

Development of a surface plasmon resonance biosensor for the identification of *Campylobacter jejuni*

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Abstract

The purpose of this study was to develop a biosensor based on surface plasmon resonance (SPR) for the rapid identification of *C. jejuni* in broiler samples. We examined the specificity and sensitivity of commercial antibodies against *C. jejuni* with six *Campylobacter* strains and six non-*Campylobacter* bacterial strains. Antigen–antibody interactions were studied using enzyme-linked immunosorbent assay (ELISA) and a commercially available SPR biosensor platform (Spreeta™). *Campylobacter* cells killed with 0.5% formalin had significant lower antibody reactivity when compared to live cells, or cells inactivated with 0.5% thimerosal or heat (70 °C for 3 min) using ELISA. The SPR biosensor showed a good sensitivity with commercial antibodies against *C. jejuni* at 10³ CFU/ml and a low cross reactivity with *Salmonella* serotype *typhimurium*. The sensitivity of the SPR was similar when testing spiked broiler meat samples. However, research is still needed to reduce the high background observed when sampling meat products.

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1. Introduction

Campylobacter is an important cause of acute bacterial gastroenteritis in humans worldwide (Akitoye et al., 2002). *Campylobacter jejuni* infections can also cause Reiter syndrome, a reactive arthropathy, and Guillian–Barre syndrome, an acute neuromuscular paralysis (Tauxe, 1992). From 1996 to 2000, there were an estimated 2.4 million *Campylobacter* infections each year in the US, with 21.9 cases reported per 100,000 people (Nachamkin and Blaser, 2000). Although the trend of *Campylobacter* infections appears to be downward, there were still 5215 *Campylobacter* infections reported for 2003, which represented an incidence of 12.60 cases per 100,000 people (CDC, 2004).

Recent studies show that a high percentage of processed broiler carcasses (Oyarzabal et al., 2004) and retail broiler

products (Dickins et al., 2002; Kramer et al., 2000) are contaminated with *Campylobacter*. The level of *C. jejuni* and *C. coli* contamination per ml of carcass rinse is between 3 to 3.7 logs CFU/ml immediately after evisceration, but it drops after the chiller (Oyarzabal, 2005; Yang et al., 2001). Therefore, a suitable methodology for rapid screening of carcass rinses for the presence of *C. jejuni* should have a sensitivity of approximately 2 to 3 log CFU/ml. This number of cells could be potentially identified with a sensitive biosensor.

Currently, all conventional microbiological methods for the identification of *Campylobacter* species take three to four days. Newest detection methods for *Campylobacter* include enzyme-linked immunoassays coupled with an enzyme electrode (Che et al., 2001), an indirect competitive ELISA for detection of somatic antigen O:23 of *C. jejuni* in foods (Hochel et al., 2004), an electrochemical biosensor based on supported planar lipid bilayers (Ivnitski et al., 2000), polymerase chain reaction (PCR) assays with microarray (Keramas et al., 2004; Szalanski et al., 2004; Wang et al., 1999), a robotic DNA purification protocol

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coupled with real-time PCR (Oliveira et al., 2005), a combination of immunoassays and PCR protocols (Bolton et al., 2002; Sails et al., 2002), and an array biosensor based on total internal reflection fluorescence coupled to a sandwich immunoassay for antigen detection (Sapsford et al., 2004). Quantification of *Campylobacter* spp. has been achieved by a real-time PCR combined to a discontinuous buoyant density gradient method (Wolffs et al., 2005) and immunocapture PCR assays (Waller and Ogata, 2000). However, most of these protocols require a sample preparation step (Waller and Ogata, 2000; Wang et al., 1999; Wolffs et al., 2005), enrichment and centrifugation steps (Oliveira et al., 2005), or have been tested only under laboratory conditions (Ivnitski et al., 2000).

Surface plasmon resonance (SPR) biosensors are one of the most sensitive optical biosensor widely applied for chemical sensing and biosensing characterizations (Homola et al., 1999). SPR biosensors do not need reagents or labels for the detection of a target analyte, the immobilized biological recognition element (bioreceptor) can be regenerated and reused for continuous or multiple detection, and because the bioreceptor and transducer are integrated into one single sensor, on site detection can be easily achieved (Quinn et al., 2000). Although various biological recognition elements are available as receptors (such as enzymes, antibodies, microbes, and organelles), antibodies are widely used as effective binding partner in SPR biosensor. Therefore, improvements in the immunoaffinity of antibody–antigen reactions can enhance the sensitivity and specificity of SPR biosensors (Mandrell and Wachtel, 1999).

