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In-field detection of multiple pathogenic bacteria in food products using a portable fluorescent biosensing system

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ABSTRACT

Foodborne diseases caused by pathogenic bacteria have increasingly become a major public health issue worldwide. Rapid, simple, and accurate detection methods are urgently needed for in-field screening of bacterial pathogens. In our previous work, rapid detection methodologies have been established based on fluorescent nanobiosensors for simultaneous separation and detection of multiple foodborne pathogenic bacteria. In this research, a portable fluorescent biosensing system was designed and built and further assessed for in-field detection of three main types of bacterial pathogens that have been associated with the outbreaks of foodborne illness. Using the developed fluorescent nanobiosensor coupled with nanobead-based immunomagnetic separation, we conducted blind tests with the portable device to simultaneously detect *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium in different food products in three cities selected from three big agricultural provinces in China. Specificity tests showed low interference of this multiplex biosensor from non-targets in food samples. The detection could be done from sampling to results within 60 min. Limits of detections of this method for *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium were determined to be 10^2, 10^3, and 10^3 CFU/mL in lettuce, shrimp, and ground beef, respectively. Recovery tests were also investigated and this method was evaluated to be accurate comparing with the gold standard culturing method. Therefore, it is feasible for this portable fluorescence biosensing system to be used in rapid and in-field screening of multiple foodborne pathogenic bacteria in foods, such as vegetables, livestock meat, and sea food. And together with fluorescent nanobiosensors, it provides a promising alternative tool to traditional culturing method, or even conventional ELISA and PCR based methods.

1. Introduction

Foodborne pathogens are responsible for many severe outbreaks of foodborne diseases and recalls of contaminated food products. The burden of foodborne illnesses is substantial domestically, regionally and globally. According to WHO, every year 1 in 10 people fall ill and 33 million healthy life years lost due to foodborne diseases worldwide. Norovirus, *Escherichia coli*, *Campylobacter* and non-typhoidal *Salmonella* are key global causes of diarrhoeal diseases, which accounts for 50% global burden of foodborne diseases caused by 31 hazards (WHO, 2015). In USA, each year roughly 1 in 6 Americans (48 million people) gets sick, 128,000 are hospitalized, and 3000 die of foodborne infections, among which pathogenic bacteria are one of the main causes, such as *Salmonella*, *Campylobacter* spp., *E. coli* O157, *Listeria monocytogenes*, and so on (CDC, 2011). In China, *Salmonella*, *Vibrio parahaemolyticus*, *Bacillus cereus*, *Staphylococcus aureus*, and *E. coli* are the leading foodborne pathogenic bacteria (NHFPC, 2016). In a globalized world foodborne diseases can spread quickly along the food chain and across borders, which highlights urgent need for rapid and accurate pathogen detection and overall disease

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surveillance.

The gold standard in pathogen diagnosis is traditional culturing methods. Though reliable and accurate, they are extremely time-consuming and labor-intensive. In-field pathogen detection in food safety demands the instant, specific, and sensitive determination of infections (Valimaa, Tilsala-Timisjarvi, & Virtanen, 2015). Hence, those culture-based techniques are far from satisfying the urgent needs of point-of-care test (POCT). Immunologic and polymerase chain reaction (PCR)-based molecular methods are well accepted nowadays as a rapid method, however, they are costly, may yield false results, and still takes 4–8 h and 3–6 h from sampling to results, respectively (Omiccioli, Amagliani, Brandi, & Magnani, 2009; Velusamy, Arshak, Korostynska, Oliwa, & Adley, 2010). Recently, many biosensor-based methods have been reported and gradually become effective alternative tools either for routine detection and fast screening of foodborne bacteria, documented to lower the limit of detection, improve the sensitivity, and shorten time to results (Wang, Wang, Chen, Kinchla, & Nugen, 2016; Jiang et al., 2015; Syed, 2014; Arora, Sindhu, Dilbaghi, & Chaudhury, 2011). To facilitate biosensing methods with simple and portable instrumentation will significantly enhance their ability to detect pathogenic bacteria in field or on site.

With respect to detecting targets, many methods developed have been focused on the determination of a single type of foodborne bacterium (Mandal, Biswas, Choi, & Pal, 2011). However, due to the fact that there can be multiple kinds of bacterial pathogens in a food sample, current pathogen detection trends emphasize the application of single sensor platform for detection of multiple pathogens/toxins in a cost-effective manner (Song et al., 2016). Simultaneous analysis of more than one foodborne pathogens in a single detection run also helps to reduce the assay time, cut the assay cost, and enable large-scale screening (Gehring & Tu, 2011; Wu, Duan, Shi, Fang, & Wang, 2014). High throughput nano/biosensor-based screening tools such as the fiber optic, cell-based and light scattering have been developed for detection of multi-pathogens and toxins from food. (Abdelhaseib et al., 2016; Bhunia, Kim, & Taitt, 2015).

