RAPID DETECTION OF BACTERIA IN FOOD BY SURFACE PLASMON RESONANCE SENSORS

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Abstract- There is an increasing development towards rapid pathogenic detection techniques in food, though most research done were on the specific testing of selected food ingredients which limits the application of these techniques. The ability of a surface plasmon resonance biosensor to detect *Escherichia coli* in inoculated complex food matrices was investigated and the results were close to that obtained from plate counting method. Prior to the SPR detection, the optimization of the sample preparation process was performed to render the complex food matrices suitable for application onto the SPR.

Keywords- Rapid Detection Of Bacteria; Food; Surface Plasmon Resonance Sensors.

I. INTRODUCTION

Disease-causing microorganisms, also known as pathogens, are found everywhere and reached every aspect of life. Potentially threatening microbes can be found in food, soil and in water and have historically resulted in many unwarranted deaths and illnesses worldwide. In fact, an estimate of 76 million illnessesare caused by the occurrence of food borne diseases yearly. The World Health Organisation (WHO) defines food borne illnesses, usually either infectious or toxic in nature, as those diseases caused by agents that enter the body through the ingestion of food. Several bacterial pathogens that are responsible for the majority of foodborne outbreaks are found to be *Campylobacter jejuni*, *Clostridium perfringens, Salmonella* and *Escherichia coli O157:H7.*

The detection of numerous foodborne pathogens such as *L. monocytogenes*, *Salmonella*, *E. coli O157:H7* and *C. jejun* 'using surface plasmon resonance (SPR)-based biosensor have been previously reported in various literatures. In a more recent work by Waswa*et al.*, they demonstrated the use of a SPR-based biosensor to detect *E. coli O157:H7* and *Salmonella* spiked in milk. A direct assay was obtained in real-time by injecting pasteurized milk samples inoculated with increasing concentrations ($0–10^7$cfu/mL) of pathogens onto the sensor surface immobilized with antibodies.This study suggested that the use of SPR biosensor for bacterial detection looked promising and hence could be employed for rapid and label-free detection of different bacterial pathogens. In addition to that, the detection of *E. coli* in other food matrices like apple juice and beef extract was also attempted by Waswa and co-workers using a different SPR biosensor. The results were consistent and the technique was found to have a low limit of detection with high specificity.

From the various rapid detection methods available, surface plasmon resonance biosensors have been proven to be a promising technique for bacteria detection due to their portability, sensitivity, speed and possible onsite detection. SPRsensors are directlabel-free optical sensors using special electromagnetic waves (surface plasmon-polaritons) to investigate interactions between an analyte in solution and the biomolecular recognition element immobilized on the SPR sensor surface.

Thus, the goal of this proposed work is to further develop SPR as a rapid method for the detection of pathogenic microorganisms in complex food matrices. Prior to detection, we will be focusingon the usage of filtration and centrifugation for sample preparation. These techniques are simple to perform, reliable and have a short running time with low cost. In filtration, bacteria is separated from the food particles based on its size as food matrices are forced through filters with varying pore sizes. In centrifugation, the interfering particles are pelletized leaving the bacteria in the supernatant fluid. These are to obtain suitable samples for SPR analysis by minimising the interfering particles. Subsequently, the SPR biosensor will be used for the bacterial detection in food and for this work, detection of *Escherichia coli*(*E. coli*) in food matrices will be addressed.

II. MATERIALS AND METHODS

The reagents were obtained from the following sources: *E. coli* DH5α™ was obtained from Invitrogen, anti *E. coli* goat polyclonal biotinylated antibody was from Thermo Scientific (Rockford, IL, USA);avidin from chicken egg, bovine serum albumin (BSA), LB broth, LB agar plates, phosphate buffered saline (PBS, pH 7.4) were from Sigma; gold disposable sensor chip was from Sensi Q Discovery. Food ingredients, raw chicken and oatmeal, were purchased from a local supermarket. Filter papers with pore sizes of 2.7 µm and 11 µm were purchased from What man™. Membrane filters of pore size 0.45µm were obtained from Merck Millipore. All other regents were all chemical analytical grades.

