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Effects of Pretreatments on Detection of *E. coli* O157:H7 by SPR Biosensor

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Escherichia coli (*E. coli*) O157:H7 was detected by using a surface plasmon resonance (SPR) biosensor and two antibodies with different characters. The lower limit of detection of *E. coli* O157:H7 samples after pretreatments was determined by SPR. Seven pretreatment methods for preparing *E. coli* O157:H7 samples for SPR detection; beads disruption, sonication, and heat shock, osmotic shock, lysozyme, alkali, and boiling treatments were compared for SPR signal with untreated cells as a control.

In the case of the antibody raised against intracellular substance, β–D–galactosidase (β–gal), was used for the detection, the lower limit of detection was 4.9×10^5 CFU/ml for both sonicated and alkali treated samples. The lower limit of detection was 8.3×10^6 CFU/ml for beads disrupted samples, and 8.2×10^8 CFU/ml for both lysozyme treated and untreated samples. In contrast, significant SPR signal was not obtained for heat shocked, osmotic shocked and boiled samples even at 8.2×10^8 CFU/ml. Sonication pretreatment improved the lower limit of detection for *E. coli* O157:H7 by three orders of magnitude compared with that of untreated sample when anti–β–gal antibody was used for detection by SPR biosensor. In the case of antibody raised against lipopolysaccharide (LPS), the cell surface substance, was used, sonicated *E. coli* O157:H7 sample was detected by SPR at 1.3×10^5 CFU/ml. The lower limit of detection was 1.1×10^6 CFU/ml for heat shocked, lysozyme treated, alkali treated, boiled and untreated samples, and 7.7×10^7 CFU/ml for beads disrupted samples, respectively. After the osmotic shock treatment, *E. coli* O157:H7 was not detected by SPR even at 2.1×10^8 CFU/ml. These results show that sonication was the most effective pretreatment method for the detection of *E. coli* O157:H7 by SPR using both antibodies recognizing intracellular β–gal, and cell surface LPS.

Key words: antibody, *E. coli* O157:H7, pretreatment, SPR

INTRODUCTION

Food poisoning is a common, distressing, and sometimes life–threatening problem for millions of people around the world (Ikeda *et al*., 2006). In the United States, food poisoning causes approximately 76 million illnesses, accounting for 325,000 hospitalizations, 5000 deaths and costs $23 billion annually (Mead *et al*., 2000). In Japan, 700~1850 incidents of food poisoning occurred annually from 1995 to 2002, 80% of which were caused by bacteria (Ikeda *et al*., 2006). Although public health conditions have been improving recent years, food poisoning incidents still occur. Rapid and sensitive detection of food poisoning bacteria is critical and effective for preventing the outbreak of food poisoning. At present, the methods that are commonly used in food industry for the detection of food poisoning bacteria are conventional methods, based on culture. These conventional methods are considered to be the "gold– standard" and are known for their cost effectiveness, sensitivity, ability to confirm cell viability and ease of standardization (Dwivedi and Jaykus, 2011). However, because of the complicated procedures, they are time consuming, taking at least 2–3 days for results and 7–10 days for confirmation, and also labor–intensive (Bai *et al*., 2010). With the rapid development of food industry, these methods could not meet the demands of this new age anymore. Thus the needs for rapid and simple detection methods for food poisoning bacteria are growing in food industries and government agencies.

In recent years, there has been much research activity in the area of development of biosensors for detecting food poisoning bacteria (Velusamy *et al*., 2010) . They have been shown to have potential to provide sensitive, rapid and specific detection of bacteria. In this study, an optical biosensor based on surface plasmon resonance (SPR), which allows for real–time and label–free detection, was used for detection of food poisoning bacteria. SPR biosensors have been used by many researchers for the detection of food poisoning bacteria and it was shown that the lower limit of detection for bacteria using a SPR biosensor is not only dependent on the sensitivity of the instrument and the specificity and affinity of the surface chemistry, but also on the sample preparation method. Taylor *et al*. (2005) compared three pretreatment methods of *E. coli* O157:H7 cells:

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untreated, heat–and–ethanol treatment, and detergent treatment. The lower limit of detection for detergent–treated samples was 10^5 CFU/ml, while they were 10^6 and 10^7 CFU/ml for the heat–and–ethanol treated and untreated samples, respectively. The results indicated that the pretreatment method used to prepare samples for SPR detection affects on the lower limit of detection. However, there is a lack of systematic studies on the method of sample preparation for SPR detection of bacteria (Taylor *et al*., 2005) .

