DEVELOPMENT OF A SURFACE PLASMON RESONANCE BIOSENSOR FOR

THE IDENTIFICATION OF *CAMPYLOBACTER JEJUNI*

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Dong Wei

Certificate of Approval:

_____	_____
Donald E. Conner Professor Poultry Science	Omar A. Oyarzabal, Chair Assistant Professor Poultry Science
_____	_____
Aleksandr L. Simonian Associate Professor Mechanical Engineering	Tung-Shi Huang Assistant Professor Nutrition and Food Science

_____

Stephen L.McFarland
Acting Dean
Graduate School

DEVELOPMENT OF A SURFACE PLASMON RESONANCE BIOSENSOR FOR THE IDENTIFICATION OF *CAMPYLOBACTER JEJUNI*

Dong Wei

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Dong Wei

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Signature of Author

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Date of Graduation

VITA

Dong Wei, son of Qinghe Wei and Heai Wang, was born on February 18, 1972, in Chongqing, China. He entered Shanghai Fisheries University, Shanghai, China, and graduated with a Bachelor of Engineering degree in Food Science and Technology in July 1995. After completing his undergraduate degree, he worked as a practicum teacher in his alma mater. He began graduate study in the Department of Fisheries & Allied Aquacultures at Auburn University, Alabama, USA, in January 2002, where he worked as a graduate research assistant, and graduated with a Master of Science degree in August 2004. He started to pursue a PhD degree in the Department of Poultry Science in Auburn University in 2004. For some family reason, he changed his program to M.S. and would earn another Master of Science degree in Food Safety in August 2006. He married Yueli Liu, PhD, daughter of Shixun Liu and Yulian Yang of Xuchang, Henan, China, in November, 2003. Their lovely daughter, Katherine L. Wei, was born on January 17, 2005 in Auburn, AL.

THESIS ABSTRACT

DEVELOPMENT OF A SURFACE PLASMON RESONANCE BIOSENSOR FOR THE IDENTIFICATION OF *CAMPYLOBACTER JEJUNI*

Dong Wei

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(B.S., Shanghai Fisheries University, China, 1995)

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Bacteria are the most common foodborne pathogens, which can cause common, distressing, sometimes life-threatening foodborne diseases to any human around the world, especially elderly, debilitated, compromised people (cancer, AIDS, and transplant patients) and infants. Therefore, rapid, specific, and sensitive routine monitoring and reliable detection and identification for bacterial pathogens are necessary in order to reduce their impacts upon human health.

Campylobacter is one of the most common foodborne pathogens, which can cause acute bacterial enteritis in humans throughout the world. The purpose of this study was to examine the sensitivity and specificity of commercial antibodies against *C. jejuni* for the development of a biosensor based on surface plasmon resonance. Six *Campylobacter*

strains and six non-*Campylobacter* bacterial strains were tested for reactivity with the antibodies. Antigen-antibody interactions were studied using enzyme-linked immunosorbent assay (ELISA) and a commercially available surface plasmon resonance (SPR) biosensor platform (Spreeta™). The reactivity to antibody of *Campylobacter* cells kept in phosphate buffer solution at 4 °C for up to 24 days were similar to the reactivity of 24-h-cultured cells. *Campylobacter* cells killed with 0.5% formalin had significant lower antibody binding reactivity when compared to live cells, or cells inactivated with 0.5% thimerosal or heat (70 °C for 3 min). The SPR biosensor showed low reactivity with *Salmonella* serotype Typhimurium and a sensitivity of 10^3 CFU of *C. jejuni* per ml. Although the average assay time was 45 min, this time could be easily shortened to an assay time of no more than 30 min. The sensitivity and specificity of SPR biosensor could be enhanced by incorporating a DNA-DNA biorecognition. This SPR biosensor could be further developed for rapid identification of *C. jejuni* in broiler products.

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Style manual or Journal used Biosensors and Bioelectronics.

Computer software used Microsoft Office 2003, Origin 7.5, SAS 9.1.

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1. LITERATURE REVIEW

1.1 Foodborne diseases

Foodborne diseases are caused by pathogens contained in foods. These kinds of disease are common, distressing, sometimes life-threatening disease, which can affect any human in the world, especially elderly, debilitated, immunodeficient people (cancer, AIDS, and transplant patients) and infants. The Centers for Disease Control and Prevention (CDC) estimates 76 million illnesses, 325,000 hospitalizations, and 5,200 deaths caused by foodborne pathogens in the United States each year (1). More than 250 foodborne diseases are known (2). The most widespread foodborne diseases are caused by recognized pathogens such as bacteria, viruses, multicellular animal parasites, protozoa, fungi and others (natural and manufactured chemicals). The common foodborne pathogens are briefly described in Table 1 (3, 4).

Bacteria are the most common foodborne pathogens, and according to the CDC, a total of 2,751 outbreaks of foodborne diseases that resulted in 86,058 people becoming ill have been reported for the US during 1993-1997 (5). Bacterial pathogens caused the largest percentage of outbreaks (75%) and the largest percentage of cases (86%) among all of the outbreaks (5). In 1996, an outbreak of *Escherichia coli* O157:H7 in Japan affected over 6,300 school children and resulted in 2 deaths. Two outbreaks of *Listeria monocytogenes* in France in 2000 and in the USA in 1999 were caused by contaminated

pork tongue and hot dogs respectively (6). An outbreak of *Campylobacter* is still under investigation, which occurred in New Richmond, Indiana in February, 2006 (7). The Economic Research Service (ERS) of the U. S. Department of Agriculture (USDA) estimates that the annual economic costs of medical care, productivity losses, and premature deaths due to foodborne illnesses caused by five major pathogens, *Campylobacter* spp., *Salmonella* (nontyphoidal serotypes only), *E. coli* O157:H7, *E. coli* non-O157:H7 Shiga toxin-producing strains, and *Listeria monocytogenes*, are $6.9 billions for the US (8).

1.2 Campylobacteriosis

Campylobacter is one of the most common foodborne pathogen, which can cause acute bacterial enteritis in humans throughout the world. More than 1 million people are estimated to be affected by *Campylobacter* in the US each year (7). The largest outbreak of *Campylobacter* enteritis occurred in a Vermont town where about 20% of the population (~2,000 persons) got infected in 1978 (14). The direct and indirect economic lost caused by *Campylobacter* enteritis has been estimated to be nearly $1 billion each year in the US alone (9).

The consistent and prominent clinical features of *Campylobacter* enteritis include fever, nausea and vomiting, abdominal cramps, headache, muscle pain, and diarrhea (often bloody). Although most of the *Campylobacter* infections are mild within gastrointestinal tract, extraintestinal symptoms including meningitis (10), cholecystitis (11), urinary tract infection (12), bacteremia, and septic arthritis (13) have been reported.

Complications that have been associated with *Campylobacter* infection include Reiter syndrome, which is a reactive arthropathy, and Guillian-Barré syndrome (GBS), which is a demyelating disorder resulting in acute neuromuscular paralysis (15). There is an estimated 120 to 360 deaths each year due to *Campylobacter* infections in the US (9). Death from *Campylobacter* infection is more common when it occurs in infants, the elderly, and patients with other diseases (e.g., cancer, liver disease, etc.).

Campylobacter species have been reported over more than a century. In 1886, Escherich observed organisms resembling campylobacter in stool samples of children with diarrhea. *Campylobacter* spp. was initially categorized as *Vibrio* spp. In 1909, *Vibrio* (now *Campylobacter*) *fetus* was first isolated from spontaneous abortions in livestock. In 1947, *Vibrio fetus* was first cultured from human blood. Since then, it was recognized as an opportunistic pathogen of debilitated patients. In 1957, Elizabeth King isolated *Vibrio* from blood samples of children with diarrhea (13). *Campylobacter*, as a new genus in the family *Spirillaceae*, was created to include *Vibrio fetus* and related organisms in 1963 (14). In 1972, clinical microbiologists in Belgium first isolated *Campylobacter* from stool samples of patients with diarrhea (18). When selective growth media were developed for culturing *Campylobacter* from human feces in the 1970s, *Campylobacter* spp. were established as common human pathogens (13).

There are 16 species and six subspecies of *Campylobacter* currently classified. Twelve of them are associated with human disease (15), especially *C. jejuni*, *C. coli*, *C. lari*, and *C. fetus* (14). More than 95% of the entire campylobacteriosis cases in the US are caused by *C. jejuni* and *C. coli* (16). Table 2 shows the current classification for the

genus *Campylobacter* (17).

Campylobacter are Gram-negative, microaerophilic bacteria with slender, spiral-shaped cells. They may have more than one helical turn. They also appear S-shaped and gull-wing-shaped when two cells form short chains. Coccoid-shaped Cells may predominate in old cultures (19). There is a single polar flagellum at one or both ends of the *Campylobacter* cell. The size of *Campylobacter* cells various in length from 0.5 to 5 µm and in width from 0.2 to 0.5 µm (20). All of the *Campylobacter* species can grow at 37 ºC. 42 ºC is the best growth temperature for *Campylobacter jejuni*. Although *Campylobacter* are sensitive to freezing, drying, acidic conditions (pH $\leq$ 5.0), and salinity, they are widely distributed in most warm-blooded animals and birds. Poultry is one of the major reservoirs of *Campylobacter* in food animals. Undercooked meats and meat products, raw or contaminated milk, contaminated water or ice are recognized as primary sources of infection in humans, with as few as 500 CFU of *Campylobacter* needed to cause disease (14).

Due to the prevalence of *Campylobacter* species in the food supply, routine monitoring and reliable detection and identification for these pathogens are necessary in order to reduce their impact upon human health.

1.3 Detection methods for bacterial pathogens

1.3.1 Conventional microbiological methods

These methods were developed more than a century ago (21). The general procedure of these methods includes enrichment, isolation, and confirmation. Usually,

enrichment steps consist of preenrichment, which is performed by the blending of the food product with a selective enrichment medium to allow growth of all organisms, and secondary enrichment, which allows growth of the organism under investigation and increase bacterial population to a detectable level. The isolation step is completed by using selective agar plates to get pure cultures. The final identification of a particular pathogenic organism can be achieved by performing some serological and biochemical tests (22).

Campylobacter species are microaerophilic bacteria. Therefore, traditional detection methods for these pathogens do not only need selective medium, but they also need to be incubated under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2). Currently, quite a few conventional cultural methods for enumerating and detecting *Campylobacter* are available such as enrichments, most probable number, direct plating (27, 28), thin agar layer (24), drop plate method (25), and Kapadnis–Baseri medium method (26).

Although conventional cultural methods achieve the most reliable and highly sensitive pathogen detection, they are laborious, costly, and time consuming. According to the Microbiology Laboratory Guidebook (3rd edition, 1998) from USDA FSIS, at least five days are needed for detecting *Campylobacter jejuni*/*coli* from meat and poultry products (23). Thus, novel detection methods, which can reduce time without compromising speed, specificity, and sensitivity, are necessary for improving the traditional method.

