Ecotoxicology and Environmental Safety 163 (2018) 274–278

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Sensitive detection of *Campylobacter jejuni* using one-step strategy based on functional nanospheres of immunomagnetic capture and quantum dots

Kuo He[a], Xiuyuan Zhang[a,b,*], Lin Chen[c], Ruiping Zhao[a], Lixia Wang[a]

[a] Food Safety Centre, Hebei North University, Zhangjiakou 075000, China
[b] College of Animal Science, Inner Mongolia Agricultural University, Hohhot 010018, China
[c] College of Food Engineering, Henan University of Animal Husbandry and Economy, Zhenzhou, 450046, China

ARTICLE INFO

Keywords:
Campylobacter jejuni
Immunofluorescent nanospheres
Immunomagnetic nanospheres
One-step strategy

ABSTRACT

Campylobacter jejuni has emerged as the most common bacterial foodborne illness in the developed world. Here, we demonstrate a convenient one-step strategy for detecting *C. jejuni*. Immunomagnetic nanospheres (IMNS) and immunofluorescent nanospheres (IFNS, quantum dots) were used for the simultaneous, sensitive capture and recognition of *C. jejuni*. After magnetic separation with the IMNS, detection of *C. jejuni* was achieved with fluorescence measurement of the IFNS in the sandwich complexes (IMNS–bacteria–IFNS). The limit of detection of this assay was 10^3 CFU/mL, and the linear range was from 10^5 to 10^7 CFU/mL (R^2 = 0.9994). When compared with a conventional two-step detection strategy, in which *C. jejuni* was first captured with the IMNS and then detected using the IFNS, this one-step detection strategy enhance sensitivity and save time. This suggested that the developed method has the potential for use as an alternative to the standard method for food quality assurance, as it provides rapid detection of *C. jejuni* in foodstuffs and the environment.

1. Introduction

Campylobacter jejuni is recognized as the major cause of human acute bacterial foodborne gastroenteritis around the world (Suzuki and Yamamoto, 2009). In the European Union, the reported incidence of *C. jejuni* cases is estimated to be more than 2.5 million per year (Bardon et al., 2009; Dasti et al., 2010). The spread of this disease has significantly increased in the last 10 years and contributes substantially to the economic and public health burden (Janssen et al., 2008). The establishment of a rapid and sensitive detection method is essential for preventing and controlling potential infections with this bacteria. However, conventional culture techniques and immunosorbent assays (ELISAs) for bacterial detection are time-consuming and labor-intensive, and polymerase chain reaction (PCR) analyses often require intensive and careful sample prepurification and skilled technical staff (Che et al., 2001; Chen et al., 2003; Klena et al., 2004).

In recent years, various nanomaterials have been applied towards the detection of biological molecules. These include quantum dots (QDs), magnetic nanoparticles (MNP), silver nanoshells, carbon nanotubes, and gold nanorods, all of which show superior sensitivity and/or simplify the detection process (Chen et al., 2011; Liu et al., 2007; Singh et al., 2017; Vikesland and Wigginton, 2010). Magnetic nanoparticles can easily be controlled by applying an external magnetic field. They offer significant potential for numerous biomedical applications such as cell separation (Yu et al., 2001), automated DNA extraction (Patolsky et al., 2003), gene targeting (Morales-Rayas et al., 2008), magnetic resonance imaging (Patolsky et al., 2003), and immunoassays. In contrast, magnetic nanoparticles can easily be coupled with other detection methods such as PCR (Ridley et al., 2008), fluorescence observation (Sun et al., 2004), and electrochemical detection (Bamrungsap et al., 2012). However, the detection strategies mentioned above usually consist of two or more steps, which makes the manipulations tedious. Moreover, multiple steps can lead to increased interference and target loss, both of which can affect the reliability of detection.

