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Detection of *Listeria monocytogenes* based on combined aptamers magnetic capture and loop-mediated isothermal amplification

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ABSTRACT

Rapid detection of *Listeria monocytogenes* (*L. monocytogenes*) is important to ensure timely treatment and to prevent outbreaks of listeriosis. Here, we reported an efficient and accurate system using aptamers magnetic capture and loop-mediated isothermal amplification (AMC-LAMP) for sensitive detection of *L. monocytogenes*. A set of combined aptamers (including four individual aptamers with high binding affinity to *L. monocytogenes*) was conjugated to magnetic beads, used for capture *L. monocytogenes*. After incubation at room temperature for 45 min, the captured *L. monocytogenes* cells were directly used for DNA extraction (1 h). Subsequently, the LAMP assays were carried out at 63 °C for 40 min, and the amplification products were visualized via SYBR Green® I staining. The AMC-LAMP had a detection limit of 5 CFU/mL, and the total assay time was approximate 3 h. By the protocols developed in this study, 17 food samples was test for *L. monocytogenes* without prior culture enrichment. The accuracy of AMC-LAMP was shown to be 100% when compared with culture-biotechnical examination, while PCR failed to detect *L. monocytogenes* in one of five positive samples. These results indicate the developed AMC-LAMP is a potential useful method for rapid screening and on-site detection of *L. monocytogenes* in food samples.

1. Introduction

Listeriosis is a severe disease in humans which remains a major public health concern, mainly due to its high mortality rate (30%) for susceptible individuals (EFSA/ECDC, 2015; Lomonaco, Nucera, & Filipello, 2015). *Listeria monocytogenes* (*L. monocytogenes*), the causal agent of listeriosis, is a pathogen which can survive in stressful environmental conditions and has been isolated from many kinds of foods (Swaminathan & Gerner-Smidt, 2007; Wieczorek & Osek, 2017).

To evaluate the potential human health risk from foods contaminated by *L. monocytogenes*, a specific, reliable, and sensitive detection method is needed. Currently, cultivation of pathogen on selective/differential agar media and identifying colonies with biochemical tests are still used as the standard method for *L. monocytogenes* detection in China (National food safety standard GB 4789.30–2010). Unfortunately, this examination is time-consuming (5–7 days), laborious, and have shown limitations in specificity and sensitivity for accurate detection (Lee et al., 2015; Xiao et al., 2014; Xu et al., 2012).

With the development of modern molecular diagnosis technologies, various PCR-based method with improved detection resolution and significantly shortened analysis time have been used to screen *L. monocytogenes* in food samples, including multiplex PCR (Jamali et al., 2015; Wieczorek & Osek, 2017), immunomagnetic separation PCR (Ayaz, Ayaz, Kaplan, Dogru, & Aksoy, 2009), real-time PCR (Traunsek et al., 2011), and multiplex real-time PCR (Xiao et al., 2014). However, these methods require expensive apparatuses and complicated procedures which limit their field applications (Mao, Qiu, Zheng, Chen, & Yang, 2012; Wu, Liu, Guo, Chen, & Wang, 2014). The loop-mediated isothermal amplification (LAMP) is a relatively simple and field-adaptable technique (Notomi et al., 2000). This technique employs a set of specially designed primers based on six or eight distinct regions of target DNA, and the entire process can continuously yield millions of long DNA concatamers under isothermal conditions within 1 h (Nagamine, Kuzuhara, & Notomi, 2002; Notomi et al., 2000). It requires only a *Bst* DNA polymerase and a laboratory heating block (or a water bath) for reaction, and the LAMP results are visualized with naked eye by adding fluorescence intercalation dye or metal ion

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indicators (Tomita, Mori, Kanda, & Notomi, 2008). These characters make it cost-efficient and applicable for on-site detection. Moreover, the LAMP technique has superior advantages in terms of sensitivity, reaction speed, and amplicon yield (Abdulmawjood et al., 2014; Saull, Duggan, Hobbs, & Edwards, 2016; Ye et al., 2017). Up to date, several studies reported the detection of *L. monocytogenes* utilizing LAMP method (Tang et al., 2011; Wu et al., 2014). However, like other kinds of molecular detection methods, prior cultural enrichment is still needed in these assays, which have become a bottleneck for rapid or "real-time" detection (Suh & Jaykus, 2013; Suh, Dwivedi, Choi, & Jaykus, 2014).

In order to accelerate the detection process, approaches have been developed to separate, concentrate, and purify target pathogens from sample matrix (Dwivedi & Jaykus, 2011; Suh & Jaykus, 2013; Suh, Brehm-Stecher, & Jaykus, 2013, 2014). Among them, the aptamer-based system has been reported a reliable method for capture of target pathogens. Aptamers are synthetic oligonucleotides that can bind to a wide range of targets through their 3-dimensional structure and intermolecular hydrogen bonding (Ma, Zhao, Ge, & Shi, 2012). Owing to their high selectivity, stability, versatile target binding and easy regeneration capabilities over traditional antibodies, there has been a considerable interest in employing aptamers as recognition ligands for microbial agents' detection (Suh et al., 2013).