1.3.2 PCR-based detection methods

Because small quantities of the genetic material (DNA/RNA) of the target antigens can be amplified by the polymerase chain reaction (PCR) technique within 1 h, PCR-based assays have been developed for rapid detection of *Campylobacter* species currently (35-39). Although PCR-based methods are rapid, specific and sensitive, the main disadvantages of these methods are labor intensity, need for skilled personnel, use of harmful chemicals such as ethidium bromide, and high cost. Also, PCR-based analysis must be performed under highly controlled conditions to obtain reliable and consistent results (39).

1.3.3 Immunological detection methods

Antigen and antibody reactions have been used as capture mechanism for detection of microorganisms and their components in medical and diagnostic microbiology for decades. Antibodies are produced in the immunesystem of human and animals when foreign particles (antigen) invade their body system. Each antibody can be specific to one particular antigen. Hence, antibodies can be used for detecting the corresponding antigen. There are several immunological detection methods reported that have been used for detecting *Campylobacter* spp., such as enzyme-linked immunosorbent assays (ELISA), latex agglutination (29), immunomagnetic separation (32, 33), specific colony-lift immunoassay (30), and hydrophobic grid membrane filters enzyme immunoassay (31).

ELISA tests are one of the most prevalent immunological detection methods in

foods, and have evolved on the late 1960s from radioimmunoassay. Briefly, after antibodies coupled with indicators (enzymes linked to dyes) bind to target antigens, an enzyme-catalysed color change can be measured by monitoring the change in absorbance. Currently, four types of ELISA are commonly used, which are termed as indirect, direct competitive, avidin-biotin complex, and antibody sandwich ELISA (34). Although there are some combined PCR-immunological based detection methods developed for detecting *Campylobacter* species, drawbacks still exist such as time consuming (~8 h - 3 days), high cost and labor intensity (64-66). Real-time and on-site detection methods for foodborne pathogens need to be developed for commercial applications.

1.3.4 Biosensor detection methods

Biosensors are real-time detection method. A biosensor is made up of an analytical device composed of a biological recognition element coupled to a physicochemical transducing microsystem, which generates an electronic signal to detect a biological or chemical agent (40). Basically, the target organisms are recognized by a biological recognition element, and then according to the particular characteristics of certain physicochemical transducer, an electronic signal can be monitored. The signal can correspond to the concentration of the target organisms and therefore the biosensor can be quantitative and determine the amount of the analyte in the sample (Fig 1.1).

Biosensor can be classified into various basic groups in terms of the different biorecognition elements and physicochemical transducers (41). Compared to other detection methods, biosensors with several outstanding merits are very promising in the

commercial market of detection methods for medical, environmental, military, and food purposes. The best merit of a biosensor is that the total detection time is shortened from days or hours to minutes. In addition, there are no reagents or labels needed for many of biosensors. Because the two main components of biosensors (bioreceptor and transducer) are integrated into one single sensor, on-site detection can be accomplished. The immobilized biological recognition element such as enzyme or antibody can be regenerated and reused for continuous or multiple detections (42).

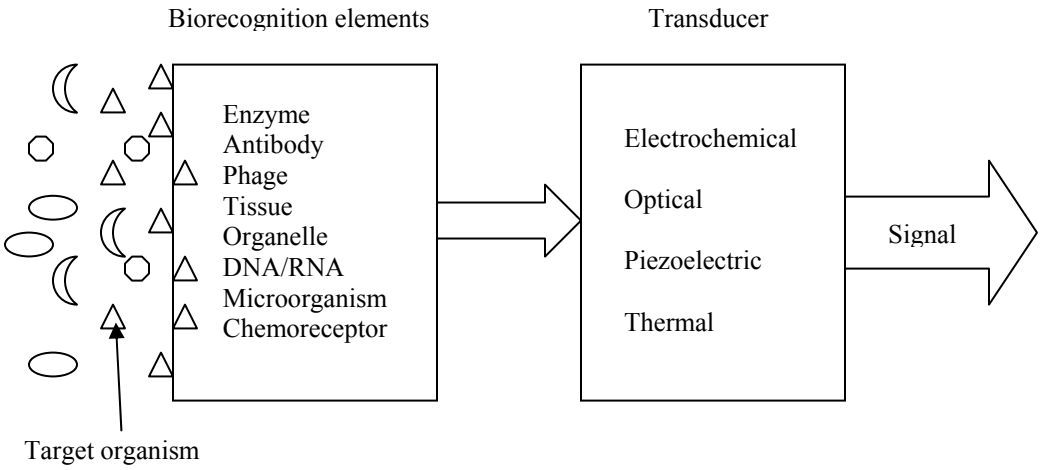

Fig. 1.1: Components and categories of biosensor.

More and more applications of biosensor methods have been reported lately for the detection of bacterial pathogens. With new technologies, biosensors may incorporate higher sensitivity for commercial real-time, portable pathogen detection. Surface plasmon resonance (SPR), evanescent-wave fiber optic biosensor, and PCR-acoustic wave sensor have been used for detecting *E. coli* O157:H7 (43-45). Immunochemical potentiometric

alternating biosensor and amperometric tyrosinase-based biosensor were used for detecting *E. coli* (49, 50). A SPR biosensor was reported to detect *Salmonella* Enteritidis and *Listeria monocytogenes* (46, 51, 52) and *Staphylococcal* enterotoxin B (SEB) in milk (54). Quartz crystal acoustic wave biosensor has been shown to detect *Salmonella* Typhimurium (47). *Aeromonas hydrophila* was identified by the method of DNA piezoelectric biosensor assay (48). Sapsford et al. (53) reported an array biosensor, which is based on the principle of total internal reflection fluorescence (TIRF), to detect *Campylobacter* and *Shigella* species.

1.4 SPR biosensor

In 1971, SPR was initially suggested as practical and common method to study organized monolayer and multilayer organic compounds on metal (such as silver and gold) surfaces. SPR was primarily demonstrated as a suitable method to detect gas and biomolecular in 1982 (55). Since then, SPR biosensors have been developed as one of the most sensitive optical biosensors widely applied in medical, environmental, drug, food, and military applications (55).

1.4.1 Principles of SPR biosensor

SPR is based on the total internal reflection (TIR) optical phenomenon. When the incident light is traveling within the more optically dense medium (refractive index n_1) towards the less optically dense medium (refractive index n_2), the ray of light is bent away from the normal line to the boundary ($\theta_2 > \theta_1$). The exit angle (θ_2) can approach 90º

when the incident angle (θ_1) increases to certain critical angle (θ_c). Once incident angles (θ_1) are equal to or greater than the critical angle (θ_c), the light will be totally internally reflected (Fig 1.2).

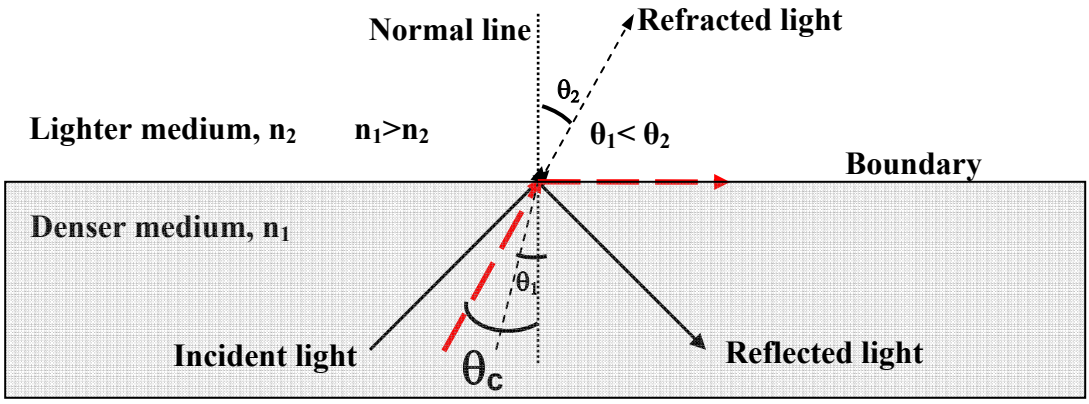

Fig. 1.2. Total internal reflection (TIR) of light.

Although the incident light is totally reflected, the electromagnetic field component of the incident light penetrates a short distance (tens of nanometers) into the less optically dense medium creating an exponentially decaying evanescent wave (Fig 1.3).

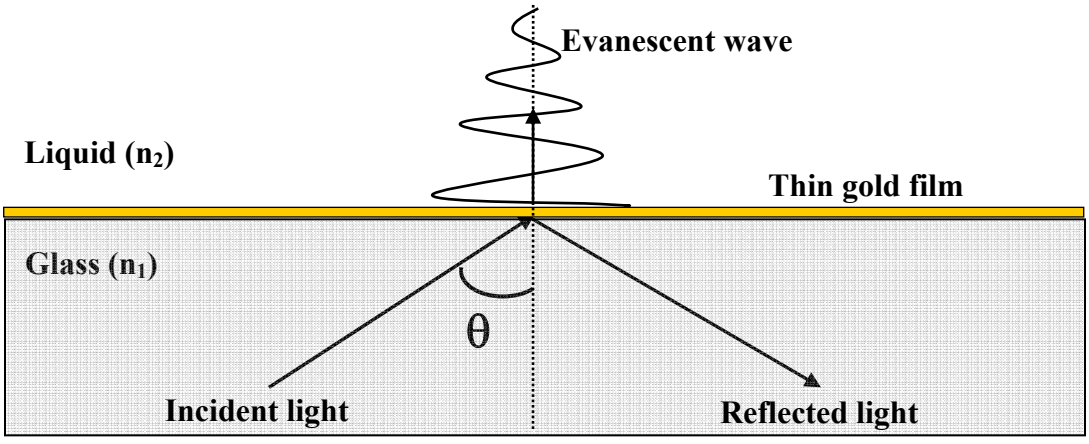

Fig. 1.3. Evanescent field.

If the incident light is monochromatic and plane-polarized, and a thin film of metal (gold) is coated at the interface between the two different optically dense media inside the evanescent wave, the photon of the evanescent wave will resonance with free oscillating electrons (plasmons) in the metal film. A sharp decrease of the intensity of the reflected light can be measured at a specific incident angle termed resonance angle, which is greater than the critical angle (θ_c), due to the resonance energy transfer from evanescent wave to surface plasmons. This resonance angle is just dependent on the refractive index of the medium close to the metal film surface. When capturing macromolecules (antibodies) at the surface bind to target molecules (antigen), the resonance angle is changed due to the changes of that refractive index. There is a linear relationship between the shift of the resonance angle and the concentration of the bound target molecules on the metal film surface. According to this linear relationship, the analyte and ligand association and dissociation can be observed (56). The principle and typical signal of SPR are shown in Fig 1.4 and Fig 1.5 (58).

