control strains and isolates obtained in this study are listed in Table 1. *Salmonella enterica* serotypes Typhimurium (American Type Culture Collection [ATCC] 13311, Manassas, Va.), Enteritidis (ATCC 13076), and Paratyphi A (ATCC 9150) were used as positive controls. *Salmonella enterica* serotypes Kentucky, Stanley, Typhi, Typhimurium, and Worthington, kindly provided by Edmundo Calva (Instituto de Biotecnología, Universidad Nacional Autónoma de Mexico, Cuernavaca, Morelos, México) also were included in the study as positive controls. Biochemical tests and serotyping of all of the above-mentioned strains were performed at the institution from which they were obtained. In addition, 22 isolates obtained from the two agricultural production systems that were not serotyped were included, for a total of 30 isolates subjected to PCR-RFLP analysis.

Data analyses. The test of difference between two binomial proportions *(19)* was performed to see whether the difference between the proportions of positive samples from the agricultural production systems was significant.

DNA extraction. Each isolate was streaked onto Trypticase soy agar plates and incubated at 35°C for 24 h. Colonies from each agar plate were removed with a loop and then suspended in 3 ml of buffered peptone water and incubated at 35°C for 24 h. The entire 3 ml of the culture was centrifuged (Sigma 1-15K, Sigma Laborzentrifugen, Gottingen, Germany) three times, 1 ml each time, at 3000 × *g* for 5 min in 1.5-ml Eppendorf tubes. DNA extraction was performed on the resulting cell pellet according to the CTAB method, but omitting the use of polyvinylpyrrolidone and β-mercaptoethanol *(5).* The DNA samples were stored at −20°C until used.

TABLE 1. Salmonella *reference strains used in this study and isolates from cantaloupe and chile pepper production systems*

	Salmonella serotype	Origin/source[a]
	Reference strains	
1	Paratyphi A	ATCC 9150
2	Enteritidis	ATCC 13076
3	Typhimurium	ATCC 13311
4	Kentucky	IBT-UNAM
5	Typhi	IBT-UNAM
6	Worthington	IBT-UNAM
7	Stanley	IBT-UNAM
8	Typhimurium	IBT-UNAM
	Serotype determined by PCR-RFLP	
	Cantaloupe production system	
9	Typhimurium	Matamoros, Coahuila/fruit rinse
10	Typhimurium	Matamoros, Coahuila/fruit rinse
11	Typhimurium	Matamoros, Coahuila/fruit rinse
17	Typhimurium	Matamoros, Coahuila/fruit rinse
18	Typhimurium	Matamoros, Coahuila/fruit rinse
19	Typhimurium	Matamoros, Coahuila/fruit rinse
12	Typhimurium	Matamoros, Coahuila/water from channel
13	Typhimurium	Matamoros, Coahuila/water from channel
14	Typhimurium	Matamoros, Coahuila/water from furrow
15	Typhimurium	Matamoros, Coahuila/water from furrow
16	Typhimurium	Matamoros, Coahuila/water from furrow
20	Typhimurium	Matamoros, Coahuila/workers' hands
	Chili pepper production system	
21	Typhimurium	Culiacán, Sinaloa/water from channel
22	Typhimurium	Culiacán, Sinaloa/water from channel
23	Typhimurium	Culiacán, Sinaloa/water from channel
24	Typhimurium	Culiacán, Sinaloa/water from channel
25	Typhimurium	Culiacán, Sinaloa/water from channel
26	Enteritidis	Culiacán, Sinaloa/water from channel
27	Enteritidis	Culiacán, Sinaloa/fruit rinse
28	Typhimurium	Culiacán, Sinaloa/fruit rinse
29	Typhimurium	Culiacán, Sinaloa/fruit rinse
30	Typhimurium	Culiacán, Sinaloa/fruit at packing

[a] ATCC, American Type Culture Collection; IBT-UNAM, Instituto de Biotecnología Universidad Nacional Autónoma de México.

