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Simultaneous detection of three foodborne pathogenic bacteria in food samples by microchip capillary electrophoresis in combination with polymerase chain reaction☆

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ABSTRACT

A rapid and sensitive PCR based strategy in combination with microchip capillary electrophoresis (MCE) was employed to simultaneously detect three foodborne pathogenic bacteria. Three pairs of primers were specially designed for the amplification of target genes from *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*) and *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium). The PCR products along with standard DNA fragments were employed to optimize the separation conditions in MCE. Under optimal conditions, detectable separation of the PCR products (1.6–3.5 ng μL^{-1}) from the three foodborne pathogenic bacteria was achieved within 135 s. The limits of detection of the three bacteria were concluded to be as low as 45 CFU mL^{-1} for *E. coli*, 62 CFU mL^{-1} for *S. aureus* and 42 CFU mL^{-1} for *S.* Typhimurium. The RSD of migration time was in the range of 0.5–0.8%. We conclude that MCE along with PCR holds real potential for rapid analysis and detection of nucleic acids from routine foodborne pathogenic bacteria.

1. Introduction

Foodborne diseases that result from consumption of food contaminated with pathogenic bacteria have been of vital concern to public health. *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) are considered major foodborne pathogens [1–3]. As a typical inhabitant in the human intestinal tract, *E. coli* is often used as an indicator organism reflecting fecal contamination and water pollution [4–6]. *S. aureus* is a Gram-positive coccoid bacterium found in dust, soil, air, and water and is currently a leading cause of infections in hospitalized patients [7,8]. *S. aureus* enterotoxin contamination, which is an important cause of food-borne disease, occurs during food preparation and handling [9]. Although *S.* Typhimurium does not have a food animal reservoir, it has been found in unpasteurized milk, eggs, meat, poultry, etc. This indicates that the primary reason for the spread of *Salmonella* infections is probably by eating food that has been incorrectly handled by infected individuals [1,10]. These three organisms are the most common foodborne pathogens, causing the largest number of outbreaks and deaths. To better control microbial contamination of meat products and to reduce foodborne illnesses and ensure food safety, rapid and accurate bacterial detection methods are needed to effectively monitor the quality of raw meat product supplies [11–13]. Traditional detection methods involve inoculating into a medium, culturing, and staining, along with biochemical and/or serological tests. These are time-consuming and extremely labor intensive, and often take several days to complete for achieving a certain result. Therefore, the requirement of public health emergencies for accurate and rapid detection of foodborne pathogenic bacteria cannot be satisfied by these methods.

DNA is an ideal molecule for detection of specific foodborne bacteria and provides clear biological information. Polymerase chain reaction (PCR) based techniques in combination with separation technologies have been promising strategies for rapid amplification and detection of many different bacteria in the microbiological research field. Therefore, the detection of specific DNA fragments using PCR in combination with separation techniques has been very popular [14–17]. Currently, capillary electrophoresis (CE) and microchip capillary electrophoresis (MCE) have been developed as

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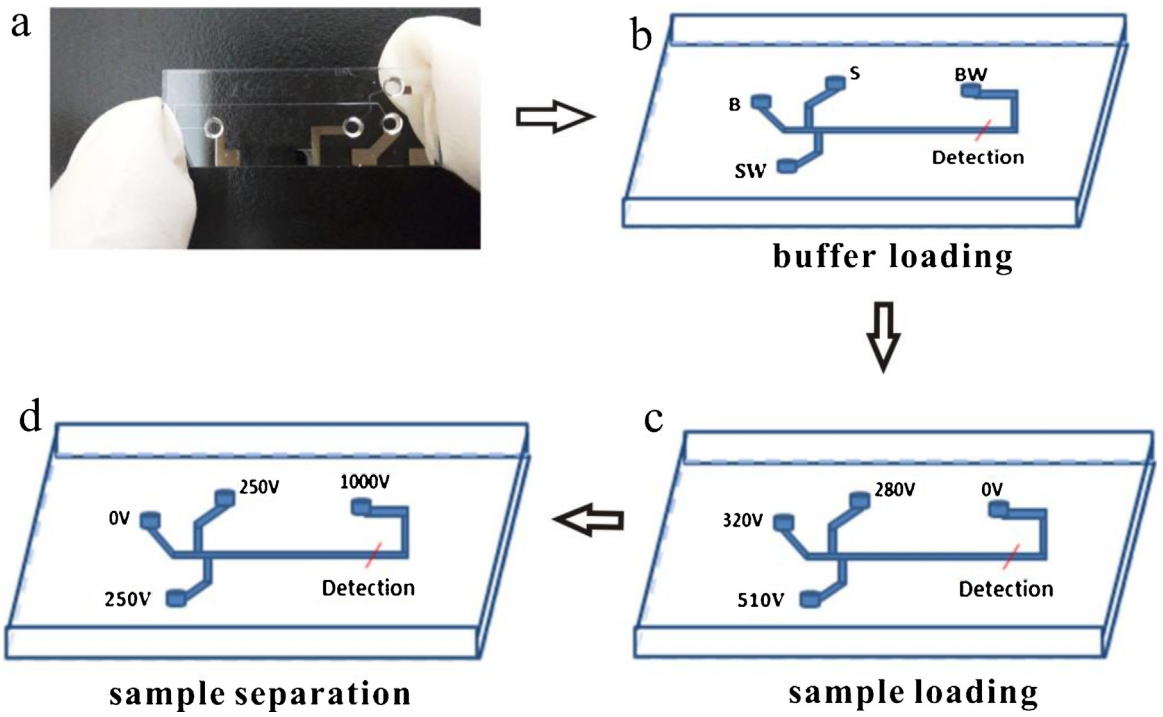