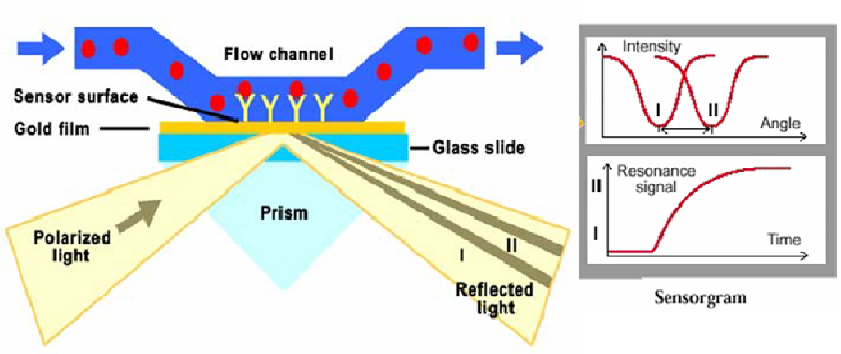

Fig. 1.4. The working principle of SPR method.

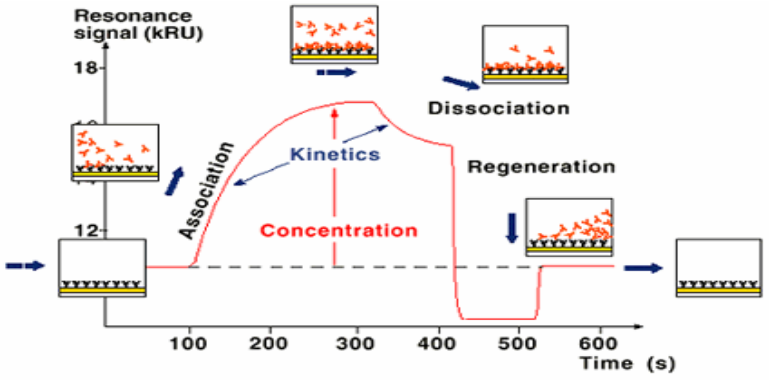

Fig. 1.5. Typical signal from the SPR measurement.

1.4.2 SPR biosensor for detecting bacterial pathogens

SPR biosensors can be used to characterize binding reactions of any biological system from proteins, oligonucleotides, oligosaccharides, lipids, phages, viruses, bacteria, and cells (59). Several configurations of SPR are in development, which include SPR fiber optic probes, SPR planar probe sensors, multichannel sensing devices and combination of SPR sensor with other methods, such as anodic stripping voltammetry and critical angle refractometry (57). SPR biosensors are becoming commonly used in real-time detection of bacterial pathogens.

The first bacterial pathogen identified by SPR biosensor was *Eschirichia coli* O157:H7. Fratamico et al. (45) used an angular-modulation commercial SPR biosensor (BIAcore) and a sandwich assay to detect *E. coli* O157:H7. They used monoclonal antibodies immobilized on the sensor surface for capturing *E. coli* and polyclonal secondary antibodies for enhancing the specific sensor response. The sensitivity of this sandwich assay SPR biosensor for detecting *E. coli* O157:H7 was 5×10^7 CFU/ml. Meeusen et al. used Spreeta^{TM} SPR biosensor and biotinylated antibodies to get a similar

detection limit, 10^7 CFU/ml, for *E. coli* O157:H7 and *Salmonella* Typhimurium (60, 61). An alkanethiol self-assembled monolayer based SPR biosensor and sandwich assay with monoclonal and polyclonal antibodies were conducted by Subramanian et al. for detecting *E. coli* O157:H7 in 2005. Direct detection, protein G detection, and sandwich detection were investigated, and the lowest detection limits for these three methods were 10^6, 10^4 and 10^3 CFU/ml, respectively (62).

Seo et al. used direct and sandwich assay of SPR biosensor to get the lowest detection limits of 1×10^7 and 1×10^5 CFU/ml, respectively for detecting *Salmonella* Typhimurium (63). Similar results were obtained for *Salmonella* by Bokken et al. (52). A 10^6 CFU/ml of the lowest detection limit was demonstrated for *Salmonella* Enteritidis and *Listeria monocytogens* by Koubová et al who used a laboratory wavelength-modulated SPR biosensor and monoclonal antibodies (46). Similarly, a detection limit of 1×10^5 CFU/ml was demonstrated for *Listeria monocytogens* by Leonard et al. (51). A time of less than 30 min is what the SPR biosensor needed to obtain positive result.

The purpose of this study was to develop a surface plasmon resonance biosensor for the identification of *Campylobacter jejuni*. In chapter II, enzyme-linked immunosorbent assay (ELISA) method was used to examine the sensitivity and specificity of commercial polyclonal antibodies against *C. jejuni* for further SPR biosensor developments. In chapter III, SPR biosensor was employed to examine the sensitivity and specificity of commercially polyclonal antibodies against *C. jejuni* using in pure cultures. Our final goal is to develop a SPR system for rapid identification of *C. jejuni* in boiler products.

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Table 1. Classification of foodborne pathogens.

Flatworms
- Flukes
  - *Fasciola*
  - *Fasciolopsis*
  - *Paragonimus*
  - *Clonorchis*
- Tapeworms
  - *Diphyllobothrium*
  - *Taenia*

Roundworms
- *Trichinella*
- *Ascaris*
- *Anisakis*
- *Pseudoterranova*
- *Toxocara*

Protozoa
- *Giardia*
- *Entamoeba*
- *Toxoplasma*
- *Sarcocystis*
- *Cryptosporidium*
- *Cyclospora*
- *Acanthamoeba*
- *Microsporidia*

Fungi-mycotoxin producers
- *Aflatoxins*
- *Fumonisins*
- *Alternaria toxins*
- *Ochratoxins*

Toxigenic phytoplanktons
- *Paralytic shellfish poison*
- *Domoic acid*
- *Pfiesteria piscicida*
- *Ciguatoxin*

Bacteria
- Gram positive
  - *Staphylococcus*
  - *Bacillus cereus*
  - *B. anthracis*
  - *Clostridium botulinum*
  - *C. perfringens*
  - *Listeria monocytogenes*
  - *Mycobacterium*
- Gram negative
  - *Salmonella*
  - *Shigella*
  - *Eschirichia*
  - *Yersinia*
  - *Vibrio*
  - *Campylobacter*
  - *Arcobacter*
  - *Aeromonas hydrophila*
  - *Helicobacter pylori*
  - *Legionella spp.*
  - *Brucella*
  - *Plesiomonas*
  - *Cyanobacteria*

Viruses
- *Adenoviruses*
- *Astrovirus*
- *Coxsackieviruses*
- *Echoviruses*
- *Enteroviruses*
- *Hepatitis A*
- *Norwalk/Caliciviruses*
- *Rotaviruses*

Prions
- Creutzfeldt-jakob disease (new variant form)

Table 2. *Campylobacter* classification.

Campylobacter jejuni	*Campylobacter concisus*
C. jejuni subsp. *jejuni*	*Campylobacter upsaliensis*
C. jejuni subsp. *doylei*	*Campylobacter curvus*
C. jejuni subsp. *fetus*	*Campylobacter rectus*
Campylobacter coli	*Campylobacter helveticus*
Campylobacter lari	*Campylobacter lanienae*
Campylobacter fetus	*Campylobacter mucosalis*
C. fetus subsp. *fetus*	*Campylobacter showae*
C. fetus subsp. *venerealis*	*Campylobacter hominis*
Campylobacter hyointestinalis	*Campylobacter gracilis*
C. hyointestinalis subsp. *lawsonii*	*Campylobacter sputorum*
C. hyointestinalis subsp. *hyointestinalis*	*C. sputorum* subsp. *bubulus*
	C. sputorum subsp. *sputorum*

II. IMMUNOREACTIVITY OF COMMERCIAL ANTIBODIES AGAINST *CAMPYLOBACTER* SPP.

2.1 Introduction

Campylobacter is an important cause of acute bacterial gastroenteritis in humans worldwide (1, 2). *Campylobacter* infections can also cause Reiter syndrome, a reactive arthropathy, and Guillian-Barre syndrome, an acute neuromuscular paralysis (13). From 1996 to 2000, there were an estimated 2.4 million *Campylobacter* infections each year, with 21.9 cases per 100,000 people reported in the US (6). Although the trend of *Campylobacter* infections appears to be downward, there were still 5,215 *Campylobacter* infections reported for 2003 that represented an incidence of 12.6 per 100,000 people (3). There is an estimated 120 to 360 deaths each year due to *Campylobacter* infections in the United States. The direct and indirect economic lost caused by *Campylobacter* enteritis has been estimated nearly $1 billion each year in the US alone (13).

Poultry is one of the major reservoirs of *Campylobacter* in food animals. Since undercooked meats and meat products, raw or contaminated milk, contaminated water or ice are recognized as primary sources of infection in humans, with as few as 500 CFU of *Campylobacter* needed to cause disease (14), specific and sensitive methods are necessary to both enumerate and detect these pathogens in food products. Enzyme-linked immunosorbent assay (ELISA) test is one of the most prevalent immunological

detection methods in foods, and has evolved in the late 1960s from radioimmunoassay. Briefly, after antibodies coupled with indicators (enzymes linked to dyes) bind to target antigens, an enzyme-catalysed color change can be measured by monitoring the change in absorbance. Currently, four types of ELISA are commonly used as shown in Fig. 2.1, which are termed indirect, direct competitive, avidin-biotin complex, and antibody sandwich ELISA (10). Avidin-biotin complex ELISA is used for current project.

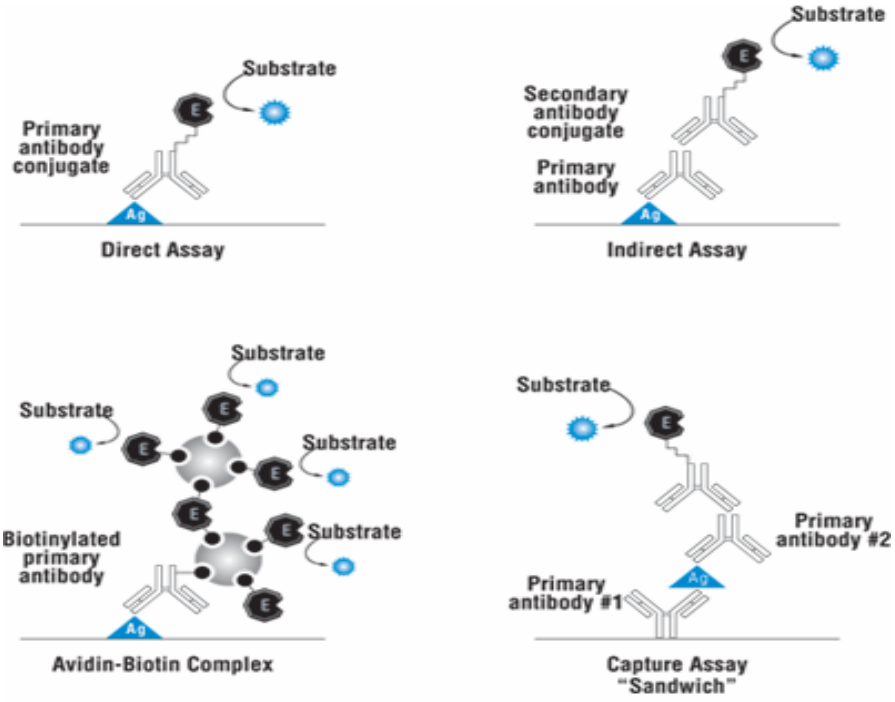

Fig. 2.1. Four types of immunoassay.

