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Analytical Methods

Monitoring of *Escherichia coli* O157:H7 in food samples using lectin based surface plasmon resonance biosensor

Yixian Wang, Zunzhong Ye, Chengyan Si, Yibin Ying *

College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, PR China

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ABSTRACT

A novel surface plasmon resonance (SPR) biosensor using lectin as bioreceptor was developed for the rapid detection of *Escherichia coli* (*E. coli*) O157:H7. The selective interaction of lectins with carbohydrate components from bacterial cells surface was used as the recognition principle for the detection. Five types of lectins from *Triticum vulgaris*, *Canavailia ensiformis*, *Ulex europaeus*, *Arachis hypogaea*, and *Maackia amurensis*, were employed to evaluate the selectivity of the approach for binding *E. coli* O157:H7 effectively. A detection limit of 3×10^3 cfu mL^{-1} was obtained for determination of *E. coli* O157:H7 when used the lectin from *T. vulgaris* as the binding molecule. Furthermore, the proposed biosensor was used to detect *E. coli* O157:H7 in real food samples. Results showed that the lectin based SPR biosensor was sensitive, reliable and effective for detection of *E. coli* O157:H7, which hold a great promise in food safety analysis.

1. Introduction

Enterohaemorrhagic *Escherichia coli* (EHEC) is one of the food-borne pathogenic bacteria that are of most concern today. EHEC infection may lead to Haemolytic uraemic syndrome (HUS) which damage blood cells, lead to kidney failure and even death (Buchanan & Doyle, 1997). Among these EHEC strains, *E. coli* O157:H7 is the most common kind. According to the Center for Disease Control and Prevention (CDC), an estimated 73,000 cases of infection and 61 deaths associated with *E. coli* O157:H7 occur in the United States each year (Mead et al., 1999). Traditional methods for the bacteria detection involves microbiological culturing and isolation of the pathogen, followed by confirmation with biochemical or serological tests. However, such a procedure is always time-consuming and labour intensive (Yang & Bashir, 2008). Therefore, the development of a sensitive and simple method for the rapid detection of *E coli* O157:H7 is highly desirable for food safe applications.

In recent years, biosensors play an increasingly important role in the detection of bacteria due to portability, sensitivity and selectivity. The commonly used bioreceptors in biosensors are antibodies (Abu-Rabeah, Ashkenazi, Atias, Amir, & Marks, 2009; Baccar et al., 2010; Liebana et al., 2009) and nucleic acid probes (Farabullini, Lucarelli, Palchetti, Marrazza, & Mascini, 2007; Liu, Elsholz, Enfors, & Gabig-Ciminska, 2008; Martinez-Paredes, Gonzalez-Garcia, & Costa-Garcia, 2010). However, the intrinsic disadvantages of these bioreceptors, such as expensive and instable, have limited their wide applications. More recently, the use of lectin as the bioreceptor in biosensor has been proven to be promising and effective. Lectins are plant or animal proteins or glycoproteins, which can bind selectively and reversibly with mono- and oligosaccharide components of polysaccharide structures that are major structural components of bacterial cells surface. Recognition of these carbohydrates on the surface of bacteria can be used for specific identification of target bacteria (Safina, van Lier, & Danielsson, 2008). Such a recognising system is superior to antibodies or nucleic acids based systems, since the antibodies or nucleic acids based systems always require some knowledge about the targets, which become increasingly problematic when the targets are unknown (Ngundi, Kulagina, Anderson, & Taitt, 2006). Furthermore, the molecule size of lectins are much smaller than antibodies, thus they allow higher densities of carbohydrate-sensing elements leading to higher sensitivity and lower nonspecific adsorption (Gamella, Campuzano, Parrado, Reviejo, & Pingarron, 2009; Mandenius et al., 2008).

To date, there are few reports on using lectin as the bioreceptor for bacteria detection (Huang, Chen, Shiang, Lin, & Chang, 2009; Lu et al., 2009; Serra, Gamella, Reviejo, & Pingarron, 2008; Shen et al., 2007; Wan, Zhang, & Hou, 2009; Yakovleva, Moran, Safina, Wadström, & Danielsson, 2011). Shen et al. proposed a QCM biosensor by using the lectin (Con A) as the bioreceptor, which showed a low detection limit of a few hundred bacterial cells for detection of *E. coli* (Shen et al., 2007). Yakovleva et al. used four kinds of lectins to discriminate seven *Campylobacter jejuni* (*C. jejuni*) strains based on QCM measurements of dissipation shift (Yakovleva et al., 2011).