PCR amplification of the *fliC* gene. Primers ABMS1 (5′-GCACAAGTCATTAATACAAACAGCC-3′) and ABMS2 (5′-TTAACGCAGTAAAGAGAGGACG-3′) described by Dauga et al. *(4)* were modified (by adding the bases that are underlined) on the basis of analysis with Amplify 1.2 software (University of Wisconsin, Madison) and used to amplify a fragment of approximately 1.5 kb from the *fliC* gene in each *Salmonella* strain. PCR amplification of the target sequence was performed in a PCR Express thermal cycler (Thermo Hybaid, Ashford, Middlesex, UK). The PCR mixture contained 25 pmol of each of the primers, 250 μM of a mix of deoxynucleoside triphosphates (Invitrogen, Carslbad, Calif.), 1 mM $MgCl_2$, 1× reaction buffer (200 mM Tris-HCl, pH 8.0, 500 mM KCl), 2.5 U *Taq* DNA polymerase (Bioline,

TABLE 2. *Samples positive for* Salmonella *from the cantaloupe and chile pepper production systems showing the number and type of samples and serotype of isolates*[a]

Type of sample	No. of positive samples/total analyzed (proportion)		*Salmonella* serotype found (no. of positive samples containing the serotype/total analyzed)	
	Cantaloupe	Chile pepper	Cantaloupe	Chile pepper
Fruit rinses	6/15 (0.4)	3/5 (0.6)	Typhimurium (6/15)	Typhimurium (2/5), Enteritidis (1/5)
Water from irrigation channel	2/4 (0.5)	6/14 (0.43)	Typhimurium (2/4)	Typhimurium (5/14), Enteritidis (1/14)
Water from farm	3/3 (1.0)	—	Typhimurium (3/3)	
Workers' hands	1/6 (0.16)	—	Typhimurium (1/6)	
Fruit rinses at packing house	—	1/8 (0.12)	—	Typhimurium (1/8)
Total	12/28 (0.43)	10/27 (0.37)	Typhimurium (12/28)	Typhimurium, (8/27), Enteritidis (2/27)

[a] —, none of these types of samples were obtained.

Taunton, Mass.), 100 ng of DNA template, and deionized water for a final volume of 25 μl. The reaction mixture was subjected to PCR under the following conditions: heat denaturation at 95°C for 1 min and then 35 cycles of heat denaturation at 94°C for 30 s, primer annealing at 60°C for 30 s, and DNA extension at 72°C for 30 s. After the last cycle, the PCR tubes were maintained at 72°C for 10 min to complete synthesis of all strands. Five microliters of each PCR reaction was loaded onto a 1.5% agarose gel (Promega, Madison, Wis.), and electrophoresis was performed with the use of 1× SB buffer (0.2 M NaOH, 0.7278 M boric acid). The gel was stained with ethidium bromide, examined with a UV transilluminator (Spectroline Transilluminator, model 7C-254R, Electronics Corporation, Westbury, N.Y.), and photographed with a Polaroid camera (adapted with UV filter, film A667). A 250-bp ladder (Invitrogen) was used as a molecular weight standard.

RFLP analysis. For the PCR-RFLP analysis, the unpurified amplified PCR product of the *fliC* gene was cleaved with *Sau*3AI (Promega) according to the manufacturer's instructions. The digestion reaction consisted of 10 μl of PCR product, 2 μl of 10× reaction buffer B (60 mM Tris-HCl [pH 7.5] 500 mM NaCl, 60 mM $MgCl_2$, 10 mM dithiothreitol), 0.2 μl of acetylated BSA (10 μg/μl), 5 U of enzyme, and deionized water for a final volume of 20 μl. The reaction was gently vortexed and then incubated at 37°C for 1 h. After incubation, 6 μl of sample was mixed with 3 μl of loading buffer (0.25% bromophenol blue, 0.25% xylene cyanole, 30% glycerol) and electrophoresed on a 10% acrylamide gel for 2.5 h at 100 V in 1× Tris-borate-EDTA buffer. The 100-bp Hyperladder (Bioline) was used as the molecular weight standard for determining the molecular weight of the restriction fragments. Gels were stained and photographed as described above. The degree of variability between two strains was determined on the basis of the Dice coefficient, and a dendrogram was made by the unweighted pair group method with arithmetic mean *(22).* The SPSS (Statistical Product for Service Solutions, v 10.0, SPSS Inc., Chicago, Ill.) statistical package was used for the analyses.