Immunoassays are frequently used to detect pathogens based on antibody-pathogen binding. In this study, quantum dots and magnetic nanospheres were conjugated with antibodies to construct immunofluorescent nanospheres (IFNS) and immunomagnetic nanospheres (IMNS), respectively. Using these nanospheres, a convenient detection strategy was devised to detect *C. jejuni* using simultaneous immunomagnetic capture and quantum dot-based detection in a single step. The IMNS possessed excellent separation and enrichment properties for *C. jejuni*, and the IFNS encapsulated hundreds of QDs into single nanospheres for sensitive detection. This one-step procedure could be completed within one hour, with a limit of detection of 10^3 CFU/mL. Compared with the two-step detection strategy, which

* Corresponding author.
E-mail address: zhangxiuyuan917@163.com (X. Zhang).

https://doi.org/10.1016/j.ecoenv.2018.07.050
Received 5 December 2017; Received in revised form 4 July 2018; Accepted 12 July 2018

included IMNS capture and subsequent detection of the IFNS, the one-step strategy not only improved the sensitivity but also reduced interference, which indicate its significant potential for practical application.

2. Materials and methods

2.1. Bacterial strains, culture conditions, and reagents

C. jejuni strain NC002163 was purchased from the National Institutes for Food and Drug Control (Beijing, China). The *C. jejuni* strain was grown at 37 °C on Columbia agar under microaerobic conditions, and the number of colony-forming units per milliliter (CFU/mL) was determined by counting the colonies grown on the plates. The recombinant plasmid PEB1-pET-28a was previously established in our laboratory (briefly, the PEB1 genes were amplified and the PCR products and the pET-28a vector were digested with *BamHI* and *XhoI*; after ligation, the recombinants were transformed into *E. coli* BL21). Nano-γ-Fe_2O_3 and CdSe/ZnS QDs were purchased from Suzhou Xingshuo Nanotech Co., Ltd (Suzhou, China). Bovine serum albumin (BSA), 1-(3-dimethylaminopropyl) − 3-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), isopropyl-β-D-thiogalactopyranoside (IPTG), and all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). All reagents were of analytical quality.

2.2. Expression of the PEB1 protein

The recombinant plasmid (PEB1-pET-28a) was extracted and transformed into *E. coli* BL21 competent cells. Positive colonies were selected and cultured in LB (Luria-Bertani) medium supplemented with 2 mM IPTG when the OD_{600} reached 0.8–1.0 to induce the production of the PEB1 protein. The cultures were further incubated with shaking at 180 rpm for another 6 h. The cultures were then centrifuged at 5000 rpm for 5 min and the supernatant was discarded. The bacterial cell pellets were resuspended in Tris-buffered saline (TBS; 0.01 M, pH 7.4) and subsequently sonicated at 300 W, with a 3 s pulse and a 1 s pause for 99 cycles while bathed in ice-cold water. The cell debris was removed using centrifugation at 12,000 rpm for 10 min at 4 °C. The supernatant was collected for purification of the target protein (He et al., 2016a, 2016b).

2.3. Preparation of anti-C. jejuni polyclonal antibody

Two New Zealand male rabbits (2–2.5 kg) were immunized subcutaneously at multiple sites (12–15 sites) on the back with a 1:1 mixture (v/v, 1 mL) of 1.5 mg PEB1 protein in PBS (0.01 M, pH 7.4) and Freund's complete adjuvant. Subsequent injections with the same mixture were given at 2-week intervals. The booster immunizations were given every 3 weeks. Rabbits were bled using the ear vein at 1 week after each injection. From these test bleeds, serum was collected and the antibody titer was determined by indirect ELISA (n = 3), and PBS was applied as the control. After the immunizations were complete, the rabbits were euthanized with ether to collect blood samples, which were left to coagulate for 1 h at room temperature and then overnight at 4 °C. The blood samples were then centrifuged at 12,000 rpm for 10 min to obtain the antiserum. The antiserum was purified with Protein A montage spin columns to produce the mAb (polyclonal antibody) (Xu et al., 2009, 2011).The purified pAb was used for colony immunoblotting to test its reactivity towards *C. jejuni* and other common foodborne pathogens (PBS was applied as the control), using the procedure previously reported (Yang et al., 2016).

