Journal of Food Protection, Vol. 68, No. 11, 2005, Pages 2264–2268

Methods for Recovering *Escherichia coli* O157:H7 from Cattle Fecal, Hide, and Carcass Samples: Sensitivity and Improvements†

GENEVIEVE A. BARKOCY-GALLAGHER,‡ KELLY K. EDWARDS,§ XIANGWU NOU,‖ JOSEPH M. BOSILEVAC, TERRANCE M. ARTHUR, STEVEN D. SHACKELFORD, AND MOHAMMAD KOOHMARAIE*

U.S. Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska 68933-0166, USA

MS 05-75: Received 14 February 2005/Accepted 10 June 2005

ABSTRACT

The Meats Research Unit (MRU) methods, developed by MRU scientists of the U.S. Meat Animal Research Center, have been used to study the prevalence of *Escherichia coli* O157:H7 in cattle carcass, hide, and fecal samples. The sensitivity of these methods for recovery of injured *E. coli* O157:H7 cells from inoculated and uninoculated samples was determined, and potential improvements to these methods were evaluated. When using the conventional MRU methods, 91% of the pre-evisceration carcass samples tested positive for *E. coli* O157:H7 when inoculated with 5 to 10 CFU, 100% of hide samples tested positive for *E. coli* O157:H7 when inoculated with 30 to 50 CFU, and 96% of the fecal samples produced positive results when inoculated with 300 to 400 CFU per 10 g. The addition of a phosphate buffer to the tryptic soy broth enrichment improved recovery of *E. coli* O157:H7 from feces. Using the modified enrichment, 92% of the samples were identified as positive when inoculated with 10 to 30 CFU per 10 g. Substituting a commercially available wash buffer for the phosphate-buffered saline (PBS) plus Tween 20 wash buffer during immunomagnetic separation of hide samples improved recovery of the target organism at lower inoculum concentrations. When comparing uninoculated samples, substituting a PBS buffer plus a zwitterionic detergent for PBS plus Tween 20 also had a positive effect on recovery of *E. coli* O157:H7 from hide samples. Data presented here indicate that the MRU methods are highly effective at recovering injured *E. coli* O157:H7 from fecal, hide, and beef carcass samples; however, modifications can be added to increase the sensitivity.

Escherichia coli O157:H7 is a human pathogen common to cattle and, therefore, a potential contaminant of red meat. Clinical cases of *E. coli* O157:H7 infection are often associated with consumption of undercooked ground beef. The source of the organism in ground beef is likely beef carcass contamination that survives antimicrobial interventions. Bacterial contamination of carcasses comes primarily from the hide *(3, 7, 15).* Cattle also can carry *E. coli* O157:H7 in their feces, which can contaminate hides and carcasses (for review, see Barkocy-Gallagher et al. *(2)*).

Methods were developed by scientists in the Meats Research Unit (MRU) of the U.S. Meat Animal Research Center to detect *E. coli* O157:H7 in samples from cattle carcasses, hides, and feces *(5).* These methods compare favorably with the methods used previously by Elder et al. *(11).* However, the sensitivity of these and other methods for recovery of *E. coli* O157:H7 from beef processing samples has not been reported. The sensitivity of the methods used to recover *E. coli* O157:H7 can greatly affect the apparent prevalence of the organism *(5, 10, 12).* In this study, the sensitivity of the MRU methods for recovery of *E. coli* O157:H7 from cattle fecal, hide, and carcass samples was determined and modifications to improve sensitivity were evaluated.