Fig. 1. Scheme illustration of DNA fragments analysis based on MCE with (a) the image of microchip; (b) buffer loading; (c) sample loading; (d) sample separation. The four reservoirs are shown as follows: Sample Reservoir (S); Sample Waste Reservoir (SW); Buffer Reservoir (B); Buffer Waste Reservoir (BW).

powerful analytical techniques for analyzing food owing to their lower sample consumption and better separation efficiency, and proven versatility and potentiality. It is noteworthy that the application of CE is relatively excellent in microbiological research. Alarcón et al. exploited a PCR procedure combined with CE for the detection of *Listeria monocytogenes, Salmonella* spp. and *Staphylococcus aureus* in artificially inoculated food [18]. Meanwhile, MCE is used more frequently than CE for the analysis of microorganisms. Li et al. successfully applied a rapid and sensitive strategy based on MCE and multiplex PCR with condition optimization of genetic algorithm-support vector regression (GA-SVR) to simultaneously detect four foodborne pathogenic bacteria [11]. Jiang et al. successfully developed a microfluidic device that combines high-throughput continuous flow PCR and DNA hybridization for the detection of various bacterial pathogens [19]. Ohlsson et al. described an integrated microsystem for rapid separation, enrichment, and detection of bacteria from blood, thereby addressing the unmet clinical need for rapid diagnostics of sepsis [20]. Ramsey et al. described a microfluidic chip integrating DNA extraction, amplification, and detection for the identification of bacteria in saliva [21]. When compared with gel electrophoresis (GE), their work demonstrated that the use of MCE for analyzing PCR products was more sensitive and had better resolution. Therefore, MCE is the preferred method for the detection of pathogenic bacteria.

In this study, a rapid and sensitive detection strategy based on MCE was developed and applied to simultaneously detect PCR products of three foodborne pathogenic bacteria. The primer pairs for *E. coli* and *S. aureus* were as described earlier [9,22], while the primer pairs for *S.* Typhimurium were designed in the current study (The sections of 2.4 and 3.1 were in the detailed introduction). Under optimal conditions, the separation of the PCR products of three foodborne pathogenic bacteria (*E. coli*, *S. aureus* and *S.* Typhimurium) was achieved within 135 s with limits of detection (S/N = 3) of 1.6, 2.7 and 3.5 ng μL^{-1}, respectively. The levels of detection were as low as 45 CFU mL^{-1} for *E. coli*, 62 CFU mL^{-1} for *S. aureus* and 42 CFU mL^{-1} for *S.* Typhimurium, respectively. Also, our assay facilitated the sensitive analysis of the three pathogenic bacteria in artificially contaminated raw food meat samples. With this procedure, shorter analysis times and smaller amounts of reagents than in GE are needed, making it more budget-friendly and therefore better suited for routine food microbial analysis.

2. Materials and methods

2.1. Instrumentation and reagents

DNA was separated and detected by MCE instrument (MCE-202 MultiNA, Shimadzu, Japan). The quartz microchip was purchased from Shimadzu Company. A blue LED (470 nm; 20 mA) apparatus is installed in the MCE unit. Fig. 1 is the scheme illustration of MCE parameters for DNA fragments analysis. The microchannel is 23 mm in the length for separation, 104 μm in width and 48 μm in depth. Briefly, the samples were introduced into the channel at different voltages: $V_1 = 280$ V, $V_2 = 510$ V, $V_3 = 320$ V, and $V_4 = 0$ V for 50 s and the samples were separated and detected for 135 s at $V_1 = 250$ V, $V_2 = 250$ V, $V_3 = 0$ V, and $V_4 = 1000$ V (Fig. 1). After MCE analysis, the microchannel was washed several times with ultrapure water in preparation for the subsequent experiment.

All chemicals and reagents used were at least of analytical grade. Ethanol, hydrochloric acid, tris(hydroxymethyl)aminomethane (Tris) and ethylenediaminetetraacetic acid (EDTA) were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Both DNA ladder and SYBR gold solution were purchased from Invitrogen Corporation (Karlsruhe, Germany), and diluted with Tris–HCl buffer (TE buffer, 10.00 mM Tris–HCl, pH 8.0, 1 mM EDTA). Taq DNA Polymerase and Rapid Bacterial Genomic DNA Isolation Kit were purchased from Sangon Biotech (Shanghai, China). All oligonucleotides were purchased from Sangon Biotech Co. (Shanghai, China) and purified by HPLC. DNA-1000 kit was purchased from

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