Biosensors and Bioelectronics 64 (2015) 171–176

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Membrane filter-assisted surface enhanced Raman spectroscopy for the rapid detection of *E. coli* O157:H7 in ground beef☆

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ARTICLE INFO

Article history:
Received 30 July 2014
Accepted 26 August 2014
Available online 28 August 2014

Keywords:
E. coli O157:H7
Rapid detection
Immunomagnetic separation
SERS nanoprobes
Surface enhanced Raman spectroscopy

ABSTRACT

Consumption of food contaminated with *Escherichia coli* O157:H7 is one of the major concerns in ensuring food safety. Techniques that are simple and suitable for fast screening to detect and identify pathogens in the food chain is vital to ensure food safety. In this work, we propose a simple and rapid technique to detect low levels of *E. coli* O157:H7 using membrane filtration and silver intensification steps in combination with surface enhanced Raman spectroscopy (SERS) analysis. The target bacteria can be captured and separated efficiently by two different antibody bearing nanoparticle complexes (magnetic nanoparticles and gold nanoparticles with a Raman reporter). After centrifugal filtration, the bacteria–nanoparticle complexes left on the filter membrane were localized by silver intensification process before probing by SERS. Extremely low concentration of *E. coli* O157:H7 (~10 CFU/mL) could be detected within 1 h and 3 h from both pure culture and ground beef samples, respectively. This method can potentially be used as an effective pathogen screening tool for routine monitoring.

1. Introduction

Escherichia coli O157:H7 (*E. coli* O157:H7) is one of the key foodborne pathogens that can cause severe illness such as diarrhea and acute kidney failure (Karch et al., 2005) upon consumption of contaminated food. A report from the Centers for Disease Control and Prevention states that around 70,000 cases of *E. coli* infection occurs annually in the United States (Eagle, 2011). Availability of sensitive and accurate techniques that can rapidly detect and identify the causative agent can help in reducing the spread of infection, and in ensuring the safety of food at source.

Numerous conventional methods based on cultural enrichment and selective plating exist for the detection and identification of specific pathogens from foodstuffs (Reissbrodt, 2004; Velusamy et al., 2010). Increasing numbers of alternative rapid methods, including polymerase chain reaction (PCR) (Malorny et al., 2008; Navas et al., 2006) and enzyme-linked immunosorbent assay (ELISA) (Delehanty and Ligler, 2002) using specific antibodies and recombinant phages have also been reported. Although highly sensitive, the conventional methods are time-consuming (up to seven days for final results), laborious and cumbersome (Cho et al., 2014). In addition to PCR and ELISAs, there are other alternate approaches that can overcome some of these hurdles and includes lateral-flow immunochromatography (Pengsuk et al., 2013), electrochemical and optical methods (Banada et al., 2009; Setterington and Alocilja, 2012; Subramanian et al., 2006; Varshney and Li, 2007; Waswa et al., 2006), microelectromechanical system (MEMS)-based microfluidic device (Zelada-Guillén et al., 2010), and nanoparticles-based biosensing (Cho and Irudayaraj, 2013; Ravindranath et al., 2011; Su et al., 2013; Wang et al., 2010, 2011).

In this study, we have investigated the potential of a simple and rapid SERS technique assisted by membrane filtration and silver intensification, for the detection of low levels of *E. coli* O157:H7 in ground beef. The silver intensification technique, also known as autometallography, is employed to visualize the target bacteria via gold nanoparticle-assisted catalytic reaction (Cho et al., 2010) that can result in metallic silver deposition around the bacterial surface and increase its optical density for microscopic visualization.

2. Materials and methods

2.1. Materials

Monoclonal (mAb) and polyclonal (pAb) antibodies specific to *E. coli* O157:H7 were purchased from Abcam (Cambridge, MA) and

☆Work was completed at Purdue University and the first author is now at Eulji University.

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http://dx.doi.org/10.1016/j.bios.2014.08.063
0956-5663/

KPL (Gaithersburg, MD), respectively. Goat anti-mouse IgG and mouse anti-goat IgG antibodies coupled to horseradish peroxidase were purchased from Pierce (Rockford, IL). Iron oxide (Fe_3O_4) magnetic nanoparticle (30 nm in diameter) and magnetic separator (SuperMag Multitube Separator™) were purchased from Ocean Nanotech (Springdale, AR). Reference cultures of *E. coli* O157:H7, *Salmonella typhimurium*, *Salmonella enteritidis*, and *Listeria monocytogenes* were obtained from the culture collection at the Center for Food Safety Engineering consortium at Purdue University. Centrifugal nylon filter membranes (0.45 μm) were obtained from VWR (Radnor, PA). Sodium citrate, tannic acid, chloroauric acid ($HAuCl_4$), casein (sodium salt type, extracted from milk), 4-mercapto benzoic acid (MBA), Tween 20, silver enhancer A and B were obtained from Sigma (St. Louis, MO).

2.2. Preparation of mAb-magnetic nanoparticle conjugate

Synthesis of this conjugate was accomplished per manufacturer's instructions. Briefly, 1 mL of the activation buffer was added to pre-weighed EDAC/NHS mixture and dissolved to obtain 2 mg/mL of EDAC and 1 mg/mL of NHS as the final concentrations, respectively. Iron oxide nanoparticles (0.2 mL) were added to 1.5 mL Eppendorf tube, followed by 0.1 mL of the activation buffer and 100 μL of the EDAC/NHS solution and mixed for 10 min at ambient temperature. The activated iron oxide particles were mixed sequentially with 0.5 mL of coupling buffer and 50 μg of monoclonal anti-*E. coli* O157:H7 antibodies, and allowed to react for 2 h at ambient temperature. To block the residual surface of the iron oxide nanoparticles, 10 μL of the quenching buffer was added and incubated with gentle mixing for 10 min at ambient temperature. Unreacted antibodies were removed using the magnetic separator. After discarding the supernatant, 1 mL of the storage buffer was added. The resulting mAb-magnetic nanoparticle complexes were stored at 4 °C until further use. When required, the mAb-magnetic nanoparticles were filtered through 0.2 μm pore size syringe filter membrane just before use in order to remove potential aggregated particles.

