Journal of Biotechnology 160 (2012) 123–128

Contents lists available at SciVerse ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

# Label-free and high-sensitive detection of *Salmonella* using a surface plasmon resonance DNA-based biosensor

Decai Zhang[a,1], Yurong Yan[a,1], Qing Li[a], Tianxiao Yu[a], Wei Cheng[a,c], Long Wang[a], Huangxian Ju[a,b], Shijia Ding[a,*]

[a] *Key Laboratory of Clinical Laboratory Diagnostics (Ministry of Education), Department of Laboratory Medicine, Chongqing Medical University, Chongqing 400016, China*
[b] *State Key Laboratory of Analytical Chemistry for Life Science, Department of Chemistry, Nanjing University, Nanjing 210093, China*
[c] *Molecular Oncology and Epigenetics Laboratory, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China*

ARTICLE INFO

*Article history:*
Received 8 November 2011
Received in revised form 20 February 2012
Accepted 23 March 2012
Available online 4 April 2012

*Keywords:*
*invA* gene
Surface plasmon resonance
Biosensor
Polymerase chain reaction
*Salmonella*

ABSTRACT

A method based on surface plasmon resonance (SPR) DNA biosensor has been developed for label-free and high-sensitive detection of *Salmonella*. A biotinylated single-stranded oligonucleotide probe was designed to target a specific sequence in the *invA* gene of *Salmonella* and then immobilized onto a streptavidin coated dextran sensor surface. The *invA* gene was isolated from bacterial cultures and amplified using a modified semi-nested asymmetric polymerase chain reaction (PCR) technique. In order to investigate the hybridization detection, experiments with different concentration of synthetic target DNA sequences have been performed. The calibration curve of synthetic target DNA had good linearity from 5 nM to 1000 nM with a detection limit of 0.5 nM. The proposed method was applied successfully to the detection of single-stranded *invA* amplicons from three serovars of *Salmonella*, i.e., Typhimurium, Enterica and Derby, and the responses to PCR products were related to different *S. typhimurium* concentrations in the range from $10^2$ to $10^{10}$ CFU $mL^{-1}$. While with this system to detect *E. coli* and *S. aureus*, no significant signal was observed, demonstrating good selectivity of the method. In addition, the hybridization can be completed within 15 min, and the excellent sensor surface regeneration allows at least 300 assay cycles without obvious loss of performance.



## 1. Introduction

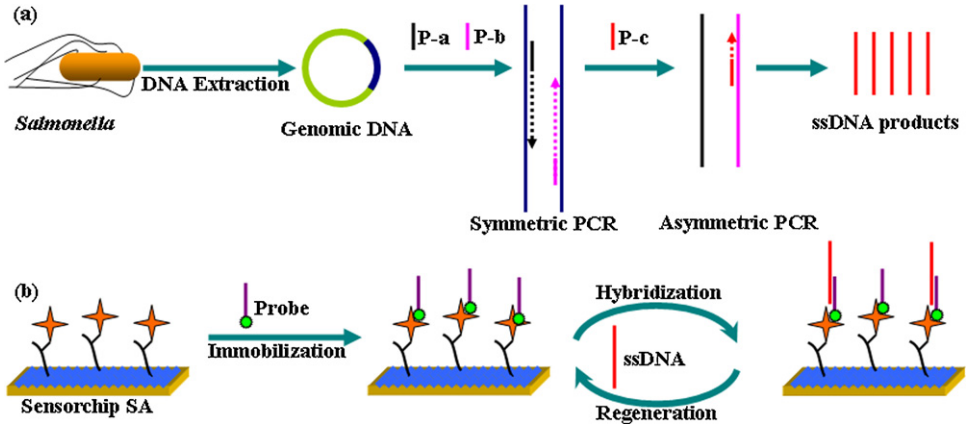
Salmonellosis is considered to be one of the most widespread foodborne zoonosis posing a public health risk. It is a global disease in both industrialized and developing countries, although the incidence varies (Pieskus et al., 2008). Rapid diagnosis of *Salmonella* infection could prevent epidemic outbreak as well as allow earlier control interventions and corresponding therapy (Sparbier et al., 2011).

Traditional culture-based methods are usually precise, but cumbersome and time-consuming, typically requiring 3–4 days to indicate negative results and up to 7 days for a confirmed positive result (Bhagwat, 2004). Most immunoassay techniques are also widely used, the methods of radioimmunoassay, fluorescence labeled antibody assay, enzyme-linked immunosorbent assays (ELISA) and immunoaffinity column assay have some limitations, such as the labeled antibody, the radiation hazards, the complicated wash procedure, and a long analysis time (Yang et al., 2009). Molecular techniques, in particular the polymerase chain reaction (PCR), can circumvent many of these limitations. The PCR-based amplification of pathogen-specific DNA provides a promising means for achieving the rapid and reliable identification of microbes, avoiding cumbersome, time-consuming culturing steps, unfortunately, PCR products are traditionally detected by agarose gel electrophoresis. While this method is simple, it lacks sensitivity (Ahn and Walt, 2005). Alternative methods including real-time PCR (Hoorfar et al., 2000) and dot blot hybridization (Lida et al., 1993) provide higher sensitivity, but are rather unwieldy and require a procedure of complex labeling. Thus, a simple, label-free, and sensitive method needs to be developed to overcome the drawbacks of these existing methods.