The objective of the present study was to examine the sensitivity and specificity of commercial polyclonal antibodies against *C. jejuni* conducted by enzyme-linked immunosorbent assay (ELISA) for further SPR biosensor developments.

2.2 Materials and methods

2.2.1 Bacterial strains and culture conditions

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. Strains were identified by using API Campy tests (bioMerieux, Hazelwood, MO) and RiboPrinter® (DuPont Qualicon, Wilmington, DE).

Two *Arcobacter* strains, *A. butzleri* ATCC 49616 and *A. skirrowii* ATCC 51399, three *Salmonella* serotypes, *S.* Enteritidis ATCC 13076, *S.* Typhimurium ATCC 13311, and *S.* Heidelberg isolated from chickens (8), 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. Sub-cultures of *Campylobacter* and *Arcobacter* strains were grown on the modified Campy Cefex (mCC) plates under microaerophilic condition (5% O_2, 10% CO_2 and 85% N_2), incubated at 37°C for 24 hours and 35°C for 48 hours, respectively. Sub-cultures of *Salmonella* strains were grown on brilliant green sulfa agar plates, and *E. coli* 48-2 strain was grown on MacConkey agar plates, incubated at 37°C for 24 hours before use.

All cultures were prepared by transferring colonies from plates into phosphate buffer saline (PBS). Centrifugation/washing procedure was used three times. The optical densities (OD) of suspensions were adjusted to between 1.700A and 2.000A at 600 nm

wavelength (~10^8 - 10^9 CFU/ml). The antigen concentrations were confirmed by plate counting after 24 h. Before use, all of the antigen suspensions were stored at 4°C overnight.

2.2.2 Antibodies against Campylobacter

Four commercial antibodies against *Campylobacter* were used as primary antibody in ELISA method.

- Ab1: polyclonal rabbit antibody to *C. jejuni* ATCC 29428, biotin conjugated (Biodesign International, Saco, Maine).
- Ab2: polyclonal rabbit antibody to *C. jejuni* ATCC 29428, biotin conjugated (Biogenisis Ltd., Brentwood, New Hampshire).
- Ab3: polyclonal rabbit antibody to *C. jejuni* ATCC 29428, biotin conjugated (Biotrend Chemikalien Gmbh, Koln, Germany).
- Ab4: polyclonal rabbit antibody to *C. jejuni*, biotin conjugated (Fitzgerald Industries International, Inc., Concord, Massachusetts).

2.2.3 ELISA procedures

A 96-well microplate (polystyrene plate, Costar, Cambridge, MA) was coated with 100 µl of antigen suspensions per well and incubated at 37°C for 1 h. The wells were washed three times with PBS with 0.1% Tween 20 and 0.02% sodium azide (PBST) and blocked with 200 µl of 1% bovine serum albumin and 0.01% sodium azide in PBS (BSA-PBS) at 37°C for 1 h. After washing the microplate three times with PBST, 100 µl

of diluted primary antibodies (Ab) were added to the wells and the plate was incubated at 37°C for 2 h. Following another three-time washing with PBST, 100 µl of diluted (1:5000) streptavidin-alkaline phosphatase conjugated Ab (Pierce Biotechnology, Inc. Rockford, IL) were added to the wells and incubated at 37°C for 1 h. The wells were subsequently washed five times with PBST. After 100 µl of *p*-nitrophenyl phosphate (Sigma, St. Louis, MO) substrate solution was added to each well, the plate was incubated at room temperature for 15 min in dark for color development. The absorbance was measured at 405 nm by ELISA microplate reader (ThermoLabsystems, Helsinki, Finland), and recorded for analysis. For the non-specific control, no antigens were coated to the wells of the microplate while blanks were performed using the substrate p-NPP on uncoated wells.

2.2.4 Specificity of commercial antibodies using ELISA

Optical densities were obtained at 405 nm of wavelength with four commercial antibodies using ELISA. An antigen solution containing 10^9 CFU/ml was prepared from each bacterium. Two trials were conducted to determine the specificity of commercial antibodies against *C. jejuni*. *C. jejuni* ATCC 35918, *C. coli* ATCC 43473, *C. lari* ATCC 35223, *A. butzleri* ATCC 49616, and *A. skirrowii* ATCC 51399 were used in the first trial. *C. jejuni* ATCC 35918, *E. coli* 48-2, *S.* Enteritidis ATCC 13076, *S.* Typhimurium ATCC 13311, and *S.* Heidelberg were used in the second trial.

To determine the antigen reactivity and antibody titers, *C. jejuni* ATCC 35918 at concentration of approximately 10^9 CFU/ml was examined with two antibodies by

ELISA. The antigen suspensions were serially diluted (1:10) from 10^9 to 10^6 CFU/ml in PBS. Antibodies were serially diluted in PBS to 1:25, 1:50, 1:100, 1:200, 1:400, 1:800, and 1:1600.

2.2.5 Reactivity of 24-h, 10-day and 24-day cultures, and of live vs. dead cells using ELISA

C. jejuni strains (ATCC 35918, post 5, CDC 370 and CDC 410) were tested with Ab2. Suspensions of 10^8 CFU/ml were kept in refrigeration for 24 h, 10 day and 24 days. To compare the reactivity between live and dead cells, *C. jejuni* ATCC 35918 (~10^9 CFU/ml) was tested with Ab2. Three different methods were used to kill *C. jejuni* cells: 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. Both live and dead cell suspensions were stored at 4°C overnight before testing.

2.2.6 Statistical Analysis

Triplicate experiments were run for every ELISA experiment in this study. 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 of the

results.

2.3 Results and discussion

2.3.1 Specificity of Commercial Antibodies

According to the information obtained from the manufacturing companies, all antibodies were polyclonal that were made against *C. jejuni*. There were no significant differences ($p > 0.05$) in the reactivity among four commercial antibodies against *C. jejuni* ATCC 35918. Fig. 2.2 shows that the highest reactivity was obtained with *C. jejuni* ATCC 35918. There was also an expected low reactivity with *Campylobacter* spp. other than *C. jejuni*, and with *Arcobacter* spp., a closely related genus that belongs to the same Family (7). Fig. 2.3 shows that Ab4 had cross-reactivity with *A. skirrowii* ATCC 51399 ($p > 0.05$), while Ab2 is specific for *C. jejuni* ATCC 35918. Since Ab1 was discontinued by manufacturer, and Ab3 was imported from Germany. Ab2 was selected for further experiment as the most economical choice. In addition, the best reactivity was obtained with *C. jejuni* ATCC 35918 and Ab2. The probable reason for these results is that the immunogen used to prepare Ab2 was *C. jejuni* ATCC 29428. Other *C. jejuni* species isolated from humans and processed broiler carcasses showed lower reactivity with the same antibody (Fig. 2.5 A). These results highlights the need of preparing specific antibodies using the strains most commonly found in the samples that will be targeted for testing.

Fig. 2.4 shows the sensitivity of serially diluted Ab2 against *C. jejuni* ATCC 35918 using ELISA. There is no significant difference on the reactivity among serially

diluted antibodies of 1:50, 1:100, and 1:200. Therefore, the 1:200 dilutions are the most economical choice as antibody titer. The reactivity obtained with *C. jejuni* cultures with less than 10^7 CFU/ml were very low.

2.3.2 Reactivity of 24-h, 10-day and 24-day cultures, and of live vs. dead cells using ELISA

These experiments were intended to determine if the reactivity of the antigens remain stable over time, and if inactivated or dead cells are still reactive. There were no significant differences ($p > 0.05$) in the absorbance among solutions of different ages (Fig. 2.5 A). The shapes of the cells changed over time, with more coccoid shapes noticed in the oldest cultures (Fig. 2.5 B-3). However, the reactivity of *Campylobacter* cells kept at 4^oC in PBS using ELISA was similar for up to 24 days. These results suggest that after the collection of naturally contaminated samples with buffered peptone water, the reactivity of the target cells could be maintained for an extended period as long as the samples are immediately processed in an acceptable buffering solution and are kept at refrigeration temperatures. Many poultry processing plants are hours away from a fully-equipped microbiology laboratory and therefore improvements in the handling of the samples will results in more opportunities for *Campylobacter* detection in broiler products.

Differences existed ($p < 0.05$) on the immunoreactivities of cells inactivated with formalin and cells inactivated with thimerosal or heating (Fig. 2.6). Formalin reduced the reactivity of inactivated *C. jejuni* cells, while inactivation with thimerosal and heating did

not. It may be possible that formalin might lower the ability of the epitopes on the surface of the *C. jejuni* cells and flagella to bind with antibodies (12). Conversely, heat (70°C for 3 min) and 0.5% thimerosal may not change substantially the binding between *C. jejuni* and antibodies.

2.4 Conclusions

Four commercial antibodies against *Campylobacter jejuni* were tested with ELISA for sensitivity and specificity. Ab4 cross-reacted with *Arcobacter skirrowii* ATCC 51399. Since Ab1 was discontinued by manufacturer, and Ab3 was imported from Germany. Ab2 was selected for further experiment as the most economical choice. There is no significant difference on the reactivity among serially diluted antibodies of 1:50, 1:100, and 1:200. Therefore, the 1:200 dilutions are the most economical choice as antibody working dilution. The reactivity obtained with *C. jejuni* cultures with less than 10^7 CFU/ml were minimal. The reactivities of *Campylobacter* cells kept in PBS at 4^oC are similar for up to 24 days, which means that contaminated food products obtained from plants can be transported to close laboratory with positive results. Reactivity of *Campylobacter* can be reduced by adding 0.5% formalin while adding 0.5% thimerosal and heat (70°C for 3 min) can not.