In this study, by using two antibodies with different characters, seven pretreatment methods for preparing *E. coli* O157:H7 samples for SPR detection were compared.

MATERIALS AND METHODS

SPR instrumentation

In this work, all SPR measurements were performed at 25˚C using a SPR biosensor Biacore J (GE Healthcare Bio–Sciences AB, Sweden). SPR biosensor is able to detect minor changes in the refractive index, which occur when analyte binds to ligand immobilized on the transducer surface and it measures the change of the angle of the reflected light as a function of change of density of medium against time (Fig. 1) (Rich and Myszka, 2001). Biacore J is equipped with two flow cells, flow cell 1 and flow cell 2. Ligand is immobilized on flow cell 1, defined as a detection channel, and flow cell 2 is left unmodified and serves as a reference channel. To compensate the detection channel for variations in sample composition and non–specific adsorption, the signal from the reference channel is subtracted from that of the detection channel. This is particularly important when determining the lower limit of detection (Taylor *et al*., 2005). The unit of signal change is resonance unit (RU). 1,000 RU represents mass change of 1 ng per mm^2 on sensor chip and also represents resonance angle change of 0.1 degree. As Biacore J has a measurement noise (<2 RU), a signal change above 6 RU, three times the measurement noise, is defined as a valid signal change due to antigen–antibody binding.

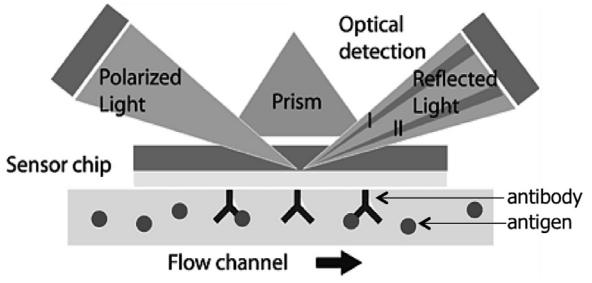

Fig. 1. Schematic representation of SPR sensor. The ligand (shown here as antibody) is immobilized on the sensor chip surface. The analyte (shown here as antigen) passes through microfluidic flow channels.

Antibody immobilization on sensor chip

As an antibody that recognizes intracellular enzyme β–D–galactosidase (β–gal), anti–*E. coli* O157:H7 β–gal rabbit polyclonal antibody (PAb) (Genenet Co., Ltd., Fukuoka, Japan) was used. For the detection of cell surface substance, lipopolysaccharide (LPS) of *E. coli* O157:H7, anti–*E. coli* O157:H7 LPS mouse monoclonal antibody (MAb) (Abcam, Tokyo, Japan) was used. This antibody recognizes LPS of *E. coli* O157:H7 specifically and has no cross–reactivity with *E. coli* O111, O125, O20, O55 and K12.

Sensor chip CM5 (GE Healthcare Bio–Sciences AB, Sweden) was used for immobilization of anti–*E. coli* O157:H7 β–gal PAb and anti–*E. coli* O157:H7 LPS MAb. Antibodies were immobilized onto sensor chips using amine–coupling chemistry (Löfås and Johnsson, 1990). The surfaces of the two flow cells were activated for 10 min with a 1:1 mixture of 0.1 M N–hydroxysuccinimide (NHS), and 0.4 M 1–ethyl–3–(3–dimethylaminopropyl)–carbodiimide hydrochloride (EDC) at the medium flow rate (approximately 30 μl/min). Antibody was immobilized on flow cell 1 at a concentration of 100 μg/ml in 10 mM acetate buffer (pH 4.0) at the medium flow rate for 18 min. Flow cell 2 was left blank to serve as a reference channel. The surfaces of both flow cell 1 and 2 were blocked with a 10 min injection of 1.0 M ethanolamine–HCl (pH 8.5) to minimize non–specific adsorption (Rich and Myszka, 2001).

