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Simultaneous detection of *Staphylococcus aureus* and *Salmonella typhimurium* using multicolor time-resolved fluorescence nanoparticles as labels

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ABSTRACT

Foodborne illnesses caused by *Staphylococcus aureus* and *Salmonella typhimurium* are common public health issues worldwide, affecting both developing and developed countries. In this study, aptamers labeled with multicolor lanthanide-doped time-resolved fluorescence (TRFL) nanoparticles were used as signal probes, and immobilized by Fe_3O_4 magnetic nanoparticles were used as the capture probes. The signal probes were bonded onto the captured bacteria by the recognition of aptamer to form the sandwich-type complex. Under the optimal conditions, TRFL intensity at 544 nm was used to quantify *S. typhimurium* ($y = 10{,}213 \times -12{,}208.92$, $R^2 = 0.9922$) and TRFL intensity at 615 nm for *S. aureus* ($y = 4803.20 \times -1933.87$, $R^2 = 0.9982$) in the range of 10^2–10^5 CFU/ml. Due to the magnetic separation and concentration of Fe_3O_4 nanoparticles, detection limits of the developed method were found to be 15, 20 CFU/ml for *S. typhimurium* and *S. aureus*, respectively. The application of this bioassay in milk was also investigated, and results were consistent with those of plate-counting method. Therefore, this simple and rapid method owns a great potential in the application for the multiplex analysis in food safety.

1. Introduction

Staphylococcus aureus (*S. aureus*) and *Salmonella typhimurium* (*S. typhimurium*) are the two most frequently reported pathogenic bacteria associated with food poisoning worldwide and pose a serious threat to human health under the appropriate conditions (Ellenberger and Krishna, 1999; Hennekinne et al., 2012). In addition, various foods can be contaminated by both of these two pathogenic bacteria simultaneously, such as poultry, egg, meat, milk, fruit and even some pre-heat or acid-treated fresh products (Callejón et al., 2015; Zweifel and Stephan, 2012). Traditional culture-based methods for *S. aureus* and *S. typhimurium* detection including the sequential steps of pre-enrichment, selective enrichment and selective different plating are time-consuming, labor-intensive and impractical for real-time application (Kramer et al., 2009; Patel et al., 2006). Studies in the past decades focused on developing new detection methods for pathogenic bacteria, such as enzyme-linked immunosorbent assay, polymerase chain reaction amplification, and ligase chain reaction (Chang and Huang, 1994; Cheng et al., 2006; Moore and Curry, 1998). However, the complex procedures and high cost limit the widespread application of these technologies. Developing new techniques with high throughput, sensitivity and selectivity, especially the simultaneous detections for multiple bacteria pathogens contamination is critical.

Aptamers are single-stranded DNA or RNA molecules isolated from nucleic acid libraries via SELEX (Tuerk and Gold, 1990). Comparing to traditional biosensors using antibodies or enzymes, aptamer-based biosensors possess the following advantages: high chemical stability, great flexibility, high specificity and purity via commercial synthesis (Ellington and Szostak, 1990; Tuerk and Gold, 1990). Because of the high specificity and affinity, aptamers can be modified with a variety of signal tags applied in aptasensors, which have become an area of research interest (Abbaspour et al., 2015; Chang et al., 2010).

Fluorescence bioassays are currently the primary analytical tool because of their convenient optical signal transduction, fast response and high sensitivity (Bünzli, 2010). However, conventional fluorescence labeling often suffers from the interferences of scattered lights and autofluorescence from cells and tissues, resulting in a low signal-to-noise ratio (S/N). One of the best approaches to decrease background interference is to use time-resolved fluorescent reagents (Hanaoka et al., 2007; Morgner et al., 2010). Lanthanide-doped (Ln^{3+}-doped) in compounds (such as commercial lanthanide-chelating agents and Ln^{3+}-doped inorganic nanoparticles (NPs)) show highly desirable spectral characteristics, including long fluorescence emission lifetimes and large Stokes'

