JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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Selection and Characterization of Aptamers against *Salmonella typhimurium* Using Whole-Bacterium Systemic Evolution of Ligands by Exponential Enrichment (SELEX)

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Supporting Information

ABSTRACT: In this paper, a high-affinity ssDNA aptamer binding to *Salmonella typhimurium* was obtained by a whole-bacterium-based Systemic Evolution of Ligands by Exponential Enrichment (SELEX) procedure. After nine rounds of selection with *S. typhimurium* as the target, a highly enriched oligonucleotide pool was sequenced and then grouped into different families based on primary sequence homology and secondary structure similarity. Eleven sequences from different families were selected for further characterization via flow cytometry analysis. The results showed that the sequence ST2P demonstrates affinity for *S. typhimurium* much more strongly and specifically than other sequences tested. The estimated K_d value of this particularly promising aptamer was 6.33 ± 0.58 nM. To demonstrate the potential use of the aptamers in the quantitative determination of *S. typhimurium*, a fluorescent bioassay with the aptamer ST2P was prepared. Under optimal conditions, the correlation between the concentration of *S. typhimurium* and fluorescent signal was found to be linear within the range of 50−10^6 cfu/mL (R^2 = 0.9957). The limit of detection (LOD) of the developed method was found to be 25 cfu/mL. This work demonstrates that this aptamer could potentially be used to improve the detection of *S. typhimurium*.

KEYWORDS: *aptamer, Salmonella typhimurium, SELEX, fluorescence*

INTRODUCTION

Among a multitude of contagious enteric bacterial infections, salmonellosis is one of the most frequently reported bacterial foodborne diseases and is a major economic and public health concern worldwide. The number of salmonellosis cases has increased significantly over the past several decades. Therefore, the inspection of food for the presence of *Salmonella* has become routine all over the world.[1] Of particular concern is salmonellosis caused by multidrug-resistant strains such as *Salmonella enterica* serovar Typhimurium or *S. enterica* serovar Newport.[2] In addition, *S. typhimurium* is a major causative agent of gastroenteritis (characterized by diarrhea, cramps, vomiting, and often fever) in many areas.[3] There is thus a real need to develop alternative molecular approaches for the identification of *S. typhimurium*.

Various methods have been developed for the detection of *Salmonella*. The conventional culture methods are time-consuming and labor intensive. Molecular techniques such as PCR can shorten the time to detection, but they are not highly sensitive or specific because residual matrix-associated inhibitors often compromise molecular detection.[4,5] Immunological assays for pathogen detection show much better detection limits as a consequence of the extraordinarily selective antigen/antibody interaction.[6−8] Unfortunately, antibody instability under room temperature limits the applications of these methods.[9]

Aptamers are single-stranded DNA or RNA oligonucleotides selected by Systematic Evolution of Ligands by Exponential Enrichment (SELEX) on the basis of their ability to bind target biomolecules with high affinity and specificity.[10,11] There are several advantages to the use of aptamers as compared to antibodies: aptamers bind target molecules with affinity and specificity equal or superior to those of antibodies, they are easily synthesized and modified, and they are more stable than antibodies. In light of these properties, studies on aptamers used as therapeutic,[12] diagnostic,[13] and analytical reagents[14,15] have become more important.

SELEX has been used against both bacterial surface molecules and live bacteria as targets.[16,17] Bacterium-based aptamer selection techniques targeting whole live bacteria in suspension have been carried out to create ssDNA aptamers against group A *Streptococcus*,[18] *Lactobacillus acidophilus*,[19] *Mycobacterium tuberculosis*,[20] *Staphylococcus aureus*,[21] and *Campylobacter jejuni*[22] without prior knowledge of a specific target molecule. Using live bacterial cells grown in suspension allows for separation of bound from unbound oligonucleotides via simple centrifugation. Our group has selected aptamers binding to *Vibrio parahemolyticus* using a whole-bacterium-based SELEX procedure.[23] On this basis, here we screened a DNA library for aptamers targeting *S. typhimurium*. In addition, to demonstrate the potential use of the aptamers in the quantitative determination of *S. typhimurium*, a fluorescent bioassay with the selected aptamer was developed. Although the selection of *S. typhimurium* aptamers has been reported, Joshi et al. selected aptamers against *S. typhimurium* outer