2.3. Preparation of pAb-AuNP–MBA complex (SERS probe)

AuNP (mean diameter of 30 nm) were synthesized using sodium citrate as the reducing agent (Hayat, 2012). Polyclonal antibodies (5 μg; specific to *E. coli* O157:H7) were dissolved in 10 mM phosphate buffer, pH 7.4, (PB) followed by addition of 1 mL of the AuNP solution and reacted with gentle shaking for 1 h at ambient temperature. For labeling the Raman reporter, 10 μL of 100 μM MBA in ethanol was added and the mixture incubated for 2 h at ambient temperature. Next, casein dissolved in PB (5% w/v) was added and reacted further for 30 min to block any residual sites on the surface of AuNP. The mixture was then centrifuged (12,000 rpm, 15 min) and the supernatant was discarded. The SERS probe was further washed three times with the casein solution.

2.4. Preparation of bacterial cultures

E. coli O157:H7, *S. typhimurium*, *S. enteritidis* and *L. monocytogenes* were each cultured in 500 mL of Luria-Bertani (LB) media at 37 °C with shaking for 18 h and then harvested by centrifugation (5000 rpm, 20 min), followed by re-suspension in 50 mL of sterilized PBS. The concentration of bacteria in these stock solutions was determined by enumeration in triplicate on the LB agar plate incubated at 37 °C for 24 h.

2.5. Enzyme immunoassay for E. coli O157:H7

High concentration (1×10^7 cells/mL) of the cultures (*E. coli* O157:H7, *S. typhimurium*, *S. enteritidis*, and *L. monocytogenes*) were each added (100 μL) to wells of a microtiter plate and incubated at 37 °C for 1 h to allow the cells to coat the wells. The subsequent steps involved: (i) addition of casein (200 μL) and incubation of the plate (37 °C for 1 h) to block any unreacted sites; (ii) addition (100 μL) of mAb-magnetic nanoparticles and incubation (37 °C for 1 h); (iii) addition of the SERS probe (100 μL) and incubation (37 °C for 30 min); (iv) addition (100 μL) of goat anti-mouse IgG-HRP and mouse anti-goat IgG-HRP conjugates for binding to the mAb-magnetic nanoparticle and SERS probe, respectively, followed by incubation (37 °C for 1 h). The HRP-labeled conjugates were diluted 5000-fold in 0.5% casein containing 0.1% (v/v) Tween 20 prior to use; and (v) finally, the substrate solution (50 mM sodium acetate: 1% TMB: hydrogen peroxide = 1000:10:1 ratio) was added (200 μL). The optical density was measured at 450 nm after incubation (ambient temperature for 15 min).

2.6. Analytical procedure for the detection of E. coli O157:H7

2.6.1. Assessment in pure culture

The bacterial samples ($\sim 1.3 \times 10^{10}$ CFU/mL) were prepared by diluting in PBS containing 0.5% casein (Casein–PBS). The samples (1 mL) were mixed with the mAb-magnetic nanoparticles (10 μL) and incubated with gentle mixing for 15 min at ambient temperature. The captured bacteria were separated and concentrated in the magnetic separator. The pellet was re-suspended in 900 μL of Casein–PBS containing 0.1% (v/v) Tween 20 (Casein–PBS–Tw). The SERS probe (5 μL), dissolved in 95 μL of Casein–PBS–Tw, was then added and mixed for 15 min at 37 °C. To remove the unbound reactants, the mixture was placed in a centrifugal filter tube (0.45 μm, nylon filter) pre-treated (1 h) with 200 μL Casein–PBS and centrifuged at 2000 rpm for 5 min. The process was repeated by sequentially washing with Casein–PBS–Tw, Casein–PBS, and DI-Water. For the silver intensification step, 200 μL of silver solution (prepared by mixing 1:1 silver enhancer A with B) was added to the filter membrane and reacted for 10 min at ambient temperature. After centrifugation at 2000 rpm for 3 min, 200 μL of DI-Water was added and centrifuged again to stop the reaction. The stained membrane was used for the Raman measurement.

2.6.2. In ground beef

A standard sample preparation protocol was followed (Muldoon et al., 2012). Ground beef (1 g) was homogenized in 10 mL of autoclaved PBS followed by addition of *E. coli* O157:H7 to give a final level of 10 CFU/mL. An unspiked control was also included. These were incubated for 2 h at 37 °C and subjected to the identical procedure as described above (Section 2.6.1).

2.7. Raman signal measurement

The filter membrane was analyzed using the Senterra confocal Raman system (Bruker Optics Inc., Billerica, MA) with 100× air objective (NA 0.9, infinity and flat field corrected). The excitation laser source was provided by 633 nm diode laser at 10 mW power and the integration time was set at 10 s. Raman spectra obtained from the sample were processed to assess the Raman vibration modes in the 800–1800 cm^{-1} range and normalized using the Min/Max option. A rubberband method was applied for baseline correction. All these manipulations were conducted using the OPUS 6.5 software.

2.8. TEM/EDX analyses

Transmission electron microscopy (TEM) images of the samples were obtained using a Tecnai T20 transmission electron microscope equipped with an energy dispersive X-ray detector to perform energy-dispersive X-ray spectroscopy (EDS) compositional analysis. A freshly prepared nanoparticles-bacteria