For L. *monocytogenes*, several candidate single-stranded DNA ((ss)DNA) aptamers with high binding specificity have been identified (Bruno et al., 2015; Duan et al., 2013; Lee et al., 2015; Ohk, Koo, Sen, Yamamoto, & Bhunia, 2010; Suh & Jaykus, 2013; Suh et al., 2013, 2014; Zhang et al., 2016). Although all of them displayed affinity for *L. monocytogenes*, each aptamer had different binding target. For example, the aptamer identified by Bruno et al. (2015) targeted listeriolysin O, a well-characterized cytolysin expressed in the pathogenic species of *Listeria*. Aptamer A8 used by Ohk et al. (2010) and NPC-aptamer used by Zhang et al. (2016) targeted internalin A, an invasive protein of *L. monocytogenes*. Four aptamers (LM6-2, LM6-116, LM12-6, and LM12-13) reported by Suh et al. (2014) bound to cell surfaces of *L. monocytogenes*, yet they targeted two different surface moieties. In other studies, the targets of identified aptamers remained unknown, such as Lbi-17 reported by Suh et al. (2013), LMCA2 and LMCA26 reported by Lee et al. (2015), and A15 reported by Duan et al. (2013). When these reported aptamers were analyzed, Lbi-17, LMCA2, LMCA26 and A15 had smaller dissociation constants after binding to *L. monocytogenes*. Therefore, they were chosen and used as a combined aptamers set for *L. monocytogenes* capture in pre-analytical processing of this study. Subsequently, the LAMP assay was carried out using specific primers designed according to the actin polymerization gene (*act*A) of *L. monocytogenes*. The sensitivity, specificity, and reproducibility of the method were assessed, and the application of this assay for detection of *L. monocytogenes* in real food samples was also evaluated.

2. Materials and methods

2.1. Bacterial strains, culture conditions and genomic DNA extraction

L. monocytogenes ATCC 19112, *Escherichia coli* ATCC 25922, *Salmonella enteritidis* ATCC 15611, *Staphylococcus aureus* ATCC 6538, and *Sligella flexneri* ATCC 12022 were kindly donated by Dr. Ke Li, Zhejiang Entry-exit Inspection and Quarantine Bureau, Hangzhou, China. *L. seeligeri* ATCC 35976, *L. ivanovii* ATCC 19119, *L. welshimeri* ATCC 35897 were obtained from the American Type Culture Collection (ATCC). The *Listeria* strains were grown in Tryptone Soy Broth (TSB) or Tryptone Soy Agar (TSA), at 37 °C for 6–12 h. Non-*Listeria* strains were grown overnight in TSB or LB broth/agar plates at 37 °C. All media were purchased from Beijing Land Bridge Technology Co. Ltd (Beijing, China). These bacteria were harvested from fresh cultures, and were decimal diluted by double-distilled H_2O (ddH_2O). 1 mL serial dilutions were plated onto appropriate agar plates, and bacterial counts were obtained and reported as CFU per mL. Genomic DNAs were isolated from 1 mL of each tested bacterial culture using "Bacterial Genomic DNA Purification Kit" (Takara Biotechnology, Dalian, China), according to the manufacturer's instructions. DNA samples were resuspended in 50 μL ddH_2O, and stored at −20 °C.

2.2. Preparation of aptamers conjugated magnetic particles

According to previous reports, four ssDNA aptamers (Lbi-17, LMCA2, LMCA26 and A15) with higher binding affinity to *Listeria* were selected and used in this study (Table 1). These aptamers were synthesized, and 5′-biotinylated by Sangon Biotech (Shanghai, China). 40 pmol of each the synthesized aptamers was denatured at 90 °C for 5 min, flash cooling on ice for 10 min, and mixed with 50 μg (50 μL) Streptavidin MagneSphere® Paramagnetic Particles (approximately 1.0 μm in diameter and irregularly shaped, Promega Corp., Madison, WI) in an individual tube. The reaction tube was incubated at room temperature for 10 min, by gently mixing the solution every 2 min. Then, the tube was placed on a Dynal Mag™-2 magnetic particle concentrator (Invitrogen, Shanghai, China) for approximately 30 s, and the magnetic beads were collected at the side of the tube. Subsequently, supernatant was carefully removed and the obtained aptamers-conjugated magnetic particles were washed three times in 1 × Tris-EDTA buffer (10 mM Tris/Tris-HCl, 1 mM EDTA), capturing the particles using the magnetic concentrator and carefully removing the supernatant each time. After final wash, the aptamers-conjugated magnetic particles were resuspended in 50 μL 1 × PBS buffer (pH 7.4), and stored at room temperature. Successful immobilizing of aptamers to the magnetic particles was proved by PCR. According to the manufacturer's instructions, the biotin-streptavidin interaction was very stable under nondenaturing conditions. Thus, no shelf life study was needed for the prepared aptamers-conjugated particles, and they were directly used in following procedures.

2.3. Aptamers magnetic capture (AMC) assay

Capture of L. *monocytogenes* by aptamers-conjugated magnetic particles was performed as follow: fresh cultured *L. monocytogenes* cells were harvested and ten-fold serially diluted. At the same time, four kinds of aptamers-conjugated magnetic particles were mixed with equal amounts (200 nM of each aptamer). Then, 1 mL of each diluted cell suspension was mixed with 50 μL aptamers-conjugated magnetic beads, and the mixture was incubation for 45 min at room temperature with gentle rotation. The successfully captured *L. monocytogenes* cells were then pulled-down using Dynal Mag™-2 magnetic particle concentrator (Invitrogen). The unbound and non-specifically bound cells (supernatant) were discarded by three consecutive washings, and finally the aptamer-bacteria complexes were resuspended in 200 μL PBS, and stored at room temperature.

To determine the capture efficiency (CE), the entire 200 μL of aptamers-bacteria complexes was used for cell enumeration by plating on TSA plates (about 24–48 h). In parallel, 1 mL of *L. monocytogenes* cells without AMC treatment was also enumerated, and named as control group. Then, CE% was calculated as the total CFU in AMC treatment group divided by the total CFU in control group, multiplied by 100. These procedures were carried out triplicate, and data were expressed as mean ± standard deviation.