The objectives of the present study were: i) to examine the sensitivity and specificity of commercial polyclonal antibodies against *C. jejuni*; ii) develop a SPR biosensor based on antibody/antigen binding for rapid identification of *C. jejuni* in pure cultures; and iii) test the specificity and sensitivity of the SPR biosensor for the rapid identification of *C. jejuni* in spiked broiler meat rinse.

2. Materials and methods

2.1. Bacterial strains, culture conditions and ELISA experiments

Six *Campylobacter* strains obtained from the American Type Culture Collection (*C. jejuni* ATCC 35918, *C. coli* ATCC 43473, *C. lari* ATCC 35223), isolated from processed broiler carcass (*C. jejuni* post 5) or from human infections (*C. jejuni* CDC 370 and CDC 410) were used in this study. Species were identified by using a multiplex PCR assay (Oyarzabal et al., 2005). Two *Arcobacter* species, *A. butzleri* ATCC 49616 and *A. skirrowii* ATCC 51399, three *Salmonella* serotypes, *S. enteritidis* ATCC 13076, *Salmonella typhimurium* ATCC 13311, and *S. heidelberg* isolated from chickens, and one non-pathogenic *Escherichia coli* isolated from a cow (College of Veterinary Medicine, Auburn University) were used for exclusivity studies. All strains were stored in broth with 20–30% glycerol at -80°C . *Campylobacter* and *Arcobacter* strains were grown on the modified Campy Cefex (mCC; Oyarzabal et al., 2005) plates under microaerophilic condition (5% O_2 ,

10% CO_2 and 85% N_2), and incubated at 37°C for 24 h and 35°C for 48 h, respectively. *Salmonella* strains were grown on brilliant green sulfa agar (Difco, Detroit, MI) or modified lysine iron agar plates, and the *E. coli* 48-2 strain was grown on MacConkey agar plates (Difco) that were incubated at 37°C for 24 h.

All cultures were prepared by transferring colonies from plates into phosphate buffer saline (PBS). Centrifugation/washing procedures were repeated three times. The bacteria were resuspended in PBS and the optical densities of the bacterial suspensions were adjusted to O.D._{600 nm} at 1.7–2.0 to obtain bacterial concentrations of 10^8 – 10^{10} CFU/ml. The bacterial concentrations were confirmed by spread plate method. All bacterial suspensions were stored at 4°C overnight for later use.

Four commercial antibodies against *Campylobacter* were used as primary antibody in the ELISA experiments. Antibody 1 (Ab1) was a polyclonal rabbit antibody to *C. jejuni* ATCC 29428 (Biodesign International, Saco, Maine). Antibody 2 (Ab2) was a polyclonal rabbit antibody to *C. jejuni* ATCC 29428 (Biogenesis Ltd., Brentwood, New Hampshire). Antibody 3 (Ab3) was a polyclonal rabbit antibody to *C. jejuni* ATCC 29428 (Biotrend Chemikalien GmbH, Koln, Germany). Antibody 4 (Ab4) was a polyclonal rabbit antibody to *C. jejuni* (Fitzgerald Industries International, Inc., Concord, Massachusetts). All four antibodies were biotin conjugated. ELISA experiments were performed in 96-well microplate (polystyrene plate, Costar, Cambridge, MA). The optimal antibody dilution factor of the commercial antibodies was determined with *C. jejuni* ATCC 35918. In specificity tests using ELISA, the antigens were different bacteria at 10^9 CFU/ml with the optimal antibody concentration.

2.1.1. Reactivity of 24-h, 10-day and 24-day cultures, and dead cells using ELISA

To compare the reactivity between live and dead cells, *C. jejuni* strains (ATCC 35918, post 5, CDC 370 and CDC 410) were tested with Ab2. Suspensions of 10^9 CFU/ml were kept in refrigeration (4°C) for 24 h, 10 day and 24 days for use as live cells. Three different methods were used to prepare the dead cells of *C. jejuni*: 1) heating at 70°C for 3 min, 2) addition of 0.5% formalin (Fisher, Fair Lawn, NJ) with the subsequent incubation at room temperature for 1 h, and 3) addition of 0.5% thimerosal (Sigma, St. Louis, MO) with the subsequent incubation at 37°C for 1 h. Live and dead cell suspensions were coated, independently on 96-well microplate for reactivity test.