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shift, has been introduced in biomedical analysis (Weibel et al., 2004; Xiang et al., 2011). Comparing to Ln^{3+}-chelates, Ln^{3+}-doped NPs exhibit stability, high resistance to photobleaching, low cytotoxicity and favorable biocompatibility have attracted reviving interest in a variety of bioapplications (Chen et al., 2007; Ju et al., 2011). In recent years, magnetic nanoparticles (MNPs) have been receiving considerable attention. Due to their magnetic properties, low toxicity, and biocompatibility, MNPs are useful for the separation of target cells from a mixture of bacteria and matrix substances. Additionally, MNPs help to concentrate the separated cells into a small volume, which is suitable for impedance measurements (Brandão et al., 2015; Dosev et al., 2007; Joshi et al., 2009).

In the present study, a new designed time-resolved fluorescence aptasensor was developed for simultaneous detection *S. aureus* and *S. typhimurium*. Two types of Ln^{3+}-doped inorganic NPs were synthesized and modified with aptamers as signal probes. Gd^{3+} and Ce^{3+} were used as sensitizers, to strengthen the emission fluorescence intensity of Eu^{3+} and Tb^{3+}, thereby yielding a higher detection sensitivity compared to conventional TRFL bioassays. Moreover, Fe_3O_4 MNPs immobilized with aptamers were used as the capture probes. It is demonstrated that this established method is featured as high-throughput, sensitivity, rapid, and has the potential in daily detection work, especially for simultaneous detections of various pathogenic factors.

2. Materials and methods

2.1. Materials and instrument

Gadolinium (III) chloride hexahydrate ($GdCl_3 \cdot 6H_2O$), europium (III) chloride hexahydrate ($EuCl_3 \cdot 6H_2O$) were purchased from Sigma-aldrich Co. LLC (USA). Terbium (III) nitrate pentahydrate ($Tb(NO_3)_3 \cdot 5H_2O$), Cerium (III) nitrate hexahydrate ($Ce(NO_3)_3 \cdot 6H_2O$) and Yttrium (III) nitrate hexahydrate ($Y(NO_3)_3 \cdot 6H_2O$) were purchased from Aladdin Chemistry Co. Ltd. (China). 2-aminoethyl dihydrogen phosphate (AEP) and polyethylenimine (PEI) (ca. 30% in water) were purchased from TCI (Shanghai) Development Co., Ltd. Ethylene glycol, $FeCl_3 \cdot 6H_2O$, NaCl, ethanol, glutaraldehyde aqueous solution (25% in V/V) and the chemicals used to prepare the buffers and solutions were purchased from Sinopahrm chemical Reagent Co. Ltd. (China). Avidin was purchased from Sigma-aldrich Co. LLC (USA). The opaque 96-well microtitration microplates (300 ml/well) were purchased from Corning (USA).

S. Typhimurium ATCC 14028, *S. aureus* ATCC 29213, *Escherichia coli* ATCC 25922 were obtained from the American Type Culture Collection (ATCC). *Listeria monocytogenes* and *Bacillus cereus* were kindly provided by the Animal, Plant and Food Inspection Center, Jiangsu Entry-Exit Inspection and Quarantine Bureau (Nanjing, China). *S. Typhimurium* aptamer (reported by Raghavendra Joshi et al. (Joshi et al., 2009)) and *S. aureus* aptamer (reported by Shao et al. (Cao et al., 2009)) were synthesized by Shanghai Sangon Biological Science & Technology Company (Shanghai, China). The sequence of the *S. Typhimurium* aptamer was 5′-biotin-C6-TAT GGC GGC GTC ACC CGA CGG GGA CTT GAC ATT ATG ACA G-3′. The sequence of the *S. aureus* aptamer was 5′-biotin-C6-GCA ATG GTA CGG TAC TTC CTC GGC ACG TTC TCA GTA GCG CTC GCT GGT CAT CCC ACA GCT ACG TCA AAA GTG CAC GCT ACT TTG CTA A-3′.