RESULTS AND DISCUSSION

Samples positive for *Salmonella*. The proportions of positive samples, obtained by dividing the number of samples positive for *Salmonella* by the total number of samples, were 0.43 and 0.37 from the cantaloupe and chile pepper production systems, respectively, and the observed difference was not statistically significant ($P > 0.05$). Although the number of samples obtained from the cantaloupe farms was quite small, the observed differences among the three farms were not statistically significant ($P > 0.05$). A total of 22 *Salmonella* isolates were obtained from the two agricultural production systems: 12 from the cantaloupe farm and 10 from the chile pepper farm. From the cantaloupe production system, at least one sample from each source was positive for *Salmonella,* and water sources had higher proportions of positive samples (5 of 7) ($P \leq 0.05$) compared with the other samples (Table 2). From the chile pepper production system, a higher number of positive samples came from fruit rinses at the field and water from the irrigation channel. These results demonstrate that water from the irrigation channel was an important source of *Salmonella* contamination. Investigations examining the environmental sources of *Salmonella* contamination indicate that water is an important source, particularly irrigation water containing manure, feces from animal wildlife, or sewage effluent *(9, 20).* A likely reason why *Salmonella* was found in water samples is that the water used for irrigation of the cantaloupe farms originated from the Rio Nazas and flowed through open channels without any protection against contact with animals. In addition, the cantaloupe farms were irrigated from the same channels used to irrigate forage crops with sewage. Castillo et al. *(2)* stated that *Salmonella* and *Escherichia coli* were frequently detected in water used for irrigation of cantaloupe farms in South Texas because the water came from the Rio Grande. From the chile pepper farm, 6 of 14 irrigation water samples were positive, and 3 of 5 fruit rinse samples were positive. It is possible that insects or birds could also have transmitted *Salmonella* to the fruits. Moore et al. *(16)* mentioned the possibility that insects belonging to the genus *Chironomus* were direct or indirect vectors of enteric bacteria for water and foods. On the other hand, Sela et al. *(21)* stated that direct contact with manure-contaminated soil or dust might be a source of preharvest contamination, and indirect sources of contamination could also be due to trophic interactions between fruit and plant foragers like birds, mammals, and insects. The lowest numbers of positive samples were from workers' hands at the cantaloupe farm and from rinses of chile peppers obtained at the chile pepper packing house. Contamination in these samples could be due to worker's poor hygiene, cross-contamination from fruit to worker, as well as poor quality of the water used in the packing house to

