Article

pubs.acs.org/JAFC

Selection and Identification of a DNA Aptamer Targeted to *Vibrio parahemolyticus*

Nuo Duan, Shijia Wu, Xiujuan Chen, Yukun Huang, and Zhouping Wang*

State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi 214122, China

ABSTRACT: A whole-bacterium systemic evolution of ligands by exponential enrichment (SELEX) method was applied to a combinatorial library of FAM-labeled single-stranded DNA molecules to identify DNA aptamers demonstrating specific binding to *Vibrio parahemolyticus*. FAM-labeled aptamer sequences with high binding affinity to *V. parahemolyticus* were identified by flow cytometric analysis. Aptamer A3P, which showed a particularly high binding affinity in preliminary studies, was chosen for further characterization. This aptamer displayed a dissociation constant (K_d) of 16.88 ± 1.92 nM. Binding assays to assess the specificity of aptamer A3P showed a high binding affinity (76%) for *V. parahemolyticus* and a low apparent binding affinity (4%) for other bacteria. Whole-bacterium SELEX is a promising technique for the design of aptamer-based molecular probes for microbial pathogens that does not require the labor-intensive steps of isolating and purifying complex markers or targets.

KEYWORDS: *aptamer, Vibrio parahemolyticus, SELEX, flow cytometry*

INTRODUCTION

Vibrio parahemolyticus is a facultatively anaerobic, Gram-negative, flagellated, halophilic, asporogenous bacterium that inhabits marine and estuarine environments.[1] Fresh seafood, particularly mussels, is often contaminated with this pathogen.[2,3] Also, this pathogen is an important enteropathogen that causes acute human gastroenteritis throughout the world.[4] Due to its broad distribution, *V. parahemolyticus* infections in China commonly occur in individuals living in coastal areas.[5] In addition, cases of human gastroenteritis have been attributed to the consumption of raw or undercooked seafood or to the ingestion of contaminated water.[6] Therefore, rapid and sensitive detection methods for *V. parahemolyticus* are necessary for the proper regulation of food hygiene and for entry−exit inspection and quarantine.

In this report, we describe the identification of an aptamer for *V. parahemolyticus*. Aptamers are short, single-stranded RNA or DNA molecules that can bind with high affinity and specificity to a wide range of target molecules, such as metal ions,[7] small molecules,[8] drugs,[9] peptides,[10] and proteins.[11] Aptamers are generated in an in vitro systematic iterative process, which is known as SELEX (systematic evolution of ligands by exponential enrichment).[12,13] Compared to antibodies, aptamers possess numerous advantages, including small size, rapid and reproducible synthesis, simple and controllable modification to fulfill different diagnostic and therapeutic purposes, slow degradation kinetics, nontoxicity, and a lack of immunogenicity.[14,15] In recent years, whole-bacterium SELEX, or complete target SELEX, has emerged as an alternative to the more traditional SELEX approach applied to crude or purified extracellular surface targets. This technology has been applied to group A *Streptococcus*,[16] *Lactobacillus acidophilus*,[17] *Mycobacterium tuberculosis*,[18] *Staphylococcus aureus*,[19] and *Campylobacter jejuni*.[20] The purpose of using intact, live bacterial cells in SELEX is to negate the need for a priori purification of specific target molecules from the cell surface, and live bacterial cells as targets have the additional benefit of being able to grow in suspension, thereby allowing for simple separation by centrifugation.

In this study, whole-bacterium SELEX was employed to identify DNA aptamer sequences specific to *V. parahemolyticus* that have the potential to be further developed into a rapid isolation/identification technology to facilitate the detection of *V. parahemolyticus* in food and environmental samples. This methodology is presented both as an alternative to aptamer selection using crude membrane protein preparations and as a means of identifying aptamer sequences that complement those sequences obtained using crude preparations.

MATERIALS AND METHODS

Bacterial Strains and Culture Media. *V. parahemolyticus* ATCC 17802, used as the target for whole-bacterium SELEX, was grown in tryptone soya broth (Oxoid, Milan, Italy) with 1% NaCl at 37 °C. Other bacterial strains used in this study included the following: *S. aureus* ATCC 29213, *Listeria monocytogenes*, *Escherichia coli* ATCC 25922, *Cronobacter sakazakii*, *Streptococcus pneumoniae*, and *Salmonella typhimurium*. All of these bacteria were grown in BBL brain heart infusion media (BD Difco). All bacteria were cultured overnight under aerobic conditions at 37 °C, and all liquid cultures were shaken at 150 rpm. All bacterial strains were obtained from the American Type Culture Collection (ATCC). *E. coli* DH5α-T1^R cells (Invitrogen, Carlsbad, CA) were used for all transformations.

DNA Library. The oligonucleotide template was synthesized as a single-stranded 87-mer with the following sequence: 5′-ATAGGAGT-CACGAC GACCAGAA-N_{40}-TATGTGCGT C TACCTCTTGAC-TAAT-3′, where the central N_{40} represents random oligonucleotides based on the equal incorporation of A, G, C, and T at each position. The initial ssDNA library and the primers used for amplification were obtained from Integrated DNA Technologies (Coralville, IA). DNA

Received: January 31, 2012
Revised: March 27, 2012
Accepted: April 5, 2012
Published: April 5, 2012

library or aptamer pools were rendered single stranded by heat denaturation at 94 °C for 10 min and subsequent cooling at 0 °C for 5 min.