Received: December 4, 2012
Revised: March 10, 2013
Accepted: March 10, 2013
Published: March 11, 2013

membrane proteins.[24] However, whether the proteins are specific for *S. typhimurium* was not studied, so other bacteria with this proteins may be recognized by the aptamer, and this may bring false-positive results. Because the target moleclues of the aptamer were these outer membrane proteins only, other target molecules on *S. typhimurium* will not be recognized by the aptamer. Moreover, the binding dissociation constants (K_d) of high-affinity aptamer sequences were not estimated, either. Liu et al. reported the aptamer for the recognition of whole *Salmonella* O8 bacterial cells,[25] but the bacteria were inactivated, and whether the selected aptamer recognizes live *Salmonella* O8 bacterial cells has not been studied in the paper. In this work, we describe the selection of aptamers against live *S. typhimurium* bacterial cells, which meet the needs of the practical detection more than aptamer against dead bacteria in public health safety. We adopted flow cytometry for further research of binding affinity and selectivity of aptamers for *S. typhimurium* and a K_d value of 6.33 ± 0.58 nM was obtained, indicating the selected aptamer is more sensitive and easier to bind to *S. typhimurium* than the two reported aptamers. Although we are not clear about the target moleclues, perhaps lipopolysaccharide, or outer membrane proteins, or lipoprotein, this indicates that the target moleclues of the aptamer we selected were more extensive.

MATERIALS AND METHODS

Materials. The initial ssDNA library and the primers used to amplify it were obtained from Integrated DNA Technologies (IDT) (Coralville, IA, USA). BBL brain−heart infusion medium was purchased from BD Difco, and tryptone soya broth medium was from Oxoid. Dulbecco's phosphate-buffered saline with $CaCl_2$ and $MgCl_2$ (DPBS, D8662) and tRNA were purchased from Sigma. Bovine serum albumin (BSA) and all PCR reagents were purchased from Invitrogen. The Qiagen MinElute PCR Purification Kit was obtained from Qiagen for purifying all PCR products.

Bacterial Strains and Culture Media. *Salmonella typhimurium* ATCC 50761, *Staphylococcus aureus* ATCC 29213, *Listeria monocytogenes*, *Escherichia coli* ATCC 25922, *Cronobacter sakazakii*, *Streptococcus pneumoniae*, and *Vibrio parahemolyticus* ATCC 17802 were obtained from the American Type Culture Collection (ATCC). All *S. typhimurium*, *E. coli*, *L. monocytogenes*, *C. sakazakii*, *S. pneumoniae*, and *S. aureus* strains were grown under aerobic conditions at 37 °C in BBL brain−heart infusion medium. *V. parahemolyticus* cells were grown in tryptone soya broth plus 1% NaCl at 37 °C. All bacteria were cultured overnight under aerobic conditions at 37 °C, and all liquid cultures were shaken at 150 rpm.

DNA Library. An 87-nt oligonucleotide single-stranded DNA library consisting of a 40-nt randomized region flanked on both sides by 22-nt and 25-nt primer regions was used. The primers used to amplify the ssDNA library and subsequent aptamer pools have the following sequences: forward, 5′-ATAGGAGTCACGACGACC-AGAA-3′; reverse, 5′-TATGTGCGTCTACCTCTTGACTAAT-3′. DNA library or aptamer pools were rendered into single strands via heat denaturation at 94 °C for 10 min in Dulbecco's phosphate-buffered saline with $CaCl_2$ and $MgCl_2$ and then renatured on ice for 10 min.