MATERIALS AND METHODS

Bacterial strains. To distinguish inoculated cells from those that might be naturally occurring, *E. coli* O157:H7 strains used for inoculation were marked by inserting the gene coding for green fluorescent protein (gfp_{uv}) into the chromosome. *E. coli* O157:H7 strains were selected from a collection of isolates recovered at beef packing plants *(11).* The strains were selected on the basis of differing genotypes, one strain each from previously designated subtypes 1d, 6d, 23b, 39c, and 46b *(4).* Four of the five strains were successfully marked by insertion of the gfp_{uv} gene at the *srlA* locus on the chromosome. The *srlA* locus was chosen because it naturally contains a mutation rendering it nonfunctional in most *E. coli* O157:H7 strains. The fifth strain was not successfully marked and was used only in experiments measuring method sensitivity for fecal samples, in which an uninoculated aliquot of each fecal sample was tested to ensure that the samples were negative for *E. coli* O157:H7.

To prepare injured cell cultures, marked *E. coli* O157:H7 strains were grown overnight on Luria-Bertani agar (LB agar; Dif-

* Author for correspondence. Tel: 402-762-4221; Fax: 402-762-4149; E-mail: koohmaraie@email.marc.usda.gov.

† Names are necessary to report factually on available data; however, the U.S. Department of Agriculture neither guarantees nor warrants the standard of the product, and the use of the name by the U.S. Department of Agriculture implies no approval of the product to the exclusion of others that may also be suitable.

‡ Present address: Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30333, USA.

§ Present address: Quintiles, Inc., P.O. Box 9708, Kansas City, MO 64134-0708, USA.

‖ Present address: U.S. Department of Agriculture, Agricultural Research Service, Animal and Natural Resource Institute, Building 201, BARC-East, 10300 Baltimore Avenue, Beltsville, MD 20705-2350, USA.

co, Becton Dickinson, Sparks, Md.) at 37°C. Following overnight growth, the cultures were mixed with buffered peptone water (BPW; Difco, Becton Dickinson) to achieve an optical density at 600 nm of 0.20, resulting in approximately 2×10^8 CFU/ml. These cultures were serially diluted in BPW, and the dilutions were stored as 50% glycerol stocks at −70°C. Prior to sample inoculation, cultures were thawed on ice and then held at room temperature for approximately 30 min. Inoculum concentrations were determined on the basis of colonies formed on LB agar. The number of injured cells was established by determining the percentage of cells that formed colonies on LB agar but not on sorbitol MacConkey agar (Difco, Becton Dickinson) supplemented with cefixime and tellurite (ctSMAC; Dynal, Lake Success, N.Y.).

Inoculation. When inoculated samples were required, samples were inoculated with known concentrations of injured cells and held at room temperature for 10 to 30 min prior to the addition of enrichment broth. Marked strains were used for inoculation to ensure that results were not influenced by natural sample flora.

Construction of marked strains. All PCRs were performed in thermal cyclers (MJ Research, Inc., Watertown, Mass.) using HotStarTaq (Qiagen, Valencia, Calif.). The buffer provided by the manufacturer was used without additional $MgCl_2$. Unless otherwise noted, the amplification conditions were 94°C for 15 min, then 30 cycles of denaturing at 94°C for 30 s, annealing for 30 s, and elongation at 72°C for 30 s, and a final elongation step at 72°C for 10 min. The *srlA* 5′ and 3′ regions were amplified from *E. coli* O157:H7 strain 43895 (American Type Culture Collection, Manassas, Va.). The *srlA* 5′ region was amplified using 400-nM primers srlA5′for (5′-TTAAGCTTGCGGCCGCAACCAGGGG CAAGTATGGTAAAGC) and srlA5′rev (GCCCTTTTGGAA CAGCCCGATAAACCACTCTGCACCATG) and an annealing temperature of 67°C. The *srlA* 3′ region was amplified using 400-nM primers srlA3′for (TTGAATTCAGGAGCAACATCATGA CGCATATTCGGATCG) and srlA3′rev (TTACTAGTGC GGCCGCCAGAGACATAAATATCTTCCAC) and an annealing temperature of 53°C. The gfp_{uv} gene was amplified from plasmid pGFPuv (P. Fratamico, Eastern Regional Research Center, ARS, USDA, Wyndmoor, Pa.) using 600-nM primers srlAgfpfor (TCGGGCTGTTCCAAAAGGGCATGAGTAAAGGAGAA GAACTTTTCAC) and srlAgfprev (GATGTTGCTCCTGAATT CAACGCTCAGTTGGAATTCATTATTTGTAG), an annealing temperature of 62°C, and a 90-s elongation step. All three PCR products were purified using Qiaquick spin columns (Qiagen), and the purified srlA5′ and srlA3′ fragments were diluted 1:100.