Recently, surface plasmon resonance (SPR) technique has attracted extensive attention in the design of biosensor due to its

* Corresponding author. Tel./fax: +86 571 88982885.
E-mail address: ybying@zju.edu.cn (Y. Ying).

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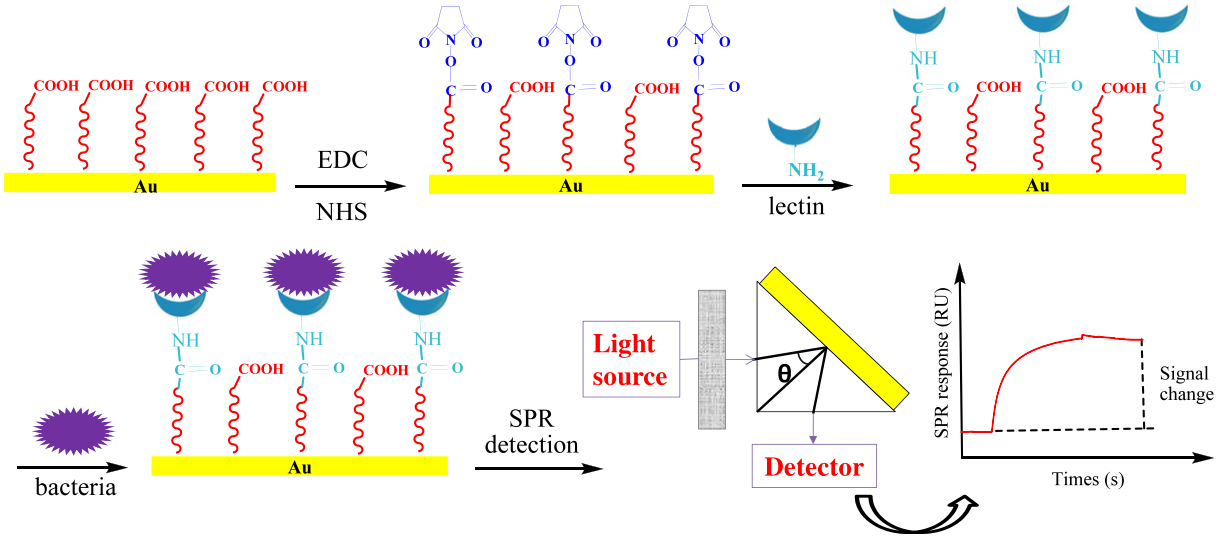

Fig. 1. Scheme of the lectin based surface plasmon resonance biosensor for *E. coli* O157:H7 detection.

label-free and less time-consuming (Linman, Abbas, & Cheng, 2010). Compared with the QCM or electrochemical measurements, SPR has some prominent advantages: firstly it can achieve real-time monitoring of biomolecular interactions, and secondly it can determine both kinetics parameters k_a and k_d as well as the thermodynamics parameters including affinity constant K_A, which is useful for analysis the biological specificity and functions of the bacteria and lectins. However, lectin based surface plasmon resonance (SPR) biosensors are rarely reported, and particularly in the area of bacteria detection there is no report until now. In this work, a novel surface plasmon resonance biosensor using lectin as the bioreceptor for rapid detection of *E. coli* O157:H7 was present. The scheme for the sensor setup used in this work is illustrated in Fig. 1. Lectins were immobilised on the CM5 chip through a self-assembly monolayer (SAM) to capture *E. coli* O157:H7 in the buffer solution. Five different lectins from *Triticum vulgaris* (WGA), *Canavailia ensiformis* (Con A), *Ulex europaeus* (UEA), *Arachis hypogaea* (PNA), *Maackia amurensis* (MAL), were used to screen the optimal lectin that bound *E. coli* O157:H7 effectively. A correlation between the SPR signal and the concentration of *E. coli* O157:H7 was found when used the lectin from WGA as the binding molecule. *E. coli* DH 5α and *Listeria monocytogenes* (*L. monocytogenes*) combined with the above five lectins were used to study the selectivity of the biosensor. Furthermore, the proposed sensor was applied to determine *E. coli* O157:H7 in real food samples.

