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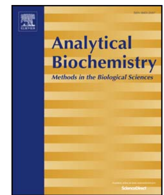

Rapid detection of foodborne pathogen *Listeria monocytogenes* by strand exchange amplification

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ABSTRACT

A strand exchange amplification (SEA) method to detect foodborne pathogen *Listeria monocytogenes* was developed. SEA is a novel nucleic acid amplification method that only requires one pair of primers. The speciespecific primers were designed by targeting the 16S rRNA gene and the amplification reaction was performed as short as 60 min at 61 °C. Notably, SEA method could not only detect genomic DNA but also detect RNA by one step without requiring extra reverse transcription. The result could be visualized by naked eyes so that water bath pot would be the only equipment needed. Moreover, culture fluids and bacteria colony could be successfully detected without any pretreatment and the method displayed good specificity and strong anti-jamming capacity. These features greatly simplified the operating procedure and made SEA method be potential for developing point-of-care testing (POCT) devices to detect viable *L. monocytogenes.*

Introduction

The gram-positive bacterium *Listeria monocytogenes* is a foodborne pathogen of global concern, which poses significant health problem to both human and domestic animals [1,2]. Despite the great efforts to decrease its incidence, *L. monocytogenes* is still an important cause of listeriosis [2,3]. Recently, an investigation of the World Health Organization (WHO) demonstrated that *L. monocytogenes* could still cause large numbers of illnesses and even deaths [4]. The mortality rate of *L. monocytogenes* is quite high compared to other foodborne pathogens, which is about 20–40% [2,5]. *L. monocytogenes* is ubiquitous in nature and easily contaminates vegetables, fruits, dairy products, meat and seafood [6,7], which significantly increases the risk of food poison [8]. Therefore, developing a rapid, specific and simple detection method of *L. monocytogenes* is of significant importance to food safety and human health.

The conventional detection method of *L. monocytogenes* is achieved by culture-based technique, which is time-consuming (about 4–7 days) [9]. In recent years, nucleic acid-based methods such as polymerase chain reaction (PCR) and immunoassay such as enzyme linked immunosorbent assay (ELISA) have been widely developed [10–12]. These approaches, however, usually require expensive equipment and are not available for point-of-care testing (POCT). Isothermal amplification provides a powerful method for nucleic acid amplification without the PCR thermocycling process and can realize detection of targets at a constant temperature [13,14]. Isothermal methods, such as nucleic acid sequence-based amplification (NASBA), and loop-mediated isothermal amplification (LAMP), have been developed to detect *L. monocytogenes* [15–17]. These methods could detect both DNA and RNA, but they need reverse transcription process when detecting RNA [18]. Here, a novel strand exchange amplification method with a simple reaction system was established by one-step detection of RNA which is more suitable for viability assays of *L. monocytogenes*.

Materials and methods

Materials and reagents

The SEA detection kit was purchased from Qingdao Navid Biotechnology Co., Ltd. (China). Ethidium bromide (EB), 20 bp DNA Marker, 6 × DNA Loading Buffer and SDS were provided by Sangon Biotech (Shanghai, China). Acrylamide and methylene diacrylamide were purchased from Sigma-Aldrich (St Louis, MO, USA). The bacterial strains including *L. monocytogenes, Staphylococcus aureus, Salmonella*

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Table 1
Sequences of nucleic acids used in this work.

Name	Sequence (5′-3′)
Listeria monocytogenes ([a] M58822.1 [b] 641–683)	GTCATTGGAAACTGGAAGACTGGAGTGCAGAAGAGGAGAGTGG
P1	GTCATTGGAAACTGGAAGACTG
P2	CCACTCTCCTCTTCTGCAC

The underlined portion was the same with primer P1. The dotted line sequence of target was complementary to the sequence of primer P2.
[a] GenBank accession number.
[b] The position of specific sequence in genomic DNA.

