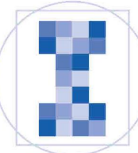


INNOVATIVE
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Application of a biosensor for rapid detection of *E. coli* O157:H7 contamination in ground beef

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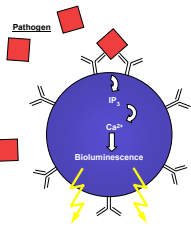
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Introduction

It is estimated that as many as 73,000 individuals are infected each year in the United States with *E. coli* O157:H7 as a result of eating contaminated food. Development of rapid, sensitive methods for detecting *E. coli* O157:H7 is of significant interest in the industry because it offers the opportunity to increase the efficiency of food testing and decrease risk to the public. We offer a novel biosensor-based system that allows for simple, sensitive, real-time detection of pathogens in a variety of food matrices. The sensor, based on the CANARY™ technology, allows for the detection of as few as 50cfu *E. coli* O157:H7 and requires only 5 minutes to perform. Our objective was to characterize the sensitivity of the CANARY™ assay in detecting *E. coli* O157:H7 in ground beef samples and to determine the specificity of the assay by examining the response of the biosensor to non-O157:H7 isolates.

CANARY™ technology consists of a cell line that is genetically engineered to recognize a specific pathogen, responding to its presence by emitting a luminescent signal that can be detected using a standard luminometer. The potential of this technology, which was developed over a period of six years at MIT (Rider et al., 2003), has been demonstrated by its application to the detection of 20 different viral and bacterial pathogens to date. In both simple and complex samples CANARY™ biosensor cells demonstrate sensitivity and specificity that rivals PCR in the detection of such pathogens as *E. coli* O157:H7 and *Bacillus anthracis*. Furthermore, this technology provides results in as little as 2 minutes and can be applied by individuals with a minimum of technical expertise, making it ideal for routine application in many settings.

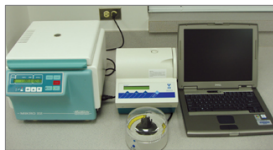
Construction of new biosensor cell lines is relatively simple and requires only that a monoclonal antibody be available for the target of interest, cDNAs encoding the light and heavy chains of the antibody are cloned into a vector that targets antibody to the cell surface. These vectors are transfected into a parental cell line expressing a bioluminescent protein, wherein antibodies are expressed and localized to the cell surface. Exposure of the biosensor cell line to its target pathogen triggers release of calcium from internal stores, thus activating the luminescent properties of the marker protein (see figure below).



Schematic of the CANARY™ Technology

BioFlash™ Instrumentation

Equipment required for detection of the CANARY™ response is off-the-shelf technology. A high-speed benchtop microfuge is used to concentrate pathogen at the bottom of a tube and the biosensor cells are brought into contact with the pathogen using a small minifuge with a horizontal rotor. A small single-tube luminometer records the response of the biosensor cells, and data is stored and analyzed on a laptop computer.



Instrumentation required for use with CANARY™ Technology

I. Experimental Methods

Measurement of pathogen in non-complex samples.

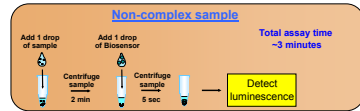
Enumerated *E. coli* were spiked in 250 µl assay buffer at various concentrations and samples were centrifuged for 2 minutes at 10,000 x g. Biosensor reagent was added to the tube containing the sample for testing and the sample was centrifuged for 5 seconds, then placed in a single-tube luminometer and luminescence measured for a total of 60 seconds.

Measurement of pathogen in ground beef.

Ground beef samples obtained from a commercial outlet were placed in sterile stomacher filter sample bags (25g of ground beef per bag) and inoculated with *E. coli* O157:H7. Samples were diluted in enrichment medium (225mL of mEC without novobiocin), agitated using a Stomacher device and incubated for various time at 37°C. After enrichment 3ml of sample was removed from the clean compartment of the filter bag, placed in a sterile tube and left undisturbed for 2-4 minutes to allow particulate material to settle. 1ml of sample was then transferred into 1.5ml centrifuge tubes and spun down at 10,000 x g for 2 minutes, then washed twice with PBS+0.05% Tween-20 and centrifuged again. The supernatant was removed and replaced with 250µL of assay buffer and samples were centrifuged again at 10,000 x g for 2 minutes and assayed using CANARY™ biosensor cells.

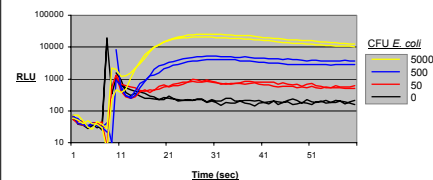
II. Procedure for detection of *E. coli* O157:H7 using CANARY™

CANARY™ provides a rapid method for detecting the presence of *E. coli* O157:H7 in complex and non-complex samples. In non-complex matrices the sample is centrifuged at 10,000 x g to pellet the pathogen. Biosensor is added and the sample centrifuged briefly to bring the biosensor in contact with the pathogen, initiating the luminescent response.