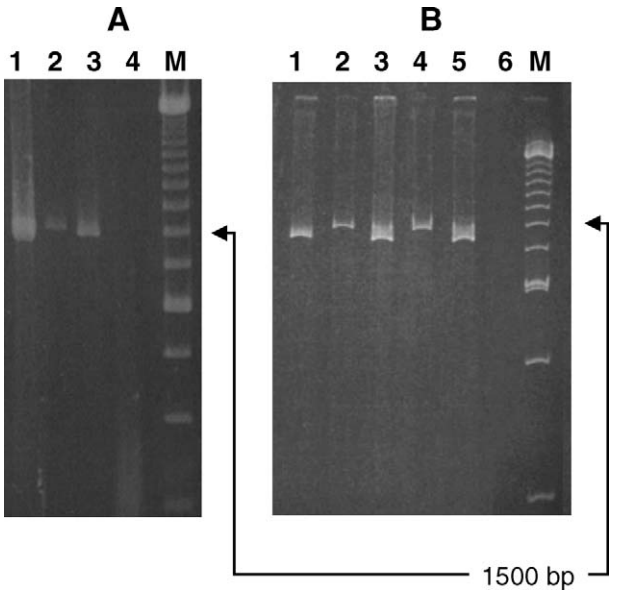

FIGURE 1. *PCR products obtained using ABMS1 and ABMS2 primers. (A) Lane 1,* Salmonella *Paratyphi A (ATCC 9150); lane 2,* Salmonella *Enteritidis (ATCC 13076); lane 3,* Salmonella *Typhimurium (ATCC 13311), lane 4, negative control. (B) Lane 1,* Salmonella *Kentucky; lane 2,* Salmonella *Typhi; lane 3,* Salmonella *Worthington; lane 4,* Salmonella *Stanley; lane 5,* Salmonella *Typhimurium; lane 6, negative control; lanes M, 250-bp ladder (Invitrogen).*

wash the fruit. The Centers for Disease Control and Prevention reported that in outbreaks that occurred in 2001 and 2002 associated with the consumption of cantaloupes from Mexico contaminated with *Salmonella* Poona, possible sources of contamination included irrigation of fields with water contaminated with sewage, processing (cleaning and cooling) produce with *Salmonella*-contaminated water, poor hygienic practices of workers who harvested and processed the cantaloupes, pests in packing facilities, and inadequate cleaning and sanitizing of equipment that came in contact with the cantaloupes *(3).*

PCR amplification. The PCR product from the reference strains (Fig. 1A) and from the different serotypes (Fig. 1B) was of the expected size on the basis of the simulated PCR product obtained with Amplify 1.2 software. As anticipated, the sizes were 1,487 bp for *Salmonella* Paratyphi A and *Salmonella* Typhimurium, 1,517 bp for *Salmonella* Enteritidis (Fig. 1A), and 1,520 bp for *Salmonella* Typhi (Fig. 1B). Figure 1B shows the PCR products of various sizes from the different *Salmonella* serotypes as evidence of the genetic variability of the *fliC* gene. The PCR products of *Salmonella* isolates obtained from both production systems were approximately 1.5 kb in size. PCR with the primer set described by Kilger and Grimont *(10)* for amplification of the *fliC* gene generated products of two different sizes: 1.24 kb for serotype Typhi and 1.5 kb for all other serotypes. The difference in the size of the PCR products obtained for the *fliC* gene can be accounted for by the variability of the central region of the open reading frame of this gene *(8).*

PCR-RFLP analysis. PCR-RFLP was carried out on 30 strains, 8 belonging to 7 different *Salmonella* serotypes and 22 of unknown serotype isolated in this study (one isolate from each positive sample) (Table 1). PCR-RFLP analyses with *Sau*3AI on *Salmonella* strains of seven different known serotypes yielded seven distinct restriction profiles for the *fliC* gene (Fig. 2A), demonstrating that *fliC* is a suitable target gene for discriminating among *Salmonella* serotypes by PCR-RFLP. Using PCR-RFLP on the *groEL* gene amplicon of *Salmonella* digested with *Hae*III, Nair et al. *(18)* found a low discriminatory capacity, because only three different profiles were obtained, and different serotypes of *Salmonella* shared the same restriction profile. However, other researchers have found good discriminatory ability with the *fliC* and *fljB* genes, particularly with the use of a double digestion *(4, 8).* A DNA sequence–based approach examining the *Salmonella* flagellin *fliC* gene revealed the existence of two groups: the g-complex, which included *Salmonella* Enteritidis and nonmotile strains, and the non–g-complex, which included motile strains such as *Salmonella* Typhimurium *(17).* Results of this study indicated that there was a high level of sequence homology between *fliC* genes of g-complex strains, and that the genetic basis for distinct antigens in this group of sequences can be due to a single amino acid substitution. Also, it has been reported that differences in the *fliC* gene sequence coding different antigenic phase 1 types could be due to 1 to 44 nucleotide substitutions, some of which result in changes in the amino acid sequence of the flagellin protein *(7).*

Comparing the restriction profiles obtained with the *Salmonella* isolates from the cantaloupe and chile pepper production systems (Fig. 2B and 2C, respectively) with those of the reference strains, all 12 isolates obtained from the cantaloupe production system had the *Salmonella* Typhimurium restriction profile. Six isolates were from rinses of cantaloupe surfaces (two from each orchard), five were from water used for irrigation, and one was from the hands of a field worker. From the chile pepper production system, eight isolates were also identified as *Salmonella* Typhimurium: five from the water used for irrigation, two from chile peppers, and one from chile peppers at the packing house. In addition, a second *Salmonella* restriction profile was found that matched that of *Salmonella* Enteritidis. It was found in two isolates: one from an irrigation water sample and one from a chile pepper rinse sample.