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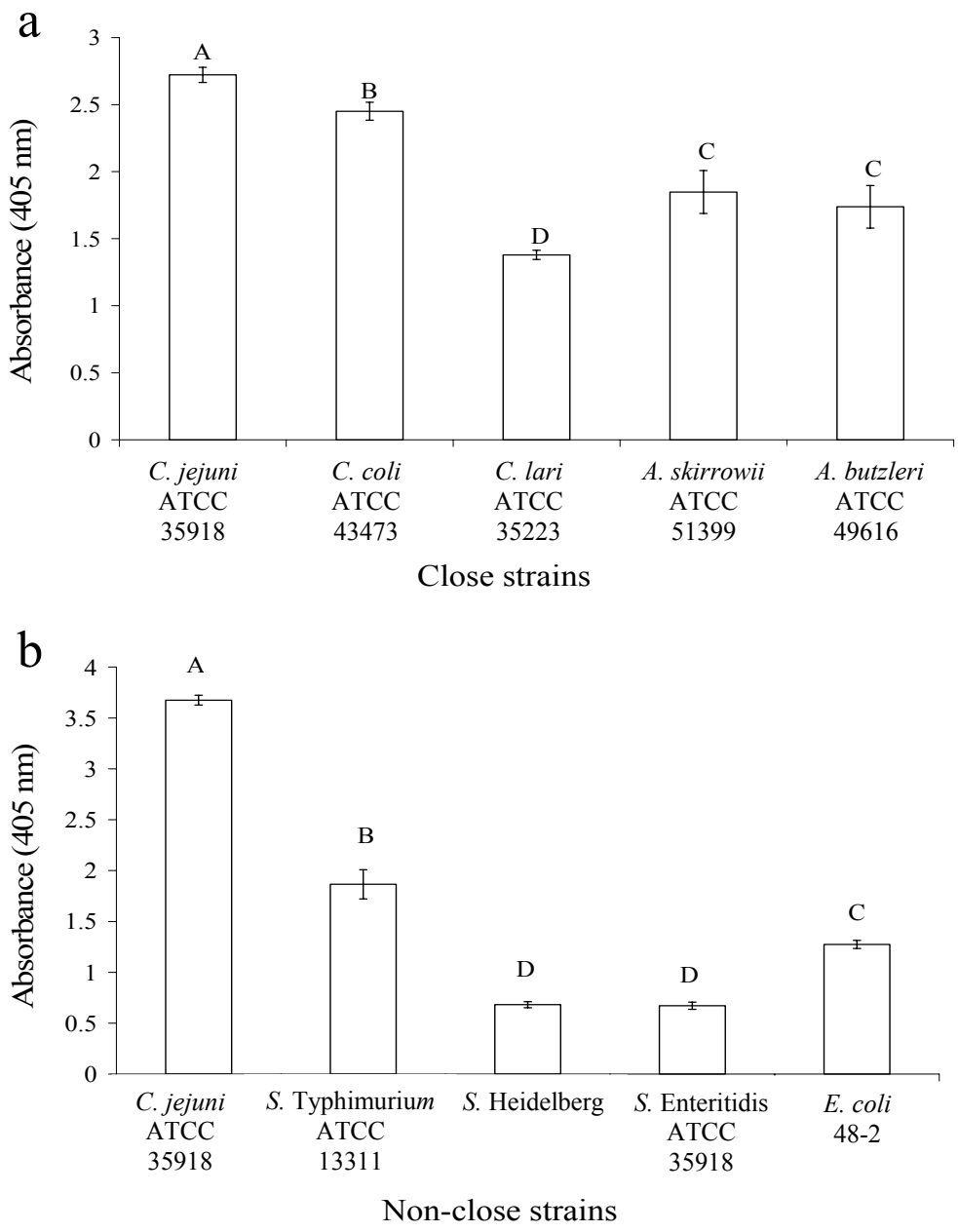

Fig. 2.2. Specificity of four commercial antibodies against *C. jejuni*. Average optical densities of triplicate experiments with ELISA for a (*Campylobacter*, *Arcobacter*), and b (*Campylobacter*, *Salmonella,* and *E. coli*). Means with different letters are significant different ($p < 0.05$). Error bars represent ±1 SEM.

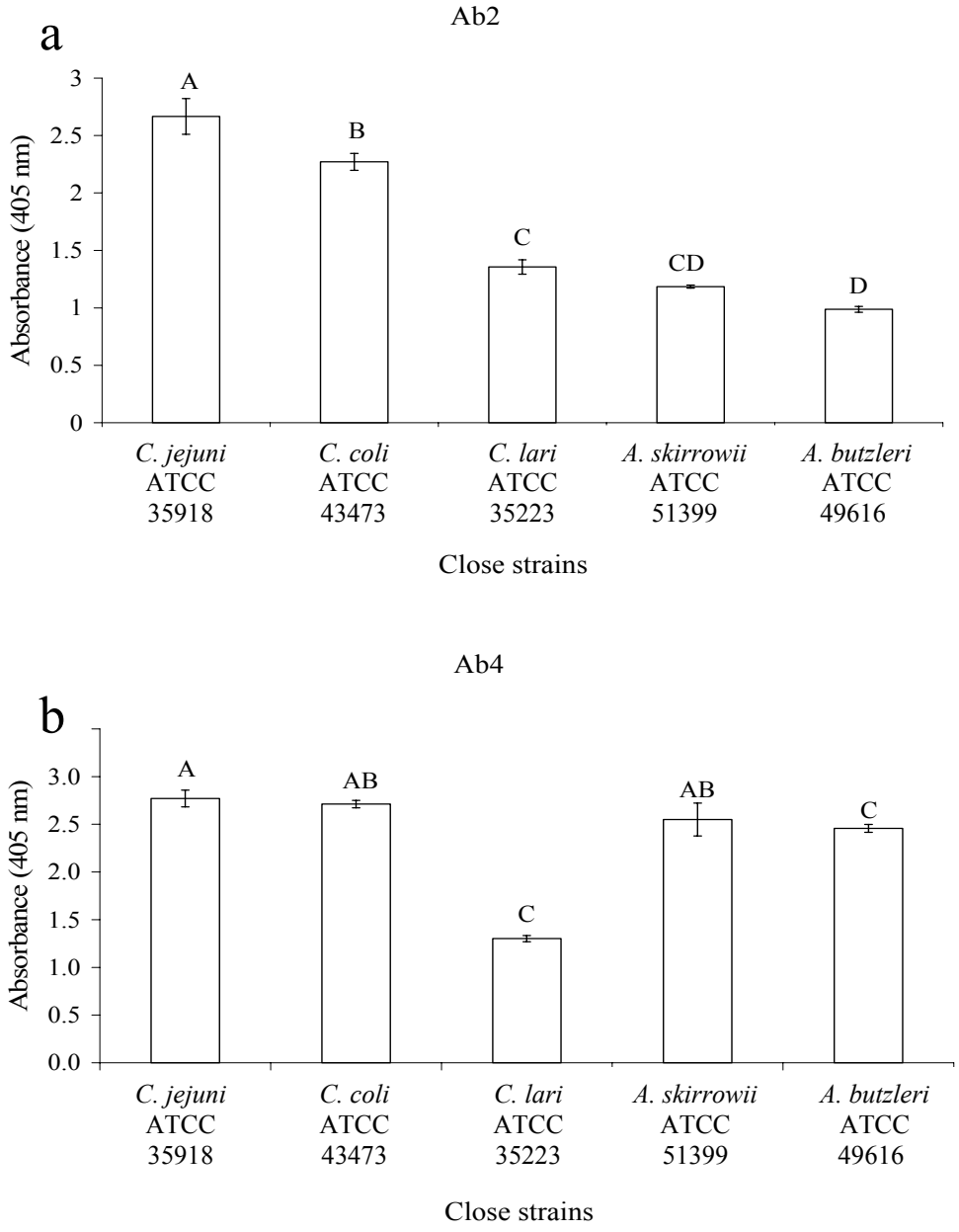

Fig. 2.3. Reactivity values of Ab2 (a) and Ab4 (b) against *Campylobacter* and *Arcobacters* strains with ELISA. Means with different letters are significant different ($p < 0.05$). Error bars represent ±1 SEM.

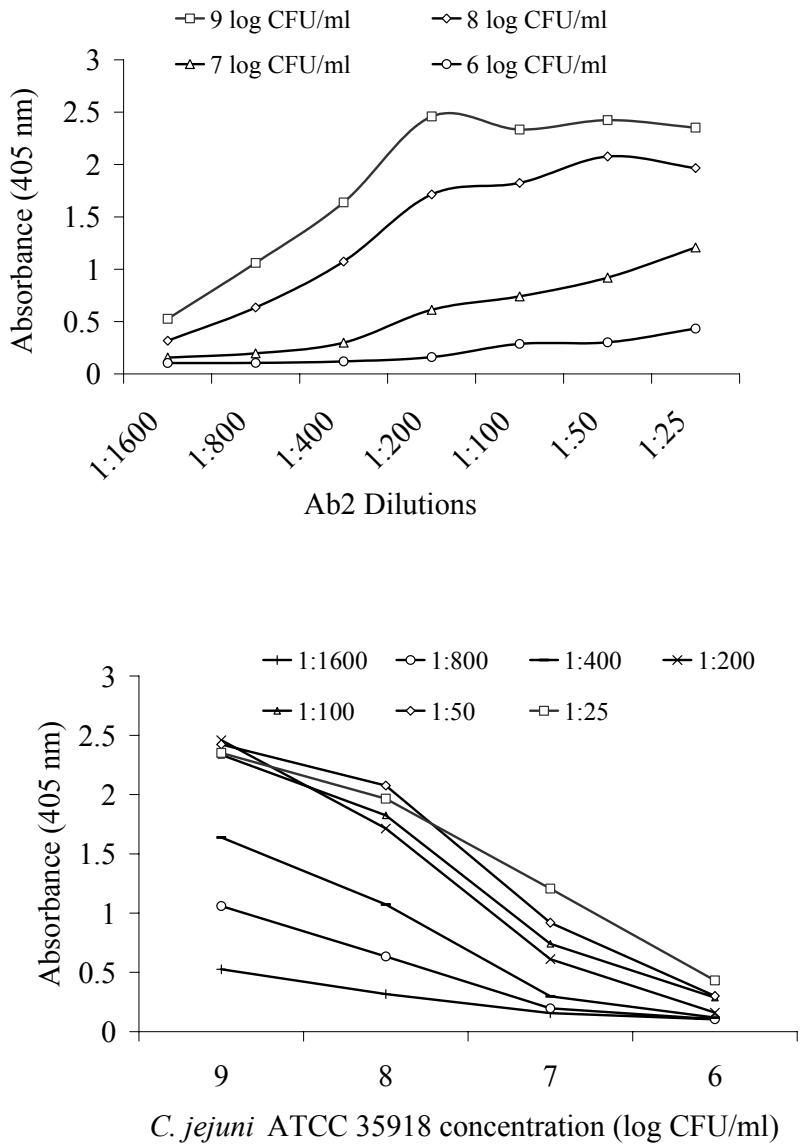

Fig. 2.4. Optical densities determined in ELISA experiments of the reactivity of serial concentrations of *C. jejuni* ATCC 35918 with serially diluted Ab2. 1:200 of antibody dilution can be selected as the most economical choice for detecting *C. jejuni*. There is low reactivity (ELISA) when *C. jejuni* concentration is lower than 10^7 CFU/ml.