2.2. Surface plasmon resonance experiments

2.2.1. Dual channel SPR

The sensors used in this work are dual channel Spreeta™ sensors (Texas Instruments, Dallas, TX) described elsewhere (Melendez et al., 1996, Balasubramanian et al., 2007). This sensor is a miniature (approximately 7 g), fully integrated surface plasmon resonance device (Fig. 1A). It is based on Kretschman's geometry (Raether, 1988; Smolyaninov, 2005) and it is fully configured with an AlGaAs light emitting diode (LED, 840 nm) with a polarizer, temperature sensor, two photodiode arrays, and reflecting mirror. Light from the LED illuminates the gold-coated

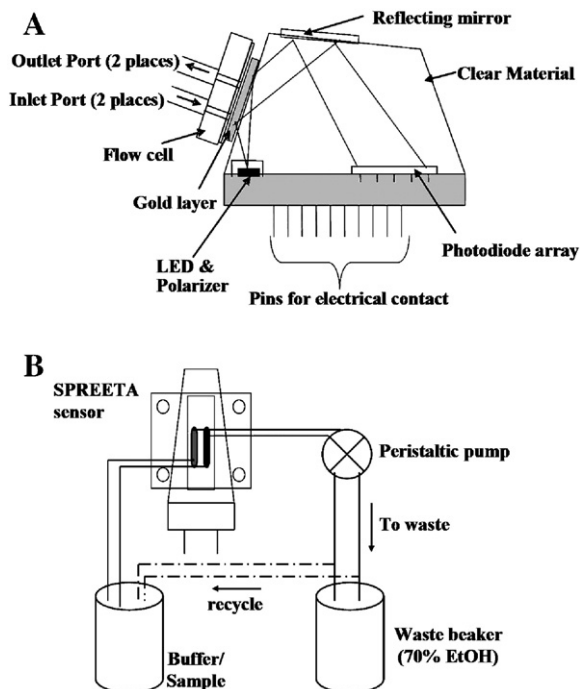


Fig. 1. A: Schematic of SPREETA™ sensor developed by Texas Instruments. Sensor is completely integrated with LED (850nm) with polarizer, 256-pixel photodiode array and pins for electrical contact. Introduction of samples were established through flow cell which comprises of two channels of volume $\sim 10 \mu\text{l}$. B: Experimental setup. A SPREETA™ sensor is docked with fluidics part to set up a flow using peristaltic pump. A polypropylene flow cell (FC) containing two independent flow channels with gasket installed on the face of SPREETA™ sensor. Silicone tubing of 0.64 mm ID was inserted to the two inlet and two outlet ports. The sensor is first initialized with air and water and the references are saved for future use. The data from the sensor is fed through the control box to the computer.

thin glass with a wide range of angles after passing through a polarizer which allows only the transverse magnetic component. After reflection from the gold-coated glass slide, the light is directed towards the two-independent linear 256 pixel Si-photodiode array with the help of mirror. The entire assembly is encased in an optically clear material while the interference from the external light is blocked with an opaque coating. The flow cell consists of a 25 mm \times 25 mm Teflon block of 4 mm thickness with inlets and outlets for each channel. The silicone rubber gasket of 0.25 mm thick with two side-by-side laser cut chambers for two channels. The flow volume of each channel is $\sim 10 \mu\text{l}$. A peristaltic pump (Cole-Parker Instruments Co., Chicago, IL) was used to establish the flow system (flow rate $\sim 100 \mu\text{l}/\text{min}$). The Teflon block is held in place by four screws (Fig. 1B). The response of the photodiode array is digitized by a 12-bit analog to digital converter and then it is transferred to a computer. The monitoring and analysis program provides the user interface for displaying and analyzing the sensor data. The software provides the user with all the information related to analysis of SPR curve, the real time binding, layer thickness and flow cell temperatures. It also provides the information related to the variation of refractive index, pixel number, angle of reflectance, probe temperature with time (Simonian et al., 2002; Naimushin et al., 2002).

The SPR sensor was assembled using a G-Clamp setup provided with sensor kit. After initialization with air and water, an in-situ 0.12N NaOH-1% Triton-x cleaning was performed to make the gold surface hydrophilic followed by recalibration in MilliQ water. Throughout the experiment PBS, pH 7.4 was used as running and washing buffer.

2.2.2. Antibodies immobilization

PBS buffer was first flowed through the sensor until a steady PBS baseline was established in the working and the control channel. On the working channel, the gold surface was modified with neutravidin (1 mg/ml) till saturation followed by PBS wash. The uncovered surface of the gold was blocked with 1 mg/ml of BSA. A solution of 22.5 $\mu\text{g}/\text{ml}$ of biotinylated Ab2 was then selectively immobilized. PBS washes were performed in between all the above steps to remove unbound molecules. An additional PBS-0.5% Tween wash was performed to restrict non-specific binding (Fig. 2). The reference channel was completely modified with BSA (1 mg/ml) to account for non-specific binding, bulk refractive index changes and temperature fluctuations during the course of the experiment.