Results from the PCR-RFLP analyses based on the *fliC* gene showed that 91% (20 of 22) and 9% (2 of 22) of the isolates from both agricultural systems matched with the *Salmonella* Typhimurium and *Salmonella* Enteritidis restriction profiles, respectively, and these were the only two serotypes found in both agricultural systems. This is in agreement with previous reports that indicate that *Salmonella* Typhimurium and *Salmonella* Enteritidis are the serotypes most commonly isolated from outbreaks of human salmonellosis linked to the consumption of contaminated animal and vegetable foods *(11, 12).* It is important that 50% of the isolates were obtained from water sources, which points out the risk associated with water used in

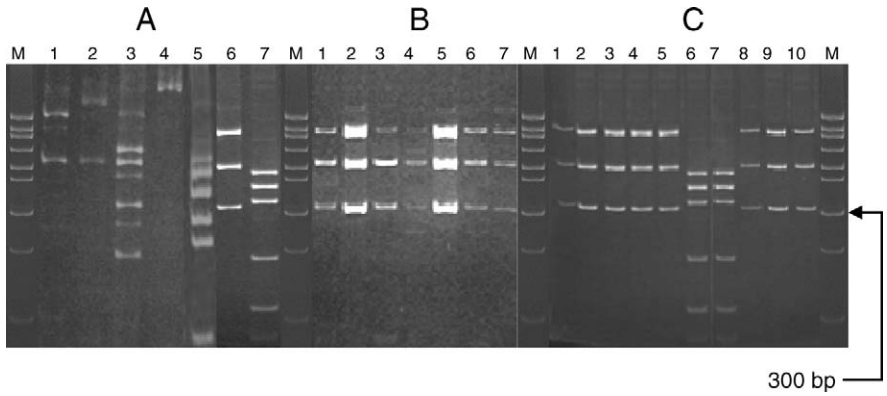

FIGURE 2. *Restriction profiles of the* Salmonella fliC *gene obtained with* Sau3A*I endonuclease digestion. (A) Lane 1,* Salmonella *Kentucky; lane 2,* Salmonella *Typhi; lane 3,* Salmonella *Worthington; lane 4,* Salmonella *Stanley; lane 5,* Salmonella *Paratyphi A (ATCC 9150); lane 6,* Salmonella *Typhimurium (ATCC 13311); lane 7,* Salmonella *Enteritidis (ATCC 13076). (B) Isolates obtained at the cantaloupe production system. Lanes 1 through 7, restriction profile of* Salmonella *Typhimurium. (C) Isolates obtained from the chile pepper production system. Lanes 1 through 5 and 8 through 10, restriction profile of* Salmonella *Typhimurium; lanes 6 and 7, restriction profile of* Salmonella *Enteritidis; lanes M, 100-bp ladder (Invitrogen).*

cantaloupe and chile pepper production systems. These results partially agree with those obtained by Castillo et al. *(2),* who reported that most isolates they recovered were from water samples; however, the *Salmonella* serovars isolated from water were different from those isolated from the melons. It is important that *Salmonella* Poona, which is a serotype that often causes outbreaks of salmonellosis in the United States associated with the consumption of melons from Mexico, was not found in this study, because the expected restriction profile for the *fliC* gene of this serotype (simulated with DNA Straider 1.2, GeneBank accession AY353467 for the *fliC* gene of *Salmonella* Poona) was not observed among the restriction profiles obtained in this study. This provides some evidence that this serotype might be present only at melon production areas in southeastern Mexico, where iguanas, which are considered a reservoir for this serotype, are found *(3).* Further studies are needed to determine this possibility.

