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

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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. During two trials of 25 samples each, *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 reaction (PCR) 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 Trial I and II 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. However the multiplex PCR performed from suspect colonies grown on Palcam indicated 48 out of 54 (89%) of the MST meat harbored *Listeria* spp. Of those, 32 of 48 (67%) were positive for *L. monocytogenes*.

Introduction

As one of the major four foodborne pathogens, *L. monocytogenes* causes over 1,500 cases annually with a mortality rate of 35%. 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 *Listeria monocytogenes* in MST meat. *Listeria monocytogenes* contaminated turkey frankfurters were incriminated in at least one human fatality (4).

Materials and Methods

Test for PCR inhibitors. Three bacterial strains of *Listeria* were obtained from the NADC Culture Collection. Pure culture *L. monocytogenes* (3086) was associated with a human fatality of listeriosis resulting from contaminated turkey meat. Pure cultures of *L. innocua* (2888) and *L. monocytogenes* (2847) also were used in this study. Suitability of the multiplex PCR to detect *Listeria* and *L. monocytogenes* was evaluated by seeding pure cultures of *Listeria* and *L. monocytogenes* to UVM and Palcam agar. Pure cultures were maintained on trypticase soy agar plates with 0.6% yeast extract and placed in 100 ml of UVM I (28°C for 24 hours). After incubation a 1.4 ml aliquot of each sample was taken for PCR, 0.2 ml was plated on Palcam (incubated under microaerophilic conditions [10% CO_2, 5% O_2, 84% N_2 for 48 hours at 37 °C]and 0.1 ml transferred to 4 ml UVM II and incubated (28°C for 24 hours). After incubation a 1.4 ml aliquot of each sample was taken for PCRs, 0.2 ml plated on Palcam, and incubated as described above.

Preparation of bacterial DNA for PCR.. The enrichment aliquots were centrifuged (10,000 rpm for one minute), supernate was decanted, and the pellet was resuspended in 1 ml of PBS (1 M, pH 7.4). The sample was recentrifuged (10,000 rpm for 1 min), supernate decanted, and the pellet was resuspended in 250 μl 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 min, followed by 25 amplification cycles. Each cycle consisted of 1 min at 94°C (denaturation), 1 min at 60°C (primer annealing), and 1 min 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.

Trial I. Twenty-eight samples of mechanically separated turkey meat were obtained from an Iowa turkey plant on July 1, 1997.

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 0.2 ml was plated onto Palcam, 1.4 ml was transferred into the 25 ml UVM II and incubated (28°C for 24 hours), and 250 μl of each sample was taken for PCR. After incubation 0.2 ml were plated on Palcam and 250 μl 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. Bacterial strains were swiped off Palcam plates and placed in 250 μl of sterile distilled water. For PCR analysis samples from both enrichments and plates were boiled and centrifuged at (10,000 rpm for 1 min).

PCR. PCR setup was done the same as in Trial I.

Trail II. Twenty-six samples of MST meat were obtained from an Iowa turkey plant on July 14, 1997.

Listeria enrichment and growth. The enrichment set up was the same as in Trial I.

Preparation of bacterial DNA for PCR. The preparation of the DNA was done the same as in Trial I with the addition of the washing technique for the enrichments.

PCR. PCR setup was performed as in Trial I.

Results and Discussion

Pure cultures of *Listeria* spp. and *L. monocytogenes* were detected from enrichments after washing (Figure 2). Pure culture strains of *L. monocytogenes* NADC 3086 and NADC 2847 yielded two PCR products 938 and 174bp. In contrast *L. innocua* NADC 2888 yielded a single 938bp amplicon.

As summarized in Table 1, in Trial I the PCR results for enrichment samples tested negative for *Listeria* spp. One out of 26 samples from UVM I tested positive for *L. monocytogenes*. The UVM enrichments were subsequently plated to Palcam agar. Suspect *Listeria* (colonies surrounded by black halos from aesculin hydrolysis) were picked and verified as such by PCR. Ninety-two percent of suspect colonies from Palcam were positive for *Listeria* spp. whereas 58% of the *Listeria* spp. were positive for *L. monocytogenes*. In Trial II, UVM enrichments samples were negative for *Listeria* spp. and *L. monocytogenes* even after washing. However, 92% of MST samples were positive for *Listeria* spp. and 71% were positive for *L. monocytogenes* from suspected colonies grown on Palcam. This indicated that *L. monocytogenes* is present in UVM but PCR inhibitors such as myoglobin and fat are present in 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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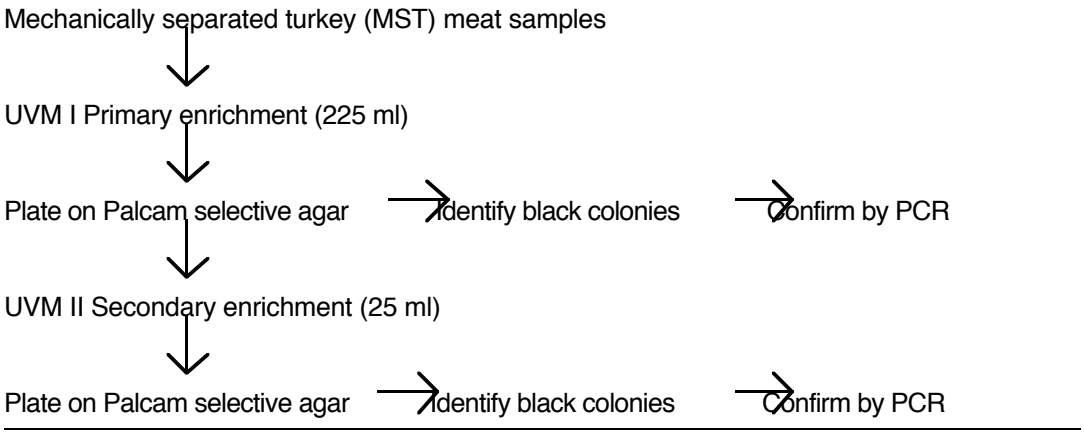

Figure 1. Listeria Enrichment and Growth.