Surface plasmon resonance (SPR) technologies have been widely used for quantitative analysis of various target biomolecules (Homola, 2003). In SPR, the refractive index of the solution close to the sensor surface changes when a detecting molecule binds to the ligand-coated sensor chip, SPR measures the change, which can be correlated to the amount of sensor surface-bound molecules. There are a number of potential advantages to the use of SPR systems. Labeling, such as fluorescence or dye tagging, is not necessary,

* Corresponding author. Tel.: +86 23 68485688; fax: +86 23 68485786.
*E-mail address:* dingshijia@163.com (S. Ding).
[1] These authors contributed equally to this work.

0168-1656/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jbiotec.2012.03.024




**Scheme 1.** Schematic illustration of the strategy for *Salmonella* detection. (a) DNA extraction from *Salmonella* cells and amplification using the second step-asymmetric PCR. (b) Probe immobilization and interaction with PCR amplicons.

the interaction of a molecule with the biosensor surface may be evaluated in real time, and only a very small sample volume is required (Hwang et al., 2005). Recently, SPR-based assays for detection of bacteria, including *Salmonella* cells, which usually employ antibodies (Abs) as recognition elements, have been described in literature (Bokken et al., 2003; Oh et al., 2004; Subramanian et al., 2006; Mazumdar et al., 2007, 2010; Waswa et al., 2007). However, the production of specific Abs is difficult, expensive and extremely time-consuming, and antibodies can easily lose their activity when environmental conditions changed (Arya et al., 2010).

In the present study, a new method based on SPR DNA biosensor for the rapid detection of the *invA* gene was proposed to detect *Salmonella*. The *invA* is a highly conserved gene located on *Salmonella* pathogenicity island 1 (SPI-1) (Malorny et al., 2003), which presents in almost all *Salmonella* serovars (Daum et al., 2002) and has been used as a potential target for *Salmonella* detection (Rahn et al., 1992; Jeong et al., 2011). Single-stranded biotinylated oligonucleotide probes containing *invA* gene sequences were immobilized onto a layer of streptavidin, which is covalently linked to carboxylated dextran fixed onto the gold surface of the chip. This procedure resulted extremely efficient in terms of sensitivity, selectivity and stability of the realized sensor chip for hybridization detection (Wang et al., 2004). The *invA* gene was isolated from bacterial cultures by a rapid and efficient boiling method and amplified using a modified semi-nested asymmetric PCR technology, then the obtained single-stranded amplicons were hybridized with the complementary probes immobilized on a sensor surface. The basic principle of the proposed method is illustrated in Scheme 1. The sensitivity, specificity and reproducibility of designed DNA sensor were evaluated in details, and the applicability of the proposed strategy was demonstrated by detecting of three serovars of *Salmonella* with a series of concentrations.

## 2. Materials and methods

### 2.1. Bacterial strains and DNA template preparation

The bacterial strains used in these experiments were kindly donated from Chongqing Center for Disease Control and Prevention (Chongqing, China). These strains were grown aerobically at 37 °C for 16 h in sterile liquid Luria-Bertani medium. Viable counts were performed by plating 100 μL of appropriate 10-fold dilutions in sterile PBS solution onto plate count agar in triplicate and incubating the plates for 24 h at 37 °C. The concentration was estimated by calculating the average number of CFU. One milliliter aliquot of each bacterial culture was centrifuged at 10,000 rpm for 5 min at 4 °C. The supernatant was carefully removed and the cell pellet was washed in 1 mL double-distilled water. After centrifugation, the cell pellet was then resuspended in 50 μL double-distilled water. The microcentrifuge tube was incubated for 15 min at 100 °C in a water bath and immediately chilled on ice. After centrifugation at 10,000 rpm for 5 min at 4 °C, the supernatant containing genome DNA was carefully transferred to a new tube. A 5 μL aliquot was used as template DNA for the PCR. All DNA preparations were stored at −20 °C prior to use.

### 2.2. Synthetic oligonucleotides

The gene of the *invA* was used to design a specific probe for *Salmonella* by exploring the GeneBank database, the specificity of primers for the PCR amplification of *invA* gene fragment had been positively verified via the BLAST search engine (http://www.ncbi.nlm.nih.gov/blast). All oligonucleotides used in the experiment were synthesized by Sangon Biotech (Shanghai, China). The corresponding nucleotide sequences of the oligonucleotides used are reported in Table 1. All oligonucleotides were dissolved with TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0) into stock solutions and stored at −20 °C. They were diluted in appropriate buffer before use.