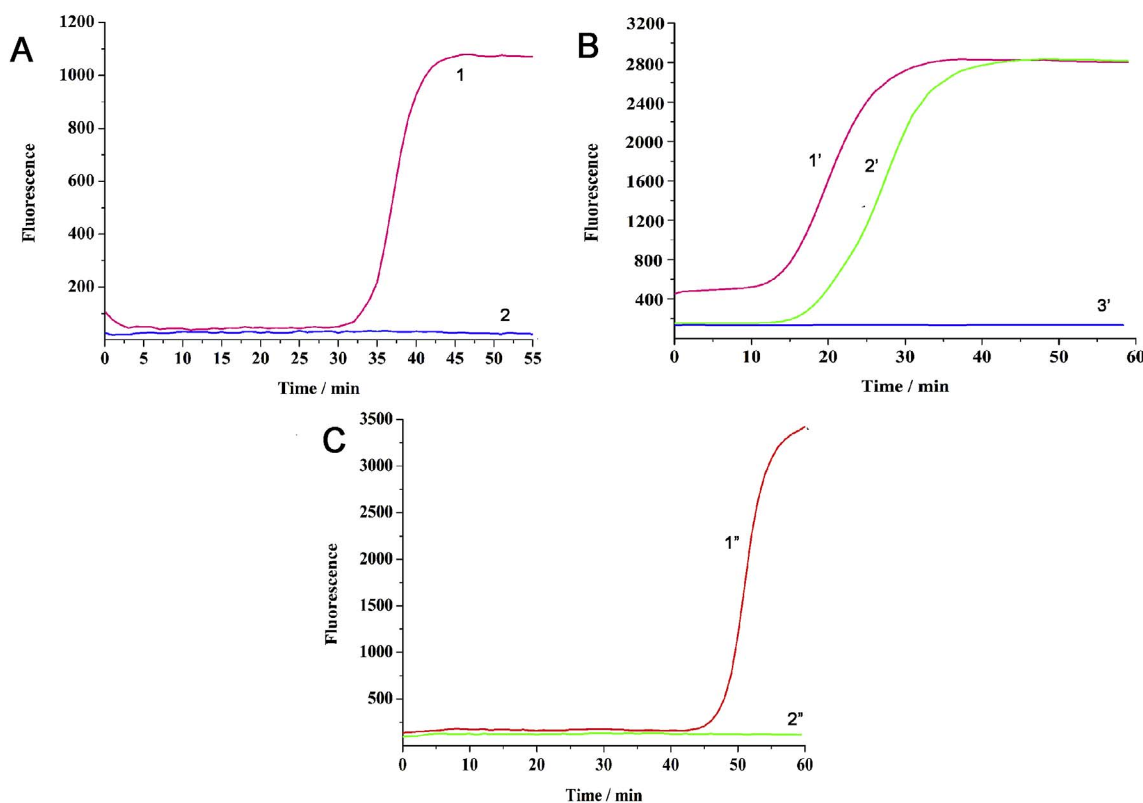

Fig. 1. The feasibility of SEA to detect *L. monocytogenes*. A represented that the targets were (1) 1.0×10^{-12} M genomic DNA and (2) no template control (NTC); B represented that the targets were culture fluids of *L. monocytogenes* diluted (1′) 100-fold and (2′) 1000-fold and (3′) NTC; C represented that the targets were (1″) bacteria colony of *L. monocytogenes* and (2″) NTC.

typhimurium, Vibrio parahemolyticus, Shigella castellani and *Escherichia coli* were saved by the laboratory.

Amplification reaction

The target was the hypervariable region from *L. monocytogenes* 16S rDNA. A pair of specific primers were designed by NUPACK software (http://www.nupack.org/) and synthesized by Sangon Biotech (Shanghai, China) and purified by HPLC (Table 1). The reaction of SEA was performed according to the manufacture's instruction. The fluorescence signal of SEA reaction was detected by CFX Connect™ Real-Time PCR System (Bio-Rad, CA, USA) at 1-min intervals. Gel images were recorded with ChampGel5000 system (Saizhi Innovation Technology Co., Ltd, Beijing, China). The reaction temperature was optimized at 55 °C, 57 °C, 59 °C, 61 °C, and 63 °C. The bacteria fluid and colony were then directly detected at the optimized temperature. The bacteria fluid cultured overnight was directly diluted 10-fold and 100-fold in water without RNase and DNase and 1 μL diluted sample was directly added to the SEA reaction as target. The bacterial colony was directly detected by adding a small amount of a colony into a PCR tube to trigger SEA reaction.

L. monocytogenes genomic DNA and total RNA extraction

The genomic DNA of *L. monocytogenes* was extracted using the bacterium genomic DNA extraction kits from Tiangen Biotech (Beijing, China) and the total RNA of *L. monocytogenes* was extracted with *TransZol* Up Plus RNA Kit from TransGen Biotech (Beijing, China) according to the manufacturers' instructions.

Results and discussion

The design of SEA to detect L. monocytogenes

The SEA is a novel isothermal nucleic acid amplification method which depends on the single-stranded denaturation bubbles of dsDNA at the reaction temperature. In this method, one pair of specific primers bind to the targets by invading to the bubbles, allowing DNA polymerase to extend [19]. The reaction requires a simple reaction system including a pair of primers and *Bst* DNA polymerase and can be

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