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Rapid detection of *Listeria monocytogenes* by a PCR assay specific for an aminopeptidase

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Specific and rapid detection of *Listeria monocytogenes* is very important with regard to food safety since all other species of *Listeria* appear to be non-pathogenic to humans. Conventional microbiological detection methods are very time consuming. The polymerase chain reaction (PCR) is one of the most promising techniques for rapid detection of micro-organisms in food products. We have developed a PCR assay, specific for *L. monocytogenes*, based on the gene encoding an aminopeptidase, which previously has not been described for this species.

The *L. monocytogenes* aminopeptidase shares strong sequence similarity with aminopeptidase C from *Streptococcus thermophilous, Lactobacillus lactis, Lactobacillus helveticus*, and with a cysteine proteinase from *Saccharomyces cerevisiae*. Polymerase chain reaction primers were synthesized based on the DNA sequence of the aminopeptidase gene. A 90 bp product was apparent with all *L. monocytogenes* strains tested but not with other species of *Listeria* or other bacterial genera. The PCR assay, which is performed directly from whole bacterial cells, does not involve DNA purification and can be conducted in 4 h. It provided positive identification of *L. monocytogenes* in mixed culture.

KEYWORDS: PCR, *Listeria monocytogenes*, detection, aminopeptidase.

INTRODUCTION

Listeria monocytogenes is a ubiquitous Gram-positive bacterium that can cause potentially serious infections in humans who ingest contaminated food.[1] Symptoms of listeriosis in humans can include meningitis, encephalitis and sepsis. In some cases fatalities have occurred. The major groups at risk for infection are pregnant women, young children, elderly and immunocompromised people.

Traditional analysis of food for the presence of micro-organisms relies on the growth of bacteria in artificial media. Viable cells that cannot be cultured will therefore not be recognized. In addition, culture techniques are often time consuming and not reliable.[2] Faster, more specific methods for detection of foodborne pathogens are needed so that contaminated products can be removed in a more timely fashion, thereby reducing the number of infections caused by these bacteria. In recent years methods of detection based on polymerase chain reaction (PCR) have been developed for several pathogenic micro-organisms.[3–6] With new rapid methods of detecting micro-organisms in food products, reduction of foodborne illness is possible.

MATERIALS AND METHODS

Bacterial growth conditions

Listeria monocytogenes and other *Listeria* were grown in tryptic soy broth (Difco Laboratories, Detroit, MI,

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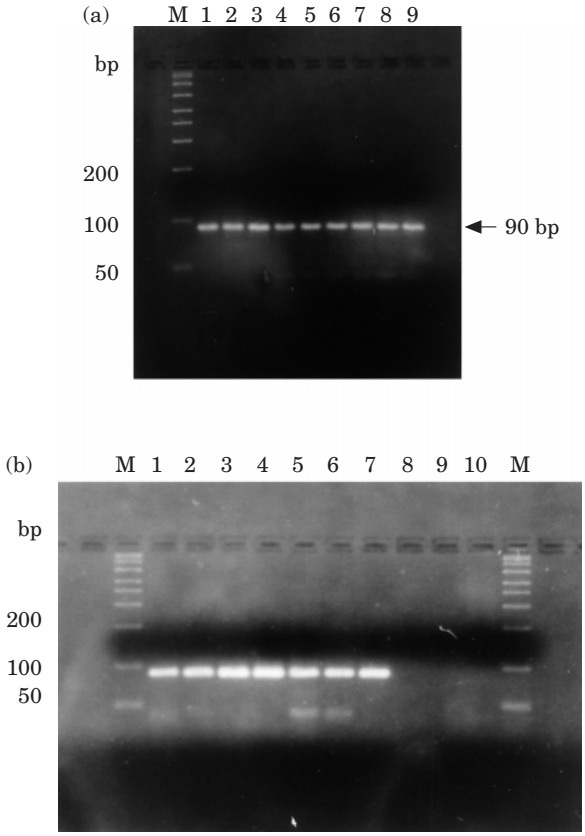