2. Experiment

2.1. Reagents

Sensor chip CM5, sodium acetate buffer (0.01 M, pH 4.5), HBS-P buffer (0.01 M, pH 7.4) and ethanolamine hydrochloride (1 M, pH 8.5) were from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). *N*-ethyl-*N*-(dimethyl-aminopropyl) carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), Lectins from *T. vulgaris* (WGA), *C. ensiformis* (Con A), *U. europaeus* (UEA), *A. hypogaea* (PNA), *M. amurensis* (MAL) were purchased from Sigma–Aldrich (St. Louis, MO). All other chemicals used were of analytical grade, and all solutions were prepared with deionized water from Millipore (Millipore-Q, 18.2 MΩ cm).

The immobilisation buffer solution was prepared by dissolving lectins into sodium acetate buffer solution (0.01 M, pH 4.5). Running buffer solution was the commercial HBS-P buffer (0.01 M HEPES, 0.15 M NaCl, 0.005% surfactant P20, pH 7.4) further adding 0.5 mM of Ca^{2+}, Mg^{2+} and Mn^{2+}.

2.2. Instruments

SPR studies were carried out on a Biacore 3000 system from BIAcore AB (Uppsala, Sweden). Sensor chip CM5 modified with carboxymethyl dextran used as the binding substrate was obtained from BIAcore AB (Uppsala, Sweden). All the experiments were conducted at around 25 °C.

2.3. Bacteria and culture plating methods

E. coli O157:H7 and *L. monocytogenes* positive control were obtained from KPL (Gaithersburg MD, USA). The freeze-dried powders of *E. coli* O157:H7 and *L. monocytogenes* were rehydrated with 1 mL of deionized water to the concentration of 3.0×10^9 cfu mL^{-1}. And then the mother liquor was serially diluted to 3.0×10^1–3.0×10^8 cfu mL^{-1} (10-fold steps) for the measurement.

E. coli DH 5α (ATCC PTA-3137) was obtained from college of food science in Zhejiang university. The pure culture of *E. coli* DH 5α was grown in Luria broth (LB) medium at 37 °C for 12 h before use. The culture was serially diluted to 10^{-7} (10-fold steps) with physiological saline solution, and viable cell number was determined by a microbial plate count method through applying 0.1 mL of dilutions on LB agar, respectively, and incubating for 24 h at 37 °C. At the same time, the culture was then heated in a 100 °C water bath for 15 min and was serially diluted to 3.0×10^1–3.0×10^8 cfu mL^{-1} for the measurements.

2.4. Fabrication of lectin based SPR biosensor

The simple scheme for the fabrication of SPR biosensor is shown in Fig. 1. Firstly, the surface of sensor chip was activated by injecting 70 μL mixture of 0.1 M NHS with 0.4 M EDC at 10 μL min^{-1} for 7 min. Then, lectins (0.1, 0.5, 1.0 mg mL^{-1}) in 0.01 M sodium acetate buffer (pH 4.5), were injected over the chip surface at 10 μL min^{-1} for 15 min, respectively. Unreacted sites were subsequently deactivated by injecting 1 M ethanolamine hydrochloride (pH 8.5) at 10 μL min^{-1} for 7 min. Then, different concentrations of diluted *E. coli* O157:H7 (3.0×10^1–3.0×10^8 cfu mL^{-1}) were flowed for 5 min at 10 μL min^{-1} for direct detection. After each detection step, the chip surface was regenerated by injecting 10 μL of

1 mM NaOH every time. The specificity test was performed by injecting 50 μL *E. coli* DH 5α or *L. monocytogenes* with concentration of 3.0×10^8 cfu mL^{-1} onto the lectins coated chip. All of these experiments were done randomly in triplicate.

The rate constants were determined as described by Mehta, Cummings, and McEver (1998). Affinity constants were calculated from the experimental values of the rate constants (k_{on} and k_{off}).

