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Detection of *Salmonella enteritidis* Using a Miniature Optical Surface Plasmon Resonance Biosensor

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**Abstract.** The frequent outbreaks of foodborne illness demand rapid detection of foodborne pathogens. Unfortunately, conventional methods for pathogen detection and identification are labor-intensive and take days to complete. Biosensors have shown great potential for the rapid detection of foodborne pathogens. Surface plasmon resonance (SPR) sensors have been widely adapted as an analysis tool for the study of various biological binding reactions. SPR biosensors could detect antibody-antigen bindings on the sensor surface by measuring either a resonance angle or refractive index value. In this study, the feasibility of a miniature SPR sensor (Spreeta, TI, USA) for detection of *Salmonella enteritidis* has been evaluated. Anti-*Salmonella* antibodies were immobilized on the gold sensor surface by using neutravidin. *Salmonella* could be detected by the Spreeta biosensor at concentrations down to $10^5$ cfu/ml.

1. Introduction

*Salmonella enteritidis* is one of the major foodborne pathogens of concern. It is a gram-negative rod-shaped bacterium that causes severe illness in the elderly, infants, and those with weak immune systems. A person infected with this pathogen shows symptoms of fever, abdominal pain, nausea and vomiting, diarrhea, dehydration, weakness, and loss of appetite. *Salmonella enteritidis* outbreaks continue to occur, and *Salmonella enteritidis*-related outbreaks from various food sources have increased public awareness of this pathogen.

Conventional methods for *Salmonella* detection and identification involve prolonged multiple enrichment steps. Optical biosensors have shown great potential for rapid detection of foodborne pathogens. They are capable of direct monitoring the antigen-antibody reactions in real time without requiring additional labeling reagents. Among the optical biosensors, surface plasmon resonance (SPR) sensors have been widely adapted as an analysis tool for the study of various biological binding reactions because of their high sensitivity and reagentless operation.

SPR sensors utilizes a thin, gold-coated transparent materials exposed to a laser or a polarized beam of light that produces surface plasmon resonance phenomenon. The SPR phenomenon is highly sensitive to changes in the thickness of attached analytes on the surface of the thin metal. A SPR biosensor that has a layer of biological active binding site on the surface of the metal could detect antibody-antigen bindings on the sensor surface by measuring either a resonance angle or a refractive index value.
SPR biosensors have been used to detect various biological agents including *Salmonella* group B, D, and E [1], *Listeria monocytogenes* [2], *Salmonella enteritidis* [2, 3], *Salmonella typhimurium* [3, 4, 5], *Escherichia coli* O157:H7 [6], and *Salmonella paratyphi* [7]. Although many SPR applications were studied on biological binding reactions, expansion of SPR application has been hindered by high-cost SPR systems designed for central laboratories. Arrival of low-cost portable SPR sensor (Spreeta, TI, USA) enabled field use and increased accessibility of the SPR system. The Spreeta sensor contains all the optical components necessary to measure refractive index changes related to SPR phenomena in the compact enclosure. The feasibility of applying the Spreeta sensor to detect various biological analytes including mouse IgG [8], *Staphylococcus aureus* enterotoxin B [9], and peanut allergens [10], and *Escherichia coli* enterotoxin [11] has been demonstrated.

The purpose of this paper was to evaluate the detection and quantification of *Salmonella enteritidis* in buffer as well as in food samples using the portable miniature SPR biosensor, Spreeta.

2. Materials and Methods

2.1. Bacteria and media
*Salmonella enterica* serotype *enteritidis* (*S. enteritidis*) ATCC 11076 was used for 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 *Salmonella enteritidis* were prepared by incubating the slant cultures in BHI broth at 37°C for 14 h. In some cases, bacteria were adjusted to approximately the same concentration by using a spectrophotometer (Beckman-Coulter, Fullerton, CA, USA). For sample preparation, selenite broth was purchased from Sigma-Aldrich (MO, USA). Package of fresh chicken (boneless chicken breast) was purchased from Sigma-Aldrich (MO, USA).

2.2. Reagents and antibodies
Purified rabbit anti-*Salmonella* polyclonal antibody was purchased from Fitzgerald Industries International (MA, USA). Bovine serum albumin (BSA) was purchased from Sigma (St. Louis, MO, USA). A long-chain biotin (EZ-Link NHS-LC-Biotin; Pierce, Rockford, IL, USA) was used for biotinylation of the polyclonal capture antibody according to the manufacturer’s instructions. One milligram of biotin was dissolved in 1 ml of dimethyl sulfoxide, and 75 μl of this solution was added to 1 mg of the antibody in 1 ml of carbonate-bicarbonate solution (5.7 g of NaHCO₃, 3.4 g of Na₂CO₃ in 1 liter of water, pH 9.3). The solution was then incubated in an ice bucket for 2 h. Free biotin was removed by column chromatography (PD-10; Amersham Biosciences, Piscataway, NJ, USA). Biotinylated antibodies were stored in phosphate-buffered saline (PBS) containing bovine serum albumin (1 mg/ml) at 4°C until used.