2.2.3. Specificity tests using SPR

The specificity of the sensor was tested with *S. typhimurium* ATCC 13311 and *C. jejuni* ATCC 35918 in suspensions from 10^4 to 10^6 CFU/ml. After the final concentration of *S. typhimurium*, 100 mM glycine elution buffer (pH 2) was used to break the non-specific binding between antibodies and *S. typhimurium*. Following a new baseline with PBS buffer, three serially diluted *C. jejuni* concentrations (10^4 to 10^6 CFU/ml) were introduced through both channels. Similarly, a glycine buffer wash was performed to break the non-specific binding between antibodies and *C. jejuni* and between antibodies and *Arcobacter skirrowii*. Each concentration of *A. skirrowii*, *C. jejuni* and *S. typhimurium* was run until the signal reached a steady value. A PBS wash was performed between all the concentrations.

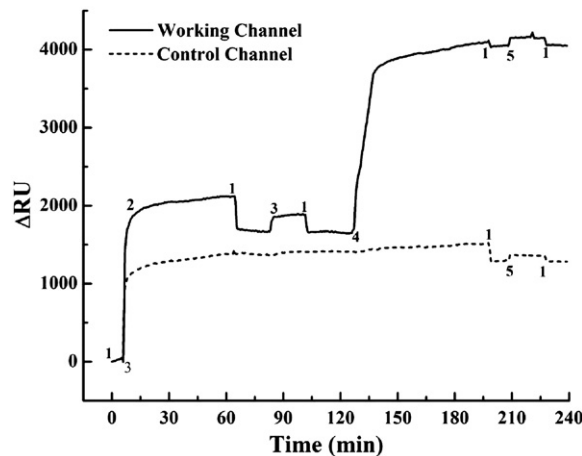


Fig. 2. Antibody immobilization for SPR experiments. Numbers show the times when various solutions were added. 1: PBS; 2: Neutravidin; 3: BSA; 4: Ab2; 5: PBS-Tween.

2.2.4. Sensitivity tests using SPR

Following immobilization of antibody on the surface of the sensor, 10 folds serially-diluted *C. jejuni* ATCC 35918 suspensions (10^1 to 10^8 CFU/ml) were introduced through both channels starting with lowest concentration (10^1 CFU/ml). Higher concentrations of bacteria were introduced once the signal from the previous low concentration reached a steady value (standard deviation < 5 RU). PBS buffer washes were performed in between each concentration to remove loosely attached bacteria.

2.3. Experiment with retail broiler meat

This experiment was aimed at determining the effectiveness of the SPR to detect *C. jejuni* in artificially inoculated chicken rinse. Boneless, skinless broiler breast was bought from a local retail food store. After 50 g of meat were added to a sterile Whirl-Pak[®] bag (Nasco, Fort Atkinson, WI) containing 200 ml of PBS, the bag with the content was vigorously shaken by hand for 1 min. The rinse was analyzed for the presence of *Campylobacter* spp. by the direct plating of the rinse in duplicate on mCC plates. Plates were incubated under microaerophilia at 42 °C for 48 h. Colony morphology and culture characteristics under phase contrast microscopy were used to determine presumptive colonies. Four and a half milliliter of the rinse were added to several sterile tubes. One tube (original) was inoculated with 10^8 CFU/ml of *C. jejuni* ATCC 35918. Then, a ten-fold, serial dilution scheme was prepared from the original tube. The contents of the tubes containing approximately 10^2 through 10^7 CFU/ml *C. jejuni* ATCC 35918 were used for the SPR analysis.

2.4. Statistical analysis

Triplicate experiments were run for every ELISA and SPR experiment in these studies. Results were statistically analyzed by ANOVA (one-way analysis of variance) to determine significant ($p < 0.05$) differences among means. Duncan's Multiple Range Test and Dunnett's Tests were performed to determine significant differences between means. All statistical analyses were applied using Statistical Analysis System (V 9.1, SAS Institute Inc. Cary, NC). Standard error of means (SEM) was calculated for all SPR results.