2.5. Preparation of food samples

The food samples including cucumber and ground beef are the most common polluted foods by *E. coli* O157:H7, which have been used as real matrix in this work. The cucumber sample was prepared as followed: firstly, 10 g of cucumber was mixed with 90 mL of the binding buffer (0.01 M HBS-P buffer), homogenised using a homogenizer and filtered through a filter paper (80–120 μm); then, 100 μL different concentrations of *E. coli* O157:H7 of 3.0×10^2–3.0×10^9 cfu mL^{-1} were added to 900 μL filtered cucumber solutions to reach the concentrations of 3.0×10^1–3.0×10^8 cfu mL^{-1}. The preparation of ground beef sample was similar with the above process. The samples without adding *E. coli* O157:H7 were used as negative control.

2.6. AFM imaging

Tapping-mode atomic force microscopy (AFM) experiments were performed on the Dimension Icon AFM equipped with a ScanAsyst (Bruker AXS, Germany). All images are presented without any subsequent data processing. Before taking AFM, each chip was washed thoroughly using water to remove nonspecific absorbed lectins and nonspecific bound *E. coli* and dried by a stream of nitrogen. The AFM was measured in open air.

3. Results and discussion

3.1. Surface modification of the sensor chip

The whole procedure for surface modification of the sensor chip could be divided into three steps (Fig. 1): activation, immobilisation and blocking. And the typical SPR response for these processes was recorded. As shown in Fig. S1 (Supplementary data I), the activation process performed by NHS-EDC induced an obvious change of SPR response (Fig. S1(a)). After rinsing with running buffer solution, lectins were immobilised onto the surface of CM5 chip through an amide bond. Immobilization of 0.1, 0.5, and 1.0 mg mL^{-1} lectins (from WGA) produced a triplicate average response of ~1865, ~2561, and ~2863 RU, respectively (data not shown). Increasing the concentration of lectins resulted in an enhanced immobilisation of lectins on the surface. Therefore, 1.0 mg mL^{-1} of lectins was chose for the immobilisation. As shown in Fig. S1(b), this process always took ~15 min and the increase of the SPR response for the immobilisation of lectin was ~2863 RU. The last process was the blocking of remained sites on the chip surface (Fig. S1(c)).

Tapping-mode AFM was used to confirm the immobilisation of the lectins on the CM5 chip. Fig. 2A shows the surface topography of the dextran modified chip, which is smooth, homogeneous with only a few surface aggregates. After immobilised with lectins, the obtained surface (Fig. 2B) displays a significantly different topography compared with the initial surface (Fig. 2A). As shown, the

