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Detection of *Listeria Monocytogenes* Using an Automated Fiber-optic Biosensor: RAPTOR

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Abstract. Fiber-optic biosensor uses light transmittable tapered fiber to send excitation laser light and receive emitted fluorescent light. The fluorescent light excited by an evanescent wave generated by the laser is quantitatively related to biomolecules immobilized on the fiber surface [1]. An automated fiber-optic biosensor based detection method for *Listeria monocytogenes* was developed in this research. Detections of *Listeria monocytogenes* in hotdog sample were performed to evaluate the method. By using the detection method with automated fiber-optic biosensor, 5.4×10^7 cfu/ml of *Listeria monocytogenes* was able to detect.

Introduction

Conventional methods for *Listeria* detection and identification involve prolonged multiple enrichment steps. Even though some immunological rapid assays are available, these assays still require enrichment steps and give results in 18 to 48 hours. Fiber-optic biosensors have shown great potential for rapid detection of foodborne pathogens. Portable types of fiber-optic biosensors (Analyte 2000, Research International, Monroe, WA, USA) have been used to detect various microorganisms including *Escherichia coli* O157:H7 [2], *Salmonella* typhimurium [3], and *L. monocytogenes* [4]. Improvements in the portability and automation of a fiber-optic biosensor (RAPTOR™, Research International, Monroe, WA, USA) increased the usefulness of this detection device. The RAPTOR can perform four assays on the same sample allowing replicate measurements of the same analyte or simultaneous detection of four different targets. The RAPTOR uses four 635 nm diodes to excite each of four, 4.5 cm long fiber-optic probes. The fibers are assembled in a coupon which has fluidic channels for automated operation. Fluorescent molecules bound on the surface of the sensing region are excited by an evenescent wave generated by the laser. Photodiodes collect emission light at wavelengths over 670 nm. The emission signal is recorded in picoamperes (pA) and related to concentration of analyte.

The purpose of this study was to develop an automated assay method for detecting *L. monocytogenes* using the RAPTOR system. A sandwich assay was devised and performed to evaluate the method.

Materials and Methods

Bacteria and regeants

Listeria monocytogenes strain V7, from our collection was used for in the experiments. The bacteria were maintained on brain heart infusion (BHI) agar (1.5%) slants (Difco Laboratories) at 25°C for the duration of this study. Fresh cultures of *Listeria monocytogenes* were prepared by incubating the slant

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cultures in BHI broth at 37°C for 16 hours. In some cases, bacteria were adjusted to approximately the same concentration by using a spectrophotometer (Beckman-Coulter, Fullerton, CA, USA).

For sample preparation, buffered *Listeria* enrichment broth (BLEB) and Oxford *Listeria* agar base were purchased from Acumedia (Baltimore, MD, USA). Modified oxford antimicrobic supplement was purchased from Becton, Dickinson (Sparks, MD, USA). Package of hotdogs was purchased from a local grocery store.

Purified anti-*Listeria* monoclonal antibody (C11E9) [5] and polyclonal antibody (LM Pab and P66) were provided by Dr. Arun Bhunia (Purdue University, IN, USA). Casein, bovine serum albumin (BSA), and phosphate buffer were purchased from Sigma (St. Louis, MO, USA). SuperBlock was purchased from Piece (Rockford, IL, USA). Antibody labeling and biotinylation was prepared according to the method described by Tims et. al [4].

Hotdog sample preparation

10 g of each hotdog sample was spiked by dropping 100 µl of 1×10^9 CFU/ml of the *Listeria monocytogenes* cell suspension onto the sample surface. The spiked hotdog samples were placed in a sterilized enrichment container filled with 30 ml of buffered *Listeria* enrichment broth (BLEB) media at 37°C for 20 hours for enrichment. The enriched cell suspensions were collected after filtration to remove any remaining food particles and used for the immunoassay. Enumeration of the enriched *L. monocytogenes* was performed using the modified oxford (MOX) plate method. After 20 hours incubation, the numbers of enriched *L. monocytogenes* cells were counted using MOX plate. The numbers of *L. monocytogenes* after 20 hr enrichment were $5.4 \pm 0.6 \times 10^7$ cfu/ml and $6.2 \pm 0.6 \times 10^8$ cfu/ml for 10 cfu/g and 100 cfu/g inoculation, respectively.