The primers used to amplify the gfp_{uv} gene complemented the primers used to amplify the 3′ end of the srlA5′ region and the 5′ end of the srlA3′ region. Overlapping PCR was then performed to amplify a fragment consisting of the srlA5′ region plus the gfp_{uv} gene plus the srlA3′ region. The amplification reaction included the srlA5′for and srlA3′rev primers at 400 nM and 1 μl each of the three PCR products. The annealing temperature was 52°C, and the elongation step was extended to 2 min. The resulting 1.4-kb PCR product was cloned into pGEM-T (Promega Corp., Madison, Wis.) using the pGEM-T ligation kit according to the manufacturer's instructions. The ligation reaction was transformed into High Competency JM109 cells (Promega). White colonies were screened by PCR for the presence of the appropriate insert.

The insert of the srlA5′ region plus gfp_{uv} gene plus srlA3′ region was subsequently removed from the pGEM-T vector by *Not*I digestion and subcloned into the suicide vector pKO3 *(13)* using standard techniques *(13, 14)*. The resulting plasmid, pGG69, was transformed into the chosen *E. coli* O157:H7 strains, and double crossover events were selected as previously described *(13)*. Although the cells did not fluoresce, the gfp_{uv} marker gene was detectable by PCR.

Sensitivity. When method sensitivity was to be determined, samples were inoculated with known concentrations of marked cells, and enrichment and detection procedures were carried out as described.

Fecal samples. For determination of sensitivity, fresh cattle fecal samples were recovered at a local feedlot, mixed, and divided into 10-g aliquots. The samples were inoculated with various concentrations (≤5 to >500 CFU per sample) of marked cells, and enrichment and detection procedures were carried out as previously described *(5)*. The number of samples was recorded for each inoculation concentration in which *E. coli* O157:H7 was detected. The addition of a phosphate buffer to the enrichment medium as a potential method improvement was evaluated for sensitivity of detection of *E. coli* O157:H7 using inoculated samples in the same manner as the standard enrichment.

The previously described MRU method and the modified phosphate buffer method also were compared for sensitivity of detection of *E. coli* O157:H7 using uninoculated samples. One hundred individual fecal samples were collected, and then each sample was homogenized by hand massaging. Two 10-g aliquots were removed for analysis using the previously described MRU method or using tryptic soy broth (TSB, Difco, Becton Dickinson) with phosphate buffer (TSB-PO_4) *(5)*, which consisted of 30 g of TSB, 2.31 g of KH_2PO_4, and 12.54 g of K_2HPO_4 per liter of solution. The amounts and ratio of the phosphate buffer components were derived from Terrific Broth *(14)*. For enrichment, 90 ml of either TSB or TSB-PO_4 were added to each 10-g fecal sample. All enrichments were incubated at 25°C for 2 h and then at 42°C for 6 h and stored at 4°C overnight as previously described *(5)*. Unless otherwise indicated, samples were processed by immunomagnetic separation (IMS) and plating as previously described *(5)*.

Hide and carcass samples. For determination of sensitivity, samples were obtained from cattle hides and pre-evisceration carcasses at large commercial packing plants as described previously for the MRU methods *(3, 5)* and then transported to the laboratory on ice. The samples were inoculated with various concentrations (≤5 to >10 to 30 CFU per sample for carcasses and ≤5 to >50 CFU per sample for hides) of marked cells, and enrichment and detection procedures were carried out as described. The number of samples was recorded for each inoculation concentration in which *E. coli* O157:H7 was detected.

