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Dead-end ultrafiltration concentration and IMS/ATP-bioluminescence detection of *Escherichia coli* O157:H7 in recreational water and produce wash

Dawn M. Hunter, Stephaney D. Leskinen, Sonia Magaña, Sarah M. Schlemmer, Daniel V. Lim *

Department of Cell Biology, Microbiology, and Molecular Biology, University of South Florida, Tampa, FL 33620-7115, United States

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ABSTRACT

The purpose of this study was to develop a detection method for viable *E. coli* O157:H7 in fresh produce and recreational water. The method was evaluated using eight samples of produce wash and recreational water with or without spiked *E. coli* O157:H7 at $\leq 10^2$ CFU·ml^{-1} and concentrated using dead-end ultrafiltration (DEUF) to produce primary and secondary retentates. Fifty-four matrix replicates of undiluted secondary retentates or dilutions (1:2 or 1:10 in buffer) were evaluated using an IMS/ATP bioluminescence assay (IMS/ATP). Combining primary and secondary DEUF yielded a 2–4 $\log_{10}$ increase in *E. coli* O157:H7 concentrations in spiked samples and resulted in signal-to-noise ratios 2–219 fold higher than controls, depending on the sample type. DEUF increased the concentration of *E. coli* O157:H7 to within the detectable limits of IMS/ATP. The combined assay provided detection of viable *E. coli* O157:H7 in produce and recreational water. Accurate detection of microbial pathogens using DEUF and IMS/ATP could reduce disease outbreaks from contaminated water sources and food products.

1. Introduction

Accurate detection of pathogens, particularly *Escherichia coli* O157:H7, is a concern shared by food and water professionals. Early detection of contaminants would enhance public health action, since *E. coli* O157:H7 has a low infectious dose and is one of the leading causes of foodborne and waterborne gastroenteritis and related morbidities (Tuttle et al., 1999). The U.S. EPA, FDA, and USDA have established goals for developing methods to detect very low levels of microorganisms in large volumes or masses of food or water, such as one *Cryptosporidium* oocyst in 10 l water or one CFU in 25–325 g of food, making some form of enrichment and/or concentration necessary for current detection technologies (Kretzer et al., 2008; NACMCF, 2010; Todd, 2002; USEPA, 2006a). The most sensitive methods have limits of detection (LOD) of 10^3 CFU·ml^{-1}; antibody-based techniques have LODs from 10^4 to 10^5 CFU·ml^{-1} depending in large part on the quality of the antibody (Brovko et al., 2004). The standard methods for detection of *E. coli* O157:H7 in produce wash and recreational water require 24–48 h of enrichment in multiple types of media and reagents (Feng and Weagant, 2009; USEPA, 2006b,c). In addition, many molecular and antibody-based methods cannot differentiate between viable and non-viable cells, which can lead to inaccurate estimates of contamination and erroneous public health decisions.

Sensitive and specific detection of viable cells can be achieved by a combination of techniques. Concentrations of pathogens in both food and water are typically too low for detection without extensive enrichment; however, this problem can be overcome by effective sample concentration (Stevens and Jaykus, 2004). Dead-end ultrafiltration (DEUF) has been shown to effectively concentrate low levels of bacteria in drinking water and recreational water (Kearns et al., 2008; Leskinen and Lim, 2008; Leskinen et al., 2009). This concentration method was coupled with detection methods including an antibody-based biosensor (Kearns et al., 2008; Leskinen and Lim, 2008) and PCR (Leskinen et al., 2010). However, complex samples can pose problems for antibody-based assays including increased background and decreased binding efficacy of the target (Squirrell et al., 2002). Target capture using IMS prior to sample analysis can increase cell concentration and decrease particulate interference, enabling specific detection and reducing or eliminating interference from non-target bacteria (Sharpe, 2003; Stevens and Jaykus, 2004).

