A Research Note

Application of a Synthetic Listeriolysin O Gene Probe to the Identification of β-Hemolytic Listeria monocytogenes in Retail Ground Beef

A. MOHAMOOD1, A. R. DATTA2, and B. E. ERIBO*1

1Department of Botany, Howard University, Washington, DC 20059
2Division of Microbiology, Food and Drug Administration, Washington, DC 20204

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ABSTRACT

The synthetic gene probe is a 20 mer oligonucleotide, derived from listeriolysin O gene sequence of Listeria monocytogenes and shown to be specific for strains of this organism. This probe was used in a DNA-colony hybridization assay to evaluate its suitability in detecting β-hemolytic L. monocytogenes in ground beef. Thirty-six ground beef samples were plated onto three media: Trypticase soy agar with 0.6% yeast extract, lithium chloride-phenylethanol-moxalactam agar and Martin’s agar, both directly and after selective enrichment in Food and Drug Administration broth. Of the 118 gram-positive and catalase-positive isolates selected from the plates, only 24 gave detectable hybridization signal with the probe. CAMP-test and standard biochemical tests also revealed that only these 24 probe positive isolates were β-hemolytic L. monocytogenes. Of the 36 samples of ground beef, 6 were positive for Listeria spp., out of which 4 were L. monocytogenes.

Listeria monocytogenes is the etiological agent of human listeriosis, a severe disease often characterized by septicemia and meningitis. In pregnant women, infection may result in abortion or neonatal listeriosis (12). Several outbreaks of foodborne listeriosis within the last decade (9,13,25) have encouraged much investigation on the incidence of L. monocytogenes in various foods which include milk, meat, poultry, seafood, and vegetables (1,8,18,20,23). These investigations, however, have been marred by a number of problems. The traditional microbiological methods (19,26,27) for isolating and identifying L. monocytogenes are very time consuming and cumbersome, especially when applied to such foods as ground beef, with heavy background contamination. The battery of morphological, biochemical and serological tests is sometimes difficult to interpret (15). This is further complicated by other factors.

For example, the production of β-hemolysis albeit, a key test in the identification of virulent strains of L. monocytogenes is shared by two other Listeria spp., L. ivanovii and L. seeligeri. There appears to be in existence, strains of L. monocytogenes that are nonhemolytic (24). The type strain ATCC 15313 has been shown to be both nonhemolytic and avirulent. The extent of hemolysis reaction varies with the animal source of blood (26).

In an attempt to minimize these problems, several methods have surfaced for the detection of foodborne L. monocytogenes (1,3,10,15). Essentially, these methods are modifications of the traditional method (26), which suffers from the limitations of long detection time and low sensitivity. However, progress has been made towards developing better methodologies. Worthy of note are the detection methods which are based on antigen-antibody reactions (2,28) and nucleic acid hybridization (5). For a while, the development of specific gene probes for nucleic acid hybridization was limited, because of scarcity of information about the virulence-related genes of L. monocytogenes. Recently, listeriolysin O gene, a virulence associated gene of L. monocytogenes (4,11,14), was cloned from several strains of L. monocytogenes (6,17,22). From the sequence data of this gene (22), a 20 mer oligonucleotide probe, AD 13 was synthesized. The synthetic probe was shown to be specific for the identification of pure cultures of β-hemolytic strains of L. monocytogenes (6). We here examine the utility of this probe for the direct identification of β-hemolytic L. monocytogenes in retail ground beef.

MATERIALS AND METHODS

Samples
A total of 36 ground beef samples consisting of ground chuck, ground round, and hamburger meat were obtained from 21 different retail outlets in the Metropolitan Washington, DC area.
The maximum number of samples obtained from any one store was limited to two.

Analysis

The ground beef samples were surface plated onto three agar media both directly, and following selective enrichment in Food and Drug Administration Listeria enrichment broth (19). The media were trypticase soy agar (BBL Microbiology Systems, Cockeysville, MD) supplemented with 0.6% yeast extract (TSAYE), and two L. monocytogenes selective agar, lithium chloride-phenylethanol-moxalactam (LPM) agar (16) and Martin’s Listeria agar (21). For direct plating, 25 g of the sample was added to 225 ml of sterile 0.1% peptone dilution blank and homogenized for 2 min in a Waring blender. A 0.1-ml portion of the sample was plated in duplicate and the plates were incubated at 30°C for 48 h. The same procedure was followed for the enrichment samples, except that 25 g of the sample was added to 225 ml of the enrichment broth and incubated at 30°C for 48 h before plating.