### 2.3. Polymerase chain reaction (PCR) amplification

The PCR reaction was carried out using a My Cycler thermal cycler (Bio-Rad Laboratories, USA) with TaKaRa Premix Taq® Version 2.0 (TaKaRa Biotechnology Co., Dalian, China). The DNA amplifications were performed in a two-step PCR. A pair of primers was used to produce double-stranded DNA (dsDNA), and an internal primer was introduced to obtain single-stranded DNA (ssDNA) products. The first step was performed in a total volume of 50 μL contained 5.0 μL of genomic DNA, 1.0 μL 20 μM of P-a, 1.0 μL 20 μM

**Table 1**
Oligonucleotides used in the present work.

| Oligonucleotide | Sequence (5′-3′) |
|---|---|
| Primer a (P-a) | TCTGGTTGATTTCCTGATCGCA |
| Primer b (P-b) | GCCAGGCTATCGCCAATAACGA |
| Primer c (P-c) | ACACCAATATCGCCAGTACG |
| Probe | biotin – TCTGGTTGATTTCCTGATCGCA |
| Target oligonucleotide | ACACCAATATCGCCAGTACGATATT-CAGTGCGAGCAGGAAATCAACCAGA |
| Non-complementary oligonucleotide | TGCCGATTTCAAGGCCGGATGCT-AGATTCCTTTGGCGGTGCGGCTACTGC |

of P-b, 25 μL of Premix Taq (1.25 U of DNA polymerase, 2 × Taq buffer, 0.4 mM of dNTPs) and 18 μL of distilled water. The cycling conditions consisted of an initial denaturation at 95 °C for 1 min, and then 35 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s. In the final cycle, extension was carried out at 72 °C for 4 min. Amplification products of dsDNA were checked using electrophoresis in 2% agarose gels and detected by GoldView (SBS Genetech, Beijing, China) staining. Gel images were captured on an imaging system (Bio-Rad Laboratories, USA). Between the first and second steps, a tube of the first PCR products was purified using DNA Fragment Purification Kit (TaKaRa Biotechnology Co., Dalian, China), dissolved in 30 μL distilled water and 5 μL of the dilutions were used as template for the second amplification of 40 cycles (95 °C for 30 s, 50 °C for 30 s and 72 °C for 30 s), the reaction performed in a volume of 50 μL (5.0 μL of template, 1.0 μL 20 μM of P-c, 25 μL of Premix Taq and 19 μL of distilled water).

### 2.4. SPR technical platform

For all the experiments, the SPR device Biacore X™ analytical system and SA chips with streptavidin covalently immobilized on a carboxymethylated dextran matrix were used (BIAcore AB, Uppsala, Sweden). There are two independent flow cells on each sensor chip (Fc1, Fc2). Running buffer was HEPES buffered saline-EP (HBS-EP), which contains 10 mM HEPES (pH 7.4), 0.15 M NaCl, 3 mM EDTA, and 0.005% (v/v) Surfactant P20 (BIAcore AB, Uppsala, Sweden). All buffers for experiments were filtered (pore size 0.22 μm) and degassed before use. All experiments were conducted at a sample flow rate of 5 μL min$^{-1}$ and at an operating temperature of 25 °C. The Biacore X™ displays results as a time course of resonance units (RU). The analytical signal was derived from the RU difference between the final value and the value (baseline value) recorded before analyte injection. All sensorgrams are displayed in subtracted trace in which non-specific binding and buffer-induced bulk refractive index changes are eliminated, displaying only the response corresponding to the true binding event. Sensorgrams were evaluated using the BIA evaluation 4.1 software (Biacore).

### 2.5. Probe immobilization

The SA chip was first cleaned with three consecutive 1-min injections of a solution of 1 M NaCl in 50 mM NaOH before the immobilization procedure. When the sensorgram reached a stable baseline after the cleaning process, biotinylated probes (1 μM in HBS-EP) were injected into the Fc2 of the SA chip for 7 min using a flow rate of 5 μL min$^{-1}$. Notably, Fc1 was not immobilized with any probes, only used as the background control for detection. The sensor chip was equilibrated with running buffer and subsequently washed with 5 μL of 50 mM NaOH to remove all solutes from the flow cell except the probes binding to the SA chip by biotin-avidin system.

### 2.6. SPR detection

Synthetic oligonucleotides were diluted to the desired concentration with hybridization buffer (30 mM sodium phosphate, 450 mM NaCl, 3 mM EDTA, and 0.25% Triton ×100, pH 7.4), amplified products from PCRs were added to the hybridization buffer in equal volumes. For each sample, 70 μL of the synthetic oligonucleotides or PCR products in hybridization buffer were injected into both the probe immobilized and reference flow cells on a SPR chip. After hybridization reaction, the chip was automatically washed with the HBS-EP buffer to remove the unbound DNA sample.