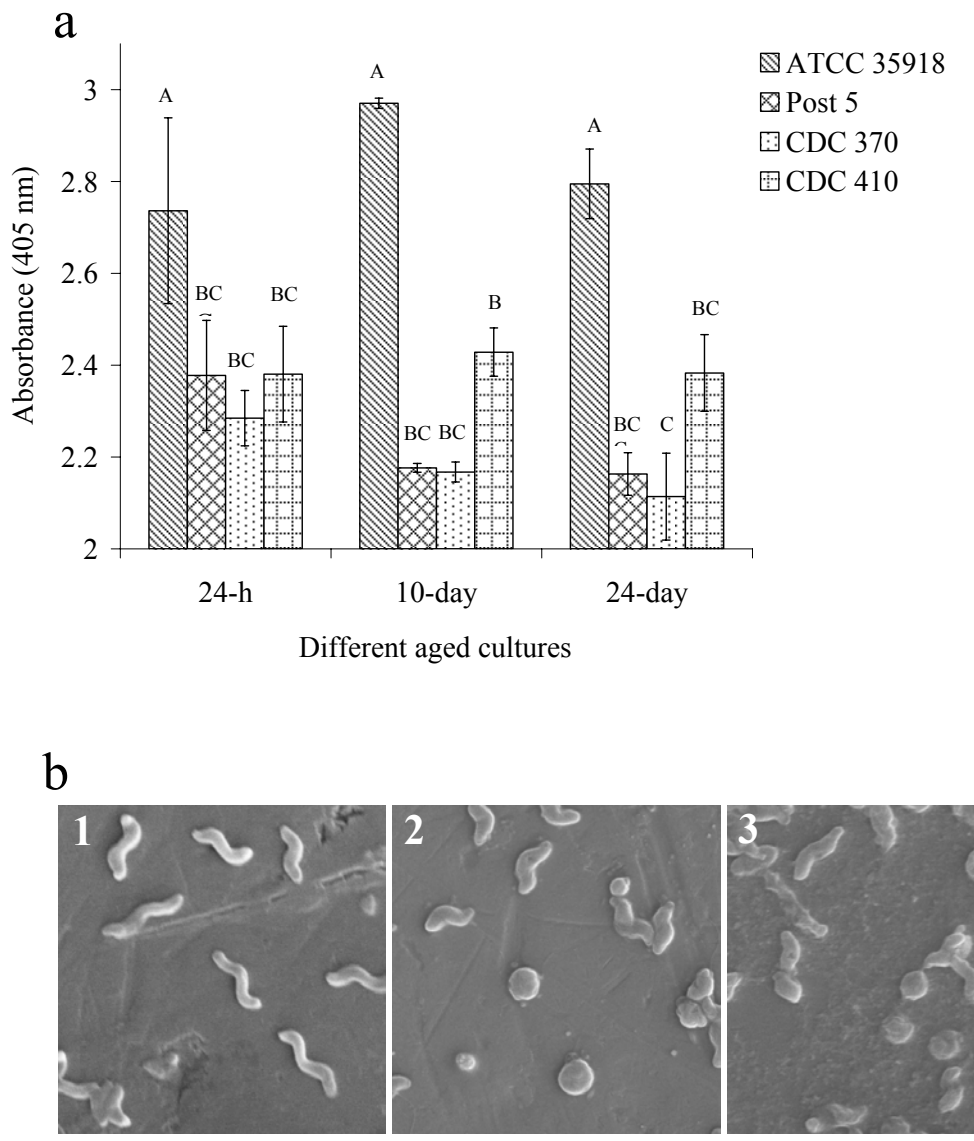

Fig. 2.5. a: Absorbance values obtained by ELISA with Ab2 of 24-h, 10-day and 24-day cultures of *Campylobacter* strains stored at 4°C. Means with different letters are significant different ($p < 0.05$). Error bars represent ±1 SEM. b: Scanning electron micrographs of different aged cells of *C. jejuni* ATCC 35918. 1: 24-h-cultured cells; 2: 10-day old; 3: 24-day old. Older cells show irregularities on their surfaces (2) and disruption of the cytoplasmic membranes (3). One ml 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.

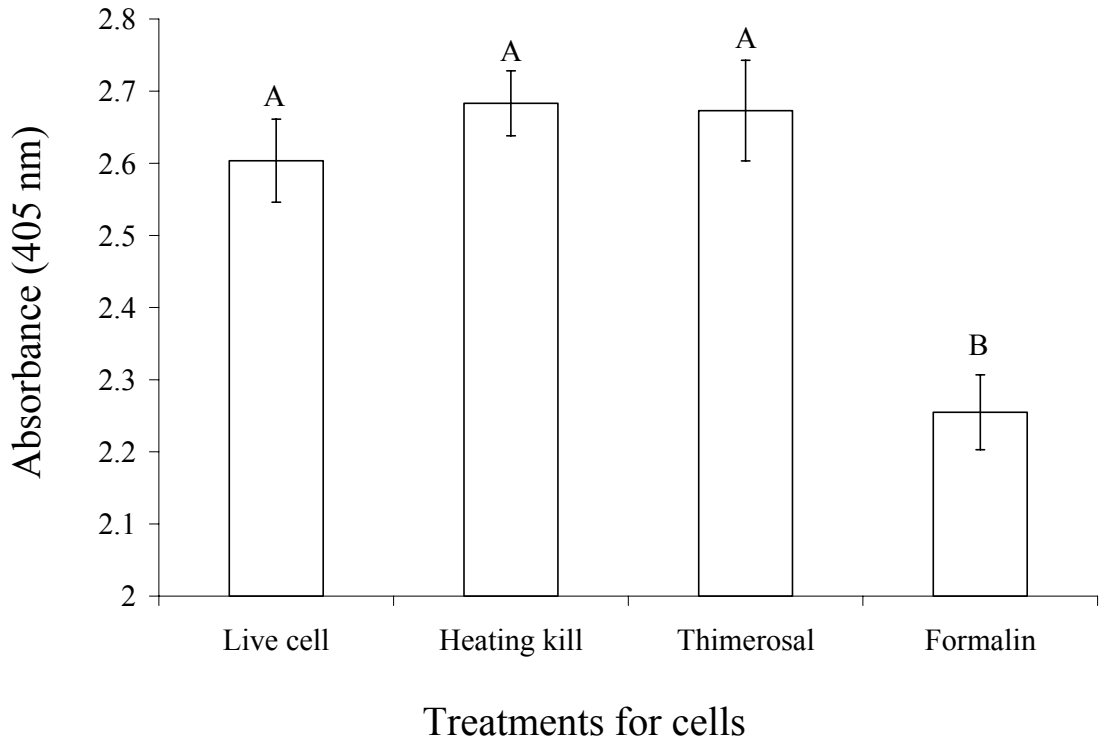

Fig. 2.6. Reactivity of different status (live cell, dead cell killed by heated at 70°C for 3 min, added 0.5% formalin and 0.5% thimerosal, respectively) of C*ampylobacter jejuni* ATCC 35918 with Ab2 using ELISA. Means with different letters are significant different ($p < 0.05$). Error bars represent ±1 SEM.

III. IDENTIFICATION OF *CAMPYLOBACTER JEJUNI* WITH COMMERCIAL ANTIBODY USING SURFACE PLASMON RESONANCE BIOSENSOR

3.1 Introduction

Currently, all conventional microbiology methods for the identification of *Campylobacter* species take three to four days. Newest detection tests for *Campylobacter* spp. include DNA-based systems, such as PCR, or antibody-based recognition systems, such as ELISA tests (1, 3, 6, 22). However, these tests are mainly performed on pure cultures, as a final identification step. Recent studies show that a high percentage of the processed broiler carcasses (15) and retail broiler products (8) are contaminated with *Campylobacter*. The level of *C. jejuni* and *C. coli* contamination per ml of carcass rinse is between 3 to 3.7 log CFU/ml immediately after evisceration and drops after the chiller (15). 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 or developed PCR based detection methods.

Although those developed PCR methods such as PCR- immunological based assay (5, 17, 23) and real-time PCR with pre-purification assay (14, 24, 25) were approved to approach this sensitivity, they can not be developed as commercially real-time and on-site methods for detecting foodborne pathogens with several drawbacks. The

main disadvantages include time consuming to complete assay (~ 8 h - 3 day), labor intensity, need for skilled personnel, use of harmful chemicals such as ethidium bromide, and high cost. Also, PCR-based analysis must be performed under highly controlled conditions to obtain reliable and consistent results. Results are very easy to be affected by any gene contaminations, which can be ubiquitous everywhere. Thus, establishing a PCR laboratory for every poultry processing plant is not practical.

The most important merit of a biosensor is that the total time for analysis of a sample can be shortened from days or hours to minutes. In addition, 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.

Surface plasmon resonance (SPR) biosensors are one of the most sensitive optical biosensor widely applied for physical quantities, chemical sensing, and biosensing characterizations (4). 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. Improvements in the immunoaffinity (antibody-antigen) reactions can enhance the sensitivity and specificity of SPR biosensors (10). The objective of the present study was to examine the sensitivity and specificity of commercially polyclonal antibodies against *C. jejuni* using SPR biosensor in pure cultures. Our final goal is to develop a SPR system for rapid identification of *C. jejuni* in boiler products.

3.2 Materials and methods

3.2.1Bacterial strains and culture conditions

One *Campylobacter* strain obtained from the American Type Culture Collection (*C. jejuni* ATCC 35918) was used in this study. One *Salmonella* serotype, *S.* Typhimurium ATCC 13311, isolated from chickens (16), was used for exclusivity studies. Both strains were stored in broth with 20-30% glycerol at -80°C. *Campylobacter* was grown on the modified Campy Cefex plates under microaerophilic condition (5% O_2, 10% CO_2 and 85% N_2), and incubated at 37°C for 24 h. *Salmonella* strain was grown on modified lysine iron agar plates 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 optical density (OD) of the suspensions was adjusted to 1.7-2.0 at 600 nm wavelength (~10^8-10^{10} CFU/ml). The antigen concentrations were confirmed by spread plate method. Before use, all antigen suspensions were stored at 4°C overnight.

3.2.2 Antibody against Campylobacter

One commercial antibody (Ab2) against *Campylobacter* was used in this study, which is a biotin-conjugated polyclonal rabbit antibody against *C. jejuni* ATCC 29428 (Biogenisis Ltd., Brentwood, New Hampshire).

3.2.3 Dual channel SPR sensor

The sensors used in this work were dual channel SPREETA ^{TM} sensors developed by Texas Instruments. The SPREETA sensor, is a miniature (approximately 7g), fully integrated surface plasmon resonance device. It is based on Kretschman geometry and it is fully configured with an AlGaAs light emitting diode (LED, 840nm) with a polarizer, temperature sensor, two photodiode arrays, and reflecting mirror. Light from the LED illuminates the gold-coated 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 a 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 25mm × 25mm Teflon block of 4mm 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 ~ 10 µl. The Teflon block is held in place by four screws. 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 (12, 19).

3.2.4 SPREETA™ cleaning and system setup

At the beginning of the SPR experiment, SPREETA™ needs to be cleaned. Initial cleaning of the gold surface was done with acid piranha solution (H_2SO_4:H_2O_2:: 3:1) for 5 min followed by rinsing with copious amount of MilliQ water. The surface is then gently wiped with ethanol using lens paper. The contaminants from the acid cleaning were removed using an ultrasonic water bath for 5 min. The gold surface was dried with Kim Wipes® and filtered air, respectively. Tubing is also cleaned with ethanol and MilliQ water. 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.

