International Journal of Food Microbiology 179 (2014) 33–37

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Short communication

Automated immunomagnetic separation for the detection of *Escherichia coli* O157:H7 from spinach

Jing Chen [a,b,1], Xianming Shi [a,*], Andrew G. Gehring [b], George C. Paoli [b,**]

[a] MOST–USDA Joint Research Center for Food Safety, Department of Food Science and Technology & Bor Luh Food Safety Center, Shanghai Jiao Tong University, 800 Dongchuan Rd., Shanghai 200240, China

[b] USDA–MOST Joint Research Center for Food Safety, Molecular Characterization of Foodborne Pathogens Research Unit, United States Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center (USDA-ARS-ERRC), 600 E. Mermaid Lane, Wyndmoor, PA 19038, United States

ARTICLE INFO

Article history:
Received 30 September 2013
Received in revised form 17 March 2014
Accepted 18 March 2014
Available online 28 March 2014

Keywords:
E. coli O157:H7
Immunomagnetic separation
Pathogen detection

ABSTRACT

Escherichia coli O157:H7 is a major cause of foodborne illness and methods for rapid and sensitive detection of this deadly pathogen are needed to protect consumers. The use of immunomagnetic separation (IMS) for capturing and detecting foodborne pathogens has gained popularity, partially due to the introduction of automated and high throughput IMS instrumentation. Three methods for automated IMS that test different sample volumes, Kingfisher® mL, Pathatrix® Auto, and Pathatrix® Ultra, were compared using microbiological detection of *E. coli* O157:H7 from buffered peptone water (BPW), in the presence of background microbial flora derived from spinach leaves, and from culture enrichments from artificially contaminated spinach leaves. The average efficiencies of capture of *E. coli* O157:H7 using the three methods were 32.1%, 3.7%, and 1.3%, respectively, in BPW; 43.4%, 8.8%, 2.9%, respectively, in the presence of spinach microbial flora; and 63.0%, 7.0%, and 6.3%, respectively, from artificially contaminated spinach. Despite the large differences in IMS capture efficiencies between the KingFisher® and two Pathatrix® methods, all three methods allowed the detection of *E. coli* O157:H7 from spinach that was artificially contaminated with the pathogen at relatively high (25 cfu/30 g sample) and low (1 cfu/30 g sample) levels after 4–6 h of culture enrichment. The differences in capture efficiency were compensated for by the differences in sample volume used by the KingFisher® mL (1 mL), Pathatrix® Auto (50 mL) and Pathatrix® Ultra (250 mL) instruments. Thus, despite the reduced capture efficiencies observed for the Pathatrix® methods, the large increase in sample volume results in a greater number of captured cells for downstream detection resulting in improved detection sensitivity.

Published by Elsevier B.V.

1. Introduction

Escherichia coli O157:H7 is a deadly foodborne pathogen originally and most often associated with the consumption of undercooked ground beef, but more recently other foods, including produce, have been reported to be a source of *E. coli* O157:H7 outbreaks (Berger et al., 2010; CDC, 2006; CDC, 2008; Hilborn et al., 1999; Rangel et al., 2005). Continued development of more rapid and reliable methods for detection of this pathogen is necessary to protect consumers. Due to its low infectious dose [as low as 10 organisms; (Feng, 2012)], sensitivity is also critical for detection of *E. coli* O157:H7 in foods. The culture enrichment of samples prior to a detection is inevitable because the contamination level often falls below the detection limit of most available rapid methods (approximately 10^2–10^3 cells/mL or g of food) (Ge and Meng, 2009). In addition, food debris and non-target organisms may interfere with subsequent detection (Stevens & Jaykus, 2004). Therefore, it is highly desirable for the sampling procedure to address both issues of increasing the density of the target pathogen and reducing or eliminating factors that inhibit detection.