Bacteria and cultivation

E. coli O157:H7 (VT1, VT2) was obtained from Fukuoka City Institute for Hygiene and the Environment, Fukuoka, Japan. *E. coli* O157:H7 was preenriched in 5 ml of sterile Tryptic soy broth (TSB, Becton, Dickinson and Company, USA) at 37˚C for 18 h with shaking at 130 rpm.

For the preparation of bacterial cells for the detection of LPS, 5 ml of *E. coli* O157:H7 culture were centrifuged at 8,000×*g* for 5 min. The pellet was resuspended in 5 ml of Phosphate buffered saline (PBS, 0.1 M phosphate, 0.15 NaCl, pH 7.2), centrifuged at 8,000×*g* for 5 min, and the pellet was resuspended in 5 ml of PBS. The suspension was 10–fold serially diluted with PBS from 10^1 to 10^5–fold and the cells were harvested from 1 ml of each of the dilutions by centrifugation at 8,000×*g* for 5 min. Pellets were used for following pretreatments.

For the preparation of bacterial cells for the detection of β–gal, *E. coli* O157:H7 was induced for β–gal by cultivation in 10 ml of Luria Broth (LB, Becton, Dickinson and Company, USA) containing 2.5 mM Isopropyl β–D–1–thiogalactopyranoside (IPTG) (Nacalai Tesque, Japan) for 6 h at 37˚C with shaking. Cells were harvested from 5 ml of the β–gal–induced *E. coli* O157:H7 culture by centrifugation and washed with PBS.

The cells were suspended in PBS and the suspension was 10–fold serially diluted with PBS from 10^1 to 10^4–fold and the cells were harvested from 1 ml of each of the dilutions by centrifugation at 8,000×*g* for 5 min. Pellets were used for following pretreatments.

Viable counts

Viable bacterial counts were determined by plating 100 μl of 10–fold serial dilutions of the bacterial suspension on CHROMagar O157 plates (CHROMagar, France). After cultivation at 37˚C for 24 h, formed colonies were counted.

Pretreatments of Bacteria

Beads disruption

Bacterial pellets were resuspended in 300 μl of HBS–EP buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% v/v Surfactant P20) and poured into ice–cold fastPROTEIN™ Blue Matrix tube (MP–Bio Japan K.K.). Bacterial cells were disrupted by using FastPrep®–24 instrument (MP–Bio Japan K.K.) at a swing speed of 4.0 m/s for 20 s, followed by adding 700 μl of ice–cold HBS–EP buffer. After centrifuging the suspensions at 15,000×*g* and 4˚C for 20 min, 700 μl of supernatants were recovered.

Sonication treatment

Bacterial pellets were resuspended in 1 ml of HBS–EP buffer and sonicated 3 times at output of 50 W for 30 s with 30 s intervals by using TOMY Ultrasonic Disruptor UD–201 (TOMY SEIKO CO., LTD, Japan) in an ice bath. The whole homogenate was directly used as a sample for detection with anti–*E. coli* O157:H7 LPS MAb. After centrifuging the suspensions at 15,000×*g* and 4˚C for 20 min, 900 μl of supernatants were recovered and used as a sample for the detection with anti–*E. coli* O157:H7 β–gal PAb.

Heat shock treatment

Bacterial pellets were resuspended in 1 ml of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0), chilled in ice bath for 1 h, kept at 42˚C for 1 min and then chilled again. The homogenate was directly used as a sample for detection with anti–*E. coli* O157:H7 LPS MAb. After centrifuging the suspensions at 15,000×*g* and 4˚C for 20 min, 900 μl of supernatants were recovered and used as a sample for the detection with anti–*E. coli* O157:H7 β–gal PAb.

Osmotic shock treatment

Bacterial pellets were resuspended in 1 ml of osmotic shock buffer (30 mM Tris–HCl, 20% sucrose, pH 8.0), vortexed for 10 min at room temperature and the cells were harvested by centrifugation at 8,000×*g* for 5 min. The pellets were resuspended in 1 ml of ice–cold deionized water (DI water). The suspension was directly used as a sample for detection with anti–*E. coli* O157:H7 LPS MAb. After centrifuging the suspensions at 15,000×*g* and 4˚C for 20 min, 900 μl of supernatants were recovered and used as a sample for the detection with anti–*E. coli* O157:H7 β–gal PAb.