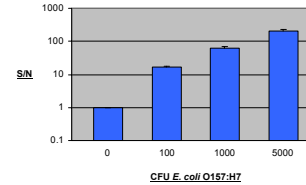
III. Reproducibility of the CANARY™ biosensor response

Various concentrations of *E. coli* O157:H7 were added to assay buffer and centrifuged at 10,000 x g to pellet the bacteria. Biosensor cells specific for *E. coli* O157:H7 were added and the sample was centrifuged for an additional 5 seconds, then placed in the luminometer to record the response.



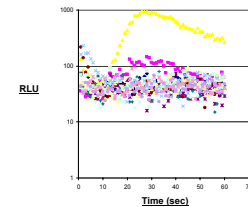
IV. Sensitivity of the CANARY™ *E. coli* O157:H7 biosensor

Various concentrations of *E. coli* O157:H7 were added to assay buffer and analyzed with CANARY™ biosensor as described in Experimental Methods. Results are expressed as signal/noise (S/N), a ratio of the luminescent signal (in RLU's) in the presence of bacteria to that in the presence of assay buffer alone.



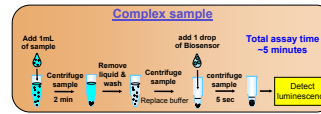
V. Specificity of the CANARY™ *E. coli* O157:H7 biosensor

Randomly selected non-O157 *E. coli* isolates were tested using the CANARY™ *E. coli* O157:H7 biosensor at densities of 10⁷cfu per reaction. The biosensor shows virtually no cross-reactivity with these strains.



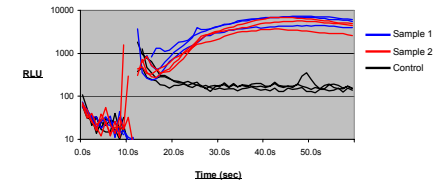
VI. Rapid processing of samples for detection of *E. coli* O157:H7 in ground beef

After enrichment (see Experimental Methods) 1ml of the sample is pelleted by centrifugation at 10,000 x g. The supernatant is then discarded and the sample washed twice with wash buffer. The sample is centrifuged again and the wash buffer replaced with a pH-neutral assay buffer. Biosensor is added and a brief centrifugation step brings the biosensor in contact with the sample, initiating the luminescent response.

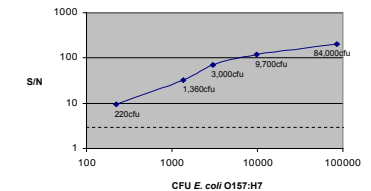


VII. Biosensor detection of *E. coli* O157:H7 in ground beef

Ground beef samples were spiked with 0.8cfu/g of *E. coli* O157:H7, followed by 6 hours enrichment as described in Experimental Methods. The samples were then processed for detection with the CANARY™ biosensor. Each sample was tested in triplicate and compared to an unspiked negative control. Results are expressed as Relative Light Units (RLU).



Ground beef samples (25g) inoculated with *E. coli* O157:H7 (0.8 cfu/g) were assayed using CANARY™ after various times of enrichment and the number of viable *E. coli* O157:H7 cells determined empirically. Results are expressed as a ratio of signal to noise for a given data point, with a value of 3 (dashed line) considered positive. Values shown are the average of at least 3 measurements.



Conclusions

Principal advantages of the CANARY™ technique include its speed and sensitivity. Even in complex samples such as ground beef, this technology enables rapid detection of pathogens. The results presented here demonstrate that CANARY™ has the capacity to detect as few as 50cfu *E. coli* O157:H7 in non-complex samples and 220cfu *E. coli* O157:H7 in ground beef samples in 5 minutes or less with a minimum of sample processing.

The CANARY™ assay shows sensitivity that is comparable to that of PCR (Cui et al., 2003; Ellingson et al., 2006; Li and Drake, 2003), with the capacity to detect low levels of *E. coli* O157:H7 contamination (<1 cfu/g) in ground beef samples after an enrichment time of 7h or less. The speed and simplicity with which CANARY™ can be performed is a significant advantage over the laborious and time-consuming steps that are required for PCR.

References

- Cui S., CM. Schroeder, D.Y. Zhang, and J. Meng. 2003. Rapid sample preparation method for PCR-based detection of *Escherichia coli* O157:H7 in ground beef. J Appl Microbiol. 95:129-134.
- Ellingson J.L., J.J. Koziczowski, J.L. Anderson, S.A. Carlson, and V.K. Sharma. 2005. Rapid PCR detection of enterohemorrhagic *Escherichia coli* (EHEC) in bovine food products and feces. Mol. Cell. Probes 19:213-217.
- Li W., and M.A. Drake. 2003. Detection of viable Shiga toxin-producing *Escherichia coli* by quantitative competitive polymerase chain reaction. J Food Prot. 66:1277-1282.
- Rider, T.H., M.S. Petrovick, F.E. Nargi, J.D. Harper, E.D. Schwoebel, R.H. Mathews, D.J. Blanchard, L.T. Bortolin, A.M. Young, J. Chen, and M.A. Hollis. 2003. A B cell-based sensor for rapid identification of pathogens. Science 301:213-215.



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