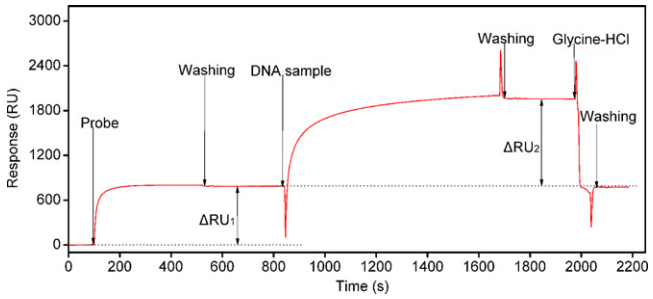



**Fig. 1.** A typical sensorgram of ssDNA probe immobilization and target DNA hybridization on the BIAcore's SA chip. At the end of each reaction step, the chip surface could be regenerated by Glycine–HCl solution.

### 2.7. Surface regeneration

After each detection, 5 μL of regeneration solution (10 mM Glycine–HCl, pH 3.0) were injected to the flow cells to dissociate the bound target DNA. The cells were equilibrated with HBS-EP buffer, the sensor chip was then ready for the next measurement. To test whether the capture activity of the SA chip was harmed or affected by the regeneration process, repeated interlaced injections of synthetic DNA sample and regeneration solution were performed. This procedure was repeated 300 times to determine the regeneration performance.

## 3. Results and discussion

### 3.1. Immobilization of biotinylated probes

Biotinylated probes can be captured on an SA sensor surface within 4–6 min and approximately 800–1000 RU could be obtained in different experiments using recommended protocols (an example is shown in Fig. 1). Continuous injections of the probe gave almost no increase of signal, indicating the SA chip surface was saturated by the probe. Even after washing the surface exhaustively (>3 h) with running buffer, the response signal remained constant without baseline drift (data not shown). Such a sensor surface is really stable enough for the hybridization of analyte DNA samples. A typical sensorgram obtained from the binding of target DNA and immobilized probes is shown in Fig. 1. As expected, the target DNA is able to specifically hybridize to the immobilized probe, generating a stable complex on sensor surface.

### 3.2. The performance of biosensor for the detection of synthesized oligonucleotides

In order to elucidate the analytical performance of the biosensor proposed in this work, experiments were carried out with different concentrations of synthetic target oligonucleotides. In Fig. 2A, the response signal increases with the increase of target nucleic acid concentration. The response was linear with the logarithm of target DNA concentration in the range form 5 nM to 1000 nM, and the detection of as low as 0.5 nM can be achieved (Fig. 2B), the corresponding regression equation was ($\Delta$RU) $Y = -145.13 + 435.51 \times \lg C$ (nM) with the correlation coefficient of 0.9994. Each point of the calibration curve was done three times, the error bars shown inset of Fig. 2B represent standard deviations. The average of the relative standard deviations (RSDs) was 4.3%, which guaranteed the precision of the biosensor. Rapidity is the most prominent advantage of this method. The time required for the hybridization is only 15 min, moreover, with this procedure no labeling is required and the hybridization interaction can be observed in real-time.

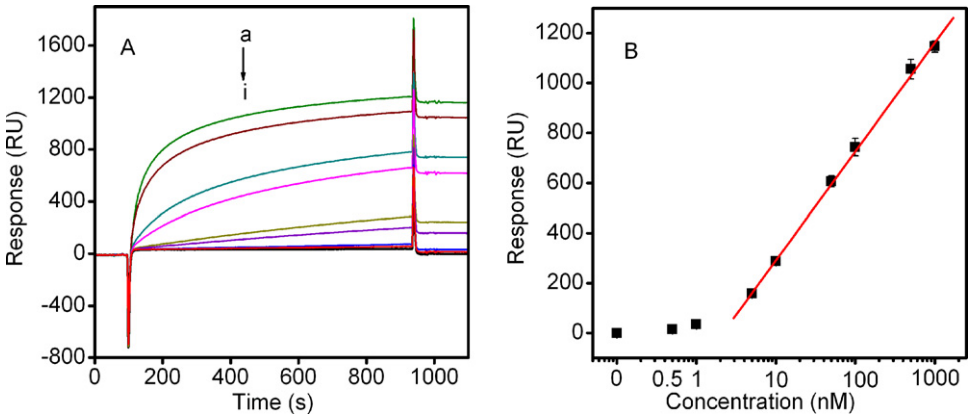



**Fig. 2.** (A) The sensorgrams obtained with synthetic target DNA concentrations of 1000 nM, 500 nM, 100 nM, 50 nM, 10 nM, 5 nM, 1 nM, 0.5 nM, 0 nM (from a to i). (B) Calibration curve of ΔRU versus synthetic target DNA concentration.