Fig. 1. (a) Nine different strains of *Listeria monocytogenes* were used in the standard polymerase chain reaction (PCR). Products from each reaction were electrophoresed through a 4% NuSieve agarose gel stained with ethidium bromide. Lane M denotes the AmpliSize molecular mass markers, purchased from Bio-Rad, that contain markers of 2000, 1500, 1000, 700, 500, 400, 300, 200, 100 and 50 base pairs. Lane 1 contains PCR products from *L. monocytogenes* V7, lane 2 is *L. monocytogenes* CAP, lane 3 is *L. monocytogenes* Scott A, lane 4 is *L. monocytogenes* Murray B, lane 5 is *L. monocytogenes* ATCC 19116, lane 6 is *L. monocytogenes* ATCC 19117, lane 7 is *L. monocytogenes* CDC F4260, lane 8 is *L. monocytogenes* CDC F4233 and lane 9 is *L. monocytogenes* CDC F4262. The product from the PCR migrated at 90 base pairs, as indicated by the arrow. (b) Seven different strains of *L. monocytogenes* and three strains of *Listeria innocua* were used in the standard PCR reaction. Products were electrophoresed through a 4% NuSieve agarose gel stained with ethidium bromide. Lane M denotes the AmpliSize molecular mass markers. Lane 1 contains PCR products from *L. monocytogenes* V7, lane 2 is *L. monocytogenes* CAP, lane 3 is *L. monocytogenes* Scott A, lane 4 is *L. monocytogenes* Murray B, lane 5 is *L. monocytogenes* CDC F4260, lane 6 is *L. monocytogenes* CDC F5069, lane 7 is *L. monocytogenes* CDC F4262, lane 8 is *L. innocua* LA-1, lane 9 is *L. innocua* CDC F4248 and lane 10 is *L. innocua* ATCC 33090. The product from the PCR migrated at 90 base pairs.

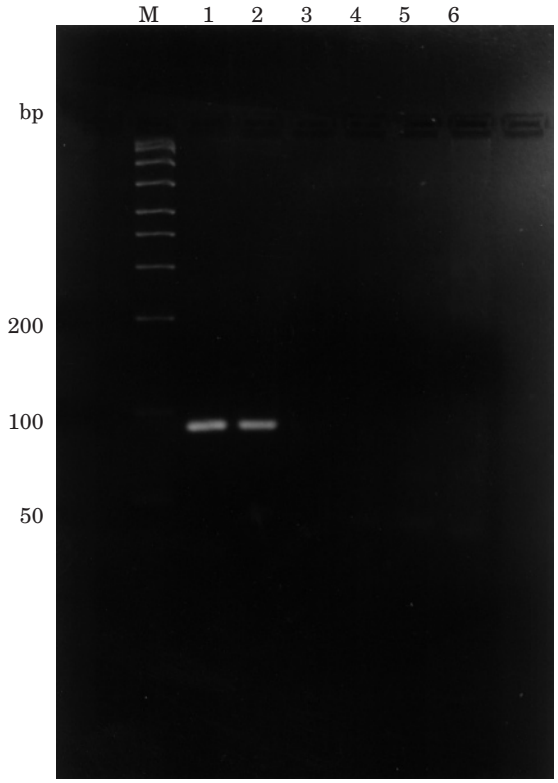

Fig. 2. Different strains of *Listeria monocytogenes, L. ivanovii, L. grayi, L. welshimeri* and *L. murrayi* were used in the standard polymerase chain reaction (PCR). Products from each reaction were electrophoresed through a 4% NuSieve agarose gel stained with ethidium bromide. Lane M denotes the AmpliSize molecular mass markers, purchased from Bio-Rad, that contain markers of 2000, 1500, 1000, 700, 500, 400, 300, 200, 100 and 50 base pairs. Lane 1 contains PCR products from *L. monocytogenes* V7, lane 2 is *L. monocytogenes* CAP, lane 3 is *L. ivanovii* KC1714, lane 4 is *L. grayi* ATCC 19120, lane 5 is *L. welshimeri* ATCC 35897 and lane 6 is *L. murrayi* ATCC 25401. The product from the PCR migrated at approximately 90 base pairs.