Lysozyme treatment

Bacterial pellets were resuspended in 50 μl of 10 mM Tris–HCl buffer containing 100 mM NaCl, 1 mM EDTA, and 1 mM PMSF (pH 8.0) and then mixed with 20 μl of 10 mg/ml of egg white lysozyme (SEIKAGAKU CORPORATION, Japan) dissolved in 10 mM Tris–HCl buffer (pH 8.0). The suspensions were kept for 60 min at 37˚C with gentle agitation every 5 min, followed by adding 930 μl of HBS–EP buffer. The suspension was directly used as a sample for detection with anti–*E. coli* O157:H7 LPS MAb. After centrifuging the suspensions at 15,000×*g* and 4˚C for 20 min, 900 μl of supernatants were recovered and used as a sample for the detection with anti–*E. coli* O157:H7 β–gal PAb.

Alkali treatment

Bacterial pellets were resuspended in 200 μl of 0.2 M NaOH solution and kept at room temperature for 5 min. After adding 600 μl of HBS–EP buffer, the mixture was mixed thoroughly by gentle pipetting. Then the suspensions were neutralized by adding 200 μl of 0.2 M HCl solution. The suspension was directly used as a sample for detection with anti–*E. coli* O157:H7 LPS MAb. After centrifuging the suspensions at 15,000×*g* and 4˚C for 20 min, 900 μl of supernatants were recovered and used as a sample for the detection with anti–*E. coli* O157:H7 β–gal PAb.

Boiling treatment

Bacterial pellets were resuspended in 1 ml of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0), kept in ice bath for 1 h and then boiled for 5 min. The suspension was directly used as a sample for detection with anti–*E. coli* O157:H7 LPS MAb. After centrifuging the suspensions at 15,000×*g* and 4˚C for 20 min, 900 μl of supernatants were recovered and used as a sample for the detection with anti–*E. coli* O157:H7 β–gal PAb.

Untreated sample

Bacterial pellets were resuspended in 1 ml of HBS–EP buffer. The suspension was directly used as a sample for detection with anti–*E. coli* O157:H7 LPS MAb. After centrifuging the suspensions at 15,000×*g* and 4˚C for 20 min, 900 μl of supernatants were recovered and used as a sample for the detection with anti–*E. coli* O157:H7 β–gal PAb.

SPR detection of *E. coli* O157:H7 samples

Sensor chip CM5 immobilized with antibody was docked into Biacore J and flow rate was set at the medium one (approximately 30 μl/min). Corresponding buffer, HBS–EP for beads disruption, sonicated and untreated samples, TE for heat shocked and boiled samples, DI water for osmotic shocked samples, 10 mM Tris–HCl (pH 8.0) containing 10 mg/ml egg white lysozyme for lysozyme treated sample, 0.2 M NaCl solution for alkali treated samples, was first injected for 5 min to establish a baseline. Then the corresponding sample was injected for 5 min for antigen–antibody binding. After measurement of each sample, regeneration solution (10 mM glycine–HCl, pH 2.0) was injected for 1 min to dissociate antigen–antibody binding. After regeneration, sensor chip surfaces were equilibrated with running buffer (HBS–EP buffer).

RESULTS

Effects of pretreatments of sample on detection of *E. coli O*157:H7 by using SPR with anti–*E. coli* O157:H7 β–gal PAb

Supernatant samples prepared from β–gal–induced *E. coli* O157:H7 by various pretreatments were applied to Biacore J equipped with anti–*E. coli* O157:H7 β–gal PAb immobilized sensor chip. Table 1 and Fig. 2 show the signal changes of supernatant samples prepared by different pretreatment methods at various cell concentrations.