Aptamer Selection. SELEX was carried out using a procedure based on previous work in our laboratory.[23] *S. typhimurium* was grown overnight in liquid cultures and harvested upon reaching the logarithmic phase (minimum OD_{600} of 0.3). Cell mixtures were centrifuged at 5000*g* at 4 °C to remove media and washed twice in 1× binding buffer (1× BB; 50 mM Tris-HCl (pH 7.4), 5 mM KCl, 100 mM NaCl, 1 mM $MgCl_2$) at room temperature. SELEX was initiated with a randomized ssDNA library (2 nmol initial round), and 100 pmol of the aptamer pool was used as the input in subsequent rounds. A total of 10^8 cells were used for each round of selection. An excess of tRNA and BSA was added to the incubation buffer (20-fold molar excess of each in the initial round up to a maximum 90-fold molar excess in round 9), and 0.05% w/v BSA was added to the wash buffer. The use of increasing amounts of BSA/tRNA increases the competition between the desired target (cells) and nontarget (BSA molecules) for aptamer molecules. The tRNA is present to compete with the aptamer sequences for target binding sites. The selection consists of nine rounds, and negative controls consisting of cells incubated with all medium components but without the oligonucleotide libraries were prepared for each round of selection. The counterselection against a mixture of related intact pathogenic bacteria, including *L. monocytogenes*, *E. coli*, *S. aureus*, *S. pneumoniae*, *V. parahemolyticus*, and *C. sakazakii*, was introduced in the third and fifth rounds, respectively, to ensure that the selected aptamers maintain high species specificity for the target, *S. typhimurium*.

All washes and incubations were carried out in 1× BB at room temperature for 45 min. An initial incubation volume of 600 μL was used for round one, and this was decreased to 350 μL for subsequent rounds. Following incubation, the cells were centrifuged at 5000*g* at 4 °C for 5 min, the supernatants were removed, and the cells were washed twice in 250 μL of 1× BB with 0.05% BSA (via resuspension and centrifugation) before a final resuspension in 100 μL of 1× PCR reaction buffer. The cells were then heated at 94 °C for 10 min and placed on ice for 10 min to denature and elute cell-bound aptamers. The mixture was then centrifuged as described above, and the supernatant was isolated and designated the cell-bound aptamer (CA) fraction. All fractions collected were amplified by PCR, and the PCR products of the CA fraction were used in the next round of selection.

The amplification of the DNA library and subsequent aptamer pools by PCR during SELEX were divided into two steps. For the first step, the reaction conditions were as follows: 10× PCR reaction buffer, 10 μM reverse primer, 25 mM dNTPs (containing Mg^{2+}), 5 U/μL Taq DNA polymerase, and either 10 ng of DNA library or 2 μL of fraction supernatant. The thermocycling parameters were as follows: initial denaturation at 94 °C for 5 min, followed by 2 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s, and extension at 72 °C for 45 s. A final extension step at 72 °C for 5 min was carried out following the last cycle (C1000 Thermocycler, Bio-Rad Laboratories, Hercules, CA). The PCR products were used as the template in the second step. The second step included 10× PCR reaction buffer, 10 μM of each primer, 25 mM dNTPs (containing Mg^{2+}), 5 U/μL Taq DNA polymerase, and 1 μL of the template. The thermocycling parameters were as follows: initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s, and extension at 72 °C for 45 s. A final extension step at 72 °C for 5 min was carried out following the last cycle.

After PCR, the reaction products were separated by 2% agarose gel electrophoresis (AGE) in 1× TAE buffer (Bio-Rad Protean III) at 60−120 V. The gels were stained with ethidium bromide and photographed under UV light.

To eliminate aptamers that bind to the tube wall, the resuspended cell solution was transferred to a fresh microcentrifuge tube between each incubation, washing, and elution step. A total of nine rounds of selection were performed using fresh aliquots of cells for each round.

Flow Cytometric Analysis of Aptamer Pool and Individual Aptamer Binding. A FACScan flow cytometer with PowerMacG4 workstation and CellQuest software (BD, USA) was used to assess the binding of the aptamer pool and individual aptamer sequences to different cell types (*S. aureus*, *L. monocytogenes*, *E. coli*, *V. parahemolyticus*, *C. sakazakii*, and *S. pneumoniae*). The aptamer pools were fluorescently labeled via PCR amplification with 5′-fluorescein amidite (FAM)-modified primers (IDT), whereas the individual aptamer sequences were purchased with the fluorescent label (5′-FAM) attached (IDT). Aptamer pools were heat denatured prior to incubation with bacterial cells. The binding assays were carried out by incubating 50 nM of fluorescently labeled aptamer/aptamer pool with 10^8 cells for 45 min, as in the SELEX process, and then washing the cells once in binding buffer prior to resuspension in binding buffer for immediate flow cytometric analysis. Forward scatter, side scatter, and fluorescence intensity (FL1-H) were measured, and the gated fluorescence intensity above the background (cells with no aptamers