IMS has been coupled to a variety of detection methods, including PCR (Mull and Hill, 2009), flow cytometry (Seo et al., 1998), electrochemiluminescence (ECL) (Shelton and Karns, 2001; Yu and Bruno, 1996), and bioluminescence (Lee and Deininger, 2004). Bioluminescence assays are an effective way to differentiate live from dead cells and are more sensitive than traditional methods such as ELISA, but have customarily been used for presence-absence testing because they lack specificity (Brovko, 2007). Recent research has examined capture and detection of target cells using ATP-bioluminescence

* Corresponding author at: Department of Cell Biology, Microbiology, and Molecular Biology, University of South Florida, 4202 E. Fowler Ave., ISA 2015, Tampa, FL 33620-7115, United States. Tel.: +1 813 974 1618; fax: +1 813 905 9940.
E-mail address: lim@usf.edu (D.V. Lim).

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immunoassays, which are sensitive and selective for specific viable targets. These assays include the detection of viable *E. coli* O157:H7 in apple juice and ground beef homogenate (Hunter and Lim, 2010), and the detection of indicator organisms in wastewater (Bushon et al., 2009b). Additional efforts to detect bacteria in complex matrices using IMS/ATP assays have encountered varying levels of success (ASM, 2005; Lee et al., 2010; Tu et al., 2000).

Linking sample concentration with capture by immunomagnetic separation (IMS) and detection using ATP-bioluminescence was the focus of this study, with the objective of addressing the problems of low levels of target cells, sample complexity, and specific detection of live cells. Sample concentration reduces time required for pathogen detection by increasing the concentration of cells in a shorter period of time relative to enrichment, while IMS/ATP detection yields rapid, specific, and semi-quantitative results. The combined method reduces the overall time from sample collection to detection, thereby reducing the potential public health impact posed by pathogens like *E. coli* O157:H7.

2. Materials and methods

2.1. Antibodies

Lyophilized, affinity-purified, biotin-labeled goat polyclonal antibody to *E. coli* O157:H7 (KPL, Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) was used as the capture antibody in the IMS/ATP assay. The antibody was rehydrated by addition of a 50% glycerol solution, and immobilized on M280 Streptavidin Dynabeads (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Antibody-labeled beads were stored at 4 °C until use. The sensitivity and specificity of the KPL antibody have been evaluated previously (Hunter and Lim, 2010).

2.2. Bacteria

Escherichia coli O157:H7 (ATCC 35150) and *E. coli* O157:H7 (ATCC 43888) were used as test organisms in the IMS/ATP assay. Working stocks prepared from frozen cultures were grown for 18 h at 37 °C in tryptic soy broth (TSB; BD, Franklin Lakes, NJ) and were maintained at 4 °C for up to 30 days.

2.3. Sample collection and processing

2.3.1. Recreational water

Duplicate 10 l samples of recreational water were collected from Lake Behnke (University of South Florida, Tampa, FL) and from Lake Carroll (Greater Carrollwood, Tampa, FL) on separate dates in April 2010. Sample quantity and volume were selected for method evaluation, and are comparable to assays evaluating similar methods (Shelton and Karns, 2001). Grab samples (500 ml) were also collected from each site, as well as physical–chemical water quality parameters using a YSI Professional Plus (YSI Incorporated, Yellow Springs, OH) during sample collection. Concentration of recreational water was performed with the Recreational Dead-End Concentrator (Rec DEC) containing an ultrafilter with molecular weight cut-off of 30–50 kDa (Leskinen and Lim, 2008). Samples were evaluated and processed the day of collection for enterococci, *E. coli* and fecal coliforms as described in USEPA Methods 1600 (2006b), 1603 (2006c), and APHA method 9222D (2005b), respectively. Pre-spike samples were also plated on sorbital MacConkey agar with cefixime and tellurite (CT-SMAC) to determine the presence or absence of *E. coli* O157:H7.