Two additional wash conditions for the IMS beads also were tested in an attempt to lower the amount of background flora on the agar plates. First, 9 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS; Sigma-Aldrich, St. Louis, Mo.) prepared in phosphate-buffered saline (PBS) was tested using uninoculated hide samples. Twenty microliters of IMS beads was added to 600 μl of PBS-CHAPS buffer and mixed on a roller drum for 10 min. One milliliter of sample enrichment was then added to the beads-PBS-CHAPS. The samples were mixed, incubated, and processed as previously described, substituting PBS-CHAPS for PBS–Tween 20 buffer *(5)*. For these experiments, the same enrichments also were evaluated by the standard MRU method.

Second, a commercially available IMS buffer (ImmTech, New Windsor, Md.) was tested as a wash buffer. Two hundred fifty microliters of IMS buffer was mixed with 20 μl of IMS beads for 10 min on a roller drum, and then 1 ml of enrichment culture

TABLE 1. *Evaluation of the MRU method for recovery of* E. coli *O157:H7 from inoculated pre-evisceration beef carcass samples*

Inoculation concentration (CFU/sample)	No. of samples analyzed	% positive samples	95% CI[a]
≤5.0	170	80	73–86
>5.0 to 10.0	46	91	79–98
>10.0 to 30.0	14	93	66–100

[a] CI, confidence interval.

was added. The sample, beads, and wash buffer then were mixed on the roller for an additional 30 min. IMS proceeded as previously described *(5)*, except 0.5× IMS buffer (diluted in sterile distilled, deionized water) was substituted for the PBS–Tween 20 wash buffer.

In both procedures (IMS buffer and PBS-CHAPS), the beads were resuspended after washing in 100 μl of PBS and plated onto ctSMAC and Rainbow agar (Biolog, Inc., Hayward, Calif.) supplemented with novobiocin and tellurite as previously described *(5)*. Up to three positive colonies were selected per plate for confirmation. Colony identity for inoculated samples was confirmed by PCR assay for the gfp_{uv} gene. When plates were crowded with growth or were derived from uninoculated samples, colonies were screened by DrySpot O157 (Oxoid, Inc., Ogdensburg, N.Y.). For uninoculated samples, suspected *E. coli* O157:H7 colonies were identified as previously described *(1)*.

Statistical analyses. The 95% confidence intervals and *P* values were calculated with PEPI software (version 2; USD, Inc., Stone Mountain, Ga.).

RESULTS AND DISCUSSION

Sensitive methods are needed to determine the prevalence of *E. coli* O157:H7 in fecal, hide, and carcass samples recovered during beef processing. However, the cells present in these samples may be injured and, therefore, difficult to grow and detect. The MRU methods were designed to allow for recovery of injured cells. Therefore, we chose to test the sensitivity of the MRU methods for recovery of injured cells to represent the worst-case scenario. On average, 44% of the *E. coli* O157:H7 cells used in this study were injured from freezing and thawing (data not shown).

Beef carcasses can become contaminated with *E. coli* O157:H7 during early processing steps. Survival of such bacteria through antimicrobial interventions leads to contamination of the finished carcass and the subsequent product *(3, 4, 11)*. When beef carcass samples were inoculated with 5 to 10 *E. coli* O157:H7 cells, the MRU method identified 91% of the samples as positive (42 of 46; Table 1). At least 80% of the samples tested positive for *E. coli* O157:H7 when inoculated with five or fewer *E. coli* O157:H7 cells. At such low inoculum concentrations, some of the samples probably did not receive any cells, so the real ability of the MRU method to detect low concentrations of *E. coli* O157:H7 may be higher.