USA) containing 0·5% yeast extract (TSB-YE) or in brain-heart infusion (BHI) media at 37°C for 16–24 h. Lactic acid bacteria were grown in MRS broth (Difco Laboratories, Detriot, MI, USA) at 37°C for 24 h. *Campylobacter* were grown using a biphasic system of Brucella agar overlayed with Brucella broth (Remel, Lenexa, KS, USA) and incubated in an atmosphere of Campy Gas (Scott Laboratories, West Warwick, RI, USA) for 24–48 h at 42°C.[7] *Bacillus cereus* was grown in BHI at 37°C for 24 h.

Polymerase chain reaction assay

The primers, SK6 and AP4, directed against the sequence for an aminopeptidase (GenBank Accession number AF067409) amplified a fragment 90 bp in

Table 1. Specificity of polymerase chain reaction (PCR) primers SK6 and AP4 for various strains of *Listeria monocytogenes*

Bacteria	Pos/Neg Reaction	Serotype
Listeria monocytogenes V7	+	$\frac{1}{2}$a
L. monocytogenes CAP	+	4b
L. monocytogenes Murray B	+	4ab
L. monocytogenes Scott A	+	4b
L. monocytogenes ATCC 19114	+	4a
L. monocytogenes ATCC 19116	+	4c
L. monocytogenes ATCC 19117	+	4d
L. monocytogenes ATCC 19118	+	4e
L. monocytogenes ATCC 2540	+	3b
L. monocytogenes CDC F4260	+	$\frac{1}{2}$b
L. monocytogenes CDC F4233	+	$\frac{1}{2}$b
L. monocytogenes CDC F4262	+	4b
L. monocytogenes CDC F5069	+	4b
L. monocytogenes SLCC2479	+	3c
L. monocytogenes SLCC2482	+	7
L. monocytogenes V3	+	3a
L. monocytogenes V4	+	4a
L. monocytogenes V8	+	4e
L. monocytogenes V23	+	7
L. monocytogenes V47	+	3a
L. monocytogenes V127	+	3a

Various *Listeria monocytogenes* strains were tested with the PCR primers SK6 and AP4 as described in Materials and Methods.

Table 2. Specificity of polymerase chain reaction (PCR) primers SK6 and AP4 for various bacterial species

Bacteria	Pos/Neg Reaction
Listeria innocua LA-1	–
L. innocua CDC F4248	–
L. innocua ATCC 33090	–
L. innocua F4248	–
L. innocua F4247	–
L. ivanovii ATCC 19119	–
L. ivanovii KC1714	–
L. grayi ATCC 19120	–
L. welshimeri ATCC 35897	–
L. murrayi ATCC 25401	–
L. seeligeri LA15	–
Lactobacillus helveticus ATCC 10797	–
Lactobacillus acidophilus NRRLB1910	–
Pediococcus acidilactici RS2	–
Pediococcus acidilactici AcH	–
Leuconostoc mesenteroides, subsp. *cremoris* ATCC 19254	–
Lactococcus lactis, subsp. *lactis* ATCC 11454	–
Campylobacter jejuni ATCC 33291	–
Campylobacter coli ATCC 33559	–
Bacillus cereus ATCC 11778	–

Various bacterial species were tested with the PCR primers SK6 and AP4 as described in Materials and Methods.