### 3.3. Amplification of target sequence and identification of PCR products

PCR amplicon, which is generally dsDNA, needs to be pre-treated to obtain ssDNA available for hybridization with the complementary immobilized probe. The most common procedure involving thermal denaturation is not compatible with SPR instrumentation using flow systems such as Biacore X$^{TM}$ (Mariotti et al., 2002). Magnetic particle separation (Mariotti et al., 2002), producing 'unilateral protruding DNA' (UPD) (Miyachi et al., 2000) or using peptide nucleic acids (PNA) probes (Kai et al., 2000) can solve the problem but they are expensive and complicated. The target ssDNA can be obtained by asymmetric PCR amplification without any pre-treatment steps (Giakoumaki et al., 2003). However, traditional asymmetric PCR relies on using unequal concentrations of primers, is difficult to optimize, often inefficient, and tends to promote non-specific amplification (Sanchez et al., 2004). In this work, in order to achieve higher sensitivity for the detection of *Salmonella*, a modified asymmetric PCR technique is introduced to produce predominantly ssDNA target from extremely low concentration of starting template. The PCR was performed in two steps by first using a "symmetric" PCR. A 231-bp fragment of the *invA* gene was successfully amplified from genomic DNA of three different *Salmonella* strains, which could be verified by agarose gel electrophoresis (Fig. 3A). However, there was no target band observed in PCR products corresponding to $10^4$–$10^2$ CFU mL$^{-1}$ of *S. typhimurium* due to the low resolution of gel electrophoresis (Fig. 3B, lanes 6–9). Then a tube of obtained dsDNA products was purified in order to remove the excess primers. The second PCR step was performed by using an internal primer and the first (symmetric) PCR product as a template. Compared with traditional asymmetric PCR, this semi-nested asymmetric PCR technology provides a more sensitive and specific means for producing ssDNA. In fact, a reduction in target length could disrupt secondary structure interference and thus greatly improve hybridization efficiency.

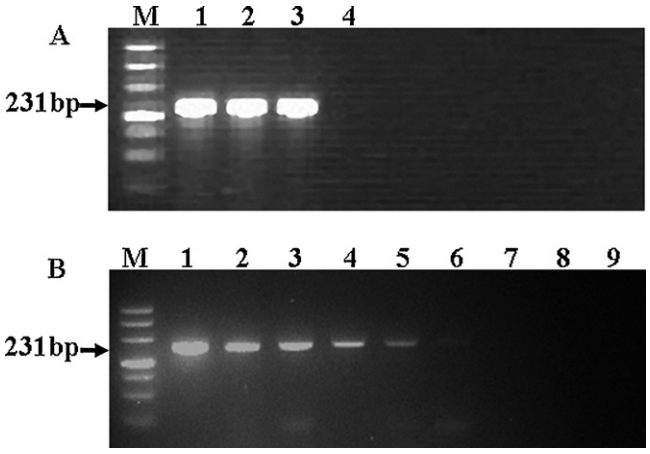



**Fig. 3.** Agarose gel electrophoresis of PCR products. The arrow on the *left* marks the expected PCR product of 231-bp *invA* gene. Size markers (M), the fragments are linear dsDNA bands of 500, 400, 300, 200, 150, 100, 50 base pairs, and (A) PCR products of $10^{10}$ CFU mL$^{-1}$ *S. typhimurium* (1), $10^{10}$ CFU mL$^{-1}$ *S. enterica* (2), $10^{10}$ CFU mL$^{-1}$ *S. derby* (3); (B) PCR products of $10^{10}$ (1), $10^9$ (2), $10^8$ (3), $10^7$ (4), $10^6$ (5), $10^5$ (6), $10^4$ (7), $10^3$ (8), $10^2$ (9) CFU mL$^{-1}$ *S. typhimurium*.

### 3.4. Detection of Salmonella

The PCR amplification products from *Salmonella invA* gene PCR reaction were diluted with buffer solution to provide high ionic strength which is necessary for hybridization and to protect the surface from nonspecific adsorption. The SPR biosensor was then applied to analyze the PCR products, as shown in Fig. 4, significant signals were obtained with *invA* amplicons from the three different *Salmonella* strains. The signal values of *S. enterica* and *S. derby* strains were also well consistent with the value of *S. typhimurium* strain at high and low concentration. These results indicated that