Cattle hides are a significant source of *E. coli* O157:H7 on beef carcasses *(3, 15)*. Using the MRU method, this organism was recovered from 100% of cattle hide samples inoculated with 30 to 50 CFU (Table 2). However, sporadic large amounts of background growth on the plates made it difficult to locate positive colonies. Additional wash buffers were evaluated for reducing nonspecific binding to the IMS beads and hence reducing background flora on plates without decreasing the number of positive colonies. One concern was that if some samples had few cells per milliliter of enrichment prior to IMS then those cells might be lost during washing with the new detergents and samples would be incorrectly scored as negative. One of the most effective washes tested was the commercially available IMS buffer used at half strength. This buffer appeared to lower the number of colonies of background bacteria without reducing the number of positive target colonies (data not shown). When 0.5× IMS buffer was substituted for the standard PBS–Tween 20 wash buffer, significantly more hide samples were identified as positive when inoculated with five or fewer CFU ($P < 0.05$; Table 2). However, when hide samples were inoculated with 30 to 50 CFU, the opposite effect was seen ($P = 0.002$). These conflicting data probably reflect the small number of samples analyzed and the variability among hide samples.

Preliminary data also suggest that a PBS-CHAPS buffer effectively reduced background growth on plates, although the number of positive colonies also was lower (data not shown). The PBS-CHAPS wash buffer and the IMS wash buffer were tested for recovery of *E. coli* O157:H7

TABLE 2. *Evaluation of MRU method with and without modifications for recovery of* E. coli *O157:H7 from inoculated hide samples*[a]

Inoculation concentration (CFU/sample)	Standard method			Modified method[b]		
	No. of samples analyzed	% positive samples	95% CI[c]	No. of samples analyzed	% positive samples	95% CI
≤5.0	15	13 A	1.7–41	32	53 B	35–71
>5.0 to 10.0	33	42 A	26–61	39	59 A	42–74
>10.0 to 30.0	105	71 A	61–79	40	83 A	67–93
>30.0 to 50.0	35	100 A	90–100	52	73 B	59–84
>50.0	15	93	68–100	ND[d]	ND	ND

[a] Within a row, values for the percentage of samples positive for *E. coli* O157:H7 that are followed by the same letter are not significantly different ($P > 0.05$).

[b] Samples were evaluated according to the MRU method *(5)* except using 0.5× IMS wash buffer.

[c] CI, confidence interval.

[d] ND, not determined.

TABLE 3. *Recovery of* E. coli *O157:H7 from uninoculated hide samples using the standard MRU method, the MRU method with a PBS-CHAPS IMS wash, or the MRU method with a commercially available IMS wash buffer*

Experiment	No. of samples analyzed	% positive samples		
		Standard method	PBS-CHAPS method	IMS buffer method
1	50	8	30	ND[a]
2	50	0	8	ND
3	50	34	72	ND
4	50	22	88	ND
5	50	98	100	ND
6	100	66	68	72
7	100	55	50	52
8	100	87	81	94
Total[b]		52	63	73

[a] ND, not determined.

[b] A total of 550 samples were analyzed using the standard PBS–Tween 20 wash buffer and the PBS-CHAPS wash buffer methods, but only 300 samples were analyzed using the IMS wash buffer method.

from uninoculated samples (Table 3). On six of eight separate occasions, PBS-CHAPS permitted identification of more positive samples than did the standard MRU method. Overall, the PBS-CHAPS wash buffer was significantly better than the standard PBS–Tween 20 wash buffer with the MRU method ($P < 0.001$). By comparison, the IMS wash buffer was slightly but not significantly more effective ($P > 0.05$) than PBS-CHAPS (72.6 versus 66.3%, respectively).

Feces also are a source of beef carcass contamination with *E. coli* O157:H7 *(3, 6, 8, 9, 11)*. The method used by Elder et al. *(11)* was more sensitive than other methods for recovery of *E. coli* O157:H7 from feces *(12)*. However, the actual sensitivity of this method has not been reported. In one study, the MRU method was at least as sensitive as the Elder et al. method *(5)*. In the present study, 96% of fecal samples inoculated with 300 to 400 CFU per sample (30 to 40 CFU/g) were identified as positive using the MRU method (Table 4). This level of sensitivity suggested poor growth of the target organism in the enrichment. Preliminary data suggest that the pH of the TSB fecal enrichments used in the MRU method dropped rapidly, possibly slowing growth of the *E. coli* O157:H7 (data not shown).