3. Results and discussion

3.1. Reactivity and specificity of commercial antibodies

There was no significant difference in the reactivity among four commercial antibodies against *C. jejuni* ATCC 35918 (data not shown). In the specificity test, the antibody 2 had the highest reactivity with *C. jejuni* ATCC 35918 and showed significant difference with other tested bacteria. There were no significant differences among these four commercial antibodies in specificity tests, except for antibody 4 that showed higher cross-reactivity with *A. skirrowii* ATCC 51399 (data not shown). However, due to the discontinuation of the antibody 1 by the manufacturer and the lengthy importation process from Germany for antibody 3, the

antibody 2 was chosen for the rest of the experiments in this study. For the sensitivity test of antibody 2 against *C. jejuni* ATCC 35918 using ELISA, the detection limit was 10^7 CFU/ml. In addition, the optimal dilution for antibody 2 to detect *C. jejuni* using ELISA was found to be 1/200.

3.2. Reactivity of antibody with live and dead cells using ELISA

These experiments were intended to determine if the reactivity of the antigens remains stable over time, and if inactivated or dead cells are still reactive with antibodies. There were no significant differences ($p > 0.05$) in ELISA results for the same samples stored for different times at 4 °C (Fig. 3A), although the shapes of cells changed over time, with more coccoid shapes noticed in older cultures (Fig. 3B–3). However, the binding efficiency of *Campylobacter* cells to antibody in PBS was similar for up to 24 days storage. These results suggest that after the collection of naturally contaminated *Campylobacter* in proper buffer and storage at refrigeration temperature, the bacteria can still be detected for more than a week using ELISA. Many poultry processing plants are hours away from a fully-equipped microbiology laboratory and the results show that the reasonable spending times of sampling, handling and processing for the detection of *Campylobacter* in broiler products shall not be affected.

The best reactivity of antibody 2 to four *C. jejuni* strains was *C. jejuni* ATCC 35918. The preparation of immunogen for antibody 2 production was from *C. jejuni* ATCC 29428, whose cell structure may be more similar to *C. jejuni* ATCC 35918 than other strains. Other *C. jejuni* species isolated from humans and processed broiler carcasses showed lower reactivity with the same antibody (Fig. 3A). These results highlights the need of preparing antibodies using the strains most commonly found in the samples that will be targeted for testing.

Immunoreactivities varied according to the method used to inactivate the cells. The reactivity of formalin inactivated cells was significant lower ($p < 0.05$) than cells inactivated with thimerosal or heating. The binding efficacy of *C. jejuni* cells inactivated by thimerosal and heating was not affected. It is possible that formalin denature the surface epitopes of the *C. jejuni* cells and/or flagella, or lower their binding properties to the antibodies (Sompuram et al., 2004). Heat treatment at 70 °C for 3 min and a solution of 0.5% thimerosal did not change substantially the binding between *C. jejuni* and antibodies. Therefore, these methods could be used as a mean of inactivating *C. jejuni* cells without substantially changing their reactivity to antibodies.

3.3. Specificity tests using SPR

The recorded response units for antibody 2 bound to *C. jejuni* was significantly higher ($p < 0.05$) than the response recorded with the binding of antibody 2 to *S. typhimurium* (Fig. 4). The binding between antibody 2 and *C. jejuni* was specific because the glycine buffer rinse did not disassociate the antibody–antigen binding returning the signal to the baseline (Fig. 5A). The binding of antibody 2 to *S. typhimurium* was of a weak