The SPR data were recorded with SensiQ Discovery instrument (Nomadics, Inc.) equipped with Qdat analysis software (Biologic Software, Inc).[11]The SPR sensor systememploys the Kretschmann SPR configuration which is based on the attenuated total reflection method and spectral investigation of SPR condition.The SPR sensor consisted of a light emitting diode source (LED), sensing chip and a photodiode array detector. The sensing surface is a planar glass chip with ~50nm gold film coating. Light from the LED is passed through the aperture and polarizing film to producemonochromatic light on the sensor surface. This light is then reflected from the sensing surface over a range of incident angles and a reflectance minimum will occur with respect to the incident angle and is detected by the photodiode array. A Teflon flow cell was pressed on the gold SPR surface. Tested samples were contacted with the SPR surface at laminar flow through a rectangular flow channel of volume 50nL. Adsorption of ligands on the sensor surface and the bacteria binding were observed as a change in response unit. Sample is injected manually *via* the use of a twelve-port, two-position injection valve. Centrifugation was performed using Hettich Universal 320R benchtop centrifuge.

2 µL from the *E. coli* (DH5α^{TM}) stock was inoculated in aliquots (5 mL) of LB broth in 25 mL conical flasks. The culture flasks were incubated at 37 °C and 200 rpm for 24 hours using an incubator shaker. A portion of each bacteria culture was killed by heating in a boiling water bath for 15 minutes before being diluted to the desired concentration with PBS.

The membrane filtration method is generally used to test large volume of liquid samples like water or filterable samples. The samples from the serial dilutions were passed through a sterile membrane filter with a pore size 0.45 µm enclosed in the filtration assembly. After which the membrane was removed using a sterile forceps and placed on the surface of a pad saturated with LB medium. The plates were inverted and incubated at 37 °C for 24 hours. The colonies were then counted through visualization by eye.

The spread plate technique is commonly used for enumerating microorganisms. 0.1 mL of aliquots from the serial dilutions was dropped onto the surface of the LB agar plates. The inoculum was spread across the surface using a sterile glass spreader to establish a dilution gradient which provides isolated colonies. The agar plates were then inverted and incubated at 37 °C for 24 hours. The colonies were subsequently counted through visualization by eye.

Gold sensor surface was cleaned with piranha solution for 15 minutes, followed by subsequent rinses with deionized water and ethanol. Finally, the surface was blown dry with nitrogen prior to the immobilization of antibody.

Antibodies were immobilized onto the gold surface by the avidin-biotin coupling method. The sensor surface was activated by injecting a concentration of 200 µg/mLavidin in PBS solution for 30 minutes at flow rate of 5 µL/min. Following that, biotinlyated anti*E. coli* antibodies diluted in PBS to a concentration of 50 µg/mL was injected for 30 minutes at 5 µL/min. Finally, the antibodies-modified substrate was treated with 1% BSA solution for 30 minutes at 5 µL/min to block the non-specific sites. PBS, pH 7.4, was used as the running (wash) buffer between injections to remove any unbound ligands.

The bacterial suspension in PBS was injected on the surface at a flow rate of 5 µL/min for 20 minutes, which allowed the bacteria to interact with the bound antibodies. The sensor surface was washed with PBS running buffer to remove any unbound *E. coli* cells. To determine sensitivity, 7-fold serial dilutions of *E. coli* prepared in PBS buffer were injected over the immobilized antibodies on the sensor surface.

A standard food matrix was prepared for experimental use. Unpasteurized fresh chicken and natural wholegrain oats both containing no preservatives or disinfectant chemical were used as the ingredients. Prior to each test, 3.75 g of raw chicken was allowed to come to laboratory room temperature (approximately 21°C) and then blended with 6.25 g of oatmeal and 90 g of deionized water for 3 minutes using a blender to create a homogeneous matrix.

The food matrices were initially tested for total bacteria count (including *E. coli*) by spread plate method on LB agar before artificially inoculated with *E. coli*. 3 samples were plated and bacterial counts for the fresh, raw untreated food matrices were around 10^6cfu/mL.5 mL samples of LB containing 10^8cfu/mL*E. coli*were mixed into 5 mL of homogeneous food matrix to achieve target inoculation of 10^8cfu/mL. LB spread plates were used to determine the initial concentration of *E. coli* in the spiked food matrices and in the food matrices after sample preparation.

10 g of inoculated food samples were subjected to a series of sample preparation processes which included filtration through pore sizes of 2.7 µm or 11 µm and centrifugation at various speeds to optimize the *E. coli* recovery and interferences elimination. The resulting samples were subsequently used for spread plate count and SPR detection for the determination of *E. coli* concentration.