The morphologies and size of nanoparticles were determined by using a JEM-2100HR transmission electron microscope (TEM, JEOL Ltd., Japan). The X-ray diffraction (XRD) pattern measurements were performed using a D8-advance (Bruker AXS Ltd., Germany) with graphite-monochromatized Cu Kα radiation (λ = 0.15406 nm). Absorption spectra were recorded on a UV-1800 spectrophotometer (Shimadzu

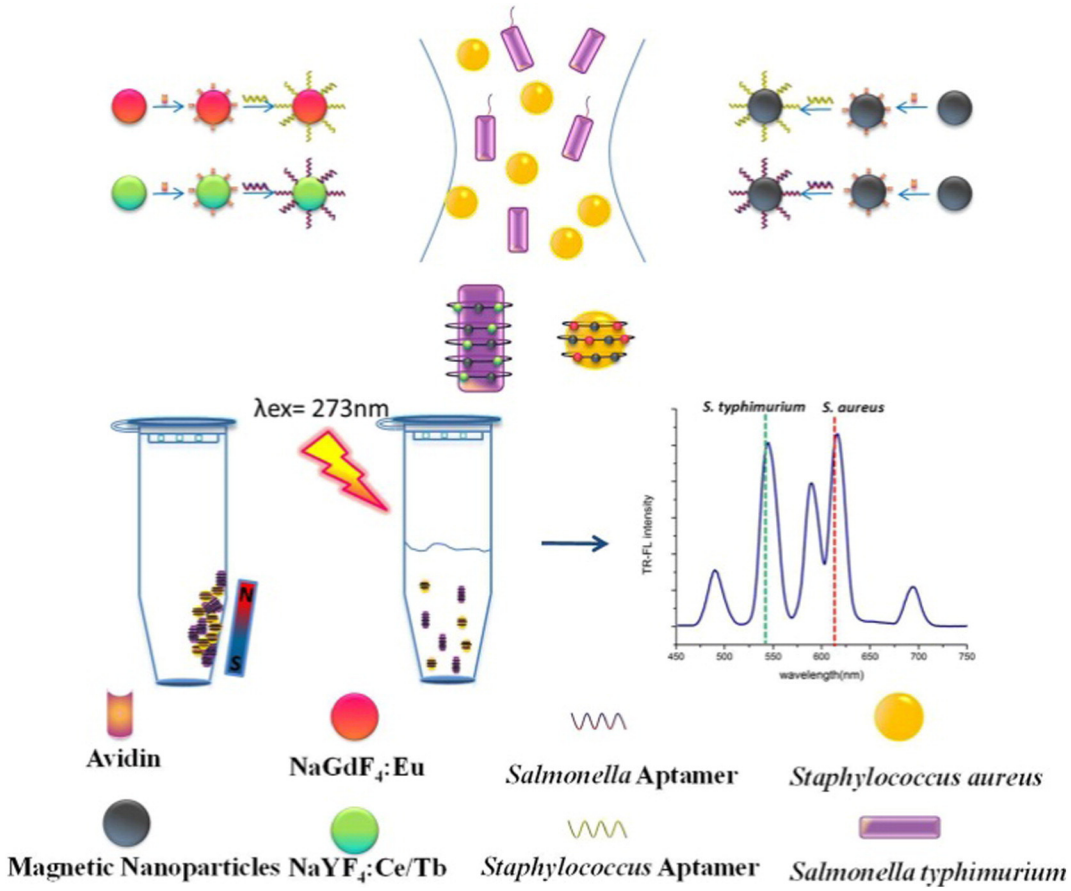

Fig. 1. Schematic illustration for simultaneous detection of two foodborne pathogens. Simultaneous detection of foodborne pathogenic bacteria using time-resolved fluorescence nanoparticles as signal probes and magnetic nanoparticles as capture probes.