2.3. Sample preparation
Three sets of sample were prepared for the experiment. First sample set was prepared with whole *Salmonella* cells. The other two sets were prepared with fragments of the *Salmonella* cells extracted by 5 min sonification. The first two sets used 20 mM phosphate-buffered saline (PBS, pH 7.2) as a buffer. To test specificity and applicability on the real food samples of the biosensor, the last sample set used chicken extracts as a buffer. For the chicken sample preparation, 250 g of chicken fresh was mixed with 250 g of 20 mM phosphate-buffered saline (PBS, pH 7.2) as a buffer. The food sample was homogenized by stomacher for 2 min and filtered through ambelite filter to remove any remaining food particles. *Salmonella enteritidis* cells were inoculated in 5 ml of BHI broth and incubated at 37°C with shaking (150 rpm). After 14 h of culture, which was the period where cell numbers reached about 1 X 10⁸ CFU/ml, the cells were diluted to appropriate numbers with PBS. The PBS sample set was spiked by dropping 100 μl of the cell suspension into the sample buffer. Then the spiked samples were serially diluted from 10⁶ cfu/ml to 10⁴ cfu/ml to complete the sets. Enumeration of the *Salmonella enteritidis* was performed using the standard plate count (SPC) method.
2.4. Apparatus
The miniature SPR sensor, Spreeta, was purchased from Texas Instruments (Dallas, TX, USA) and the controller was purchased from Normadics (Stillwater, OK, USA). A 6 mm thick Teflon flow cell (volume 8 μl) was installed on the gold-coated Spreeta sensor surface to provide interface to outside fluidic components. A variable mini peristaltic-pump (VWR, West Chester, PA, USA) was connected to the inlet of the flow cell to provide constant flow of various solutions including immobilization reagents, capture antibody, blocking agents, and washing buffer. Figure 1 shows a schematic diagram of measurement setup for the Spreeta biosensor.

2.5. Immobilization of antibody
The exposed gold surface of a Spreeta sensor was cleaned by injecting PBS (pH 7.4) about 10 min until the sensor response became stable. The running buffers were maintained at 20 μl/min throughout the cleaning and the immobilization process. When the gold surface has been cleaned, 100 μl of biotinylated BSA (BBSA; 100 μg/ml in PBS) was injected onto the sensor surface for 5 min to create a physisorbed film of BBSA. After that, 100 μl of neutravidin (100 μg/ml in PBS) was injected to form a layer of neutravidin onto the BBSA film. Once the layer of neutravidin was created, 100 μl of biotinylated polyclonal Salmonella antibody (10 μg/ml in PBS) was injected to create a layer of Salmonella binding site. Finally, 100 μl of BSA (1 mg/ml in PBS) was introduced to block the non-specific binding sites on the sensor surface. Between each step, PBS was injected for 10 min to rinse the system.

2.6. Sample assays
With the functionalized Spreeta biosensor, consecutive measurements were performed using serially diluted bacteria samples in PBS or food extract. Each sample was analyzed by injecting 100 μl at 20 μl/min. The response of the Spreeta biosensor was represented by the refraction index at which surface plasmon resonance occurred. To normalize the response, the baseline refractive index, which was measured with non-innoculated PBS or chicken buffer depending on the sample buffer, was subtracted from the responses.

3. Results and Discussion

3.1. Antibody adsorption
Figure 2 shows a response of the biosensor during immobilization. After the BBSA blocking, the first neutravidin layer was physically adsorbed on the gold surface. Responses of 1100–1500 μRIU (Refractive index unit) were observed for the neutravidin binding. The values were within the normal range of typical responses of neutravidin bindings suggested by the manufacturer. In the following step, capture antibodies were attached to the adsorbed neutravidin layer via avidin-biotin reaction. The binding responses were around
400~500 μRIU for the antibody binding events. Finally, BSA molecular layer was physically adsorbed onto the gold surface to block the non-specific binding site.

3.2. Immunoassay of Salmonella

Since SPR biosensor is affected by the matrix in the solutions, a baseline measurement was performed with a plain buffer, which was the same buffer as the sample buffer but didn’t contain the cells, prior to any assay. Responses of the Spreeta biosensor to various concentrations of whole Salmonella cells in PBS are investigated. The Spreeta biosensor couldn’t detect the bindings of Salmonella cells.

To increase the sensitivity of the biosensor, fragments of Salmonella cells, which were extracted by sonification process, were used for the experiment. Figure 3 shows responses of the biosensor to various concentrations of fragmented Salmonella cells in PBS. Detection of Salmonella enteritidis in PBS with three Spreeta biosensors was shown in Fig. 4. For each experiment, the standard deviation of the mean (SEM) signals from three Spreeta biosensors was calculated. The error bars on each graph designate ± SEM. The Spreeta biosensor could detect the Salmonella at concentrations down to 10^5 cfu/ml. The increased sensitivity over whole Salmonella cells could partly explained by the higher probability of bindings to the antibody due to a higher number of accessible epitopes near the antibody layer. Relatively big size of the antigens (1~2 μm) might hinder the matching epitopes from binding to the immobilized antibodies.

3.3. Performance of Spreeta immunoassay

The responses of biosensors to increasing concentrations of Salmonella enteritidis spiked into chicken extracts are shown in Fig. 5. The Spreeta biosensor detected Salmonella enteritidis in food samples at concentration level of 10^6 cfu/ml. Binding of Salmonella enteritidis to the antibody immobilized on the sensor surface generated noticeable response (248 μRIU).

Unlike responses for samples with PBS buffer, signal noise was observed from responses for food samples. A possible reason of these signal noise of the Spreeta biosensor in the food matrix comes from interference of antigen-antibody bindings from other competitive microorganisms or the food ingredients. To overcome the inference and to increase sensitivity of the biosensor, further development of the detection technique is required. In this research, a miniature SPR biosensor, Spreeta, was evaluated to detect Salmonella enteritidis in PBS or food samples. The biosensor was functionalized by immobilizing a neutravidin layer followed by a Salmonella specific antibody layer. The Spreeta biosensor could detect 10^5 cfu/ml of Salmonella enteritidis in PBS. Also, the sensor could
detect $10^6$ cfu/ml of *Salmonella enteritidis* in chicken samples. The Spreeta biosensor may offer rapid detection of foodborne pathogens to enable early protection of food safety. Further development of this detection technique will make a significant improvement on food safety programs.

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**References**


