Rapid Detection of *Listeria monocytogenes* in Mechanically Separated Turkey Meat

Ann M. Ramos, graduate student,
Department of Microbiology
Irene V. Wesley, research microbiologist,
USDA-Agricultural Research Service, National Animal Disease Center
James M. Dickson, associate professor,
Department of Microbiology

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Summary and Implications

The purpose of this study was to determine the level of *Listeria* spp., especially *L. monocytogenes,* in mechanically separated turkey (MST) meat samples. During four trials (n=150 samples), *Listeria* spp. were selected by using two enrichments (University of Vermont-modified I and II) and plating to selective Palcam agar base. A multiplex polymerase chain (PCR) reaction was used to confirm *Listeria* isolations. The specificity of the multiplex PCR assay was evaluated with reference strains of *Listeria* from the National Animal Disease Center (NADC) Culture Collection. The *Listeria* spp. yields a single 938-bp product, whereas *L. monocytogenes* yields the 938-bp product along with a 174-bp fragment. Results from trials I-IV indicated *L. monocytogenes* could not be detected by PCR in the UVM enrichment due perhaps to PCR inhibitors present in poultry fats and muscle myoglobin. The multiplex PCR performed from suspect colonies grown on Palcam, however, indicated 29 out of 150 (19%) of the MST meat harbored *Listeria* spp. Fifty-seven of 150 (38%) were confirmed positive for *L. monocytogenes.* Those confirmed as *L. monocytogenes* by PCR were serotyped and fingerprinted using enterobacterial repetitive intergeneic consensus (ERIC) motifs-PCR.

Introduction

As one of the major four foodborne pathogens, *L. monocytogenes* causes over 1,500 cases annually with a mortality rate of 35%. *Listeria monocytogenes* contaminated turkey frankfurters were incriminated in at least one human fatality (4). The cost of listeriosis in the United States averages $220 million per year. Finding a quick and reliable method to detect and identify *L. monocytogenes* is important in recognizing contaminated products. Previous reports show that the distribution of *L. monocytogenes* in turkey products ranges from 90% in turkey frankfurters, 76% in ground turkey, and 38% in turkey parts such as legs and wings (1,3,5). The purpose of this study was to develop and evaluate rapid methods to detect and confirm *L. monocytogenes* in MST meat.

Materials and Methods

Sample collection. Twenty-five samples were collected on July 1 (Trial I) and 14 (Trial II). Fifty samples were collected on September 9 (Trial III) and December 10 (Trial IV) 1997 from an Iowa turkey plant.

Listeria enrichment and growth. As shown in Figure 1, 25 g of each sample was placed in 225 ml of UVM I enrichment and incubated (28°C for 24 hours). After incubation 0.2ml was plated onto Palcam, 1.4 ml was transferred into the 25 ml UVM II and incubated (28°C for 24 hours), and 250µl of each sample was taken for PCR. After incubation 0.2ml was plated on Palcam and 250µl of each sample was taken for PCR. The Palcam plates were incubated under microaerophilic conditions (10% CO_2, 5% O_2, 84% N_2) for 48 hours at 37°C.

Preparation of bacterial DNA for PCR. The enrichment aliquots were centrifuged (10,000 rpm for 1 minute), supernate was decanted, and the pellet was resuspended in 1 ml of PBS (0.01 M, pH 7.4). The sample was recentrifuged (10,000 rpm for 1 min), supernate decanted, and the pellet was resuspended in 250µl of sterile distilled water.

PCR. The primers and conditions were used as described by Harmon (2). Amplification conditions were as follows: initial denaturation step at 94°C for 4 minutes, followed by 25 amplification cycles. Each cycle consisted of one min at 94°C (denaturation), 1 minute at 60°C (primer annealing), and 1 minute at 72°C (primer extension). The amplified DNA was analyzed by gel electrophoresis (120 V for 1 hour) on 1.5% agarose gels with TBE as the running buffer. The gels were stained with ethidium bromide, rinsed, visualized by UV light, and photographed.