One milliliter of *E. coli* O157:H7 ATCC 43888 at approximately 10^6 CFU·ml^{-1} was spiked into one of the duplicate 10 l samples immediately upon return to the laboratory following collection from each site, which resulted in final concentrations of 8.6×10^2 CFU·ml^{-1} (Lake Behnke) and 2.9×10^2 CFU·ml^{-1} (Lake Carroll). The spiked samples were mixed for 2 min to disperse the *E. coli* O157:H7 cells and then filtered using DEUF. Each filter was backflushed with 250 ml buffer (0.1 M sodium phosphate buffer with 0.01% sodium polyphosphate) to recover the concentrated sample (retentate). Forty milliliter aliquots of spiked and non-spiked retentates were secondarily concentrated using an InnovaPrep HSC-40 Hydrosol Concentrator (HSC-40; Drexel, MO) for use in the IMS/ATP assay. The aliquot volume was selected based on several criteria: run time, sample retention for additional analysis, and sample archival. Secondary retentate volumes were approximately 2 ml.

Viable counts for samples post-primary and secondary concentration and IMS were completed using 100 µl each of two consecutive dilutions on duplicate plates of eosin methylene blue agar (EMB) and CT-SMAC incubated for 18 h at 37 °C and 41 °C, respectively (BBL, Becton Dickinson, Sparks, MD). Plates with 30–300 CFU were used to calculate CFU·ml^{-1}.

2.3.2. Produce wash

Fresh heads of lettuce or bunches of spinach were purchased from a local wholesaler (Sanwa Growers, Inc., Tampa, FL) for same day use. Produce was washed according to the following protocol: a 1–5 kg produce sample was washed in 50 l dechlorinated tap water that was then poured through stacked stainless steel sieves of decreasing pore size. The flow-through volume was collected for concentration with an Automated Concentration System (ACS) previously described (Kearns et al., 2008) and recovered using the same buffer as the Rec DEC. Viable counts were performed to determine background and target organism levels. All produce lots were evaluated for *E. coli* O157:H7 as described in the U.S. FDA *Bacteriological Analytical Manual* (Feng and Weagant, 2009). Tap water was evaluated for total coliforms using APHA method 9222B (2005a). Produce was either not spiked or spiked with 5 ml of 2.2–2.3 CFU·ml^{-1} of *E. coli* O157:H7 ATCC 35150 in Dulbecco's phosphate-buffered saline (DPBS) directly on the leaves prior to being washed. Each retentate (250 ml) was stored at 4 °C for 5–9 days until further processing could be performed. Aliquots (40 ml) were secondarily concentrated using the HSC-40 to produce a final retentate volume of approximately 2 ml.

2.4. IMS/ATP assay

Secondary retentates (1 ml) were undiluted or diluted 1:2 or 1:10 in modified Buffered Peptone Water with pyruvate (mBPWp) for a total volume of 1 ml. Dilution of secondary retentates was performed to evaluate whether results of the IMS/ATP assay were improved by decreased viscosity and particulate matter. Twenty microliters of antibody-labeled beads were added to each sample, which were then incubated with shaking for 1 h at 41 °C. Beads were separated from the supernatant using a magnet, the supernatant was removed, and beads were washed three times with DPBS containing 0.05% Tween-20 then re-suspended in 1.2 ml (900 µl for the assay, 300 µl for plating) of Mueller Hinton II broth (MHII). Nine replicates of 100 µl per well of each suspension were added to Lumitrac 600 plates (ISC Bioexpress, Kaysville, UT), followed by addition of 100 µl/well of BacTiter Glo reagent (Promega, Madison, WI). MHII broth containing labeled beads only was used as a negative control to establish background. Contents of the plates were mixed briefly on an orbital shaker, incubated for 5 min at 25 °C, and read using a GloMax 96 microplate luminometer (Promega, Madison, WI) with no delay and 1 s integration.

Sample S:N were determined by subtracting the signal mean of the negative controls from the raw sample signal and dividing by the negative control standard deviation. The negative control mean and standard deviation were calculated from individual negative control luminescence signals across all assays for each sample type ($n=36$ for recreational water and for produce wash; or 6 background readings for each of 3 sample dilutions for 2 sample types). Sample S: