Research Note

Rapid Detection of Escherichia coli O157:H7 Inoculated in Ground Beef, Chicken Carcass, and Lettuce Samples with an Immunomagnetic Chemiluminescence Fiber-Optic Biosensor

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ABSTRACT

A biosensor was evaluated with regard to its usefulness in the rapid detection of Escherichia coli O157:H7 inoculated in ground beef, chicken carcass, and romaine lettuce samples. The biosensor consisted of a chemiluminescence reaction cell, a fiber-optic light guide, and a luminometer linked to a personal computer in conjunction with immunomagnetic separation. The samples inoculated with E. coli O157:H7 were first centrifuged and suspended in buffered peptone water and then incubated with anti–E. coli O157 antibody-coated magnetic beads and horseradish peroxidase (HRP)–labeled anti–E. coli O157 antibodies to form antibody-coated bead–bacterium–HRP-labeled antibody sandwich complexes. Finally, the sandwich complexes were separated from the samples in a magnetic field and reacted with luminol in the reaction cell. The number of E. coli O157:H7 cells was determined by collecting the HRP-catalyzed chemiluminescence signal from the bead surface through a fiber-optic light guide and measuring the signal with a luminometer. The chemiluminescence biosensor was specific for E. coli O157:H7 in samples containing other bacteria, including Salmonella Typhimurium, Campylobacter jejuni, and Listeria monocytogenes. The chemiluminescence signal was linear on a log scale from 10^2 to 10^3 CFU of E. coli O157:H7 per ml in samples. Detection could be completed within 1.5 h without any enrichment. The detection limits for ground beef, chicken carcass, and lettuce samples were 3.2 \times 10^2, 4.4 \times 10^2, and 5.5 \times 10^2 CFU of E. coli O157:H7 per ml, respectively.

According to the latest data from the Centers for Disease Control, foodborne diseases cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the United States each year (11), at an estimated cost of 7.7 to 8.4 billion dollars (3). Enterohemorrhagic Escherichia coli O157:H7 is one of the most dangerous pathogens, causing hemorrhagic diarrhea and hemolytic uremic syndrome with a very low infectious dose. It has been estimated that 73,480 cases of E. coli O157:H7–related illnesses occur each year (11), accounting for 3.1% of all foodborne disease outbreaks and 27.6% of all the deaths related to foodborne disease outbreaks in the United States (12). Conventional methods for the detection of E. coli O157:H7 are time-consuming (taking 2 to 3 days) and labor intensive, generally involving sequential preenrichment, selective enrichment, and selective plating steps and subsequent biochemical and serological confirmation (8).

In order to meet industrial and public requirements with regard to the rapid and accurate detection of small numbers of E. coli O157:H7 cells in food samples, many new methods have been investigated. The enzyme-linked immunosorbent assay (ELISA) technique has been widely studied and applied, but most ELISAs have high detection limits (ca. 10^5 CFU/ml for E. coli O157:H7 (2, 10)). The polymerase chain reaction (PCR) technique is sensitive and rapid compared with the traditional methods. However, the PCR method is a complex multistep assay with a relatively high cost, requires well-trained personnel, and takes longer than other rapid methods. A method involving immunomagnetic separation and PCR could detect 1 CFU/g of food in 10 h (7). A semiautomated fluorogenic PCR method had detection limits of 5.8 to 580 CFU and 1.2 to 1,200 CFU for beef and feces, respectively, and took 8 to 10 h to complete (18).

Biosensor technology has shown great potential for use in the development of rapid, sensitive, and portable systems. A flow-through immunofiltration assay system with porous filter membranes had a working range of 100 to 600 cells per ml (1). An amperometric biosensor based on a sol-gel thin-film electrode could detect enterotoxin at concentrations of up to 3 ppm (3.6 \times 10^{-8} M) (13). A fiber-optic evanescent wave biosensor was able to detect 3 to 30 CFU/ml in seeded ground beef samples after enrichment (5). A light-addressable potentiometric sensor could detect 7.1 \times 10^2 heat-killed bacterium cells per ml or 2.5 \times 10^4 live bacterium cells per ml (6, 19).

The immunomagnetic separation (IMS) technique has been successfully used to isolate bacteria, including E. coli (14, 16, 17, 19). The advantage of IMS lies in its ability to separate target bacteria directly from samples or from the
prenrichment medium with the application of a magnetic field without the need for filtration or centrifugation. The chemiluminescence detection method has been proven to be comparable to or better than radioactive methods (15) and much more sensitive than colorimetric and fluorescent techniques in various applications (4, 9). However, the high detection limit and nonspecific bonding of antibodies remain major problems in the application of chemiluminescence biosensors to food samples. IMS could be used with the chemiluminescence biosensor to overcome these problems. Therefore, the objectives of this study were to evaluate the usefulness of an IMS-coupled chemiluminescence biosensor for the detection of E. coli O157:H7 and to determine the detection range, assay time, and selectivity of the biosensor for ground beef, chicken carcass, and lettuce samples inoculated with E. coli O157:H7 and other bacteria.

MATERIALS AND METHODS

Bacterial culture. E. coli O157:H7 (ATCC 43888), Listeria monocytogenes (FDA 10143), and Salmonella Typhimurium (ATCC 14029) were grown in brain heart infusion broth (Difco, Detroit, Mich.) at 37°C for 18 to 20 h and then serially diluted to 10⁻⁸ with 1.0 × 10⁻² M phosphate-buffered saline (PBS) (pH 7.0). The 0.1-ml diluted solutions of E. coli O157:H7, L. monocytogenes, and Salmonella Typhimurium were plated on sorbitol MacConkey agar (Remel, Lenexa, Kans.), Oxford agar (Oxoid Ltd., Basingstoke, Hampshire, UK), and XLT4 agar (Remel), respectively, and incubated at 37°C for 24 h for enumeration of the bacteria. Campylobacter jejuni (ATCC 33291) was grown in a biphasic system in which brain heart infusion broth overlays brucella agar (Remel), and in a three-gas (5% O₂, 10% CO₂, and 85% N₂) incubator at 42°C for 18 to 20 h. The 0.1-ml diluted solutions were plated on Campy-Cefex agar and incubated in the three-gas incubator at 42°C for 48 h for the enumeration of C. jejuni cells.

Preparation of samples. Ground beef was purchased from a local grocery store, and 25-g samples were weighed. Each of the samples was mixed with 225 ml of 0.1% buffered peptone water (Difco) in a Whirl-Pak plastic bag (Nasco, Fort Atkinson, Wis.) and then stomached in a stomacher (Model 400, Seward, UK). The fluid was separated from the solid ground beef with a syringe filter (25-μm pore size; MSI, Westboro, Mass.). Romaine lettuce was obtained from a local grocery store, and 25-g samples were weighed. Each of the samples was mixed with 225 ml of 0.1% buffered peptone water in a Whirl-Pak plastic bag and stomached in a stomacher. Then the sample solution was collected from the bag. Twenty-four chicken carcasses were obtained from a commercial poultry-processing plant. Each carcass was put in a Whirl-Pak plastic bag with 100 ml of 0.1% buffered peptone water and shaken in an automated shaking device for 2 min. Then all carcass wash water was collected and mixed together for use in the experiments. Nine milliliters of the three kinds of sample fluid was thoroughly mixed with 1 ml of diluted pure culture of E. coli O157:H7 in a tube with a Vortex mixer (Shelton Scientific, Shelton, Conn.). Finally, a series of samples inoculated with E. coli O157:H7 at concentrations ranging from 10⁴ to 10⁶ CFU/ml was obtained. The 1-ml inoculated sample was placed in a microcentrifuge tube and centrifuged at 13,000 rpm for 10 min. After the supernatant was removed, the pellet was resuspended with PBS solution (pH 7.4, 0.01 M) containing 0.1% Tween 20.

Bioensor and detection of E. coli O157:H7. The setup (Fig. 1) and procedure for the chemiluminescence biosensor system were described in a previous study (20). Briefly, 50 μl of Supersignal ELISA Pico chemiluminescence reagent, a mixture of enhanced luminol and stable H₂O₂ with a ratio of 1:1 (Pierce, Rockford, Ill.), was added to the 500-μl centrifuge tube with the immunocomplexes in the reaction cell to start the reaction. A fiber-optic light guide collected the light from the enzymatic reaction, a Lumi-Tec luminometer (Model A5-2021A, St. John Associates, Beltsville, Md.) measured the signal, and a laptop personal computer with a data acquisition unit recorded the peak output of the chemiluminescence signal for 1 min.

Assay parameters. In a previous study (2), the operation parameters, including magnetic bead amount, HRP-labeled antibody concentration, incubation time, and blocking agent, were optimized on the basis of pure culture samples to maximize the chemiluminescence signal and to minimize the operation cost of the biosensor. Hence, the optimized values of the parameters—10 μl of the magnetic beads, 20 μg of the HRP antibody conjugates per ml, an incubation time of 1 h, and 0.1% Tween 20 as the blocking agent—were used in all tests for the present study.

Evaluation of the biosensor with IMS. The detection limit of the biosensor was determined on the basis of the ratio of detectable signal over noise, which should be ≥3. The detection
range was defined as the range in which the chemiluminescent signal was proportional to the number of target bacterium cells. The specificity of the biosensor was checked with the use of three co-contamination pathogens, *Salmonella* Typhimurium, *L. monocytogenes*, and *C. jejuni*, which are commonly found in beef, chicken, and lettuce. Four samples were prepared for the specificity tests. Sample 1 did not contain any bacteria, sample 2 contained three co-contamination bacteria but no *E. coli* O157:H7, sample 3 contained only *E. coli* O157:H7, and sample 4 contained *E. coli* O157:H7 as well as three co-contamination pathogens.

**Statistical analysis.** The means and standard deviations of the chemiluminescence signals for all samples were calculated on the basis of the data from three replicates, and the difference among the means was analyzed by a *t* test (α = 0.05) with JMP statistical software (SAS Institute Inc., Cary, N.C.).

**RESULTS AND DISCUSSION**

**Detection of *E. coli* O157:H7 in ground beef samples.** The ground beef wash water without any bacteria was used in the control tests. Its signal for control samples was very low. The mean control signal was 2.60 mV, with a standard deviation of 0.76 mV. The composition of the ground beef wash water did not increase false signal or background from nonspecific binding much, because the signal for buffered peptone water was only 2.10 mV. The total aerobic microbe counts for the ground beef, chicken carcass, and lettuce samples ranged from $10^3$ to $10^5$ CFU/ml. The responses of the biosensor to different concentrations of *E. coli* O157:H7 are presented in Figure 2. The biosensor signal was linear on a log scale from $3.2 \times 10^2$ to $3.2 \times 10^5$ CFU of *E. coli* O157:H7 per ml. The standard deviations at different concentrations of *E. coli* O157:H7 were low, indicating good reproducibility for the biosensor. When the *E. coli* O157:H7 concentration was $<10^2$ CFU/ml, the signal was almost the same as that for the control.

Therefore, for ground beef samples, the detection limit was $3.2 \times 10^2$ CFU/ml.

As a test of its selectivity, the chemiluminescence biosensor was used to detect *E. coli* O157:H7 in the presence of three other bacteria, *Salmonella* Typhimurium, *C. jejuni*, and *L. monocytogenes*. In Figure 3, sample BF-1 consisted of ground beef wash water without any bacteria; sample BF-2 consisted of ground beef wash water inoculated with $3.6 \times 10^5$ CFU of *Salmonella* Typhimurium per ml, $2.4 \times 10^4$ CFU of *C. jejuni* per ml, and $1.3 \times 10^5$ CFU of *L. monocytogenes* per ml but no *E. coli* O157:H7; sample BF-3 contained $1.6 \times 10^4$ CFU of *E. coli* O157:H7 per ml only; and sample BF-4 contained $1.6 \times 10^4$ CFU of *E. coli* O157:H7 per ml, $3.6 \times 10^5$ CFU of *Salmonella* Typhimurium per ml, $2.4 \times 10^4$ CFU of *C. jejuni* per ml, and $1.3 \times 10^5$ CFU of *L. monocytogenes* per ml. As shown in Figure 3, the signal for sample BF-1 was the same as that for sample BF-2. The presence of *Salmonella* Typhimurium, *C. jejuni*, and *L. monocytogenes* did not increase false signal from nonspecific binding. The signal for sample BF-4 was almost the same as that for sample BF-3, but the signals for these two samples were much higher than those for samples BF-1 and BF-2. There were no significant differences between the signals for the control (sample BF-1) and those for the samples with three other bacteria and no *E. coli* O157:H7 (sample BF-2) or between those for the samples with $1.6 \times 10^4$ CFU of *E. coli* O157:H7 per ml (sample BF-3) and those for the samples with three other bacteria and $1.6 \times 10^4$ CFU of *E. coli* O157:H7 per ml (sample BF-4) (*P > 0.05*). There was a significant difference between the signals for the samples with *E. coli* O157:H7 (samples BF-3 and BF-4) and those for the samples without *E. coli* O157:H7 (samples BF-1 and BF-2). These findings indicate that the presence of other bacteria had a minimal effect on the response of the biosensor.
Detection of *E. coli* O157:H7 in vegetable samples.

The vegetable sample without any bacteria was used in the control tests. The signal for control vegetable samples was slightly higher than that for control ground beef samples. The mean control signal was 3.34 mV, with a standard deviation of 0.44 mV. The responses of the biosensor to different concentrations of *E. coli* O157:H7 are shown in Figure 4. The biosensor signal was linear from $5.5 \times 10^2$ to $5.5 \times 10^5$ CFU of *E. coli* O157:H7 per ml on a log scale. The results for the vegetable samples were similar to those for the ground beef samples. The biosensor signal was shown to be highly reliable, and the results obtained with it were reproducible because the standard deviations at different concentrations of *E. coli* O157:H7 were low. When the *E. coli* O157:H7 concentration was $< 10^2$ CFU/ml, the signal seemed the same as that for the control test. Therefore, for vegetable samples, the detection limit was $5.5 \times 10^2$ CFU/ml.

As a test of its selectivity, the chemiluminescence biosensor was used to detect *E. coli* O157:H7 in vegetable samples in the presence of three other bacteria, *Salmonella Typhimurium*, *C. jejuni*, and *L. monocytogenes*. In Figure 5, sample ROM-1 consisted of vegetable wash water without any bacteria; sample ROM-2 consisted of vegetable wash water that was inoculated with $3.6 \times 10^5$ CFU of *Salmonella Typhimurium* per ml, $2.4 \times 10^4$ CFU of *C. jejuni* per ml, and $1.3 \times 10^5$ CFU of *L. monocytogenes* per ml but no *E. coli* O157:H7; sample ROM-3 was the sample inoculated with $2.8 \times 10^4$ CFU of *E. coli* O157:H7 per ml only; and sample ROM-4 contained $2.8 \times 10^4$ CFU of *E. coli* O157:H7 per ml, $3.6 \times 10^5$ CFU of *Salmonella Typhimurium* per ml, $2.4 \times 10^5$ CFU of *C. jejuni* per ml, and $1.3 \times 10^5$ CFU of *L. monocytogenes* per ml. As shown in Figure 5, the results for the vegetable samples were similar to those for the ground beef samples. There were no significant differences between the results for the control (sample ROM-1) and those for the sample with three other bacteria and no *E. coli* O157:H7 (sample ROM-2) or between the results for the sample with only *E. coli* O157:H7 (sample ROM-3) and those for the sample with three other bacteria and *E. coli* O157:H7 (sample ROM-4) ($P > 0.05$). There was a significant difference between the results for the samples with *E. coli* O157:H7 (samples ROM-3 and ROM-4) and those for the samples without *E. coli* O157:H7 (samples ROM-1 and ROM-2).

Detection of *E. coli* O157:H7 in chicken carcass samples.