*Serological identification of L. monocytogenes***.** A total of 66 selected *L. monocytogenes* strains confirmed by PCR as typed using the rapid slide test for serotypes 1, 4 and polyvalent Types 1, 4. Cultures were grown on trypticase soy agar plates with 0.6% yeast extract at 37°C for 24 hours. Cells were swiped and suspended in 1 ml of phosphate buffered saline (PBS) with 0.3% formalin. Samples were heated (1 hour at 80°C in water bath), centrifuged (10,000 rpm for 1 minute), and supernate was

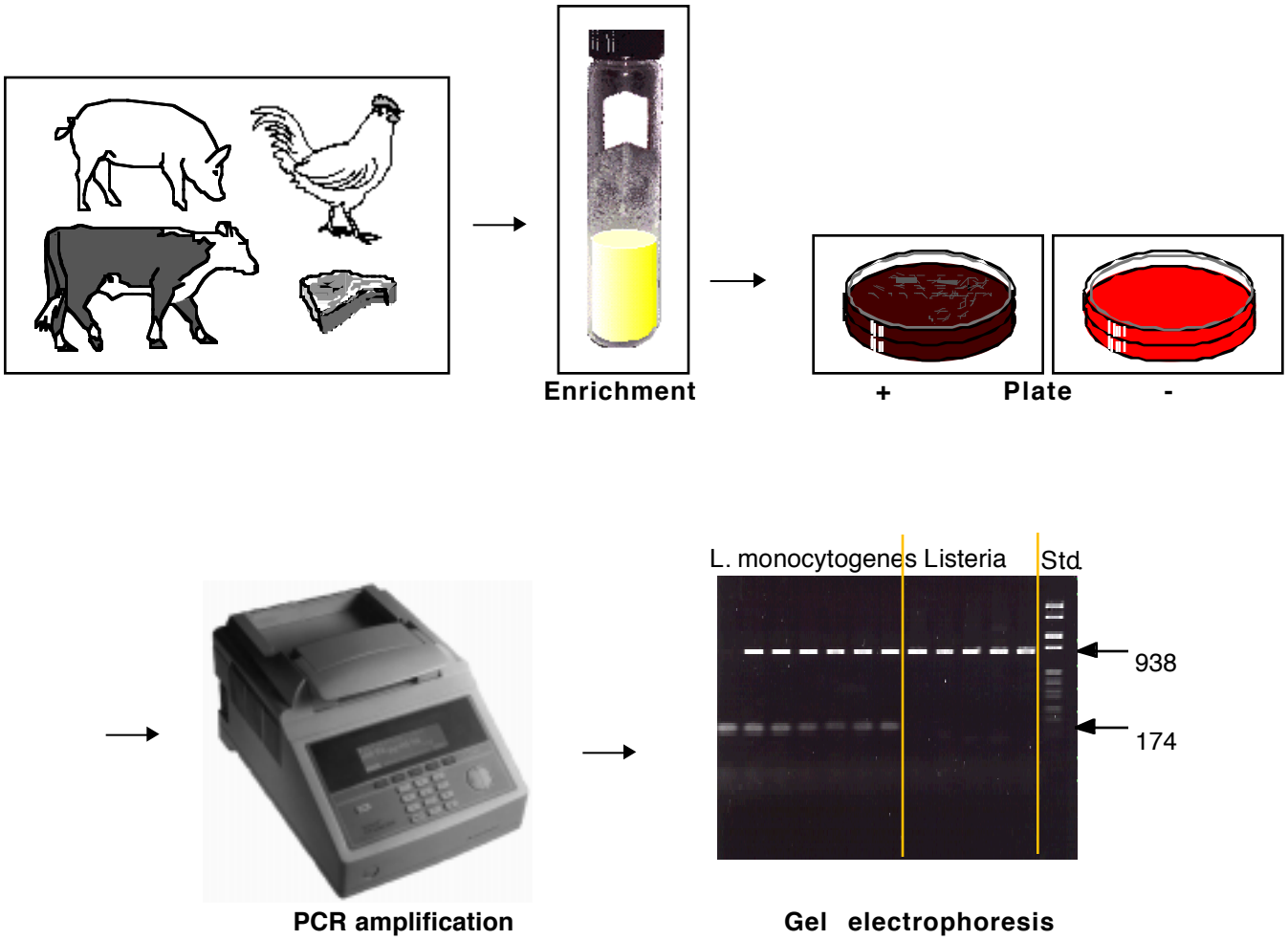

Figure 1. Enrichment and isolation of Listeria spp. and L. monocytogenes

decanted. Sample was resuspended in remaining portion of the supernate by vortexing. Positive control consisted of Bacto-Listeria O Antiserum Type 1, 4, or Polyvalent 1, 4 plus homologous O Antigen (Difco Laboratories, Detroit MI). Negative control consisted of PBS buffer with 0.3% formalin plus a drop of the organism in suspension. A drop of the sample was place on a slide then 1 drop of antisera was added. The slide was rocked back and forth (1-2 minutes). Slides were read for positive or negative agglutination.