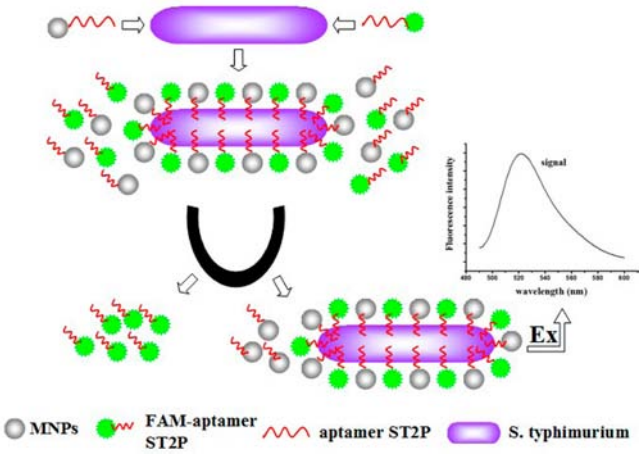
added) was quantified. The fluorescently labeled ssDNA library was used as a control for nonspecific binding in each experiment. Binding curves were used to estimate K_d values by various aptamer concentrations (0−150 nM incubation) with a fixed number of cells (10^8 cfu/mL). GraphPad Prism 5.0 software was used to perform nonlinear regression curve fitting from which K_d values were estimated. All cultures used for flow cytometric screening were harvested in the stationary phase to minimize differences in cell surface molecule expression.

Cloning, Sequencing, and Structural Analysis of Aptamers. The highest affinity aptamer pools measured via flow cytometry were chosen for sequencing analysis. Aptamer pools were cloned using a TOPO TA Cloning Kit for Sequencing and transformed into *E. coli* DH5α-T1^R cells; colonies containing the vector were selected via overnight incubation at 37 °C on LB plates containing 50 μg/mL kanamycin. From each aptamer pool, 20 colonies were chosen for screening. The plasmid DNA was purified and analyzed for the presence of an 87 bp insert via digestion with 1 U of *Eco*R1 at 37 °C for 30 min followed by 7.5% native PAGE. A total of 60 inserts were then sequenced, yielding 35 useable sequences. The secondary structure of each sequence was predicted using RNA structure 3.0 with input conditions of room temperature (21 °C) and 1 mM $MgCl_2$. The most likely structure was chosen on the basis of the lowest predicted free energy of formation (ΔG; kcal/mol).

Preparation of Aptamer-MNPs Conjugates. Amine-functionalized Fe_3O_4 magnetic nanoparticles (MNPs) and the subsequent aptamer−MNPs conjugates were prepared according to method described in our previous work.[26,27] In brief, amino-activated MNPs (1 mg/mL) were treated with 5% glutaraldehyde at room temperature for 2 h. Then the magnetic nanoparticles were magnetically separated and subsequently washed with a phosphate buffer solution three times. After the activation procedure, the surface was covered with avidin by incubating the nanoparticles in an avidin solution (125 μg/mL) overnight to form covalent bonds between the avidin and amino groups. The aptamer ST2P (5 μL, 10 μM), which was modified with biotin at its 5′ end, was activated with 1 mL of 1 mg/mL avidin-coated MNPs. After 12 h of gentle shaking, oligonucleotides were covalently attached to MNPs through avidin−biotin-specific binding followed by blocking the nanoparticles with a 2% BSA solution.

Aptamer-Based Fluorescent Bioassay. Sample solutions containing various concentrations of *S. typhimurium* diluted by 10-fold were prepared in the 1× BB buffer. In a typical test (Scheme 1), the sample solution was incubated in 80 μL aof ptamer ST2P−MNPs conjugates and 150 nM FAM-aptamer ST2P for 40 min at 37 °C followed by thorough washing with the same buffer to remove unbound *S. typhimurium* with an external magnet. Trapped particles were resuspended in 500 μL of PBS and assayed using the F-7000 fluorescence spectrophotometer.