The chicken carcass wash water without *E. coli* O157:H7 was used in the control tests. The signal for control chicken carcass samples was higher than those for control ground beef or control romaine lettuce samples. The mean control signal was 7.8 mV, with a standard deviation of 5.4 mV. The higher signal was ascribed to higher false signals due to non-specific binding resulting from the many proteins, fats, and bacteria in the chicken carcass wash water, which did not undergo filtration. With the plating method, more bacteria were detected in the chicken carcass samples than in the ground beef or the romaine lettuce samples, and the bacterium level was lowest in the ground beef samples, a finding that is consistent with the signals from the control tests. However, the signals for the control samples were lower than those for the samples with $>10^5$ CFU of *E. coli* O157:H7 per ml. The results obtained with the biosensor are presented in Figure 6. The biosensor signal was almost linear from $4.4 \times 10^2$ to $4.4 \times 10^5$ CFU of *E. coli* O157:H7 per ml on a log scale. The signal for $4.4 \times 10^2$ CFU/ml was slightly higher than that for $4.4 \times 10^1$ CFU/ml. For chicken carcass wash water samples, the detection limit was $4.4 \times 10^2$ CFU/ml.

As a test of its selectivity, the chemiluminescence bio-
sensor was used to detect \textit{E. coli} O157:H7 in chicken carcass samples in the presence of the three other bacteria, \textit{Salmonella} Typhimurium, \textit{C. jejuni}, and \textit{L. monocytogenes}. In Figure 7, sample CHK-1 consisted of chicken carcass wash water without any bacteria; sample CHK-2 contained $3.6 \times 10^5$ CFU of \textit{Salmonella} Typhimurium per ml, $2.4 \times 10^4$ CFU of \textit{C. jejuni} per ml, and $1.3 \times 10^5$ CFU of \textit{L. monocytogenes} per ml but no \textit{E. coli} O157:H7; sample CHK-3 consisted of chicken carcass wash water with $4.4 \times 10^4$ CFU of \textit{E. coli} O157:H7 per ml; and sample CHK-4 contained $4.4 \times 10^4$ CFU of \textit{E. coli} O157:H7 per ml, $3.6 \times 10^5$ CFU of \textit{Salmonella} Typhimurium per ml, $2.4 \times 10^4$ CFU of \textit{C. jejuni} per ml, and $1.3 \times 10^5$ CFU of \textit{L. monocytogenes} per ml. As shown in Figure 7, the results for the chicken carcass samples were similar to those for the ground beef and romaine lettuce samples. There were no significant differences between the results for the control (sample CHK-1) and those for the sample with three other bacteria and no \textit{E. coli} O157:H7 (sample CHK-2) or between the results for the sample with only \textit{E. coli} O157:H7 (sample CHK-3) and those for the sample with three other bacteria and \textit{E. coli} O157:H7 (sample CHK-4) ($P > 0.05$). There was a significant difference between the results for the samples with \textit{E. coli} O157:H7 (samples CHK-3 and CHK-4) and those for the samples without \textit{E. coli} O157:H7 (samples CHK-1 and CHK-2).

The chemiluminescence signals for the lettuce samples (Fig. 5) and the chicken carcass samples (Fig. 7) are 10-fold higher than that for the beef sample (Fig. 3). This finding might be attributable to the high concentrations of fat, tissue, and other organic materials in beef samples compared with those in lettuce and chicken carcass samples. The organic materials in the beef samples prevented exposure of the cells to the antibodies and/or resulted in more nonspecific binding of the antibodies.

The chemiluminescence biosensor in conjunction with IMS was successfully used to detect \textit{E. coli} O157:H7 in ground beef, chicken carcass, and vegetable samples. The biosensor showed strong selectivity and no interference from three other bacteria when it was used to detect \textit{E. coli} O157:H7 in the different types of samples. The 1.5-h detection time, the $10^2$-CFU/ml detection limit, and the $10^2$-to-$10^5$-CFU/ml working range were evaluated with these different types of samples without any enrichment. This biosensing method could detect \textit{E. coli} O157:H7 in food samples rapidly compared with ELISA and PCR methods (which take 4 to 7 h). The biosensor was more sensitive than ELISA ($10^4$ to $10^5$ CFU/ml) (2, 10), and its sensitivity was similar to that of PCR ($10^1$ to $10^2$ cells per ml) (7, 18). Its sensitivity level was at least as high as those of several reported sensors that do not require the enrichment of samples ($10^2$ to $10^4$ CFU/ml) (1, 6, 9). The results of this study indicate that the chemiluminescence biosensor with IMS has potential as a rapid, simple, and compact instrument for the detection of \textit{E. coli} O157:H7 in food samples.

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**REFERENCES**