III. RESULTS AND DISCUSSION

We began our study for the rapid bacterial detection *via* SPR by looking at the online immobilization of anti *E. coli*polyclonal antibody onto the gold sensor surface. Several methods are available for the

immobilization including avidin-biotin coupling, amine coupling, thiol coupling and self-assembly monolayerformation. The avidin-biotin coupling method was selected due to its strong interactions and simple implementation.**Figure 1**shows a schematic diagram of the proposed immobilization.

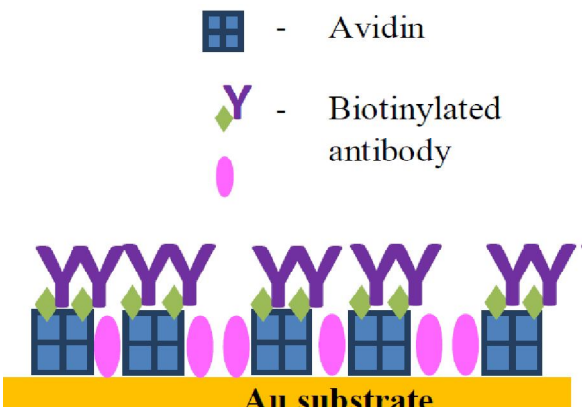


Figure 1.Schematic diagram of antibody immobilization

Following the antibody immobilization, the real-time assay for the detection of *E. coli* suspended in PBS was obtained by allowing various concentrations of the bacteria culture to bind to the immobilized antibodies on the gold sensor surface. The optical responses are shown in **Figure2**which illustrates the binding of *E. coli* at increasing concentration of 10^3 to 10^9 cfu/mL in one set of experiments.

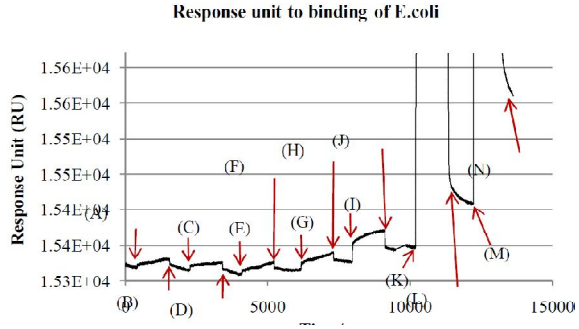


Figure 2.The RU to binding of *E. coli*. Injection of (A) *E. coli* solution (10^3 cfu/mL); (B) PBS; (C) *E. coli* solution (10^4 cfu/mL); (D) PBS; (E) *E. coli* solution (10^5 cfu/mL); (F) PBS; (G) *E. coli* solution (10^6 cfu/mL); (H) PBS; (I) *E. coli* solution (10^7 cfu/mL); (J) PBS; (K) *E. coli* solution (10^8 cfu/mL); (L) PBS; (M) *E. coli* solution (10^9 cfu/mL); (N) PBS.

Four replications were performed for each concentration and the average change in response unit (ΔRU) were calculated in **Table 1** and used to construct the calibration curve in **Figure3**. The average change in response unit to the concentrations werefound to be 4.311, 19.970, 63.219, 119.105 and 222.827 respectively. The observed discrepancies between the SPR responses could be due to the variation in sensor functionalization since the sensor surface functionalization for each experiment was done in different batches. According to **Figure 3**, the working range was found to be between 10^5 and 10^9 cfu/mL while the detection limit of the proposed method, which was found by using three times of the noise (about 1.63 RU), was about 10^5 cfu/mL for direct detection mode.

Table 1.Mean ΔRU for respective *E.coli* concentrations(mean of four sets of readings).

E.coli concentration (cfu/mL)	Mean ΔRU	Standard Deviation of ΔRU
10^3	4.311	2.064
10^4	19.970	11.794
10^5	63.219	28.817
10^8	119.105	22.398
10^9	222.827	46.277

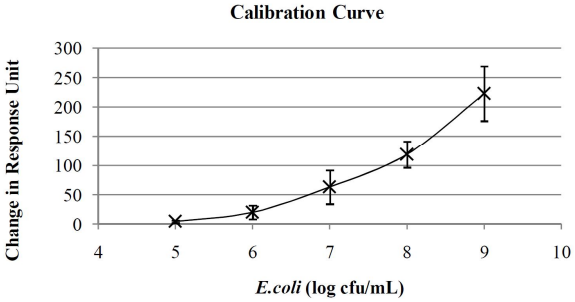


Figure 3.The change in the RU induced by the binding of *E. coli* at various concentrations.

