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Rapid and Sensitive Isolation and Identification of *Escherichia coli* O157:H7

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ABSTRACT

This study describes a rapid and sensitive method for isolation and detection of *Escherichia coli* O157 by combining immunomagnetic separation (IMS) with fluorescence immunoassay from enriched food and water samples. Rapid and sensitive immunoassays to identify presumptive positive samples are beneficial to food producers, especially for the fresh produce industry. Our goal is to improve immunoassay sensitivity using a more sensitive fluorescence detection platform.

E. coli O157:H7 bacterial cells in the water samples were first captured on the surface of the magnetic beads coated with specific monoclonal anti-O157 antibodies. Other bacteria and contaminants were removed by subsequent washes. The captured *E. coli* O157:H7 bacteria were recognized by a polyclonal anti-O157 detector antibody labeled with Cy5 fluorescent dye. The immunosandwich complex on the magnetic beads was dissociated and detected in aqueous phase using an ultra sensitive spectrofluorometer.

The capture efficiency of IMS was 100% for input *E. coli* O157:H7 concentrations of 10^2 cells, and 98.8% for 10^3 to 10^5 cells. Fluorescence immunoassay conditions have been optimized to reduce background noise and increase dissociation efficiency. 10-fold dilutions of *E. coli* O157:H7 ranging from 10 to 10^6 CFU/ml were analyzed by IMS in conjunction with fluorescence immunoassay. Based on the negative control, the threshold detection value was set at background plus 3 SD. As low as 10 CFU/ml was able to generate higher Cy5 fluorescence signal than the threshold. Thus, the detection limit for *E. coli* O157:H7 was less than 10 CFU/ml, with assay time about 3 h.

INTRODUCTION

E. coli O157:H7, the most common serotype of enterohemorrhagic *E. coli* (EHEC), is responsible for numerous food-borne and water-borne infections worldwide. Cultural methods take more than 48-h to complete multiple operations and still require further confirmation of strain identity and virulence. The objective of this study was to develop an integrated system for isolation and detection of *E. coli* O157:H7 from water samples. This system consists of two basic steps: (1) isolation and concentration of *E. coli* O157:H7 from water sample using immunoaffinity magnetic beads, and (2) detection of *E. coli* O157:H7 serotypes using immunomagnetic fluorescence assay and Creatv's Signalyte™-II spectrofluorometer. By combining immunocapture and fluorescence immuoassay, this new system was able to provide high sensitivity and specificity for rapid screening and detection of *E. coli* O157H7 in food and water samples.

MATERIALS & METHODS

ASSAY DEVELOPMENT

1. Preparation of immunoaffinty magnetic beads: Biotinylated anti-O157 monoclonal antibody (BioDesign) was immobilized on Dynal M280 beads through biotin/avidin interactions. The antibody-coated Dynal beads were blocked in PBS containing 2% BSA (PBSB) at room temperature for 1 h. The beads were resuspended in PBSB containing 0.1% NaN_3 and stored at 4°C until use.
2. Immunocapture of *E. coli* O157:H7: 1 ml of filter-concentrated water sample or pre-enriched cultural broth was used for the assay. 3 µl of immunoaffinity magnetic beads was added to the sample and incubated at room temperature for 1 h with rotation. The sample tube was placed on a magnetic separator MPC-1 (Invitrogen). Unbound bacteria and other contaminations were washed away.

Fluorescence detection: Bead-bound bacteria were recognized by Cy5-conjugated anti-*E. coli* O157 antibody (KPL). After removing unbound antibody, fluorescence signals from the antibody sandwich complex on the magnetic beads were dissociated and measured on the Signalyte™-II

SIGNALYTE™-II INSTRUMENTATION

Fig. 1. Creatv's Signalyte™-II spectrofluorometer and computer control.

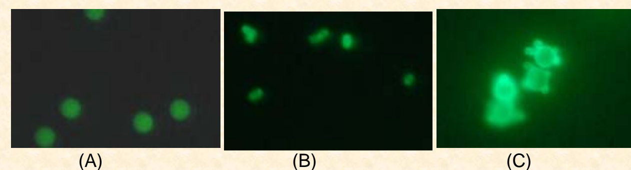
Fig. 2. Configuration of test capillary and LED illumination.