Although the number of samples analyzed in this study was small, it was demonstrated that irrigation water could be an important source of contamination of produce by *Salmonella* Typhimurium and *Salmonella* Enteritidis. *Salmonella* might have been transmitted by direct contact of the water with the melons or by contact of the melons or the water to other parts of the production systems, including the workers. It is also possible that contamination from ill personnel during the handling of the fruit or contamination through vectors, including insects and birds, could have occurred; however, these possibilities were not investigated here. The results of this investigation suggest that in production systems in which Good Agricultural Practices are not in place, as in the farms tested in this study, contamination with *Salmonella* can occur, representing a health risk for the farmers and the consumers of the contaminated produce. Although only a small number of strains were tested, this study demonstrated the utility of the PCR-RFLP technique for determining the serotypes of *Salmonella* isolates obtained from cantaloupe and chile pepper production systems by comparison to restriction profiles of known reference strains. The method is rapid, simple, and reproducible and can potentially be applied for identification of isolates obtained from other production systems. More extensive studies need to be performed examining a larger number of farms and samples to determine the prevalence of *Salmonella* in agricultural production systems. Typing of isolates by pulsed-field gel electrophoresis might be useful for source tracking of *Salmonella* on the farms. Production systems with Good Agricultural Practices and Good Manufacturing Practices in place should be compared with those that do not use Good Agricultural Practices and Good Manufacturing Practices to determine the efficacy of such practices for the prevention of *Salmonella* contamination.

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REFERENCES

1. Brenner, F. W., R. G. Villar, F. J. Angulo, R. Tauxe, and B. Swaminathan. 2000. *Salmonella* nomenclature. *J. Clin. Microbiol.* 38: 2465–2467.
2. Castillo, A., I. Mercado, L. M. Lucia, Y. Martinez-Ruiz, J. Ponce de Leon, E. A. Murano, and G. R. Acuff. 2004. *Salmonella* contamination during production of cantaloupe: a binational study. *J. Food Prot.* 67:713–720.
3. Centers for Disease Control and Prevention. 2002. Multistate outbreaks of *Salmonella* serotype Poona infections associated with eating cantaloupe from Mexico—United States and Canada, 2000–2002. *Morb. Mortal. Wkly. Rep.* 51:1044–1047.
4. Dauga, C., A. Zabrovskaia, and P. A. D. Grimont. 1998. Restriction fragment length polymorphism analysis of some flagellin genes of *Salmonella enterica. J. Clin. Microbiol.* 36:2835–2843.
5. Doyle, J. J., and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19:11–15.
6. Gutiérrez-Cogco, L., E. Montiel-Vázquez, P. Aguilera-Pérez, and M. C. González-Andrade. 2000. Serotipos de *Salmonella* identificados en los servicios de salud de México. *Salud Pública Méx.* 42:490–495.
7. Herrera-Leon, S., J. R. McQuiston, M. A. Usera, P. I. Fields, J. Garaizar, and M. A. Echeita. 2004. Multiplex PCR for distinguishing the most common phase 1 flagellar antigens of *Salmonella* spp. *J. Clin. Microbiol.* 42:2581–2586.