Though new bio-recognition elements such as aptamer, bacteriophage, and antibiotic peptide have been investigated to overcome the challenge of limitations of available biosensing materials in the assay design, when it comes to more than one targets in a multiplexed assay for foodborne pathogens, antibodies still present robust affinity to target bacterial cells and more effective in overcoming the more complex biological and chemical cross-reactions in a practical way (Edgar et al., 2006; Nandi, Ritenberg, & Jelinek, 2015; Valimaa et al., 2015; Wang et al., 2016; Wu et al., 2014). Magnetic beads (MNBs) and the magnetic separation technique have been well applied in isolation and concentration of foodborne pathogens from complex food matrices. This technique shortens analysis time, improves the specificity and sensitivity, and helps to develop automatic and large-scale analytical platforms (He, Zhou, He, Wang, & Cao, 2011; Tamanaha, Mulvaney, Rife, & Whitman, 2008). Integrated with nanomaterials, fluorescent biosensors have now become effective alternative tools for routine detection of foodborne bacteria (Qian et al., 2014). QD-based fluorescence approaches are promising due to multicolored QDs have enabled multiplexed analysis of several pathogens in a single analytical run with high sensitivity, as well as easy conjugation of the QDs' core-shell structure with various bio-receptors (Jamieson et al., 2007).

Particularly in our laboratory, several fluorescent nanobiosensor methods has been developed in couple with magnetic separation for simultaneous detection of two, three and four bacterial pathogens not only in buffer solution but also in food products (Wang, Li, Wang, & Slavik, 2011; Xu et al., 2015; Yang & Li, 2006). Taking a step further, a portable, automated and high-throughput fluorescent biochemical rapid analyzer was set up in coordination with the established methodologies, aiming to realize rapid detection of multiple foodborne pathogens in fields.

To validate our technology, the portable optical device was assessed for the feasibility to detect food samples infected with multiple foodborne pathogenic bacteria in the wild. Three typical pathogenic bacteria, *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* Typhimurium were used as model pathogens. Highly specific antibodies were applied as bio-recognition elements for the bacterial targets. Nanobead-based magnetic separation were used for sample pretreatment and target enrichment from bulk solution. With the proposed portable device and reported method, blind tests and recovery tests were conducted to detect *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium simultaneously in vegetable, livestock meat, and sea foods in Hangzhou, Nanchang, and Wuxi, three big cities located in three large agricultural provinces, Zhejiang, Jiangxi, and Jiangsu Provinces, respectively.

2. Material and methods

2.1. Portable fluorescent biochemical rapid analyzer

The in-house built portable and high-throughput fluorescent biochemical rapid analyzer (Wang, Lu, Zhang, Li, & Yu, 2016a, 2016b) was assessed in this study to detect multiple foodborne pathogenic bacteria. The analyzer mainly consists of a fluorescence device and a laptop. A spectrometer with a range of 345–1045 nm, a light source with an excitation wavelength of 450 nm, an UV–visible optical probe, and a sampling stage were housed in the fluorescence device, which was described in detail in another manuscript by our group (Lu et al., 2016). The laptop was installed with self-developed operating software. Fig. 1 shows the home screen of spectrum data acquisition and processing software.

The portable fluorescent biochemical rapid analyzer enables automatic sampling and detection of 10 samples within 4 min in one analytical run. In the process of fluorescence measurement, the disposable syringes (1.0 mL) with the ready-to-measure samples inside are placed in the holes of the cylindrical syringe holder, which can hold totally 10 syringes and housed on a rotating stage. The samples are detected one by one according to the programmed automatic rotation of the stage. The resulting fluorescent signals are then processed with the data acquisition software installed in the laptop, and the results are finally shown on the screen.

The application scope of this portable fluorescent biochemical rapid analyzer is to quantify fluorescent signals of multiple samples automatically in an analytical assay. The source of fluorescence signal includes but not limited to QDs. In this study, the portable analyzer was used for rapid and in-field detection of three pathogenic bacteria in foods coupled with a nanobead-based sample pretreatment for simultaneous separation and a QD based fluorescent aptasensor for simultaneous detection.

2.2. Reagents and supplementary

2.2.1. Immuno-magnetic nanobeads

Streptavidin-coated magnetic nanobeads (MNBs) with a diameter of 150 nm (MagCellect streptavidin ferrofluid) were purchased from Ocean NanoTech (San Diego, Cal.). They were water-soluble iron oxide nanoparticles with an amphiphilic polymer coating conjugated to streptavidin. Biotin-labeled rabbit anti-*E. coli* O+K antibodies (4–5 mg mL^{-1}), rabbit anti-*S.* Typhimurium antibodies (4–5 mg mL^{-1}), and rabbit anti-*L. monocytogenes* antibodies (4–5 mg mL^{-1}) were obtained from Biodesign International (Saco, Maine). The three types of immunomagnetic nanobeads (immuno-MNBs, MNBs conjugated with antibodies, or MNB-Ab conjugates)