Random amplified polymorhphic DNA (RAPD) technique. Primers and conditions were followed as described by Sciacchitano (4). Cultures were incubated on trypticase soy agar with 0.6% yeast extract (37°C, 24 hours). A High Pure PCR Template Preparation Kit (catalog number: 1 796828; Boehringer Mannhiem) was used to isolate bacterial DNA from samples. Primers for the ERIC-PCR (ERIC1R, 5'-ATGTAACGTCCTGGGGATTCAC-3' and ERIC2, 5'-AAGTAAGTGACTGGGGTGAGCG-3') were used as described by Versalovic et al. (5). Amplification conditions included, predenaturation; 1 cycle at 94°C, 5 minutes, followed by 40 cycles at 1 minute 94°C (denaturation), 1 minute at 25°C (annealing), 1 minute at 74°C (extension), and a final extension at 74°C for 10 minutes Samples were prepared in duplicate. The RAPD-PCR products were analyzed using 1.5% agarose gel and TBE as the running buffer at 120 V for 45 minutes. The gels were stained with ethidium bromide, rinsed, visualized, and photographed on a Gel Doc (Bio-Rad).

Results and Discussion

Suspect *Listeria* (colonies surrounded by black halos from aesculin hydrolysis) were picked and verified as such by PCR. As summarized in Table 1, for Trial I, none of MST samples as positive for *Listeria* spp., whereas 76%

(19 of 25) were positive for *L. monocytogenes*. In Trial II, 28% (7 of 25) of MST samples were positive for *Listeria* spp. and 64% (16 of 25) were positive for *L. monocytogenes*. Results for Trial III indicate a similar trend. Fourteen percent (7 of 50) of MST samples were positive for *Listeria* spp., while 24% (12 of 50) *L. monocytogenes.* In Trial IV, 30% (15 of 50) as positive for *Listeria* spp. and 20% (10 of 50) as positive for *L. monocytogenes*.

L. monocytogenes isolates (n=57) were serotyped and assigned to Type 1 (51%) and Type 4 (44%). By serotyping ≈2% as assigned to serogroups other than 1 and 4 (Table 2).

Because of the ease of generating DNA fingerprints, *L. monocytogenes* isolates were compared by ERIC analysis. The reproducibility of the resolution patterns is indicated in Figure 2.

Table 1. Summary of PCR Results. Detection of Listeriaand L. monocytogenes in UVM enrichment and plating to differential Palcam agar.

	Listeria spp.	L. monocytogenes
Trial I(n=25)	0	19 (76)
Trial II (n=25)	7 (28)[a]	16 (64)
Trial III (n=50)	7 (14)	12 (24)
Trial IV (n=50)	15 (30)	10 (20)
Total (n=150)	29 (19)	57 (38)

[a] Numbers in parenthesis are percentages

Table 2. Summary of serotyping of L. monocytogenes isolated from MST meat.

	Poly (1,4)	Type 1	Type 4	Negative
Trial I (n=19)	18	14	4	0
Trial II (n=16)	16	5	11	0
Trial III (n=12)	11	5	6	1
Trial IV (n=10)	9	5	4	0
Total (n=57)	54	29	25	0

[a] One isolate not available for testing.

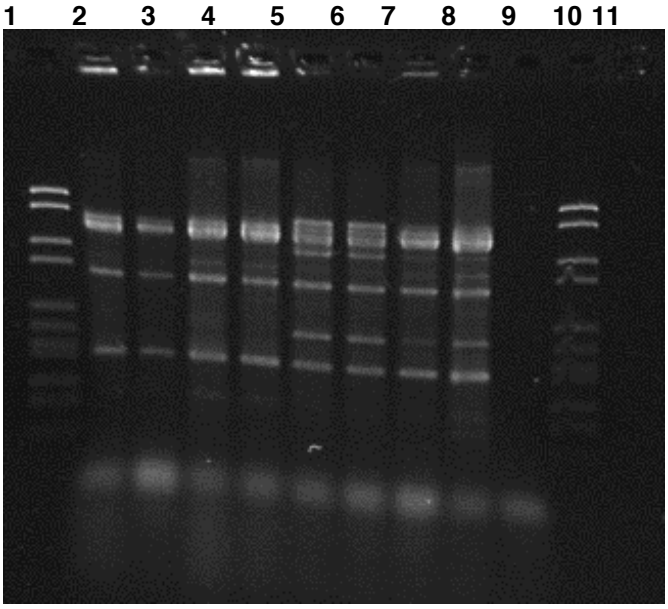

Figure 2. RAPD analysis with primers ERIC1R and ERIC2.Lanes: 1, 11, Molecular weight marker VI; 2-9, L. monocytogenes isolates in duplicate; 10, negative control.

Overall, *L. monocytogenes* was isolated from 38% of samples (57 of 150) and other *Listeria* species were isolated from 19% (29 of 150) of the meat. These results also show a high contamination of *L. monocytogenes,* indicating that it is prevalent in mechanically separated turkey meat.

References

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