3.2.5 Antibodies immobilization

As shown in Fig.3.1, SPR biosensor system should be prepared before running bacteria. First of all, PBS buffer was passed through the sensor until a steady PBS baseline was established in both working channel and control channel. For the preparation of working channel, the gold surface was modified with neutravidin (1mg/ml) until saturation, followed by PBS wash. The uncovered surfaces of the gold were blocked with 1mg/ml of BSA. The 22.5µg/ml of biotinylated Ab2 was then selectively immobilized through well characterized avidin-biotin chemistry. In all the above steps in-between PBS wash was performed to remove unbound molecules. An additional PBS-

0.5% Tween wash was performed to restrict non-specific binding. The reference channel was completely modified with BSA (1mg/ml) to account for non-specific binding, bulk refractive index changes and temperature fluctuations during the course of the experiment.

3.2.6 Specificity tests using SPR

S. Typhimurium ATCC 13311and *C. jejuni* ATCC 35918 were used in this study. Following the sensor surface preparation, *S.* Typhimurium solutions from 10^4 to 10^6 CFU/ml were serially introduced through both channels starting with lowest concentration (10^4 CFU/ml). Higher concentrations of bacteria were introduced once the signal from the previous low concentration reaches a steady value (Std. dev < 5 RU). PBS buffer wash was performed in between each concentration to remove loosely attached bacteria. After the final concentration of *S.* Typhimurium, 100mM glycine elution buffer (pH 2) was used to break the non-specific binding between antibody and *S.* Typhimurium. Following a new baseline with PBS buffer, three serially diluted *C. jejuni* from 10^4 to 10^6 CFU/ml were introduced through both channels with same procedure for *S.* Typhimurium. A glycine buffer wash was also performed to check the specificity of antibody-*C. jejuni*.

3.2.7 Sensitivity tests using SPR

Following the sensor surface preparation, eight serially diluted *C. jejuni* solutions from 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 reaches a steady value (Std. dev < 5 RU). PBS buffer wash was performed in between each concentration to remove loosely attached bacteria.

3.2.8 Statistical analysis

Triplicate experiments were run for every experiment in this study. 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 identify 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 each result.

3.3 Results and discussion

3.3.1 Specificity tests using SPR

Fig. 3.2 shows the SPR response units of Ab2 bound to *S.* Typhimurium and *C. jejuni* at serially diluted concentrations from 10^4 to 10^6 CFU/ml. Although there were response units for Ab2 bound to *S.* Typhimurium, the recorded response was lower ($p < 0.05$) than the response recorded with the biding of Ab2 to *C. jejuni* (Fig. 3.2). In addition, the binding of Ab2 to *S.* Typhimurium was of a weak association, which was demonstrated with glycine elution buffer (Fig. 3.3-A). The binding between Ab2 and *C. jejuni* was specific because the glycine buffer rinse did not return the signal to the baseline (Fig. 3.3-B). The specificity was studied with concentrations greater than the

sensitivity determined for the SPR system (see 3.3.2). To let the system attain saturation, an average assay time of 45 min was used for each concentration. However, this assay time could be easily shortened to an assay time of no more than 30 min.

3.3.2 Sensitivity tests using SPR

The signal (ΔRU) in the working and the control channels were almost the same up to solutions containing 10^3 CFU/ml of *C. jejuni*. Hence, the theoretical lowest sensitivity of *C. jejuni* by SPR biosensor was determined to be 10^3 CFU/ml. These results are in conformity with, and are even better than, the results from other researchers who used SPR biosensor to detect other bacteria. Meeusen et al. (11) demonstrated a lowest detection limit, 10^7 CFU/ml, for *E. coli* O157:H7 and *S.* Typhimurium, respectively with direct avidin-biotin-characterized SPR biosensor assay. Fratamico et al. (2) determined a lowest detection limit of 5-7 × 10^7 CFU/ml for *E. coli* O157:H7 using SPR sandwich assay with primary and secondary antibodies. Lowest detection limits of 10^6 CFU/ml, 10^4 CFU/ml, and 10^3 CFU/ml for *E. coli* were reported using a polyethylene glycol terminated alkanethiol mixed self-assembled monolayers based SPR biosensor with direct, Protein G, and sandwich assay, respectively (21). A self-assembled protein G layer based SPR biosensor was employed to detect *Legionella pneumophila* with lowest detection limit of 10^5 cells/ml (13). A 10^6 CFU/ml of the lowest detection limit was reported for *Salmonella enteritidis and Listeria monocytogens* by Koubová et al. (7).

Similarly, Leonard et al. (9) demonstrated a lowest detection limit of 1 x 10^5 CFU/ml for *Listeria monocytogens* by means of 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 x 10^2 CFU/ml of *C. jejuni* within 25 min in artificially spiked foods (18). Comparing to this array biosensor experiment, in which sandwich assay and labeled antibodies were used, simple procedure with direct assay and unlabeled antibodies were used for detecting *Campylobacter* in current project.

When the concentrations of *C. jejuni* reached 10^5 CFU/ml, the control channel showed a significant response. This response may be due to non-specific binding to the sensor surface and instrument noise (Fig. 3.4). Significant differences ($p < 0.05$) were shown between 10^3 and 10^2 CFU/ml (Fig. 3.5).

One key factor for detecting whole-cell bacterial pathogens should be taken into consideration. In a SPR biosensor, the effective evanescent wave field extension is around 0.3 µm. Since the size of *Campylobacter* cell is varying in length from 0.5 to 5 µm and in width from 0.2 to 0.5 µm, 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 1) increasing the penetration depth of evanescent wave field to locate the whole *Campylobacter* cell within this depth (26), or 2) 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 µm (20).

3.4 Conclusions

The SPR biosensor demonstrated to be very specific for identification of *C. jejuni* and exhibited a sensitive of 10^3 CFU/ml in pure cultures. However, further investigation for SPR biosensor applications in detection of *C. jejuni* is required. The sensitivity and specificity of SPR biosensor can be enhanced by improving the bioreceptor of SPR biosensor. Such as using DNA/RNA as biorecognition part of SPR biosensor. Although the average assay time was 45 min, this time could be easily shortened to an assay time of no more than 30 min.

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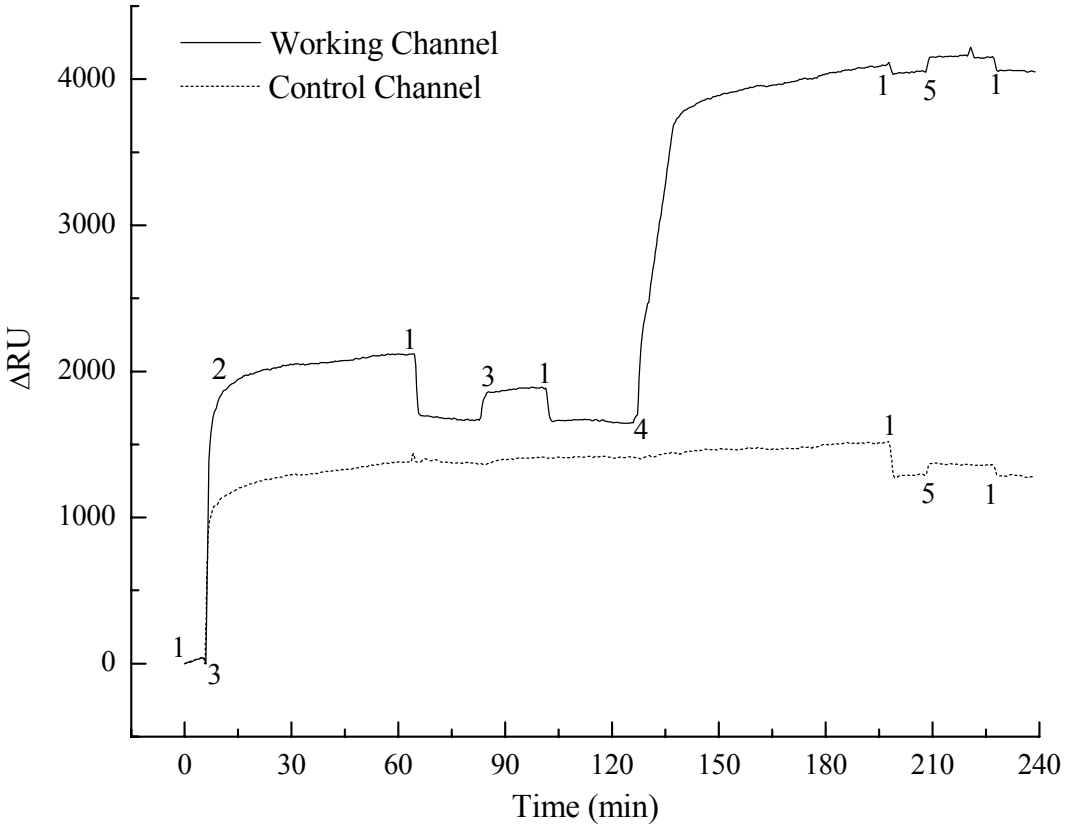

Fig. 3.1. Antibodies immobilization for SPR experiments. Numbers show the times when various solutions were added. 1: PBS; 2: Neutravidin; 3: BSA; 4: Ab2; 5: PBS-Tween.