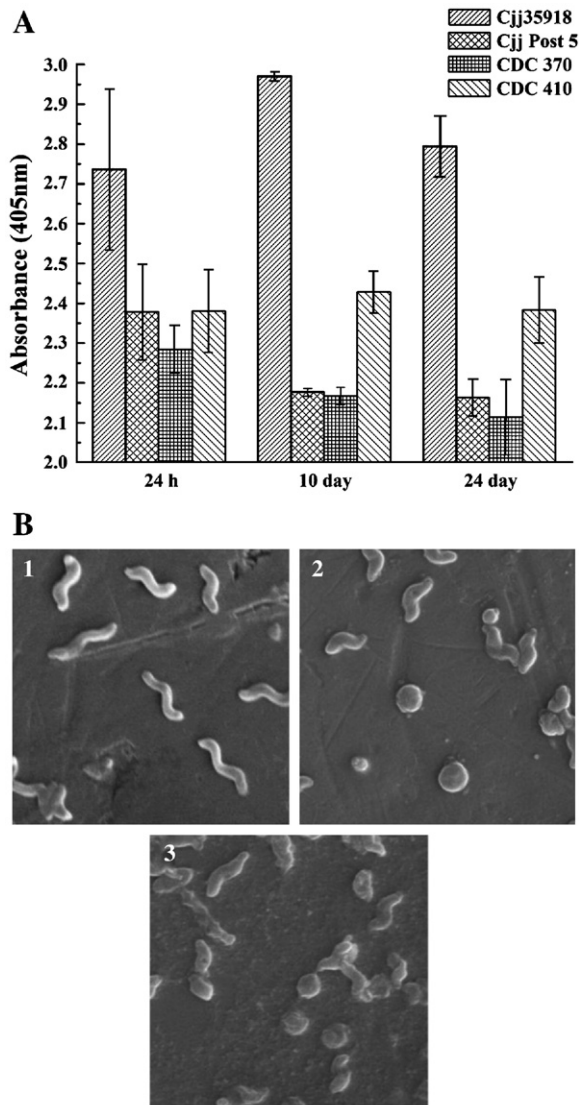


Fig. 3. A: Absorbance values obtained by ELISA with antibody 2 at 24-h, 10-day and 24-day cultures of *Campylobacter* cells stored at 4 °C. Means with different letters are significant different ($p < 0.05$). Error bars represent ± 1 SEM. B: Scanning electron micrographs of different aged cells of *C. jejuni* ATCC 35918. 1: 24-h cultured cells; 2: 10-day old; and 3: 24-day old. Older cells show irregularities on their surfaces (B-2) and disruption of the cytoplasmic membranes (B-3). One milliliter of the culture was fixed in 2% glutaraldehyde, 1% osmium tetroxide and 0.1 M cacodylate buffer (pH 7.2) for 20 min. Specimens were examined in a Zeiss DSM 940 scanning electron microscope operated at 15 kV.

association which was demonstrated the breakup of antibody–antigen binding by glycine elution buffer (Fig. 5B). Same results were obtained for the binding of antibody 2 to *A. skirrowii* (Fig. 5C). The binding of *C. jejuni* to the antibody 2 is permanent, while the bindings of *S. typhimurium* and *A. skirrowii* to the antibody 2 are temporary. To let the system attain saturation, an average assay time of 45 min was used for each concentration.

3.4. Sensitivity tests using SPR

The signal (Δ RU) in the working and the control channels were almost the same up to suspensions containing 10^2 CFU/ml

of *C. jejuni*. When the concentrations of *C. jejuni* were greater than 10^3 CFU/ml, the working channel showed an increased response. This response may be due to non-specific binding to the sensor surface and instrument noise (Fig. 6). Significant differences ($p < 0.05$) were found between 10^3 and 10^2 CFU/ml, while no difference ($p > 0.05$) was observed between 10^1 and 10^2 CFU/ml. Hence, the sensitivity of the SPR biosensor was determined to be 10^3 CFU/ml of *C. jejuni* (Fig. 6). These results are more encouraging than the results obtained by other researchers who used SPR biosensor to detect other foodborne bacteria. Meeusen et al. (2001) demonstrated a sensitivity of 10^7 CFU/ml, for *E. coli* O157:H7 and *S. typhimurium* with direct avidin–biotin-characterized SPR biosensor assays. Fratamico et al. (1998) also obtained a sensitivity of 10^7 CFU/ml for *E. coli* O157:H7 using SPR sandwich assay with primary and secondary antibodies. Lower sensitivities of 10^6 CFU/ml, 10^4 CFU/ml, and 10^3 CFU/ml of *E. coli* were reported using a SPR biosensor based on polyethylene glycol terminated alkanethiol, a self-assembled monolayers with Protein G, and a sandwich-type assay, respectively (Subramanian et al., 2006). A self-assembled protein G layer based SPR biosensor was employed to detect *Legionella pneumophila* with a sensitivity of 10^5 cells/ml (Oh et al., 2003). A sensitivity of 10^6 CFU/ml has been reported for *Salmonella* serotype *enteritidis* and *Listeria monocytogenes* (Koubová et al., 2001).

Similarly, Leonard et al. (2004) demonstrated a sensitivity of 1×10^5 CFU/ml for *L. monocytogenes* with a subtractive inhibition based SPR biosensor assay. A recent fluorescence-based array biosensor developed at the Naval Research Laboratory is the only test able to detect 9.7×10^2 CFU/ml of *C. jejuni* within 25 min in artificially spiked foods (Sapsford et al., 2004). This array biosensor experiment used a sandwich assay and labeled antibodies, while our procedure with a direct assay and unlabeled antibodies is simpler for the detection of *C. jejuni*. More research is needed to understand if the variability in the sensitivity reported by different methods is due to the methodology itself or the different sample and target preparation protocols used in the studies.