Despite the widespread use and apparent advantages of IMS in isolation of pathogenic bacteria and viruses (Hsih & Tsen, 2001; Kapperud & Vardund, 1995; Tian, Yang, & Mandrell, 2011), the target concentration effect of most IMS protocols is limited by the fact that only a small volume of sample is used (e.g., 1 mL) from a large volume of enrichment culture (e.g., 250 mL). In 2002, Matrix Microscience (Golden, CO; currently Life Technologies, Grand Island, NY) introduced a novel flow-through IMS system, Pathatrix®, which extended the sampling capacity from 1 mL to 250 mL (i.e., 25 g or mL of food sample and 225 mL buffer). This semi-automated system recirculates the entire sample over specific immunomagnetic beads (IMBs) held on the capture phase for a total of 30 min, and the washing and elution steps are integrated in the system through the use of diverter valves (Prentice et al., 2006), and protocols have been developed for isolation of a wide range of pathogens using this system (Himathongkham, et al., 2007;

* Corresponding author. Tel./fax: +86 21 34206616.
** Corresponding author. Tel.: +1 215 233 6671; fax: +1 215 836 3742.
E-mail addresses: xmshi@sjtu.edu.cn (X. Shi), george.paoli@ars.usda.gov (G.C. Paoli).
[1] Current Address: Department of Food Science and Technology, The University of Georgia, 100 Cedar St., Athens, GA 30602, United States

http://dx.doi.org/10.1016/j.ijfoodmicro.2014.03.022
0168-1605/Published by Elsevier B.V.

Jean-Gilles Beaubrun, et al., 2012; Morales-Rayas, Wolffs, & Griffiths, 2008; Isonhood, Drake, & Jaykus, 2006; Mullane, et al., 2006; Mattison, et al., 2009). In 2009 the third generation Pathatrix® system, Pathatrix® Auto, was released, which carries out an automated room temperature target capture with the sample volume and recirculation time reduced to 50 mL and 15 min.

Despite the increasing application of the large-volume recirculating IMS in foodborne pathogen detection, very few studies comparing recirculating IMS and conventional low volume automated IMS have been reported (Weagant et al., 2012; Yoshitomi et al., 2012). Therefore the purpose of this study was to evaluate and compare the two aforementioned IMS strategies, including three different protocols (Kingfisher®–Dynabeads®, Pathatrix® Ultra and Pathatrix® Auto), with respect to the limit of detection, capture efficiency, test sensitivity, and the capability of detection at low contamination levels.

2. Materials and methods

2.1. Preparation of inoculum

Twenty-five milliliters of tryptic soy broth [TSB; Becton Dickinson (BD), NJ, USA] was inoculated with *E. coli* O157:H7 (ATCC 43889) and incubated overnight at 37 °C, with shaking at 250 rpm. One milliliter of the overnight culture was harvested by centrifugation at 5000 rpm for 5 min. Cell pellets were resuspended in 1 mL of phosphate buffered saline (PBS, pH 7.0) and ten-fold serially diluted in PBS.

The cell density of the inocula was enumerated by counting cells in a Petroff-Hausser Chamber (Hausser Scientific, PA, USA) and the number of colony forming units (cfu) was confirmed by the 6 × 6 drop plating (Chen et al., 2003) on Luria-Bertani (LB; BD, NJ, USA) agar plates. For the artificial contamination experiments, inoculum size was also confirmed by the most probable number (MPN) procedure (USDA-FSIS, 2008), and at low inoculum levels, fractional positive estimates were obtained using a Poisson distribution calculation based on the IMS results (Denyer and Baird, 2006):

$$\text{Fractional positive estimate} = 2.303\,\lg(N_0/N_n),$$

where N_0 is the total number of samples, and N_n is the number of negative samples.

2.2. Limit of detection and capture efficiency

2.2.1. In buffered peptone water

One hundred microliters of PBS containing approximately 0, 10^0, 10^1, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, and 10^8 *E. coli* O157:H7 cells was thoroughly mixed with 276 mL of buffered peptone water (BPW; Oxoid, UK) in Stomacher bags (Seward, UK) to obtain final densities of 10^{-1}–10^6 *E. coli* cells/mL BPW. One, 50, and 225 mL from each bag were then subjected to Kingfisher® (Thermo Fisher Scientific, Hudson, NH, USA) automated IMB capture using anti-*E. coli* O157 Dynabeads® (Life Technologies – Invitrogen, NY, USA), Pathatrix® Auto (Life Technologies Corporation), and Pathatrix® Ultra IMS, respectively, following the protocols described in Section 2.3.

After IMS, the IMB–target complexes were resuspended in 100 μL of PBS and 10-fold serially diluted. One hundred microliters of the appropriate dilutions was surface plated on CHROMagar™ O157 plates (BD, NJ, USA), which were incubated at 37 °C for 18–24 h to enumerate the final growth of captured *E. coli* O157 cells.