8. Hong, Y., T. Liu, C. Hofacre, M. Maier, D. G. White, S. Ayers, L. Wang, and J. J. Maurer. 2003. A restriction fragment length polymorphism–based polymerase chain reaction as an alternative to serotyping for identifying *Salmonella* serotypes. *Avian Dis.* 47:387–395.
9. Islam, M., J. Morgan, M. P. Doyle, S. C. Phatak, P. Millner, and X. Jiang. 2004. Fate of *Salmonella enterica* Serovar Typhimurium on carrots and radishes grown in fields treated with contaminated manure composts or irrigation water. *Appl. Environ. Microbiol.* 70: 2497–2502.
10. Kilger, G., and P. A. D. Grimont. 1993. Differentiation of *Salmonella* phase 1 flagellar antigen types by restriction of the amplified *fliC* gene. *J. Clin. Microbiol.* 31:1108–1110.
11. Leon-Velarde, C. G., H. Y. Cai, C. Larkin, P. Bell-Rogers, R. W. Stevens, and J. A. Odumeru. 2004. Evaluation of methods for the identification of *Salmonella enterica* serotype Typhimurium DT104 from poultry environmental samples. *J. Microbiol. Methods* 58:79–86.
12. Lim, Y.-G., K. Hirose, H. Izumiya, E. Arakawa, H. Takahashi, J. Terajima, K. I. Itoh, K. Tamura, S. I. Kim, and H. Watanabe. 2003. Multiplex polymerase chain reaction assay for selective detection of *Salmonella enterica* serovar Typhimurium. *Jpn. J. Infect. Dis.* 56: 151–155.
13. Liu, G. R., A. Rahn, W.-Q. Liu, K. E. Sanderson, R. N. Johnston, and S.-L. Liu. 2002. The evolving genome of *Salmonella enterica* serovar Pullorum. *J. Bacteriol.* 184:2626–2633.
14. Mancera, M. A., N. J. Vázquez, and Z. A. Heneidi. 2004. Fagotipificación de aislamientos de *Salmonella enteritidis* obtenidos de aves de México. *Téc. Pecu. Méx.* 42:287–294.
15. McQuiston, J. R., R. Parrenas, M. Ortiz-Rivera, L. Gheesling, F. Brenner, and P. I. Fields. 2004. Sequencing and comparative analysis of flagellin genes *fliC, fljB,* and *flpA* from *Salmonella. J. Clin. Microbiol.* 42: 1923–1932.
16. Moore, B. C., E. Martinez, J. M. Gay, and D. H. Rice. 2003. Survival of *Salmonella enterica* in freshwater and sediments and transmission by the aquatic midge *Chironomus tentans* (Chironomidae: Diptera). *Appl. Environ. Microbiol.* 69:4556–4560.
17. Mortimer, C. K. B., T. M. Peters, S. E. Gharbia, J. M. J. Logan, and C. Arnold. 2004. Towards the development of a DNA-sequence based approach to serotyping of *Salmonella enterica. BMC Microbiol.* 4:31.
18. Nair, S., T. K. Lin, T. Pang, and M. Altwegg. 2002. Characterization of *Salmonella* serovars by PCR-single-strand conformation polymorphism analysis. *J. Clin. Microbiol.* 40:2346–2351.
19. Ott, L. R. 1992. An introduction to statistical methods and data analysis, 4th ed. Duxbury Press, Belmont, Calif.
20. Reilly, W. J., G. I. Forbes, G. M. Paterson, and J. C. Sharp. 1981. Human and animal salmonellosis in Scotland associated with environmental contamination, 1973–79. *Vet. Rec.* 108:553–555.
21. Sela, S., D. Nestel, R. Pinto, E. Nemny-Lavy, and M. Bar-Joseph. 2005. Mediterranean fruit fly as a potential vector of bacterial pathogens. *Appl. Environ. Microbiol.* 71:4052–4056.
22. Sneath, P. H. A., and R. R. Sokal. 1973. Numerical taxonomy. W. H. Freeman & Co., San Francisco.
23. U.S. Department of Agriculture, Food Safety and Inspection Service. 2008. Isolation and identification of *Salmonella* from meat, poultry, and egg products. Available at: http://www.fsis.usda.gov/PDF/MLG_4_04.pdf. Accessed September 2008.
24. U.S. Food and Drug Administration. 2001. Outbreaks associated with fresh and fresh-cut produce. Incidence, growth, and survival of pathogens in fresh and fresh-cut produce. Available at: http://www.cfsan.fda.gov/~comm/ift3-4a.html. Accessed 20 June 2007.
25. Wilson, R. C., P. A. Padron, and W. B. Dockstader. 1971. *Salmonella* screening procedure with tests for β-galactosidase and flagellar antigens. *Appl. Microbiol.* 21:346–349.