Omisakin et al. *(16)* suggested that 61% of cattle carrying *E. coli* O157:H7 shed the organism at <100 CFU/g. The MRU method may not be sufficiently sensitive to recover *E. coli* O157:H7 from these samples consistently. To improve recovery of *E. coli* O157:H7 by the MRU method, a phosphate buffering system was added to the TSB enrichment (TSB-PO_4). The additional phosphate buffer appeared to improve recovery of *E. coli* O157:H7 substantially. At an inoculum concentration of 10 to 30 CFU per sample (1 to 3 CFU/g), 39% (20 of 52) of the samples were identified as positive for *E. coli* O157:H7 using the standard MRU method with TSB, whereas 92% (33 of 36) of the samples were identified as positive using the TSB-PO_4 method (Table 4).

A comparison of the enrichments also was performed with split uninoculated fecal samples. The MRU method recovered *E. coli* O157:H7 from 26 of 100 samples. By incorporating the TSB-PO_4 enrichment, a slightly higher number of fecal samples ($P = 0.07$) was determined to be positive (39 of 100; $P < 0.1$). Therefore, TSB-PO_4 offers an improvement over TSB alone for recovery of *E. coli* O157:H7 from cattle fecal samples.

Data presented here indicate that the MRU method is highly effective at recovering injured *E. coli* O157:H7 from beef carcass samples. The MRU method also is effective for recovery of *E. coli* O157:H7 from hide samples; however, incorporation of IMS buffer or PBS-CHAPS as the IMS wash buffer improves the ability to correctly identify positive samples (when interference by background micro-

TABLE 4. *Evaluation of MRU method and MRU method with TSB-PO_4 enrichment for recovery of* E. coli *O157:H7 from inoculated fecal samples*[a]

Inoculation concentration (CFU/sample)	Standard method			TSB-PO_4 method		
	No. of samples analyzed	% positive samples	95% CI[b]	No. of samples analyzed	% positive samples	95% CI
≤5.0	43	30 A	17–46	47	68 B	53–81
>5.0 to 10.0	28	54 A	34–73	36	78 A	61–90
>10.0 to 30.0	52	37 A	24–51	36	92 B	78–98
>30.0 to 50.0	33	42 A	26–61	36	75 B	58–88
>50.0 to 100.0	47	49 A	34–64	4	100 A	40–100
>100.0 to 200.0	37	43	27–61	ND[c]	ND	ND
>200.0 to 300.0	25	72	51–88	ND	ND	ND
>300.0 to 400.0	52	96 A	87–100	8	100 A	63–100
>400.0 to 500.0	55	95 A	85–99	12	100 A	74–100
>500.0	12	92	62–100	ND	ND	ND

[a] Within a row, values for the percentage of samples positive for *E. coli* O157:H7 that are followed by the same letter are not significantly different ($P > 0.05$).

[b] CI, confidence interval.

[c] ND, not determined.

flora is expected). The MRU method for recovering *E. coli* O157:H7 from feces is substantially improved by the incorporation of a phosphate buffer in the TSB enrichment broth.

ACKNOWLEDGMENTS

We thank Kim Kucera for technical assistance and Carol Grummert for secretarial assistance. We also thank Dr. George Church (Department of Genetics, Harvard Medical School, Boston, Mass.) for generously providing the pK03 plasmid.