In the detection of sonicated samples and alkali treated samples, cell concentration–dependent increases in signal change were observed and valid signal changes, 24.9 RU for sonicated sample and 13.3 RU for alkali treated sample, were obtained at cell concentration of 4.9×10^5 CFU/ml. In the detection of osmotic shocked samples and lysozyme treated samples, although valid signal changes were obtained above cell concentrations of 2.1×10^3 CFU/ml. However, the signal changes were not cell concentration–dependent and thus seem not reliable. In the detection of beads disrupted samples, valid signal change of 28.2 RU was obtained at cell concentration of 8.3×10^6 CFU/ml. In the detection of untreated samples, valid signal change was not obtained until cell concentration increased to 10^8 CFU/ml. In the detection of heat shocked and boiled samples, valid signal changes were not obtained at all cell concentrations tested.

For SPR detection of *E. coli* O157:H7 with antibody recognizing intracellular β–gal, sonicated and alkali treated samples showed the lowest limit of detection, 4.9×10^5 CFU/ml. The lower limit of detection was 8.3×10^6 CFU/ml for beads disrupted sample, 8.2×10^8 CFU/ml for lysozyme treated and untreated samples. In the case of heat shocked, osmotic shocked and boiled samples, *E. coli* O157:H7 was not detected even at 8.2×10^8 CFU/ml.

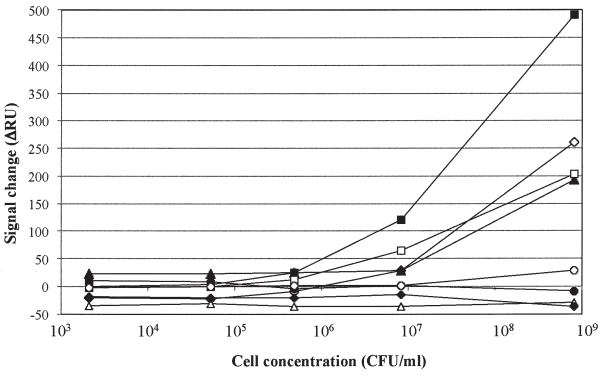

Fig. 2. Effects of pretreatments of sample on detection of *E. coli* O157:H7 by using SPR biosensor with anti–*E. coli* O157:H7 β–gal rabbit polyclonal antibody.
E. coli O157:H7 cells were suspended in buffer and untreated (○) and pretreated with beads (◇), sonication (■), heat shock (△), osmotic shock (●), lysozyme (▲), alkali (□), and boiling (◆) at various cell concentrations. After centrifugation, the supernatants were used as samples for SPR analysis.

Effects of pretreatments of sample on detection of *E. coli* O157:H7 by using SPR with anti–*E. coli* O157:H7 LPS MAb

Samples prepared from normal *E. coli* O157:H7 cells by various pretreatments were applied to Biacore J equipped with anti–*E. coli* O157:H7 LPS MAb immobilized sensor chip. Table 2 and Fig. 3 show the signal changes of samples prepared by different pretreatment methods at various cell concentrations.

At cell concentration of 10^4 CFU/ml, valid signal change was not obtained in all the samples. At cell concentration of 10^5 CFU/ml, a valid signal change of 6.8 RU was obtained only in the detection of sonicated sample. At cell concentration of 10^6 CFU/ml, *E. coli* O157:H7 was detected by using sonicated, heat shocked, lysozyme treated, alkali treated, boiled and untreated samples with valid signal change of 30.3 RU, 14.7 RU, 24.9 RU, 24.5 RU, 29.9 RU and 24.0RU, respectively. While valid signal change was not obtained at the same cell concentration on both the beads disrupted and osmotic shocked samples. For beads disrupted sample, a valid signal change of 19.9 RU was obtained at the cell concentration of 7.7×10^7 CFU/ml, meanwhile, for osmotic shocked sample, no valid signal change was obtained even at 10^8 CFU/ml.

For the detection of *E. coli* O157:H7 with antibody recognizing cell surface LPS, sonicated sample showed the lowest limit of detection, 1.3×10^5 CFU/ml. The lower limit of detection was 1.1×10^6 CFU/ml for heat shocked,

Table 1. Signal changes of samples prepared by different pretreatments at various cell concentrations determined by SPR biosensor with anti–*E. coli* O157:H7 β–gal rabbit polyclonal antibody