Fiber preparation and instrument setup

Fibers were prepared to have active binding sites for *L. monocytogenes* detection as shown in Fig. 1. A fiber was inserted into a 100 µl pipette tip and incubated overnight (18 to 22 hours) at 4 °C with 90 µl of 100 µg/ml streptavidin. Fibers were rinsed with PBS-Triton (0.02 M phosphate buffered saline (PBS) containing 0.05 % Triton X-100) and incubated with 90 µl of 100 µg/ml biotinylated capture antibody (PAb or P66) in PBS at room temperature for 1 hour. Finally, the fibers were incubated with 90 µl of SuperBlock followed by 90 µl of 1 mg/ml biotinylated bovine serum albumen (bBSA) at room temperature for 1 hour each. Prepared fibers were mounted into the disposable coupon. And then the assembled coupon was inserted into the RAPTOR and measurements were done automatically by running preprogrammed a baseline recipe for each baseline reading and an assay recipe for each sample reading (Fig. 2).

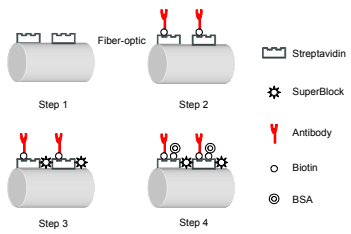

Fig. 1 Immobilization procedure for the fiber-optic biosensor.

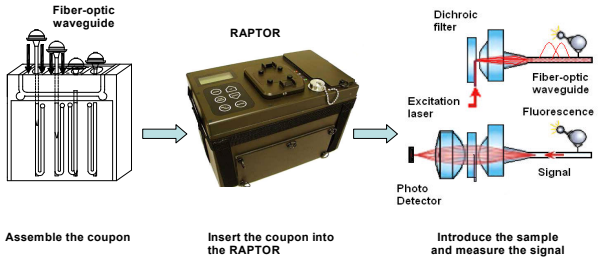

Fig. 2 Measurement procedure of the RAPTOR.

Blocking non-specific binding and assay procedure

To reduce non-specific binding, buffers were modified. The first buffer modification included the addition of BSA and casein into the detection antibody buffer. The second buffer modification included the addition of BSA and casein into the sample buffer prior to injection into the coupon. Both modified detection antibody buffer and modified sample buffer contained 2 mg/ml blocker BSA

and 2 mg/ml casein.

Four negative control signals were measured with blank samples which didn't contain *L. monocytogenes*. The negative control signals were used to calculate the detection limit. The limit of detection was designated as three times the standard deviation of the three control signals minus background signal. A change in signal above the last control signal for all samples tested was considered a positive result if the change was higher than the limit of detection. For each experiment, the standard deviation of the mean (SEM) signals from 4 fibers within a coupon was calculated. The error bars on each graph designate $\pm$ SEM.

With the same coupon, consecutive measurements were performed using serially diluted bacteria samples (10^2 to 10^9 cfu/ml) in PBS. To determine the performance difference between different capture antibody and their immobilization concentration, two *Listeria* polyclonal antibodies (LM PAb and P66 PAb) at two different concentrations (10 μg/ml and 20 μg/ml) were compared.

Results and Discussion

Using modified buffers with SuperBlock immobilization effectively reduced non-specific bindings. Adding blocking agents in the detection antibody buffer or sample buffer stabilized the background signal which had not contacted the bacteria yet. Bars in the figures show signal differences from previous assay (Fig. 3).

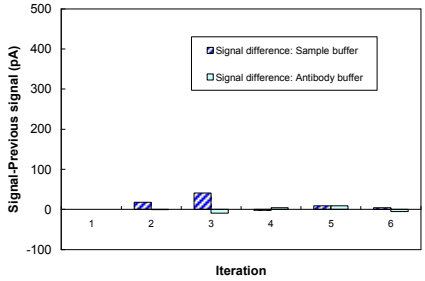

Fig. 3 Non-specific bindings of modified detection antibody buffer and sample buffer.