REFERENCES

1. Arthur, T. M., J. M. Bosilevac, X. Nou, S. D. Shackelford, T. L. Wheeler, M. P. Kent, D. Jaroni, B. Pauling, D. M. Allen, and M. Koohmaraie. 2004. *Escherichia coli* O157 prevalence and enumeration of aerobic bacteria, *Enterobacteriaceae*, and *Escherichia coli* O157 at various steps in commercial beef processing plants. *J. Food Prot.* 67:658–665.
2. Barkocy-Gallagher, G. A., T. M. Arthur, and M. Koohmaraie. 2002. Prevalence of *Escherichia coli* O157:H7 in cattle and during processing. *Proc. Recip. Meat Conf.* 55:15–20.
3. Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of Shiga toxin–producing *Escherichia coli*, including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. *J. Food Prot.* 66:1978–1986.
4. Barkocy-Gallagher, G. A., T. M. Arthur, G. R. Siragusa, J. E. Keen, R. O. Elder, W. W. Laegreid, and M. Koohmaraie. 2001. Genotypic analyses of *Escherichia coli* O157:H7 and O157 nonmotile isolates recovered from beef cattle and carcasses at processing plants in the midwestern states of the United States. *Appl. Environ. Microbiol.* 67:3810–3818.
5. Barkocy-Gallagher, G. A., E. D. Berry, M. Rivera-Betancourt, T. M. Arthur, X. Nou, and M. Koohmaraie. 2002. Development of methods for the recovery of *Escherichia coli* O157:H7 and *Salmonella* from beef carcass sponge samples and bovine fecal and hide samples. *J. Food Prot.* 65:1527–1534.
6. Bonardi, S., E. Maggi, G. Pizzin, S. Morabito, and A. Caprioli. 2001. Faecal carriage of verocytotoxin-producing *Escherichia coli* O157 and carcass contamination in cattle at slaughter in northern Italy. *Int. J. Food Microbiol.* 66:47–53.
7. Bosilevac, J. M., T. M. Arthur, T. L. Wheeler, S. D. Shackelford, M. Rossman, J. O. Reagan, and M. Koohmaraie. 2004. Prevalence of *Escherichia coli* O157 and levels of aerobic bacteria and *Enterobacteriaceae* are reduced when hides are washed and treated with cetylpyridinium chloride at a commercial beef processing plant. *J. Food Prot.* 67:646–650.
8. Chapman, P. A., and C. A. Siddons. 1994. A comparison of strains of *Escherichia coli* O157 from humans and cattle in Sheffield, UK. *J. Infect. Dis.* 170:251–252.
9. Chapman, P. A., C. A. Siddons, D. J. Wright, P. Norman, J. Fox, and E. Crick. 1993. Cattle as a possible source of verocytotoxin-producing *Escherichia coli* O157 infections in man. *Epidemiol. Infect.* 111:439–447.
10. Chapman, P. A., D. J. Wright, and C. A. Siddons. 1994. A comparison of immunomagnetic separation and direct culture for the isolation of verocytotoxin-producing *Escherichia coli* O157 from bovine faeces. *J. Med. Microbiol.* 40:424–427.
11. Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie, and W. W. Laegreid. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proc. Natl. Acad. Sci. USA* 97:2999–3003.
12. Laegreid, W. W. 2000. Diagnosis of infection by *Escherichia coli* O157:H7 in beef cattle. *Proc. Natl. Conf. Anim. Prod. Food Safe.* 2000:101–103.
13. Link, A. J., D. R. Phillips, and G. M. Church. 1997. Methods for generating precise deletions and insertions in the genome of wild-type *Escherichia coli*: application to open reading frame characterization. *J. Bacteriol.* 179:6228–6237.
14. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. Nou, X., M. Rivera-Betancourt, J. M. Bosilevac, T. L. Wheeler, S. D. Shackelford, B. L. Gwartney, J. O. Reagan, and M. Koohmaraie. 2003. Effect of chemical dehairing on the prevalence of *Escherichia coli* O157:H7 and the levels of aerobic bacteria and *Enterobacteriaceae* on carcasses in a commercial beef processing plant. *J. Food Prot.* 66:2005–2009.
16. Omisakin, F., M. MacRae, I. D. Ogden, and N. J. C. Strachan. 2003. Concentration and prevalence of *Escherichia coli* O157 in cattle feces at slaughter. *Appl. Environ. Microbiol.* 69:2444–2447.