Scheme 1. Schematic Illustration of the Fluorescent Bioassay Using the Aptamer ST2P for Determination of *S. typhimurium*



RESULTS AND DISCUSSION

Aptamer Selection. To select aptamers that potentially recognize *S. typhimurium*, nine rounds of SELEX were performed. Prior to incubation with the target cells, the double-stranded DNA aptamer pool was rendered single stranded via heat denaturation. Following incubation of the ssDNA library with *S. typhimurium* cells, most of the DNA library/aptamer pool remained unbound in the supernatant (So) as illustrated by the large number of PCR amplification products around 70−100 bp. Only a small amount of DNA was present in the first two washes of the centrifuged cells (wash). No DNA was amplified from the third wash fraction from any of the SELEX rounds. The amplification products of the heat-eluted cell-bound aptamers (CA) fraction represented the DNA sequences strongly bound to the cells. As shown in Figure 1, a

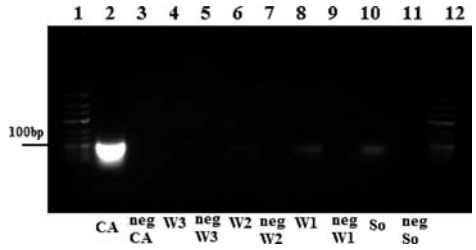


Figure 1. AGE of PCR-amplified oligonucleotide fractions after the first round of SELEX. A randomized, single-stranded DNA library was incubated with *S. typhimurium* cells in the presence of tRNA and BSA. Following incubation, the cells were centrifuged to remove the incubation supernatant (So) and repeatedly washed and centrifuged to remove DNA sequences that were nonspecifically or weakly bound (W1, W2, W3). The cells were then heated at 94 °C, the suspension was centrifuged, and the supernatant was collected containing the heat-eluted cell aptamer fraction (CA). A SELEX negative control was carried out in which cells were incubated in the absence of DNA library (negSo), washed (negW1, negW2, negW3), and heat-eluted (negCA). The different fractions collected during SELEX (So, W1−W3, CA) and the parallel negative control were PCR-amplified and analyzed via AGE. Lanes 1 and 12 on the gel contain the DNA ladder (25−750 bp).

negative control consisting of cells without added DNA was run concurrently through the entire procedure of incubation, washing, and heat elution. The negative control did not produce detectable PCR products of the expected aptamer size (87 nt). The observation of a single 87 bp band on the gel after each round of selection and PCR amplification of the CA fractions suggests that the cells were able to bind to a pool of aptamer sequences. The PCR products from the CA fraction were used in the next round of selection following purification.

Cloning and Sequence Analysis of Aptamer Pools. Aptamer pools from the ninth round of SELEX displayed the highest affinity for the target cells when screened via flow cytometry. The aptamer pools were cloned and sequenced, and 35 sequences were obtained in total. The 35 sequences were then divided into nine families based on DNA sequence homology and secondary structure similarity (data shown in Table S1 in the Supporting Information). Sequences were chosen for further screening on the basis of not only on their repetitiveness but also their predicted secondary structures and free energies of formation.

dx.doi.org/10.1021/jf400767d | J. Agric. Food Chem. 2013, 61, 3229−3234

Table 1. Tested Aptamer Sequences with (87 nt) and without (40 nt) Primers[a]

name	sequence
ST2	5′-FAM-AGTAATGCCCGGTAGTTATTCAAAGATGAGTAGGAAAAGA-3′
ST2P	5′-FAM-ATAGGAGTCACGACGACCAGAAAGTAATGCCCGGTAGTTATTCAAAGATGAGTAGGAAAAGA TATGTGCGTCTACCTCTTGACTAAT-3′
ST3P	5′-FAM-ATAGGAGTCACGACGACCAGAATTTCGGCTAAGGACGGGTGAAATACATTTAATAGGGTGGA TATGTGCGTCTACCTCTTGACTAAT-3′
ST7P	5′-FAM-ATAGGAGTCACGACGACCAGAATAAGTACAGGACTGGAGTATTAGCGGGGTCCATGCAAGGC TATGTGCGTCTACCTCTTGACTAAT-3′
ST9	5′-FAM-AATCAATAGAAGACAAAGTCCGAAACAGGTGTGACGGTAA-3′
ST9P	5′-FAM-ATAGGAGTCACGACGACCAGAAAATCAATAGAAGACAAAGTCCGAAACAGGTGTGACGGTAA TATGTGCGTCTACCTCTTGACTAAT-3′

[a]Primer sequences are underlined.