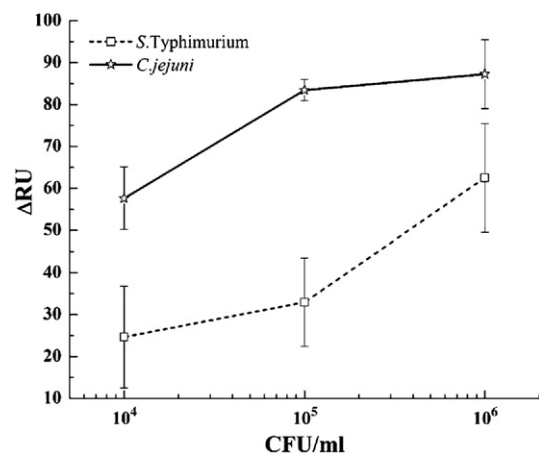


Fig. 4. Sensorgram showing the response units of antibody 2 bound to *S. typhimurium* and *C. jejuni* at serially diluted concentrations from 10^4 to 10^6 CFU/ml. Average of three independent experiments. Error bars represent ± 1 SEM.

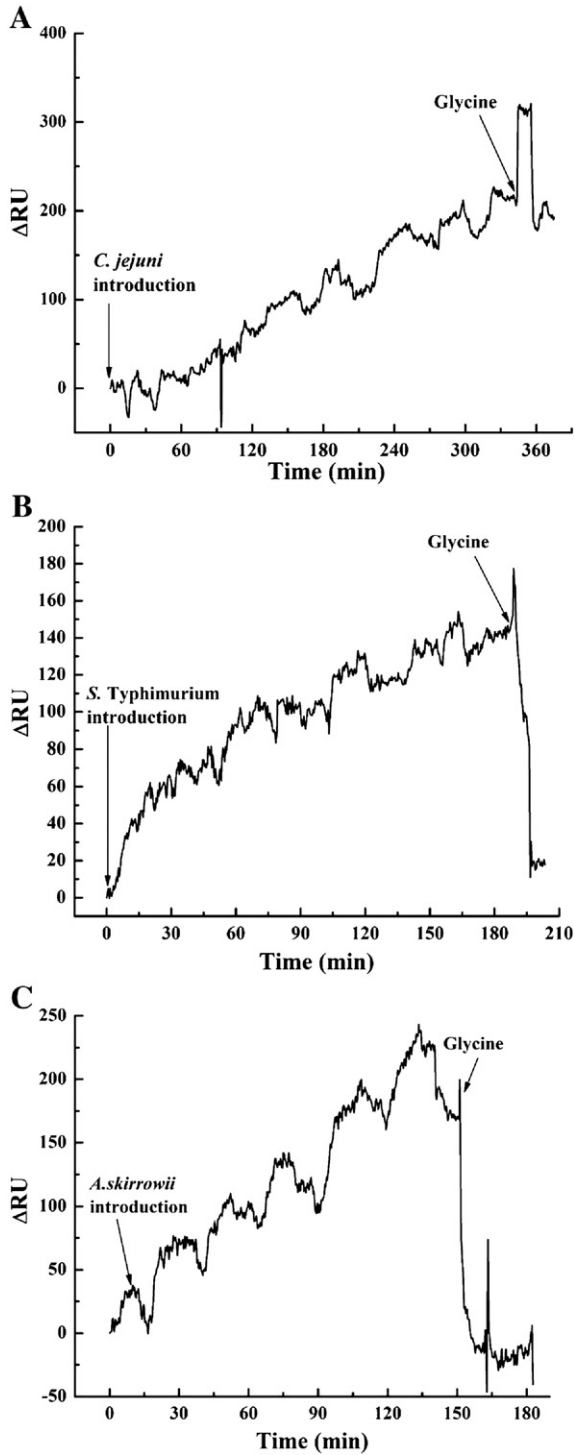


Fig. 5. Sensorgram showing the response units of antibody 2 bound to *C. jejuni* (A), *S. typhimurium* (B) and *A. skirrowii* (C) after glycine washing. Average of three independent experiments. The binding of *C. jejuni* to the antibody 2 is permanent, while the binding of *S. typhimurium* and *A. skirrowii* to the antibody 2 are temporary.