length. Primer SK6 (5′-GGTCGGTGCATTAATAAG-3′) is located between nucleotides 542 and 559 on the sequence, while primer AP4 (5′-CAA-GAGTTACAAATTACACC-3′) is located between nucleotides 612 and 631 nucleotides. Cells used for PCR were harvested by centrifugation, resuspended in 1% Triton X-100, boiled for 5 min, and cooled on ice for 10 min. The samples were centrifuged for 2 min at 8000 × ***g*** to remove debris. Polymerase chain reaction was performed on 1·5 μl samples from the super-

natant. The standard reaction mixture contained 50 mM Tris-Cl (pH 8·5), 50 mM NaCl, 1 mM $MgCl_2$, 1 mM dithiothreitol, 0·1% Triton X-100, 0·2 mM of each deoxynucleoside triphosphate, 0·28 μM of each primer, and 0·25 U of *Taq* polymerase (Perkin-Elmer, Norwalk, CT, USA). The reaction was carried out in an Ericomp Powerblock thermocycler (San Diego, CA, USA) with the following program: one cycle of 95°C for 3 min, 40 cycles of 95°C for 1 min, 52°C for 1 min, 72°C for 1 min and one cycle of 72°C for 3 min. Ten-microlitre samples were electrophoresed through a 4% NuSieve 3:1 agarose gel (FMC Bio-Products, Rockland, ME, USA). Amplification products were visualized by ethidium bromide staining and u.v. transillumination.[8,9]

Identification of mixed cultures by plating

Listeria monocytogenes V7 and *Listeria innocua* ATCC 33090 were mixed in equal proportions, serially diluted, spread on BHI and TSB-YE plates and incubated at 37°C for 16 h. Single colonies of unknown identity were subjected to PCR and were grown in BHI and checked for microbiological identity using the API Listeria Kit, as described by the manufacturer (Bio-Merieux Vitek, Inc., Hazelwood, MO, USA).

RESULTS AND DISCUSSION

The PCR primers SK6 and AP4 are based on the sequence of the gene encoding an aminopeptidase from *L. monocytogenes*.[10] The gene for aminopeptidase shares strong sequence similarity to that of lactic acid bacteria, such as *Lactobacillus helveticus*.[11,12] These primers have been evaluated for specificity in detecting *L. monocytogenes*. When *L. monocytogenes* was the test organism, a 90 base pair (bp) product was detected on an ethidium bromide-stained agarose gel with all strains tested regardless of serotype (Fig. 1a). Several strains of different serotypes were chosen including serotype $\frac{1}{2}$a, here represented by strain V7, from which the aminopeptidase gene was originally isolated.[10] Also studied were serotypes $\frac{1}{2}$b, 4b, 4ab, 4c and 4d. The primers did not amplify a product from the closely related *L. innocua* (Fig. 1b), or other *Listeria* species including *L. ivanovii, L welshimeri, L. gravi* and *L. murrayi* (Fig. 2). *Listeria seeligeri* and other bacterial genera, both Gram-positive and Gram-negative, were also tested (Table 1). None of these bacteria gave the 90 bp amplification product with the SK6 and AP4 primers. To test the accuracy of the PCR assay, cultures of *L. monocytogenes* V7 and *L. innocua* ATCC 33 090 were mixed and plated for overnight growth. Colonies were subjected to PCR and then identified by the API Listeria kit. A total of 55 colonies from five different plates of each type were subjected to the test. There was total agreement between the PCR method and the API characterization.

Both raw and processed foods, as well as dairy products, contaminated with *L. monocytogenes* have been implicated as the source of several recent outbreaks of listeriosis.[5,13,14] Due to the lack of simple, rapid and sensitive identification procedures, many questions remain about the contamination of foods by such pathogens, including the route of transmission and the epidemiology of the disease. Standard methods that rely on cultivation of presumptive pathogens are time consuming and often labour intensive. Polymerase chain reaction offers an alternative approach for the specific and rapid detection of foodborne pathogens. Since there is no need for DNA purification in this method, it can be conducted in 4 h using intact bacterial cells. Rapid methods, such as this PCR assay, would be helpful in distinguishing the pathogen *L. monocytogenes* and non-pathogenic *Listeria* when grown under non-selective conditions.

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