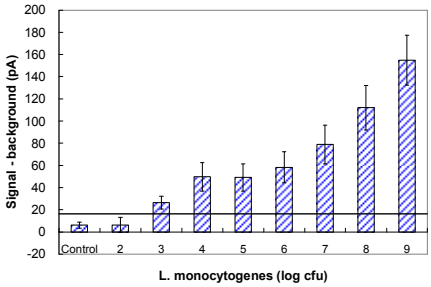

Fig. 4 Response of biosensor for different concentrations of *L. monocytogenes.*

The responses of biosensor to increasing concentrations of *L. monocytogenes* spiked into PBS by using 10 μg/ml of P66 are shown in Fig. 4. P66 PAb produced higher signal than LM PAb and using higher concentration of capture antibody at the fiber preparation step generally increased responses. Detection limits of the fibers were shown in Table 1.

Table 1 Detection limits of biosensors which have different captures immobilized on the surface.

Capture antibody	Concentration [μg/ml]	Detection limit [pA]	Lowest detectable cell numbers [cfu/ml]	Response at the lowest detectable cell numbers [pA]
LM Pab	10	62.2	10^8	85.6
	20	11.7	10^4	18.3
P66	10	10.1	10^3	26.4
	20	50.1	10^3	71.1

Even though the P66 capture antibody could detect 10^3 cfu/ml *L. monocytogenes* in PBS (Table 1), it couldn't detect the bacteria at that level in real food sample. There was no signal increase from *L. monocytogenes* presence in the food sample. A possible reason of this false negative detection of *L. monocytogenes* comes from interference of antigen-antibody bindings from complex entity of food. Other competitive microorganisms or the food ingredients could interfere with the signal measurements. Furthermore, the detection could be affected by stressful environment of food which might alter physiology and metabolism of the microorganisms thus interfering antibody binding to

bacteria. This complexity requires more sensitive and robust method to detect *L. monocytogenes* in food samples.

To increase the sensitivity of the biosensor, dye conjugated detection antibody was incubated with sample prior to injection into the sample port of the RAPTOR. Fig. 5 shows responses of *L. monocytogenes* spiked PBS samples which were incubated with 10 μg/ml of C11E9-Cy5 conjugation for 30 min at room temperature. With prior incubation of C11E9-Cy5 with sample, the biosensor could detect 1×10^8 cfu/ml of *L. monocytogenes* in PBS using P66 as the capture antibodies.

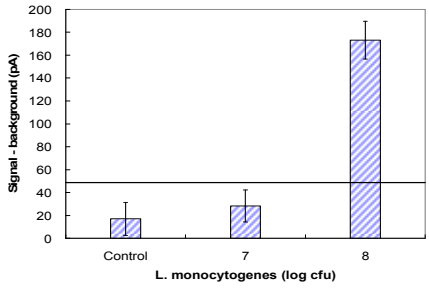

Fig. 5 Detection antibody was bound to antigen and then introduced to the coupon.

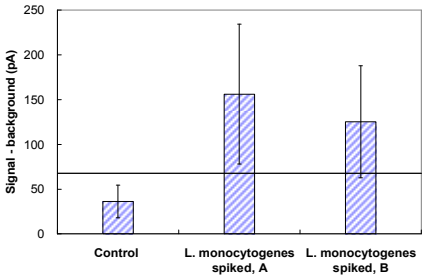

Fig. 6 Detection of *L. monocytogenes* (5.4×10^7 cfu/ml) in hotdog matrix with coupon A and B.

This method also worked for detecting *L. monocytogenes* in the hotdog sample (Fig. 6). This sensitivity increase by prior incubation of C11E9-Cy5 with antigen can be explained partly by principle of evanescent wave biosensor. Usually, the penetration depth of the evanescent wave reaches less than 300 nm [6]. Because the penetration depth of the evanescent wave covers a small fraction of the cells, average size is 1 to 2 μm, only the C11E9-Cy5 within the depth can be excited to produce the signal. Incubating the C11E9-Cy5 with antigen prior to the assay might increase the chance of binding the C11E9-Cy5 onto all surfaces of the cells.

Summary

In this research, methods for detecting *Listeria monocytogenes* in food samples were developed using an automated fiber-optic-based immunosensor: RAPTOR. Two detection methods were devised and evaluated to detect *Listeria monocytogenes* in PBS and hotdog samples. The sandwich method could detect 10^3 cfu/ml of *Listeria monocytogenes* in PBS. Incubation C11E9-Cy5 with antigen prior to the assay, which was devised to overcome low penetration depth of evanescent wave, could detect 5.4×10^7 cfu/ml of *Listeria monocytogenes* in hotdog samples.

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