The most important merit of a biosensor is that the total time for analysis of a sample can be reduced to minute. In our experiments, the actual time necessary for the sample to be tested with the biosensor was approximately 45 min. We allowed the samples to stabilize more than what is required for

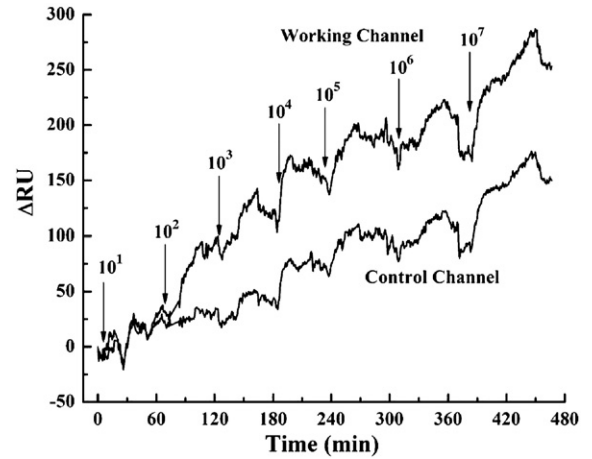


Fig. 6. SPR biosensor response to serially diluted (10^1 to 10^7 CFU/ml) *C. jejuni* in PBS. Both channels show the average response units of triplicate results. Arrows indicate the times when solutions were added in both channels.

conventional SPR sensing. Therefore, the final testing time could be reduced to less than 30 min. In an SPR biosensor, the effective evanescent wave field extension is around $0.3 \mu\text{m}$. Since the size of *Campylobacter* cell is varying in length from 0.5 to $5 \mu\text{m}$ and in width from 0.2 to $0.5 \mu\text{m}$, there is just part of the *Campylobacter* cell located within the most sensitive region of the evanescent wave field, which is bound on the sensor surface. The sensitivity of SPR biosensor could then be improved by increasing the penetration depth of evanescent wave field to locate the whole *Campylobacter* cell within this depth (Zourob et al., 2005), or by developing a capturing mechanism that target parts of the cell and are closer to the surface of the gold, such as DNA–DNA binding. A closer binding procedure would keep the target molecules within a depth of $0.3 \mu\text{m}$ (Spadavecchia et al., 2005).

3.5. Experiment with retail broiler meat

Although the SPR analysis of the tubes containing approximately 10^2 through 10^7 CFU/ml showed that the control

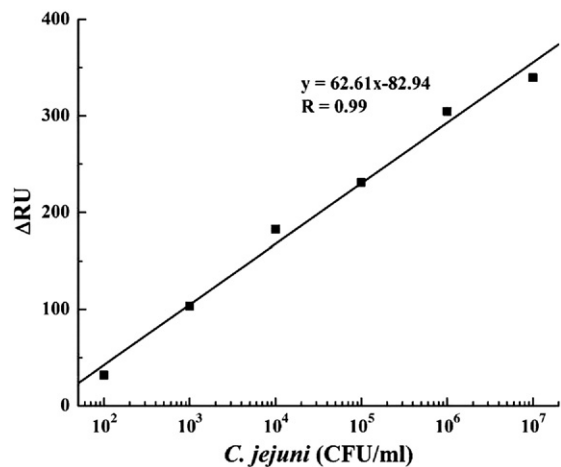


Fig. 7. Evaluation of *C. jejuni* detection by SPR biosensor on spiked rinse collected from retail broiler meat.

channel had a significant interfering background (data not shown), which was originated from the protein and lipid compounds of the broiler meat, the signal (ÄRU) in the working and the control channels were significant different when the concentrations of *C. jejuni* were more than 10^3 CFU/ml in solutions (Fig. 7). Therefore, the detection sensitivity of the SPR on inoculated samples in an individual experiment was 10^3 CFU/ml of *C. jejuni*. These results were consistent with the result obtained with pure cultures.

4. Conclusions

From four commercial antibodies against *Campylobacter*, Ab2 was chosen as the best for SPR experiments. The SPR biosensor demonstrated to be high specific for identification of *C. jejuni* and exhibited a sensitive of 10^3 CFU/ml in pure culture and broiler meat rinse spiked with *C. jejuni*. However, further investigation for SPR biosensor applications in the detection of *C. jejuni* on artificial spiked foods is required such as test of broiler meat rinse spiked with both *C. jejuni* and *Salmonella* or *Arcobacter*. Finally, a standard curve of SPR biosensor applied in detection of *C. jejuni* on the artificial spiked foods should be obtained for commercial applications. The sensitivity and specificity of SPR biosensor can be enhanced by improving the bioreceptor on SPR sensor platform, such as DNA/RNA biorecognition.

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