Binding of Individual Aptamer Sequences to Target Cells. Fluorescently labeled aptamer sequences were incubated with the *S. typhimurium* strain used for selection and then analyzed via flow cytometry. Aptamer sequences obtained after the ninth round of SELEX seemed to have the highest affinity for *S. typhimurium* (data shown in Figure S1 in the Supporting Information). A total of six sequences from that pool had >50% gated fluorescence intensity above a randomized library control. These sequences are summarized in Table 1. Sequences ST2 and ST2P (ST2 with primers) were the highest binders, with gated fluorescence above background values of around 82%.

The sequences ST2 and ST9 form hairpins both in the absence and in the presence of the primer sequences. The affinity of these two aptamers for *S. typhimurium* changed minimally upon inclusion or exclusion of primers in the sequence; gated fluorescence intensity above background remained at 82 ± 2.34% for ST2, at 82 ± 2.18% for ST2P, at 72 ± 1.57% for ST9, and at 66 ± 1.45% for ST9P (Figure 2A). These results suggest that the variable region of the 40 nt sequences, not the ∼20 nt primers flanked at either end, is likely responsible for the high-affinity binding to the target.

Selectivity of High-Affinity Aptamers for *S. typhimurium*. Fluorescently labeled aptamer sequences ST2, ST2P, ST9, and ST9P were tested against a variety of other bacteria including *L. monocytogenes*, *E. coli*, *V. parahemolyticus*, *S. aureus*, *C. sakazakii*, and *S. pneumoniae*. All selected aptamer sequences showed preferential binding to *S. typhimurium* over the other cells tested. This preferential binding confirms the selectivity of these aptamers for *S. typhimurium*. The lowest selectivity was obtained with the aptamers ST9P and ST9, which seemed to have some affinity for *L. monocytogenes*. However, this affinity was low in comparison to the *S. typhimurium* target cells. The percent gated fluorescence intensity above library background was 20% when ST9P was incubated with *L. monocytogenes* cells and 72% when incubated with *S. typhimurium* (Figure 2). When the primer sequences were excluded (ST9), the values were 17% for *L. monocytogenes* cells and 66% for *S. typhimurium*. The sequence ST2P seems to negate *L. monocytogenes* binding, bringing the percent gated fluorescence intensity above library background down to 3%. Binding of the same sequence (ST2P) to the target *S. typhimurium* cells yielded 82% gated fluorescence above background (Figure 2). It can thus be concluded that the aptamer sequences tested are specific for *S. typhimurium*.

Estimation of Binding Dissociation Constants (K_d) of High-Affinity Aptamer Sequences. Figure 3 shows a typical binding saturation curve from flow cytometric analysis of the fluorescently labeled ST2P aptamer and the *S. typhimurium* cells used for selection (10^8 cfu/mL). Estimates from a