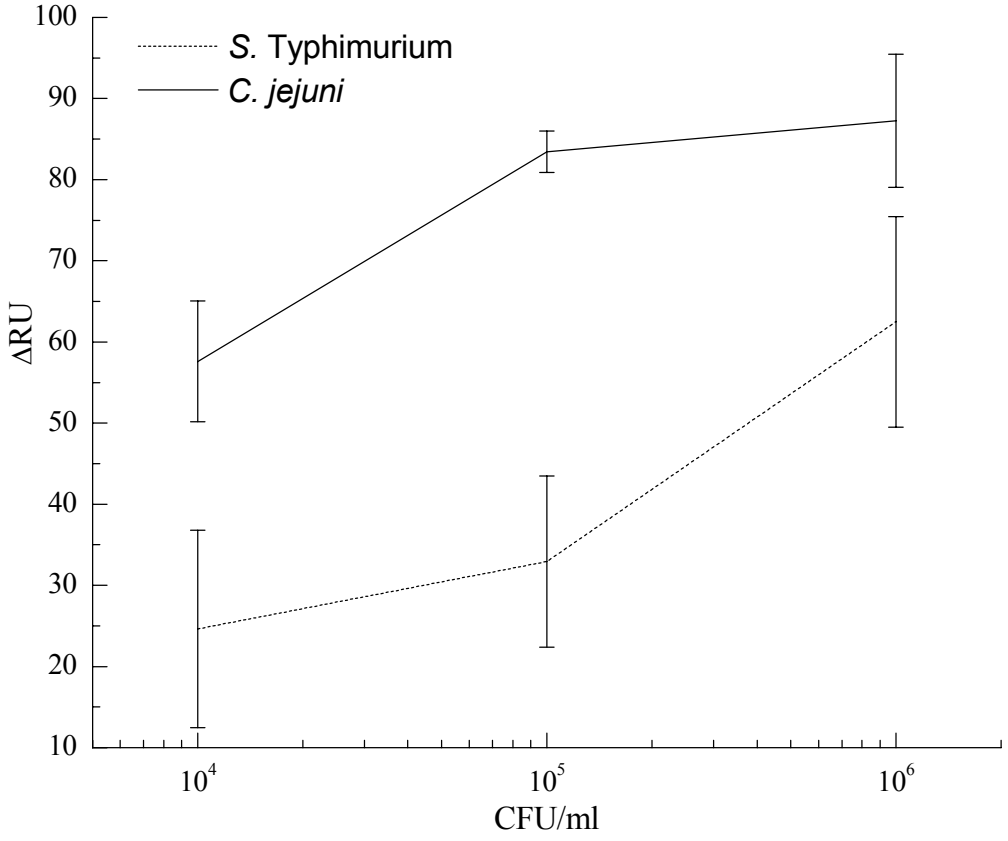

Fig. 3.2. Sensorgram showing the response units of Ab2 bound to *S.* Typhimurium and *Campylobacter jejuni* at serially diluted concentrations from 10^4 to 10^6 CFU/ml. Average of three independent experiments. Error bars represent ±1 SEM.

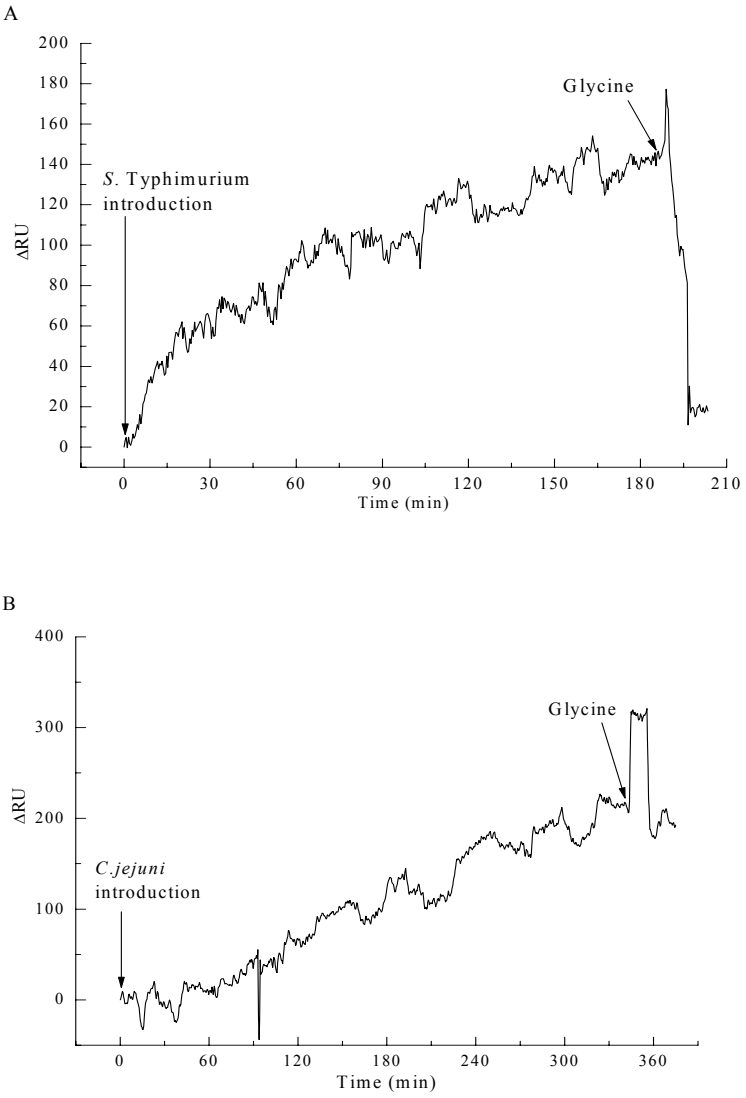

Fig. 3.3. Sensorgram showing the response units of Ab2 bound to *Salmonella* Typhimurium (A) and *Campylobacter jejuni* (B) after glycine washing. Average of three independent experiments. The binding of *C. jejuni* to the Ab2 appears to be permanent, while the binding of *S.* Typhimurium to the Ab2 are temporary.

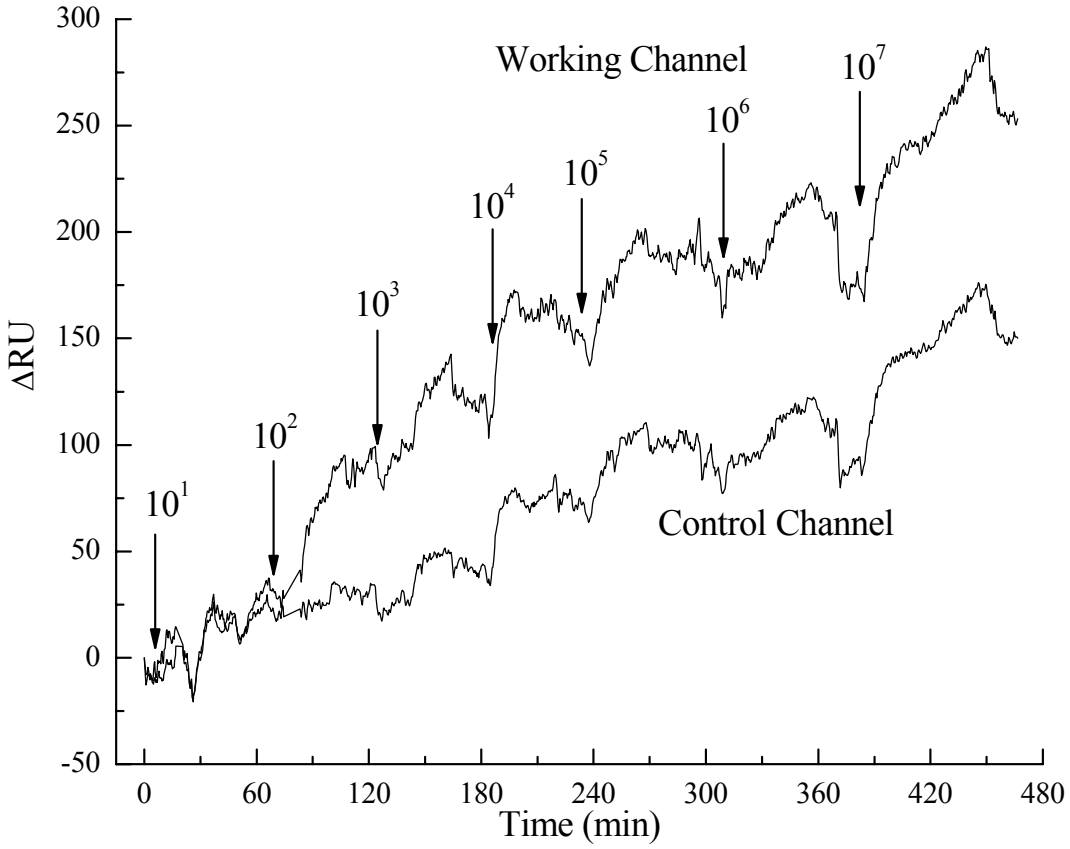

Fig. 3.4. SPR biosensor response to serially diluted (10^1 to 10^7 CFU/ml) *C. jejuni* in PBS. The working channel shows the average response units of triplicate results after accounted for non-specific responses from control channel. Arrows indicate the times when solutions were added in both channel.

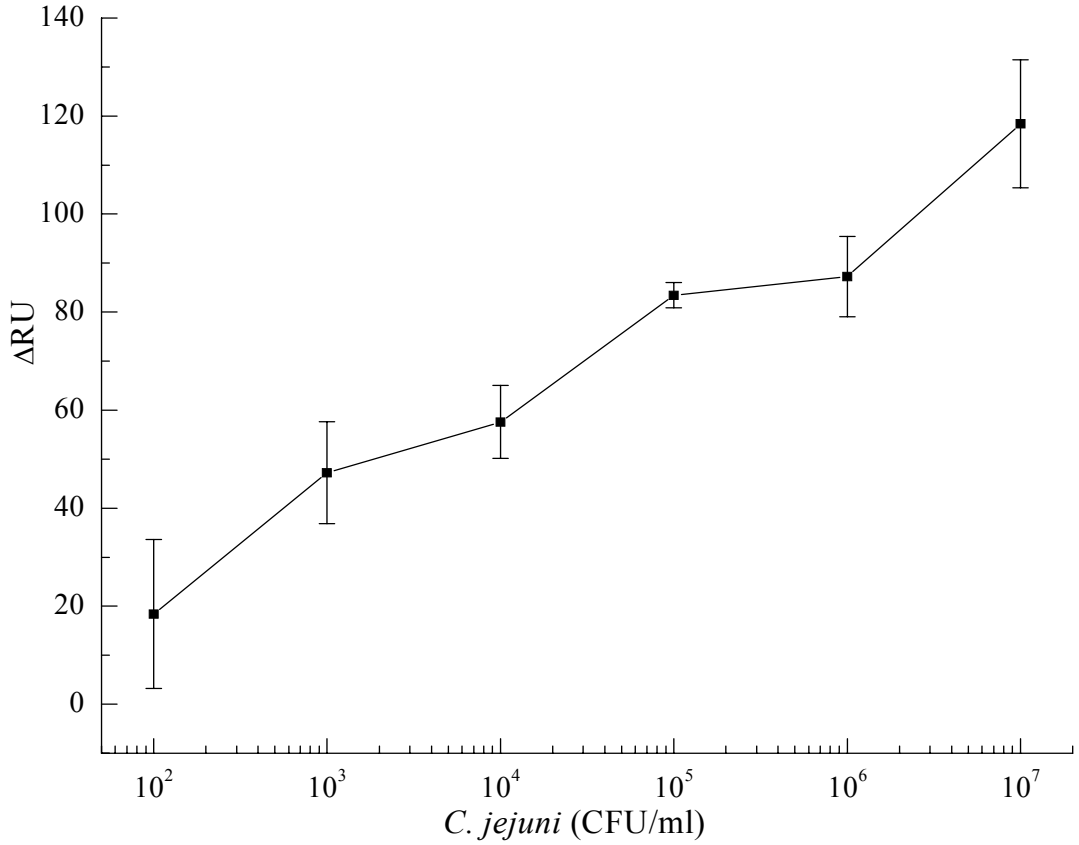

Fig. 3.5. Average response units (triplicate experiments) of serially diluted *C. jejuni* solutions. Values were obtained after deduction of the response units in the control channel. Error bars represent ±1 SEM.

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