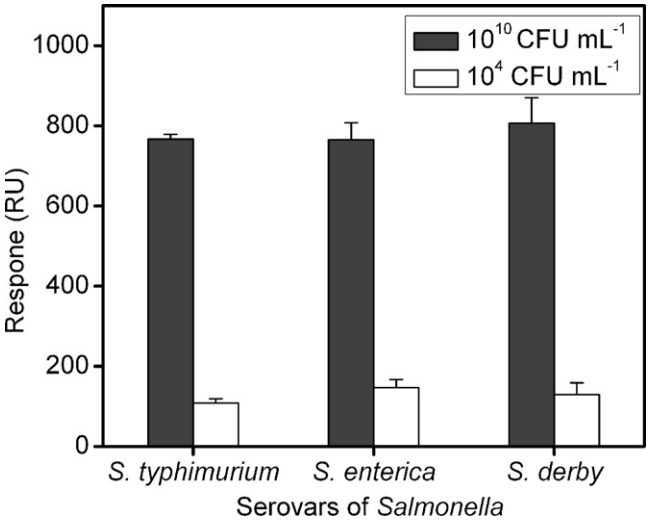



**Fig. 4.** SPR signals for the detection of 3 *Salmonella* strains at different concentrations. Data are expressed as mean ± *SD* (*n* = 3).

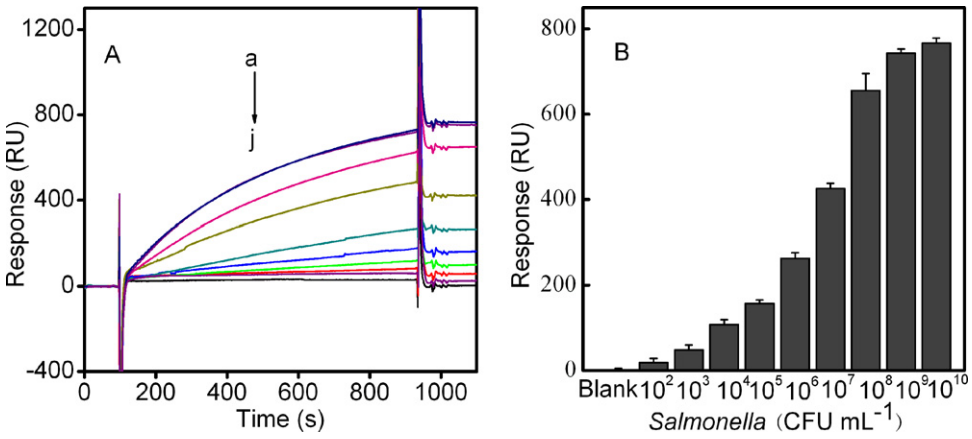



**Fig. 5.** (A) The sensorgrams obtained with PCR products of $10^{10}$, $10^{9}$, $10^{8}$, $10^{7}$, $10^{6}$, $10^{5}$, $10^{4}$, $10^{3}$, $10^{2}$ CFU mL$^{-1}$ *S. typhimurium*. (from a to j). (B) The change in response units to PCR products obtained from serial dilutions of *S. typhimurium* in the range of $10^{2}$–$10^{10}$ CFU mL$^{-1}$.

the developed method could be applied to detect different serovars of *Salmonella* contained the *invA* gene, which exists in almost all *Salmonella* serotypes. The response is proportional with the concentration of *S. typhimurium* over the range of $10^{2}$–$10^{10}$ CFU mL$^{-1}$ (Fig. 5). The high sensitivity based on the powerful amplification ability was comparable to recently reported values obtained by other methods for the detection of *Salmonella* (Oh et al., 2004; Mazumdar et al., 2007; Salam and Tothill, 2009; Kyprianou et al., 2010; Li et al., 2010). A control experiment of PCR blanks (containing all the reagents of the PCR mixture except the template DNA) indicated that the PCR blanks did not interfere with the detection. Furthermore, the whole detection process, including sensor surface generation, DNA extraction, PCR amplification and real-time detection, can be done within 4.5 h.

### 3.5. Specificity of the method

In order to assay the biosensor selectivity in the hybridization of the target DNA, two different oligonucleotides corresponding to full and non-complementary sequence to the probe were designed and used with the capture probe previously immobilized onto the sensor surface. The biosensor gave a very clear response with fully complementary oligonucleotide, and the interaction with non-complementary DNA did not result in measurable analytical signals (Table 2). Thus, the results suggest that the process of hybridization is highly selective to the sequence of the target DNA, it occurs only with the fully complementary chain not with the non-complementary one. This result provides an absolute guarantee for the follow-on specific detection of PCR amplicons from the *invA* gene of *Salmonella*. *E. coli* and *S. aurues* cells were also used to prove the selectivity of the method for *Salmonella* detection. *E. coli* and *S. aurues* cells were diluted to obtain a concentration of $10^{8}$ CFU mL$^{-1}$. No significant response, in comparison to *Salmonella*, was obtained when the *E. coli* and *S. aurues* were checked using the same assay (Table 2). The high selectivity of *Salmonella* detection was achieved due to the high specificity of the biosensor and the primers designed to *invA* gene.