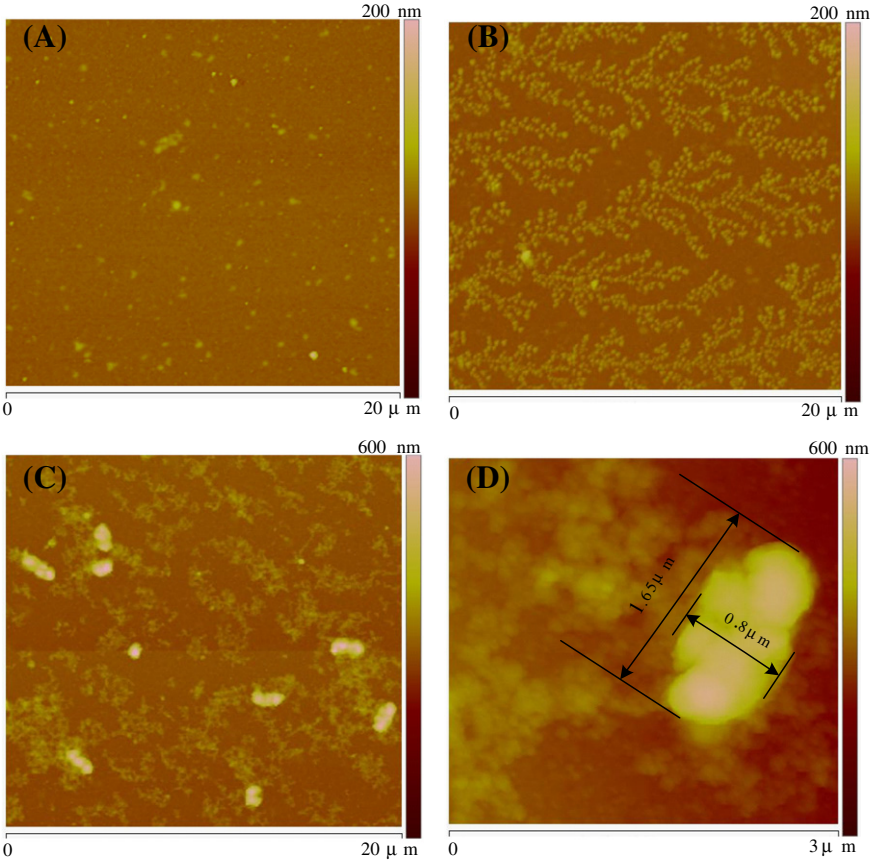

Fig. 2. AFM images of CM5 chip modified with dextran (A), then immobilised with lectins from WGA (B), captured *E. coli* O157:H7 cells (C), and a small part (D) of chip surface (C) with 45 magnifications.

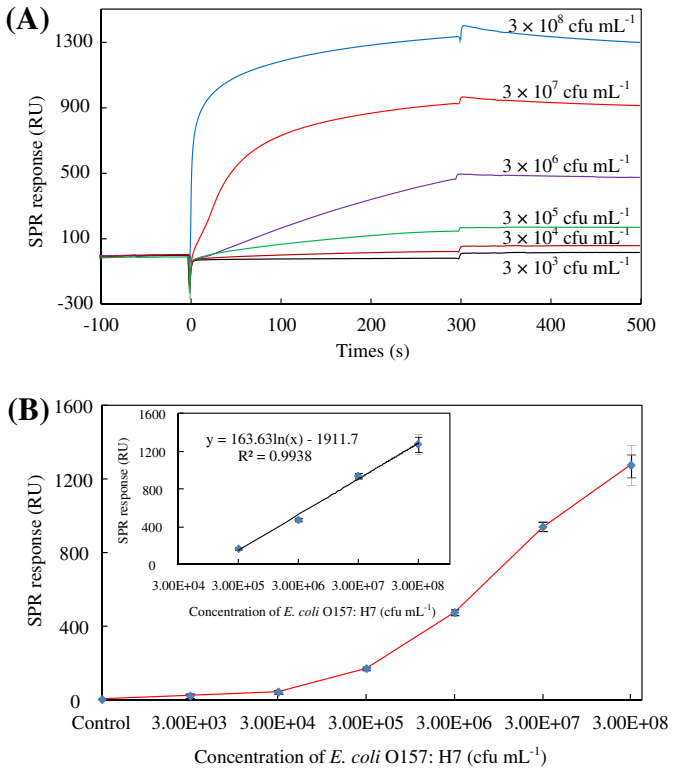

Fig. 3. (A) SPR sensorgrams obtained with binding different concentrations of *E. coli* O157:H7 from 3.0×10^3–3.0×10^8 cfu mL^{-1} using WGA chip; (B) SPR response caused by negative control and different concentrations of *E. coli* O157:H7 from 3.0×10^3–3.0×10^8 cfu mL^{-1} in buffer system, inset: a linear correlation between the SPR response and the different concentrations of *E. coli* O157:H7 from 3.0×10^5–3.0×10^8 cfu mL^{-1}.

lectins are globular due to their protein nature, indicating the immobilisation of the lectins on the dextran coated chip. Meanwhile, the lectin film is dense, smooth and homogeneous. Such a film may provide a favourable platform for high sensitive and reproducible determination of bacteria.

3.2. Monitoring of the lectin-bacteria binding

The accumulation of *E. coli* O157:H7 bound to the surface resulted in an increase in refractive index, which served as a SPR signal. As shown in Fig. 3A, cell concentration of 3.0×10^8 cfu mL^{-1} resulted in a significant SPR signal increase (1275 RU), which indicated that the lectin from WGA can bind *E. coli* O157:H7 effectively. In order to further confirm the binding of the target bacteria onto the surface of lectin modified chip, the chip used in the experiment was examined with AFM. Fig. 2C showed the surface topography of *E. coli* O157:H7 cells binding to the lectin modified chip. To make a comparison between panels B and C of Fig. 2, the distinctive difference in the topography of the surface can be observed before and after binding of *E. coli* O157:H7 cells (3.0×10^8 cfu mL^{-1}) onto the surface of lectin modified chip. As shown in Fig. 2D, a single *E. coli* O157:H7 cell firmly bound on the chip surface, confirming the binding capacity of the lectin from WGA for *E. coli* O157:H7.