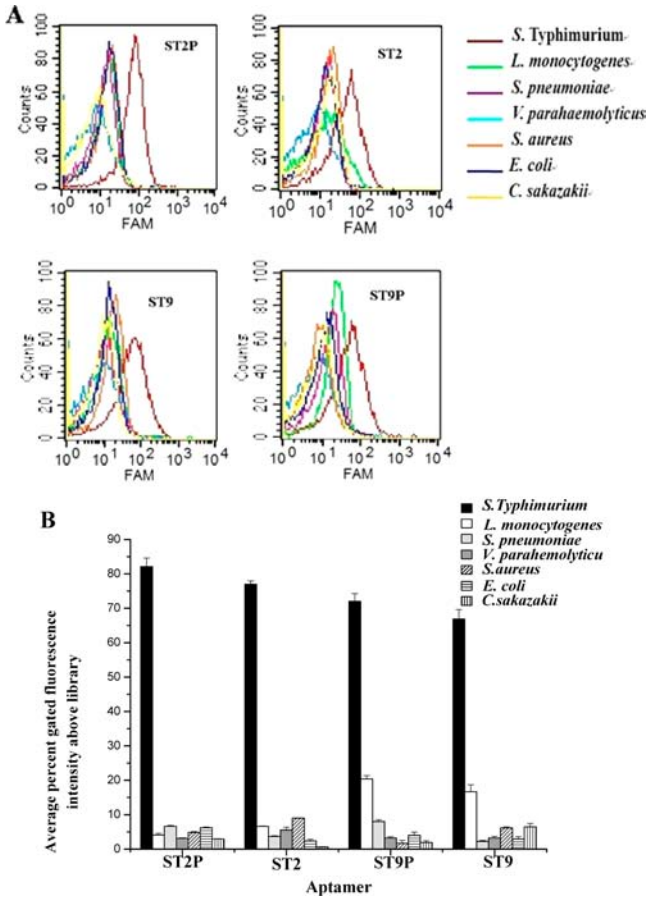


Figure 2. Characterization of specificity of individual aptamer for *S. typhimurium*: (A) flow cytometry assay for the binding of aptamers to bacteria (different color curves represent different strains of bacteria); (B) histogram of the percent gated fluorescence intensity above library background for individual aptamers. Selected aptamer sequences preferentially bind to *S. typhimurium* over the other cells tested. The 5′-FAM-labeled individual aptamers were incubated with 10^8 bacteria at 37 °C for 45 min.

nonlinear regression curve fit yield a K_d value of 6.33 ± 0.58 nM and a B_{max} of 84% gated fluorescence above background. Binding of a fluorescently labeled randomized oligonucleotide library to the *S. typhimurium* cell mixture was also examined as a negative control. In contrast, all aptamer sequences tested exhibited saturation binding kinetics.

Table 2 summarizes the estimated K_d and predicted secondary structures of the most selective aptamers for *S. typhimurium*. Sequences ST2P and ST9 had the highest

dx.doi.org/10.1021/jf400767d | J. Agric. Food Chem. 2013, 61, 3229−3234

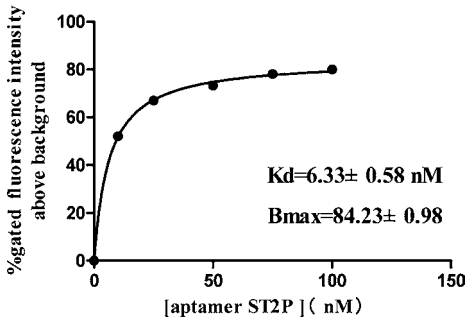
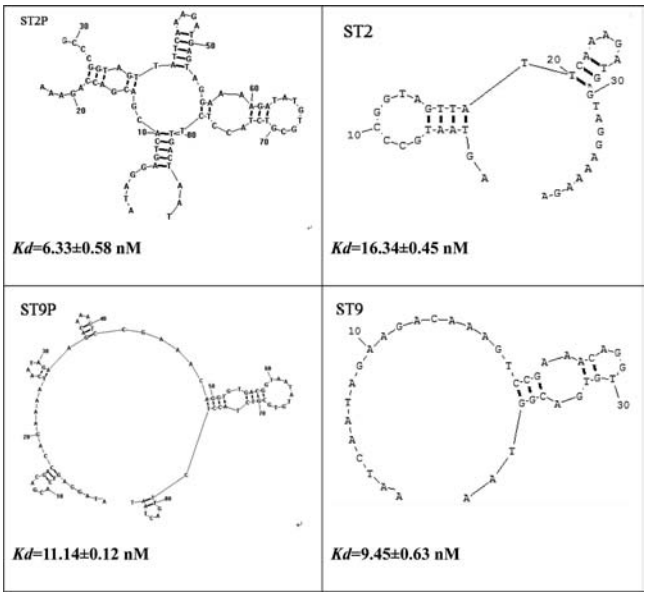


Figure 3. Binding saturation curve of aptamer ST2P to the target *S. typhimurium* cells. A nonlinear regression curve was fit to the data using GraphPad Prism 5.0. Similar experiments were run for all high-affinity aptamer sequences, and the estimated K_d values are summarized in Table 2.

Table 2. Binding Dissociation Constants (K_d) and Predicted Secondary Structures of Aptamer Sequences That Have High Affinity and Selectivity for *S. typhimurium* Cells Used in SELEX



affinities and selectivities for the target cell mixture, as all had K_d values below or equal to 10 nM (6.33 ± 0.58 nM for ST2P; 9.45 ± 0.63 nM for ST9). Sequence ST9 had a very high affinity for *S. typhimurium* but was less specific than ST2 due to its same affinity for *L. monocytogenes* cells (Figure 3).