Following the determination of the aerobic plate count, colonies showing positive in Gram-reaction and catalase test were selected as presumptive L. monocytogenes. These and three positive controls of L. monocytogenes (ATCC 19111, 19113, 19116) and two negative control strains of L. innocua and L. ivanovii were spot plated on TSAYE plates. The plates were incubated at 35°C for 24 h. From these plates, membrane filters were prepared for colony hybridization with 32P-labeled probe as previously described by Datta et al. (7). The gene probe, AD 13 (5’-GAT CCT GAA GGT AAC GAT AT 3’), was synthesized, purified, and labeled with 32P-dATP as before (7). The identity of all colonies was confirmed by standard microbiological methods (26).

RESULTS AND DISCUSSION

The aerobic plate count and number of β-hemolytic L. monocytogenes are summarized in Table 1. Of the 778 colonies examined, 118 (15%) were gram-positive and catalase-positive. When the 118 colonies were hybridized with the probe, only 24 (20%) gave detectable hybridization signals. The intensity of the signals as observed visually was comparable among the isolates (Fig. 1). This result was not affected by the amount of hemolysin produced by the isolates in liquid assay. The amount of hemolysin, ranged from <1 unit to 266 units/ml of supernatant (Table 2). Similar results were obtained by Datta et al. (6). The 24 isolates were confirmed to be β-hemolytic L. monocytogenes by CAMP test with Rodococcus equi and Staphylococcus aureus, tumbling motility, and standard biochemical tests (Table 3).

TABLE 1. Bacterial load of the retail ground beef samples.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TSA-YE</th>
<th>MLA</th>
<th>LPM</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean APC (X10⁶)</td>
<td>3.6</td>
<td>0.4</td>
<td>0.2</td>
<td>1.4</td>
</tr>
<tr>
<td>No. colonies examined</td>
<td>147</td>
<td>50</td>
<td>73</td>
<td>270</td>
</tr>
<tr>
<td>No. Gram (+), catalase (+)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No. L. monocytogenes</td>
<td>0</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No. Listeria spp.</td>
<td>0</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*TSA-YE: Trypticase soy agar-yeast extract.
*MLA: Martin’s Listeria agar (21).
*LPM: Lithium chloride-phenylethanol-moxalactam (16).
*D: Direct plating, E: Enriched.
*APC: Aerobic plate count per ml.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>CAMP</th>
<th>Catalase</th>
<th>MR/VP</th>
<th>Nitrate</th>
<th>Esculin</th>
<th>Mannitol</th>
<th>Xylose</th>
<th>Rhamnose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus</td>
<td>R. equi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>+</td>
<td>-</td>
<td></td>
<td>+/+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+/+</td>
</tr>
<tr>
<td>L. seeligeri</td>
<td>+</td>
<td>-</td>
<td></td>
<td>+/+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+/+</td>
</tr>
<tr>
<td>L. welshimeri</td>
<td>-</td>
<td>-</td>
<td></td>
<td>+/+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+/+</td>
</tr>
<tr>
<td>L. murrayi</td>
<td>-</td>
<td>-</td>
<td></td>
<td>+/+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L. grayi</td>
<td>-</td>
<td>-</td>
<td></td>
<td>+/+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The method as described by Datta et al. (6) was used for the liquid hemolysin assay.

* The remainder of L. monocytogenes isolates produced <1 unit of hemolysin, but were hemolytic on SBA.
** SBA: Sheep blood agar.
*** Supernatants of Listeria culture.

With regards to the efficacy of the media, LPM was highly effective in suppressing the background colonies. Thirty-five percent of the 62 gram-positive and catalase-positive isolates from LPM was probe-positive, compared to 3.4% of the 29 colonies from Martin’s Listeria agar and 3.7% of the 27 colonies from TSAYE.

In addition to the 24 isolates of L. monocytogenes, 31 other Listeria spp. were identified by standard microbiological methods (26). Twenty-six of the 31 isolates were identified to four species: L. seeligeri, 13; L. welshimeri, 2; L. murrayi, 2; and L. grayi, 6. Five isolates could not be placed. The Listeria spp. were isolated from 6 of the 36 samples of ground beef, 4 of which contained L. monocytogenes. It was of interest to note that none of the 31 Listeria spp. and the other bacterial isolates which grew on these plates, hybridized with the probe.

In this study, we have demonstrated that the synthetic gene probe, AD 13, is suitable for the identification of β-hemolytic L. monocytogenes in ground beef. Of special advantage are its relative rapidity, specificity, and the synthetic nature which has the potential for allowing the generation of large amounts of probe for routine screening of foods. The efficiency of the probe, however, depends largely on the ability of the media to suppress the background colonies and the stringency of the hybridization conditions. We found LPM agar and the stringent hybridization conditions employed in this study to be highly complementary.

ACKNOWLEDGMENTS

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REFERENCES