2.2.2. In the presence of a natural background microflora from spinach

Pre-packaged spinach purchased from a local grocery store was portioned into batches of 30.5 ± 0.5 g and mixed with 276 mL BPW in separate Stomacher bags. After incubation at 42 °C for 4 h without agitation, spinach leaves were removed from the bags and 50 μL of the culture was collected in order to enumerate the background flora using the 6 × 6 drop plating method (Chen et al., 2003) on LB agar. Then, serial dilutions of *E. coli* O157:H7 were spiked into the BPW containing the spinach flora. IMS using the Kingfisher®, Pathatrix® Auto and Ultra was performed immediately. After IMS, captured cells were enumerated using CHROMagar™ O157 plates. All experiments were done in triplicate.

2.3. IMS protocols

2.3.1. Kingfisher®–Dynabeads®

For each sample, a Kingfisher® mL strip was prepared as follows: Tube 1, 20 μL Dynabeads® anti-*E. coli* O157, and 1 mL of pre-enriched sample; Tube 2, 1 mL PBST (PBS + 0.05% Tween 20); Tube 3, 1 mL PBS; and Tube 4, 100 μL PBS.

The tube strips were placed in a Kingfisher® mL instrument. Automated IMS was then conducted as recommended by the manufacturer. Briefly, the bacterial cells in the pre-enriched sample were allowed to bind with the anti-*E. coli* O157 antibodies in Tube 1 with agitation. Sheathed magnetic rods were then inserted into the tube to collect and transfer the obtained IMB–bacteria complex to the next two tubes for washing in PBST and PBS, respectively. Finally, the washed IMB–bacteria complex was resuspended in PBS.

2.3.2. Pathatrix® Ultra (2nd generation Pathatrix®)

For each sample, 50 μL of anti-*E.coli* O157:H7 IMBs (Matrix MicroScience, CO, USA) was pre-blocked with 100 μL of 1% (*w*/*v*) skim milk solution and mixed at room temperature on an end-over-end rotator at 35 rpm for 1 h prior to the IMS. IMS by Pathatrix® Ultra was performed according to the manufacturer's instructions. Briefly, Stomacher bags containing approximately 224 mL of the sample enrichments were placed into the incubation pots. A 150-μL aliquot of pre-blocked IMBs was transferred to the Pathatrix® tubing and fixed onto a magnetized slanted surface in the capture vessel within 1 min of recirculation. After a 30-min recirculation at 37 °C, the IMB–target complexes were collected on the magnetic capture vessel and washed with 50 mL of PBST to remove the food particles and non-target microorganisms. Then the capture vessels were disconnected from the magnets, allowing the IMBs to be eluted into the collection tube. In the final step the collection tube was placed on a magnet rack to remove the remaining PBST and further concentrate the IMBs. The captured IMBs were resuspended in 100 μL of PBS.

2.3.3. Pathatrix® Auto (3rd generation Pathatrix®)

The samples were prepared for the Pathatrix® Auto by transferring 50 mL of the samples to the sample vessel, 35 mL of PBS to the elution vessel, and 50 μL of Pathatrix® anti-*E. coli* O157 IMBs into the spout on the sample vessel. After the capture phase kit was connected to the Pathatrix® Auto instrument, the vessels were placed into the cartridge, and the automated IMS was carried out using the manufacturer's program, which included a 15-min continuous capture cycle and a wash cycle with PBS. Like the Pathatrix® Ultra, capture of *E. coli* O157 in the Pathatrix® Auto took place on a slanted surface coated with the anti-*E. coli* O157 IMBs. At the end of program, the IMBs were eluted in 100 μL of PBS.

2.4. Artificial contamination of spinach

Pre-packaged spinach was portioned into batches of 30.5 ± 0.5 g. Samples were inoculated at two levels: high (10–50 cfu/30.5 g, 5 replicates) and low (1–5 cfu/30.5 g, 20 replicates). For each experiment, a blank control was prepared by adding 1 mL of PBS into a spinach sample. The controls and artificially contaminated samples were manually mixed in the Stomacher bags for 30 s, and stored at 4 °C overnight to allow for sufficient adherence of the *E. coli* cells onto spinach leaves. MPN tubes were prepared for the estimation of inoculum size and placed in the refrigerator with the samples.