2.4. Fabrication of IMNS and IFNS

Approximately 5 mg each of the MNS or QDs were activated in 100 mM EDC and 100 mM NHS in l mL PBS (0.01 M, pH 6.8) at 25 °C by gentle shaking. After incubation for 30 min, the MNS were magnetically separated and the QDs were centrifuged and rinsed three times with PBS (0.01 M, pH 7.2). Then, the QDs and MNS were respectively dispersed in 1 mL PBS (0.01 M, pH 7.2) to react with 2.5 mg of NH_2–PEG–CM for 5 h at room temperature with gentle shaking (Xie et al., 2012). Afterward, the resultant QD–PEG–COOH and MNS–PEG–COOH were rinsed with PBS (0.01 M, pH 7.2) five times to remove unreacted reagents. Then, QD–PEG–COOH and MNS–PEG–-COOH were conjugated with anti-*C. jejuni* polyclonal antibody (pAb), and the same activation method described above was followed except that NH_2–PEG–CM was replaced by the antibodies (Xie et al., 2007).

2.5. One-step sandwich strategy for C. jejuni detection

The IFNS and IMNS were synchronously added to 1 mL bacterial sample solution (bacteria were suspended in a 0.1% skim milk (w/v) solution containing 0.05% Tween 20% and 0.9% NaCl), and the same solution without bacteria was applied as the control. The mixtures were incubated at 37 °C for 1 h with continuous shaking. Then, the sandwich immunocomplexes were magnetically separated with a magnetic scaffold and washed with the 0.1% skim milk, 0.05% Tween 20%, and 0.9% NaCl solution four times and once with 0.9% NaCl. Finally, sandwich immunocomplexes were resuspended in 0.9% NaCl (400 μL) for fluorescence spectrum measurements using a fluorescence spectrometer. To develop a visual fluorescence detection method, the sandwich immunocomplex solutions were filtered with filter membranes, and the filter membranes were then placed under ultraviolet light. The minimum bacterial concentration that produced a visible difference in fluorescence compared with the blank was defined as the limit of detection. Serial dilutions of bacterial samples were used to determine the linearity of detection.

2.6. Two-step sandwich strategy for C. jejuni detection

For comparison with the developed one-step sandwich method for *C. jejuni* detection, a conventional, two-step procedure was performed. First, the IMNS were added to 1 mL bacterial solution and incubated for 1 h to capture the bacteria. Second, the IMNS–bacterial complexes were magnetically separated with a magnetic scaffold and the complexes were then resuspended in 0.1% skim milk–0.05% Tween 20–0.9% NaCl. Third, the IFNS were added to the IMNS–bacterial complex solution followed by incubation for another 1 h. Finally, the sandwich complexes were rinsed by magnetic separation and detected using a fluorescence spectrometer. To allow direct comparison, the detection conditions were the same as for the one-step method.

2.7. Detection of C. jejuni in spiked samples

Water, beverages, and milk (from a local supermarket, Hebei province, China) were chosen for the spiking study. Beforehand, the samples were tested with the plating method to validate that they were not contaminated by *C. jejuni*. Afterward, the bacterial suspensions were mixed (9:1 by volume) with the water, beverage, and milk samples. IMNS and IFNS were synchronously added to the samples to detect *C. jejuni* (the control experiments contained no bacteria).

3. Results and discussion

3.1. Fusion expression and purification of PEB1 protein

To obtain pure PEB1 for immunization, the constructed recombinant PEB1-pET-28a plasmid was used for the expression of this periplasmic protein. The molecular mass of the recombinant PEB1 was ~ 30 kDa as determined by SDS-PAGE (Fig. 1A), which agreed with the expected size. The purity of the fusion PEB1 protein was satisfactory, as no other bands were visible (Fig. 1B).