Cell concentration (CFU/ml)	Signal changes (ΔRU)							
	Pretreatment							
	Beads	Sonication	Heat shock	Osmotic shock	Lysozyme	Alkali	Boiling	Untreated
2.1×10^3	–19.5	0	–35.1	11.8	23.3	–2.1	–17.7	–1.7
5.3×10^4	–21.9	3.7	–30.4	9.2	23.3	–0.3	–20.5	0.7
4.9×10^5	–8.3	24.9	–36.1	–3.7	25.7	13.3	–19.1	1.5
8.3×10^6	28.2	119.7	–35.5	1.9	29.0	65.2	–14.5	1.2
8.2×10^8	261.7	492.1	–29.3	–8.4	192.6	204	–37	27.9

Table 2. Signal changes of samples prepared by different pretreatments at various cell concentrations determined by SPR biosensor with anti–*E. coli* O157:H7 LPS mouse monoclonal antibody

Cell concentration (CFU/ml)	Signal changes (ΔRU)							
	Pretreatment							
	Beads	Sonication	Beads Sonication	Osmotic shock	Lysozyme	Alkali	Boiling	Untreated
1.3×10^4 1.4×10^{4}*	–0.1	1.6	–3.4	1.3	–0.2	0.9	–6.8	0.8
1.3×10^5 8.3×10^{5}*	1.9	6.8	2.4	1.4	3.7	5.0	4.4	3.1
1.1×10^6 4.6×10^{6}*	4.4	30.3	14.7	0.8	24.9	24.5	29.9	24
1.6×10^7 7.7×10^{7}*	19.9	30.9	17.8	2	40.5	29.1	16.1	40.5
2.1×10^8 7.8×10^{8}*	68.7	99.5	82	5.5	98.9	48.4	49.8	141.4

* Cell concentrations of beads treated samples

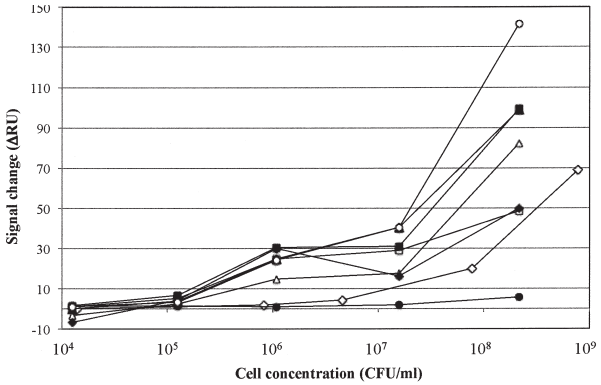

Fig. 3. Effects of pretreatments of sample on detection of *E. coli* O157:H7 by using SPR with anti-*E. coli* O157:H7 LPS mouse monoclonal antibody.
E. coli O157:H7 cell were suspended in buffer and untreated (○) and pretreated with beads (◇), sonication (■), heat shock (△), osmotic shock (●), lysozyme (▲), alkali (□), and boiling (◆) at various cell concentrations. After pretreatments, the whole suspensions were used as samples for SPR analysis.

lysozyme treated, alkali treated, boiled and untreated samples, and 7.7×10^7 CFU/ml for beads disrupted sample. In the case of osmotic shocked sample, *E. coli* O157:H7 was not detected even at 2.1×10^8 CFU/ml.