Creatv MicroTech, Inc. has developed an ultrasensitive spectrofluorometer (Fig. 1). Signalyte™-II combines light emitting diode (LED) illumination at 470 nm, 530 nm, 590 nm and 635 nm with spectrometer detection for 350-800 nm. Signalyte™-II is able to perform simultaneous tests of 8 samples plus a reference in Roche glass capillary tubes (Fig. 2).

RESULTS

IMMUNOCAPTURE Using MAGENTIC BEADS

The magnetic beads (2.8 µm in diameter, Fig. 3A) used in this study were larger than the *E. coli* O157:H7 bacteria (Fig. 3B). The beads were coated with biotin-conjugated monoclonal O157-specific antibody through biotin/avidin interactions. Fluorescence immunostaining showed that one magnetic bead could bind many *E. coli* O157:H7 cells (Fig. 3C).



(A) (B) (C)

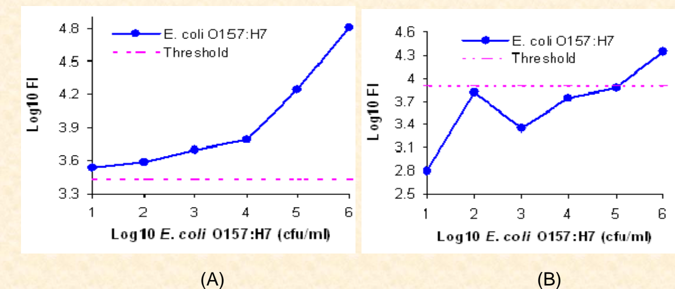
Fig. 3. Micrography of immunocapture of *E. coli* O157:H7 with immunoaffinity magnetic beads. The *E. coli* cells were stained with fluorescence-conjugated O157-specific polyclonal antibody and photographed by using a Olympus BX51W1 microscope with 100× water immersion objective. (A) Immunoaffinity magnetic beads; (B) *E. coli* O157:H7 bacteria; (C) Attachment of *E. coli* O157:H7 on the magnetic beads.

DETECTION SENSITIVITY FOR *E. COLI* O157:H7

Serial 10-fold dilutions of *E. coli* O157:H7 ranging from 1 to 10^6 cells per ml were tested by IMS and fluorescence immunoassay. At the last assay step, 40 µl of the supernatant sample, containing the dissociated-detector antibody, was transferred to a glass capillary tube (Roche) and its Cy5 fluorescence signal was measured using Signalyte™-II spectrofluorometer at excitation/emission wavelengths 635/670 nm. Based on the negative control (2,429±11), a threshold (average+3S.D.) was set 2,463 (Fig. 4A). The estimated limit of detection was less than 10 CFU/ml for *E. coli* O157:H7. The dynamic range of the detection system was over six magnitudes from 10 to 10^6 cells/ml (Fig. 4A).

The detection sensitivity was also compared between Creatv's Signalyte™-II and BMG microplate reader. 100 µl of the supernatant sample was transferred to the well of a 96-well microtiter plate (Nunc) for fluorescence measurement with BMG FLUOstar microplate reader. The results showed that the detection sensitivity of the plate reader (Fig. 4B) was much lower than that of Signalyte™-II.

To explore alternative fluorescence dye to Cy5, Dylight 649-conjugated polyclonal anti-O157 antibody (provided by KPL) was used as a detector in IMS-FIA. Serial dilutions of *E. coli* O157:H7 were tested and the same level of detection sensitivity (<10 cfu/ml) was obtained, suggesting that Dylight 649 is a suitable replacement for Cy5 dye in IMS-FIA method.



(A) (B)

Fig. 4. Fluorescence immunoassay for the detection of a 10-fold serial *E. coli* O157:H7 dilution. (A) Signalyte™-II; (B) BMG FLUOstar fluorometer. The y-axis indicates the log10 fluorescence intensity (FI), while x-axis indicates log10 concentration of *E. coli* O157:H7 (cfu/ml).

CONCLUSIONS

A simple fluorescence immunoassay has been developed for the detection of *E. coli* O157:H7 for food and water. This method is based on a combined use of immunomagnetic separation, fluorescence antibody sandwich assay and Creatv's Signalyte™-II spectrofluorometer, providing a specific detection of very low levels of bacterial cells in water samples. This method can also be used for screening of *E. coli* O157:H7 in the pre-enrichment samples. Furthermore, this method can be adapted to perform diagnostics of other microbial pathogens currently detected by using culture and ELISA methodology.

ACKNOWLEDGEMENT

This work is supported by SBIR grant 2007-02430 from the U.S. Department of Agriculture.

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