3.3. Sensor performance for detection of E. coli O157:H7

Fig. 3A presents the SPR response of the lectin from WGA based biosensor towards different concentrations of *E. coli* O157:H7 from 3×10^3 to 3×10^8 cfu mL^{-1}. It can be seen that the SPR response increased with the increasing concentrations of *E. coli* O157:H7. The results demonstrated that the more cells bound on the chip surface, the higher was the SPR response of the lectin based SPR biosensor. A calibration curve using response units plotted against the *E. coli* O157:H7 concentrations, was constructed (Fig. 3B). As shown, the range of detection was found to be 3.0×10^3–3.0×10^8 cfu mL^{-1} and a linear relationship ($R^2 = 0.9938$) between the SPR response and the concentrations of *E. coli* O157:H7 in a

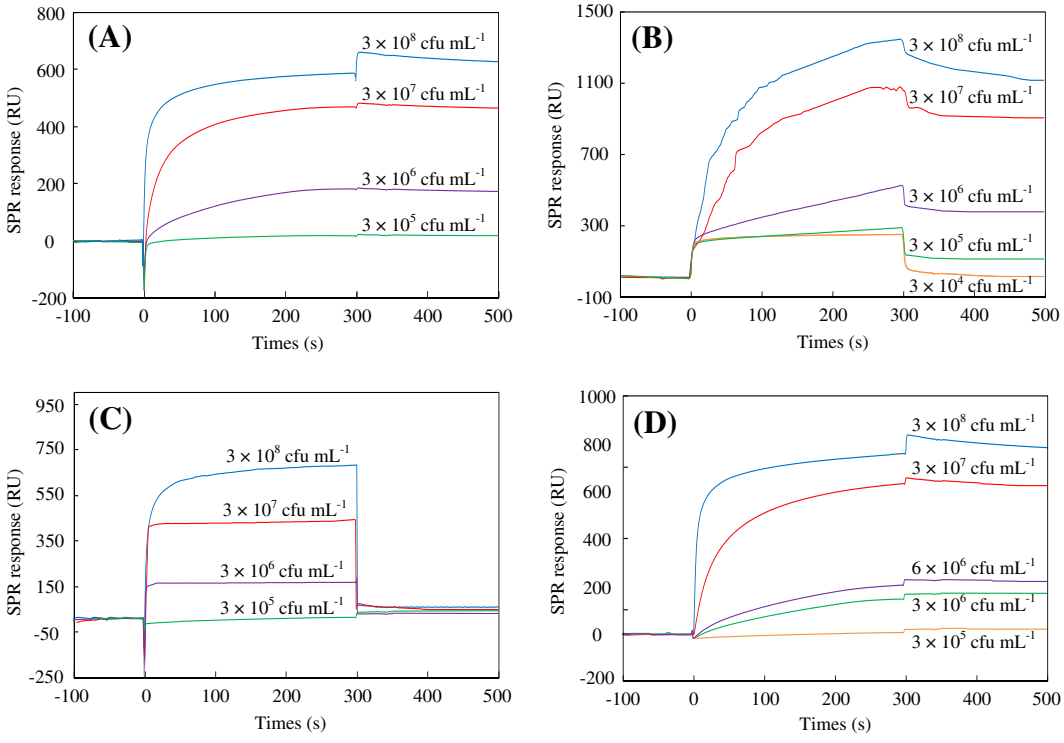

Fig. 4. SPR sensorgrams obtained with binding different concentrations of *E. coli* O157:H7 using four kinds of lectins modified chips, (A) Con A, (B) UEA, (C) PNA, (D) MAL.

range from 3×10^5 to 3×10^8 cfu mL^{-1} was established. Triplicate tests were done for each concentration of bacteria and the standard deviation (SD) for each point of the standard curves (3.0×10^8, 3.0×10^7, 3.0×10^6, 3.0×10^5, 3.0×10^4, 3.0×10^3 cfu mL^{-1} and control) was 53, 26, 16, 11, 8, 11 and 0.4, respectively, which demonstrated the good reproducibility of the lectin based SPR biosensor.