**Table 2**
Test the specificity of the method.

| | Concentration | ΔRU | |
|---|---|---|---|
| | | av[a] | *SD* |
| Target DNA | 10 nM | 287.4 | 14.76 |
| | 500 nM | 1055.8 | 39.74 |
| Non-complementary | 10 nM | 7.5 | 20.53 |
| DNA | 500 nM | 9.9 | 11.58 |
| *S. typhimurium* | $10^{8}$ CFU mL$^{-1}$ | 655.8 | 39.25 |
| *E. coli* | $10^{8}$ CFU mL$^{-1}$ | 7.6 | 9.29 |
| *S. aurues* | $10^{8}$ CFU mL$^{-1}$ | 6.4 | 13.94 |

[a] Average of 3 values.

### 3.6. Regeneration performance of sensor surface

10 mM Glycine–HCl (pH 3.0) was used as regeneration solution, one or two 1-min injections could remove the binding target DNA completely form the SA chip surface without irreversible loss of its activity (Fig. 1). Up to 300 cycles of binding/regeneration processes on the same chip, the SPR response decreased <20% (data not shown). These results indicated that a well-immobilized SA chip can be regenerated more than 300 times. Therefore, the sensor chip can be reused by simply washing the analyte off from the surface, resulting in a reduced detection cost and a rapid detection process.

## 4. Conclusions

We developed a SPR-based DNA biosensor for detection of *Salmonella*, an important foodborne pathogen. The highly specific DNA probe for *invA* gene sequence recognition was immobilized on a sensor surface using biotin-streptavidin binding system. The *invA* gene was isolated from bacterial cultures employing a simple DNA extraction and amplified using a modified semi-nested asymmetric PCR technique. The use of PCR allows achieving a highly sensitive and specific detection of *Salmonella*. As the results indicated, this DNA biosensor can detect as low as $10^{2}$ CFU of *Salmonella* cells form 1 mL sample within 4.5 h, and excellent regeneration of sensor surface could reduces the cost of the approach greatly. The strategy proposed in this report performs the advantages of rapid detection, free label, high sensitivity and specificity, and general applicability to almost all *Salmonella* serotypes, which has potential application in *Salmonella* detection. Future investigation will be focused on the optimization of a standardized sample preparation procedures and the method could be potentially developed as a pragmatic tool for *Salmonella* monitoring in real samples. Moreover, we deduce that this method could detect various other pathogens by immobilization of appropriate probes and amplification of corresponding specific gene fragments.

## Acknowledgment

This work was supported by the National Natural Science Foundation of China (21075141).

## References


Ahn, S., Walt, D.R., 2005. Detection of *Salmonella* spp. using microsphere-based, fiber-optic DNA microarrays. Anal. Chem. 77, 5041–5047.

Arya, S.K., Singh, A., Naidoo, R., Wu, P., McDermott, M.T., Evoy, S., 2010. Chemically immobilized T4-bacteriophage for specific *Escherichia coli* detection using surface plasmon resonance. Analyst 136, 486–492.

Bhagwat, A.A., 2004. Rapid detection of *Salmonella* from vegetable rinse-water using real-time PCR. Food Microbial 21, 73–78.

Bokken, G.C.M.B., Corbee, R.J., Knapen, F., Bergwerff, A.A., 2003. Immunochemical detection of *Salmonella* group B, D and E using an optical surface plasmon resonance biosensor. FEMS Microbiol. Lett. 222, 75–82.

Daum, L.T., Barnes, W.J., McAvin, J.C., Neidert, M.S., Cooper, L.A., Huff, W.B., Gaul, L., Riggins, W.S., Morris, S., Salmen, A., Lohman, K.L., 2002. Real-time PCR detection of *Salmonella* in suspect food from a gastroenteritis outbreak in Kerr County, Texas. J. Clin. Microbiol. 40, 3050–3052.

Giakoumaki, E., Minunni, M., Tombelli, S., Tothill, I.E., Mascini, M., Bogani, P., Buiatti, M., 2003. Combination of amplification and post-amplification strategies to improve optical DNA sensing. Biosens. Bioelectron. 19, 337–344.

Homola, J., 2003. Present and future of surface plasmon resonance biosensors. Anal. Bioanal. Chem. 377, 528–539.

Hoorfar, J., Ahrens, P., Padstrom, P., 2000. Automated 5′nuclease PCR assay for identification of *Salmonella enterica*. J. Clin. Microbiol. 38, 3429–3435.

Hwang, S.Y., Yoo, C.H., Jeon, J.Y., Choi, S.C., Lee, E.K., 2005. Quantitative assay of hepatitis B surface antigen by using surface plasmon resonance biosensor. Biotechnol. Bioproc. 10, 309–314.

Jeong, E.S., Lee, K.S., Heo, S.H., Seo, J.H., Choi, Y.K., 2011. Triplex PCR for the simultaneous detection of *Pseudomonas aeruginosa*, *Helicobacter hepaticus*, and *Salmonella typhimurirm*. Exp. Anim. 60, 65–70.