3.1 Detection of *E. coli* in food samples*via* SPR

Figure 4showed the SPR response from the 11 different food samples. It was observed that the response change between the pure *E. coli*sample and the prepared food sample differed.The difference is attributable to the interfering particles in the inoculated food matrix.The response unit changes for the prepared food samples(**Table 2**) fell close to the 10^7cfu/mL response range within the calibration curve (shown in **Table 1** and **Figure 3**). Hence, this provided the evidence that the sample preparation techniqueused wasquite effective in recovering the *E. coli* and removing most of the interferences from the food matrix. Nonetheless, the sample preparation process could still be further optimized to obtain a more accurate response.

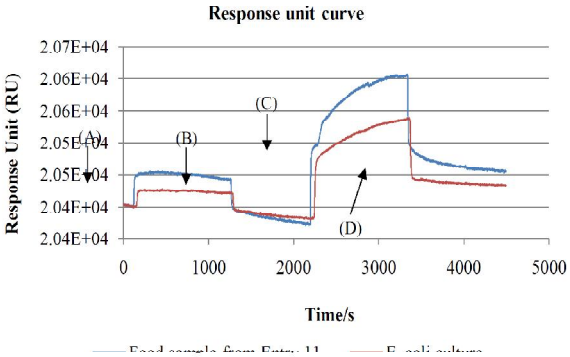


Figure4. The RU to binding of *E. coli*. in food sample and bacteria culture respectively. Injection of (A) Sample/culture(10^6 cfu/mL); (B) PBS; (C) Sample/culture(10^7cfu/mL); (D) PBS.

Table 2. Comparison of ΔRU between *E. coli* culture and prepared food samples for 2 replications.

No.	Concentration of *E. coli* (cfu/mL)	ΔRU for *E. coli*	ΔRU for food sample
1	10^7	26.460	70.041
2	10^7	61.327	100.700

CONCLUSION AND FUTURE WORK

In summary, the development of amethod for bacterial detection *via* the SPR sensor was developed. The results suggestedthe possibility of rapid sample preparation and detection of bacteria directly from complex food homogenates to facilitate the 'real time' screening in food production. Future research and development could focus on the further optimization of the sample preparation process to obtain more accurate SPR response and also improve the sensitivity of the detection method.

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REFERENCES

[1] Mandal, P. K.; Biswas, A. K.; Choi, K.; Pal, U. K. Methods for Rapid Detection of Foodborne Pathogens: An Overview. Am. J. Food Techno. 6.2011, 2, 87-102.

[2] Benoit, P. W. Non-cultural enrichment: Methods for Rapid Separation and Concentration of Bacteria in Food. Ph.D. Dissertation, University of Maine, 1998.

[3] Velusamy, V. et. al. An Overview of Foodborne Pathogens Detection: In the Perspective of Biosensors. Biotechnol. Adv.2010, 28, 234-254.

[4] Bhunia, A. K.; Geng, T.; Lathrop, A.; Valadez, A.; Morgan, M. T. Optical Immunosensors for Detection of Listeria Monocytogenes and Salmonella Enteritidis from Food. In: Bennedsen BS, Chen YR, Meyer GE, Senecal AG, Tu SI, editors. Monitoring food safety, agriculture, and plant health.2004, 1–6.

[5] Taylor, A. D.; Ladd, J; Yu, Q. M.; Chen, S. F.; Homola, J.; Jiang, S. Y. Quantitative and Simultaneous Detection of Four Foodborne Bacterial Pathogens with a Multi-Channel SPR Sensor. Biosens.Bioelectron.2006, 22, 752–758.

[6] Waswa, J. W.; Debroy, C.; Irudayaraj, J. Rapid Detection of Salmonella Enteritidis and Escherichia Coli Using Surface Plasmon Resonance Biosensor.J. Food Process Eng.2006, 29, 373-385.

[7] Waswa, J. W.; Debroy, C.; Irudayaraj, J. Direct Detection of E. coli O157:H7 in Selected Food Systems by a Surface Plasmon Resonance Biosensor. LWT Food Sci. Technol. 2007, 40, 187-192.

[8] Homola, J. Present and Future of Surface Plasmon Resonance Biosensors.Anal.Bioanal. Chem. 2003, 377, 528-539.

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