The limit of detection here is defined as the concentration of cells resulting in a detection signal that is the average value of the detection signal obtained due to the control signal plus 3 times its standard deviation (Mazumdar, Hartmann, Kampfer, & Keusgen, 2007). The control signal and the standard deviation were 7 RU and 0.4, respectively. The signal of 3×10^3 cfu mL^{-1} was 26 RU, which was larger than the control signal plus 3 times the standard deviation. Therefore, the limit of detection of the lectin-based SPR biosensor was 3×10^3 cfu mL^{-1}, which was comparable with the antibody and nucleic acid based biosensors for detection of bacteria in previous literatures (see Table S1 in Supplementary data II), nevertheless our developed biosensor was inexpensive, stable and easy to be prepared.

The complete regeneration of biosensor was achieved by using 10 μL of 1 mM NaOH solution every time without degradation of binding properties. Under these regeneration conditions, the biosensing surface remained stable during 50 measurements intraday (data not shown).

3.4. Screen the lectin for detection of E. coli O157:H7 with strong affinity

Five lectins from WGA, Con A, UEA, PNA and MAL, were selected according to their specific affinity to the main classes of the sugar structures (Supplementary data III). Con A and PNA are specific to bind mannose and galactose, MAL and WGA are specific to bind sialic acid and β-N-acetylglucosamine, UEA is able to bind fucose specifically. Fig. 3A and Fig. 4 show the SPR response of the five lectins for binding *E. coli* O157:H7. The most significant responses were obtained from WGA surface, indicating the lectin from WGA had a high binding capacity for binding *E. coli* O157:H7. WGA is a dimeric protein with two identical 18 kDa subunits, each consisting of four homologous domains of 43 amino acids. It is reported that the WGA can bind the GlcNAc form on the surface of *E. coli* O157:H7 at two high affinity and two low affinity sites per subunit (Mandenius et al., 2008). This may explain the observed strong binding capacity of WGA with *E. coli* O157:H7. Lectin from UEA showed a slightly weaker binding capacity compared with the WGA (Fig. 4B). UEA has two subunits, which can bind the fucose specifically, and hence it can bind the *E. coli* O157:H7 through the fucoses of cell surface. The interaction was much weaker when using Con A (Fig. 4A) and MAL (Fig. 4D) as the binding molecules. For the PNA, the binding was almost negligible (Fig. 4C).

Kinetic and affinity data for binding of *E. coli* O157:H7 to the lectins were used to evaluate the specific interaction. The kinetic parameters (K_{on} and K_{off}) indicate what fraction of the molecules in the initial compartment is travelling through the pathway per unit time. Affinity parameter (K_A) is a useful way to present the affinity of lectins and bacteria. The higher the association constant (K_A) is, the tighter the *E. coli* O157:H7 binds to the lectins. The WGA bound with *E. coli* O157:H7 exhibited high association rate constants (k_{on}) of 3.36×10^5 M^{-1} s^{-1} and low dissociation rate constant (k_{off}) of 1.63×10^{-3} s^{-1} leading to strong affinity binding with a K_A of 2.07×10^8 M^{-1} probably due to multimeric binding sites. The kinetic data also showed similar strong affinity binding with the Con A, UEA and MAL with the K_A of 1.81×10^8, 5.01×10^7, 8.22×10^7 M^{-1}, respectively. While the PNA exhibited high association rate constant (k_{on}) of 2.89×10^5 M^{-1} s^{-1} and high dissociation rate constant (k_{off}) of 5.31 s^{-1} leading to low affinity binding with *E. coli* O157:H7 with a K_A of 5.44×10^4 M^{-1}.

3.5. Specificity testing

The selectivity of the approach was evaluated by checking the SPR response of binding between five different lectins and three different bacteria (*E. coli* O157:H7, *E. coli* DH 5α and *L. monocytogenes*) at the same concentration level (3×10^8 cfu mL^{-1}). The SPR response values were classified in three ranges: the SPR response value >1000 RU was denoted as (++), the SPR response value ranging between 20 and 1000 RU was denoted as (+), and the SPR response value <20 RU was denoted as (0). As summarized in Table 1, different lectins exhibited different affinities with different bacteria and gave different SPR response. Such a result is in a good agreement with the previously published data (Gamella et al., 2009; Serra et al., 2008). Different bacteria response profiles were

Table 1
SPR response obtained between the five kinds of lectins and three types of microorganisms.