Application of the Aptamer ST2P in *S. typhimurium* Determination. To demonstrate the potential use of the aptamers in the quantitative determination of *S. typhimurium*, we prepared a fluorescent bioassay with the aptamer ST2P. The bioassay system was fabricated by immobilizing aptamer ST2P onto the surface of MNPs, which were implemented to capture and concentrate *S. typhimurium*. Due to the high affinity of aptamer to the bacteria, the aptamer ST2P−MNPs−*S. typhimurium* complex subsequently binds to FAM-labeled aptamer ST2P. The fluorescent signal was effectively amplified with the help of both magnetic separation and concentration.

Sample solutions containing various concentrations of *S. typhimurium* diluted by 10-fold were prepared in the 1× BB buffer. In a typical test, the sample solution was incubated in 80 μL of aptamer ST2P−MNPs conjugates and 150 nM FAM−aptamer ST2P for 40 min at 37 °C followed by thorough washing with the same buffer to remove unbound *S. typhimurium* with an external magnet. Trapped particles were resuspended in 500 μL of PBS and assayed using the F-7000 fluorescence spectrophotometer. In the absence of pathogenic bacteria, the fluorescent intensity was at a minimum, and in the presence of the different concentrations of *S. typhimurium*, the fluorescent signal varied. Under optimal conditions, the concentration of bacteria is proportional to the increased fluorescent intensity (ΔI) in which ΔI represents the difference of FAM fluorescent intensity in the absence and in the presence of *S. typhimurium*. Various intensities of fluorescent spectra obtained in the presence of different concentrations of *S. typhimurium* (log cfu/mL) are shown in Figure 4. A strong

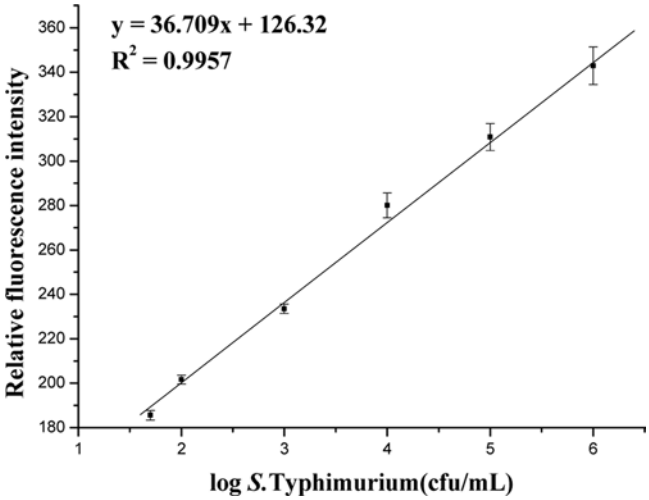


Figure 4. Standard curve of the increased fluorescent intensity (ΔI) versus *S. typhimurium* concentration measured by the fluorescent bioassay.

linear correlation (R^2 = 0.9957) was obtained between 50 and 10^6 cfu/mL *S. typhimurium* concentration. The sensitivity of the developed bioasssay was investigated, and the LOD of the proposed method for *S. typhimurium* was found to be 25 cfu/mL.

Although new rapid test methods appear frequently, these almost always focus on the detection aspect and neglect the need for preanalytical sample processing prior to detection. The fact remains that the detection of pathogens in these complex matrices would be more sensitive if the agent was concentrated and purified from the matrix prior to detection. One important requirement for development of such preanalytical sample processing is the availability of high-affinity ligands that can aid in the selective capture and concentration of the pathogen. The aptamers generated here for *S. typhimurium* could be used to improve these assays. Further work is ongoing to broaden the application demonstrated here with the development of sensors or other analytical systems for field and laboratory determination of this pathogenic bacterium in food products.

■ ASSOCIATED CONTENT

Supporting Information

Random sequence of 9 families of the 35 sequences and results from screening of the sequenced *S. typhimurium* candidate aptamer pools. This material is available free of charge via the Internet at http://pubs.acs.org.

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Funding

This work was partly supported by National S&T support program of China (2012BAK08B01), S&T Supporting Project of Jiangsu (BE2010679), NCET-11-0663, and Doctor Candidate Foundation of Jiangnan University (10054).

Notes

The authors declare no competing financial interest.

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