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

	UVM I	
	Listeria spp.	*L. monocytogenes*
Trial I (n=26)	0	1
Trial II (n=28)	0	0
Total Positive (n=54):	0	1

	UVM II	
	Listeria spp.	*L. monocytogenes*
Trial I (n=26)	0	0
Trial II (n=28)	0	0
Total Positive (n=54):	0	0

	Palcam	
	Listeria spp.	*L. monocytogenes*
Trial I (n=26)	24(92%)	15(58%)
Trial II (n=28)	24(92%)	17(71%)
Total Positive(n=54):	48	32

Multiplex PCR from UVM II

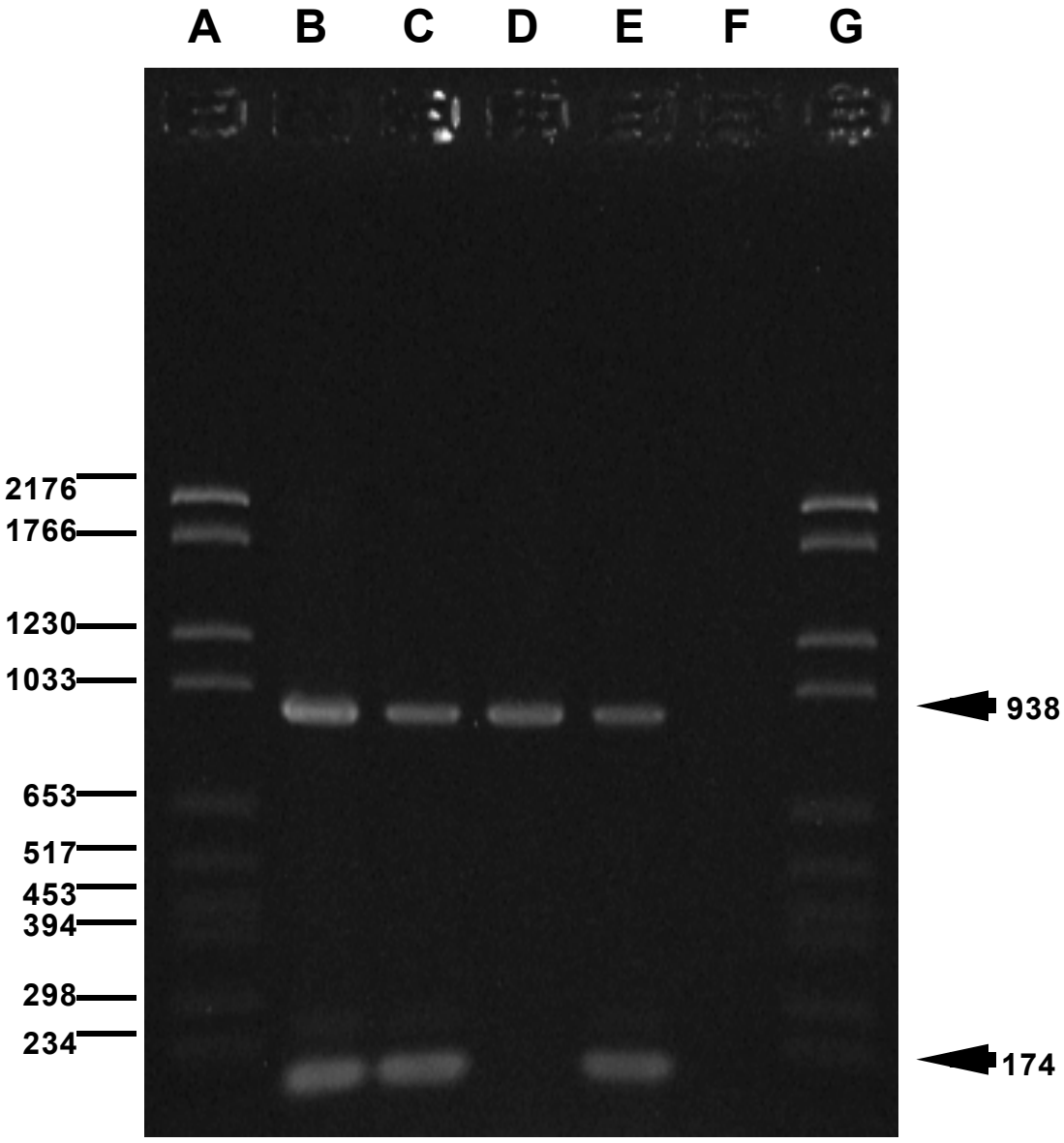

Figure A. 1.5% agarose gel electrophoresis of PCR-amplified DNAs from different *Listeria* reference strains. (A) and (G) Molecular weight marker VI. (B) Positive control: Genomic DNA from strain *L.* 3086 *monocytogenes*. (C) Strain NADC 3086. (D) *L. innocua* NADC 2888. (E) *L. monocytogenes* strain NADC 2847. (F) Negative control contains a sample without DNA. Note that *L. monocytogenes* generates both a 938 and 174-bp amplicon. A single 938 bp product is visible in *Listeria* strains other than *L. monocytogenes*.