Lectin	Microorganism		
	E. coli O157:H7	*E. coli* DH 5α	*L. monocytogenes*
Triticum vulgaris (WGA)	++	+	++
Concanavlin A (Con A)	+	+	+
Ulex europaeus (UEA)	++	+	0
Arachis hypogaea (PNA)	0	0	+
Maackia amurensis (MAL)	+	+	+

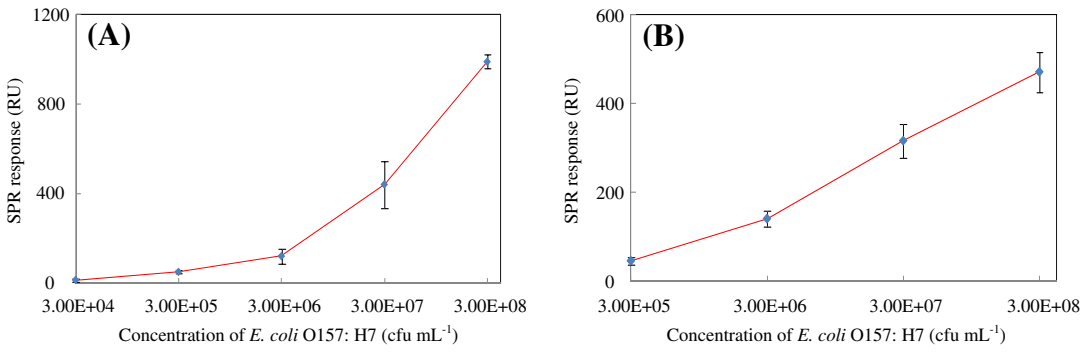

Fig. 5. (A) SPR response of cucumber sample inoculated with different concentrations of *E. coli* O157:H7 from 3.0×10^4–3.0×10^8 cfu mL^{-1} using WGA modified chip; (B) SPR response of ground beef sample inoculated with different concentrations of *E. coli* O157:H7 from 3.0×10^5–3.0×10^8 cfu mL^{-1} using WGA modified chip .

achieved when different lectins were used, which made it possible for the lectin based SPR biosensor for detection of *E. coli* O157:H7 with high specificity.

3.6. Detection of E. coli O157:H7 in food samples

The feasibility of the proposed SPR biosensor for use in measuring *E. coli* O157:H7 in real food samples was investigated. Two food samples, including cucumber and ground beef, were employed. The calibration curves using the SPR response (the value of samples minus the negative control) plotted against *E. coli* O157:H7 concentration, were constructed (Fig. 5). As shown, the limit of detection in case of *E. coli* O157:H7 contaminated cucumber samples and ground beef samples was 3.0×10^4 and 3.0×10^5 cfu mL^{-1}, respectively. The depressed sensitivity of the WAG based SPR biosensor in cucumber samples could be attributed to the non-specific adsorption of dietary fibre, carbohydrate, vitamin, etc. (Li et al., 2011). While for ground beef samples, the substantial number of non-pathogenic *E. coli* cells may hinder the transport of *E. coli* O157:H7 to the sensor surface (Campbell, Uknalis, Tu, & Mutharasan, 2007). Nevertheless, a correlation between the SPR response and the concentration of *E. coli* O157:H7 cell in these two samples could be achieved, which demonstrated the possibility of the lectin based SPR biosensor for detection of bacteria in complex food samples.

4. Conclusion

A novel type of SPR biosensor using the lectin as the bioreceptor for the determination of *E. coli* O157:H7 was proposed. This new sensor configuration combined the advantages of SPR technique with the lectin, which brought out an advanced and high-performance sensing platform for *E. coli* O157:H7 detection. When using the lectin from WGA as the binding molecule, the obtained SPR biosensor exhibited a wide linear range from 3×10^5 to 3×10^8 cfu mL^{-1} and a low detection limit of 3×10^3 cfu mL^{-1}. Different response profiles were achieved for three different bacteria (*E. coli* O157:H7, *E. coli* DH 5α, *L. monocytogenes*) and five different lectins (WGA, Con A, UEA, PNA and MAL), which demonstrated the lectin based SPR biosensor for detection of bacteria with high specificity. Furthermore, the developed SPR biosensor was successfully used to determine bacteria in real food samples including cucumber and ground beef. This work firstly reveals that the lectin could be a favourable and promising bioreceptor material for constructing SPR-based bacteria biosensors, which may have wide applications in food safety analysis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2012.09.069.

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