DISCUSSION

In this study, a SPR biosensor was used to detect samples containing *E. coli* O157:H7, which were prepared by seven pretreatment methods; beads disruption, sonication, heat shock, osmotic shock, lysozyme, alkali, and boiling treatments. These methods are commonly used either for lysing bacterial cells to obtain intracellular proteins or for disrupting the bacterial cells into small pieces. In beads disruption, FastPrep®–24 instrument, a high–speed homogenizer, was used to crush bacterial cells through the multidirectional, simultaneous beating of specialized lysing matrix beads on the sample material. FastPrep®–24 instrument can lyse bacteria thoroughly and quickly and thus allows easy isolation of active proteins. Sonication is often used to disrupt cell membranes and release cellular contents, which is called sonoporation process. Sonication appears to weaken microbial membranes through cavitation induced by ultrasonic shock waves, which making microorganisms more vulnerable to external stresses (Wong *et al*., 2012). Heat shock treatment looses cell membrane and thus causes the leakage of intracellular substances. Osmotic shock is a sudden change in the solute concentration around a cell, causing a rapid change in influx of water across its cell membrane. Under the condition of low concentrations of solutes, water enters into the cell in large amounts; causing it to swell and either burst or undergo apoptosis (Lang *et al*., 2005) . Since bacterial cell is protected by cell wall composed of peptidoglycan, even in the state of low osmotic shock, bacterial cell does not break down thoroughly. If this cell wall undergoes partial enzymatic degradation, structure of bacterial cell will become loose and bacterial cell will collapse completely in hypotonic solution. Lysozyme, also known as muramidase or N–acetylmuramide glycanhydrolase, is glycoside hydrolase, an enzyme that usually be used to degrade bacterial cell wall by catalyzing hydrolysis of 1,4–beta–linkages between N–acetylmuramic acid and N–acetyl–D–glucosamine residues in a peptidoglycan and between N–acetyl–D–glucosamine residues in chitodextrins. In the case of *E. coli* O157:H7, since it has outer membrane, it is necessary to use EDTA together with lysozyme to degrade cell wall. Alkali also can be used for lysis of *E. coli* O157:H7, since the cell wall of *E. coli* O157:H7 is thinner than that of Gram–positive bacteria. Boiling can break *E. coli* O157:H7 cell bodies and make them into spherical from rod shape (Taylor *et al*., 2005) . In this study, when using antibody recognizing intracellular enzyme β–gal, sonication and alkali treatment were the most effective method to extract intracellular enzyme from *E. coli* O157:H7 cells. In the case of SPR detection using

antibody recognizing cellular surface substance, LPS, valid signal change was obtained only on sonicated sample at a low cell concentration of 10^5 CFU/ml. At 10^6 CFU/ml, valid signal changes were obtained for all the other samples except osmotic shocked and beads disrupted samples. In the case of beads disruption, supernatants were used as samples for SPR detection since it was difficult to separate beads from the bacterial pellets after the treatment. In this treatment, only a little amount of antigen, LPS, was released into supernatant from the bacterial surfaces and most of them were still present in the bacterial bodies and was discarded together with beads after centrifugation. This can explain why the signal changes in the detection of beads disrupted samples were lower than that of other treatments. Based on these results, it is clear that for the detection of bacteria by SPR with specific antibody at a low cell concentration such as 10^5 CFU/ml, sonication is the most effective pretreatment method to prepare sample for SPR. Meanwhile, if detection is performed at a high cell concentration (above 10^6 CFU/ml), untreated sample is preferable as it is simple to prepare and has almost the same valid signal change as sonicated sample.

Recent years, SPR based biosensors have been reported by several researchers for the detection of *E. coli* O157:H7 (Meeusen *et al*., 2005; Subramanian *et al*., 2006; Taylor *et al*., 2005). By using a miniaturized SPR biosensor, Meeusen *et al*. (2005) detected *E. coli* O157:H7 at 8.7×10^6 CFU/ml. Subramanian *et al*. (2006) applied self–assembled monolayers based SPR biosensor for the rapid and specific detection of *E. coli* O157:H7 and showed that the lower detection limit for direct assay was 10^6 CFU/ml. Taylor *et al*. (2005) reported the SPR detection of *E. coli* O157:H7 at 10^7 CFU/ml. These lower detection limits were not satisfactory, since samples used in these studies were untreated *E. coli* O157:H7 cells only. To improve the detection sensitivity by SPR, some researchers adopted sandwich assay method, in which secondary antibody was used to amplify the signal of detection. By using secondary antibody, Taylor *et al*. (2005) improved the lower limit of detection of *E. coli* O157:H7 by one order of magnitude, from 10^7 CFU/ml to 10^6 CFU/ml. However, the usage of secondary antibody will increase the cost of detection by a large margin because of high price of antibody. In this study, by using sonicated samples, the lower limit of detection for *E. coli* O157:H7 was improved by three orders of magnitude compared with that of untreated sample when the antibody recognizing intracellular β–gal was used for detection by SPR biosensor. Sonication treatment of bacteria is an effective and low–cost way to improve the lower detection limit of *E. coli* O157:H7 by SPR. In future study, other bacteria will also be used to investigate effectiveness of sonication treatment in bacteria detection by SPR biosensor.

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