Kai, E., Ikebukuro, K., Hoshina, S., Watanabe, H., Karube, I., 2000. Detection of PCR products of *Escherichia coli* O157:H7 in human stool samples using surface plasmon resonance (SPR). FEMS Immunol. Med. Microbiol. 29, 183–288.

Kyprianou, D., Guerreiro, A.R., Nirschl, M., Chianella, I., Subrahmanyam, S., Turner, A.P.F., Piletsky, S., 2010. The application of polythiol molecules for protein immobilisation on sensor surfaces. Biosens. Bioelectron. 25, 1049–1055.

Li, S., Li, Y., Chen, H., Horikawa, S., Shen, W., Simonian, A., Chin, B.A., 2010. Direct detection of *Salmonella typhimurium* on fresh produce using phage-based magnetoelastic biosensors. Biosens. Bioelectron. 26, 1313–1319.

Lida, K., Abe, A., Matsui, H., Danbara, H., Wakayama, S., Kawahara, K., 1993. Rapid and sensitive method for detection of *Salmonella* strains using a combination of polymerase chain reaction and reverse dot-blot hybridization. FEMS Microbiol. Lett. 114, 167–172.

Malorny, B., Hoorfar, J., Bunge, C., Helmuth, R., 2003. Multicenter validation of the analytical accuracy of *Salmonella* PCR: towards an international standard. Appl. Environ. Microbiol. 69, 290–296.

Mariotti, E., Minunni, M., Mascini, M., 2002. Surface plasmon resonance biosensor for genetically modified organisms detection. Anal. Chim. Acta 453, 165–172.

Mazumdar, S.D., Hartmann, M., Kampfer, P., Keusgen, M., 2007. Rapid method for detection of *Salmonella* in milk by surface plasmon resonance (SPR). Biosens. Bioelectron. 22, 2040–2046.

Mazumdar, S.D., Barlen, B., Kampfer, P., Keusgen, M., 2010. Surface plasmon resonance (SPR) as a rapid tool for serotyping of *Salmonella*. Biosens. Bioelectron. 25, 967–971.

Miyachi, H., Yano, K., Ikebukuro, K., Kono, M., Hoshina, S., Karube, I., 2000. Application of chimeric RAN-DNA oligonucleotides to the detection of pathogenic microorganisms using surface plasmon resonance. Anal. Chim. Acta 407, 1–10.

Oh, B.K., Lee, W., Kim, Y.K., Lee, W.H., Choi, J.W., 2004. Surface plasmon resonance immunosensor using self-assembled protein G for the detection of *Salmonella paratyphi*. J. Biotechnol. 111, 1–8.

Pieskus, J., Kazeniauskas, E., Butrimaite-Ambrozeeviciene, C., Stanevicius, Z., Mauricas, M., 2008. *Salmonella* incidence in broiler and laying hens with the different housing systems. J. Poult. Sci. 45, 227–231.

Rahn, K., De Grandis, S.A., Clarke, R.C., McEwen, S.A., Galán, J.E., Ginocchio, C., Curtiss III., R., Gyles, C.L., 1992. Amplification of an *invA* gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella*. Mol. Cell Probe 6, 271–279.

Salam, F., Tothill, I.E., 2009. Detection of *Salmonella typhimurium* using an electrochemical immunosensor. Biosens. Bioelectron. 24, 2630–2636.

Sanchez, J.A., Pierce, K.E., Rice, J.E., Wangh, L.J., 2004. Linear-After-The-Exponential (LATE)-PCR: an advanced method of asymmetric PCR and its uses in quantitative real-time analysis. Proc. Natl. Acad. Sci. U.S.A. 101, 1933–1938.

Sparbier, K., Weller, U., Boogen, C., Kostrzewa, M., 2011. Rapid detection of *Salmonella* sp. by means of a combination of selective enrichment broth and MALDI-TOF MS. Eur. J. Clin. Microbiol., http://dx.doi.org/10.1007/s10096-011-1373-0.

Subramanian, A., Irudayaraj, J., Ryan, T., 2006. A mixed self-assembled monolayer-based surface plasmon immunosensor for detection of *E. coli* O157:H7. Biosens. Bioelectron. 21, 998–1006.

Wang, R., Tombelli, S., Minunni, M., Spiriti, M.M., Mascini, M., 2004. Immobilisation of DNA probes for the development of SPR-based sensing. Biosens. Bioelectron. 20, 967–974.

Waswa, J., Irudayaraj, J., Chitrita, D., 2007. Direct detection of *E. Coli* O157:H7 in selected food systems by a surface plasmon resonance biosensor. Lwt-food Sci. Technol. 40, 187–192.

Yang, G.J., Huang, J.L., Meng, W.J., Shen, M., Jiao, X.A., 2009. A reusable capacitive immunosensor for detection of *Salmonella* spp. based on grafted ethylene diamine and self-assembled gold nanoparticle monolayers. Anal. Chim. Acta 647, 159–166.