PCR Amplification and Gel Electrophoresis. The primers used to amplify both the ssDNA library and the subsequent aptamer pools had the following sequences:

forward, 5′-ATAGGAGTCACGACGACCAGAA-3′

reverse, 5′-ATTAGTCAAGAGGTAGACGCACATA-3′

PCR for amplification of the DNA library and the subsequent aptamer pools during SELEX was performed in two steps. The first step included 10× PCR reaction buffer, 10 μM of the reverse primer, 25 mM dNTPs (containing Mg^{2+}), 5 U/μL of Taq DNA polymerase, and either 10 ng of the DNA library or 2 μL of the fraction supernatant (all reagents were from Invitrogen). Thermocycling parameters were 94 °C for 5 min for denaturation 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 with a final extension step at 72 °C for 5 min (C1000 Thermocycler, Bio-Rad Laboratories, Hercules, CA). The PCR products generated in the first step were used as the template for the second step. The second step included 10× PCR reaction buffer, 10 μM of each primer, 25 mM dNTPs, 5 U/μL of Taq DNA polymerase, and 1 μL of the template. Thermocycling parameters were 94 °C for 5 min for denaturation 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 followed the last cycle.

Following PCR, the reaction products were separated by 2% agarose gel electrophoresis in 1× TAE (Tris-acetate−EDTA) buffer (Bio-Rad Protean III) at 60−120 V. Gels were stained with ethidium bromide and photographed under UV light. All PCR products were purified using a Qiagen MinElute PCR Purification Kit (Qiagen Inc., Valencia, CA).

Aptamer Selection. The SELEX procedure used in this work was based on the method by Hamula et al.[17] with several modifications. *V. parahemolyticus* was grown overnight in liquid culture and harvested upon reaching logarithmic phase (minimum OD_{600} of 0.3). Cells were centrifuged at 5000*g* and 4 °C to remove media and were washed twice in 1× binding buffer (1× BB, 50 mM Tris-HCl (pH 7.4), 5 mM KCl, 100 mM NaCl, and 1 mM $MgCl_2$) at room temperature. SELEX was initiated with a randomized ssDNA library (2 nmol), and 100 pmol of the aptamer pool was used as the input in subsequent rounds of selection. A total of 10^8 cfu/mL cells were used for each round of selection. An excess of tRNA (Sigma) and bovine serum albumin (BSA; Invitrogen) was added to the incubation buffer (20-fold molar excess of each in the initial round, up to a maximum of 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. SELEX was performed a total of nine rounds. 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; this volume was decreased to 350 μL for subsequent rounds. Following incubation, cells were centrifuged at 5000*g* and 4 °C for 5 min; next, the supernatants were removed, and cells were washed twice in 250 μL of 1× BB with 0.05% BSA before a final resuspension in 100 μL of 1× PCR reaction buffer (TAKARA). The cells were incubated at 94 °C for 10 min and placed on ice for 10 min in order to denature and elute cell-bound aptamers. The mixture was centrifuged as described above, and the supernatant was isolated and designated as the cell-bound aptamer (CA) fraction. All collected fractions were amplified by PCR, and the PCR products of the CA fraction were used in the next round of selection. Between each incubation, wash, and elution step, the resuspended cell solution was transferred to a fresh microcentrifuge tube to eliminate aptamers that bound to the tube wall. A total of nine rounds of selection were performed using fresh aliquots of cells for each round. Negative controls, consisting of cells incubated with all medium components except the oligonucleotide libraries, were prepared for each round of selection.

In the last selection cycle, aptamer pools were cloned using a TOPO TA Cloning Kit for Sequencing (Invitrogen) and transformed into *E. coli* DH5α-T1[R] cells (Invitrogen), and colonies containing the vector were selected by overnight incubation at 37 °C on LB plates containing 50 μg/mL of kanamycin. From each aptamer pool, 20 colonies were chosen for screening. The plasmid DNA was purified (QIAEX II Gel Extraction Kit; Qiagen, Mississauga, ON, Canada) and analyzed for the presence of an 87-bp insert by 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 sequenced (The Applied Genomics Centre, Department of Medical Genetics, University of Alberta), yielding 26 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 sequence was chosen on the basis of the lowest predicted free energy of formation (Δ*G*, kcal/mol).

Binding Assays. A FACScan flow cytometer with a PowerMac G4 workstation and CellQuest software (Flow Cytometry Facility, Faculty of Medicine and Dentistry, University of Alberta) was used to assess the binding of the aptamer pool and individual aptamer sequences to different species of bacteria (*S. aureus*, *L. monocytogenes*, *E. coli*, *S. typhimurium*, *C. sakazakii*, and *S. pneumoniae*). The aptamer pools were fluorescently labeled by PCR amplification with 5′-FAM-modified primers (IDT), and 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. Binding assays were carried out by incubating 50 nM fluorescently labeled aptamer/aptamer pool with 10^8 cells for 45 min, as in the SELEX process, and 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 gated fluorescence intensity above background (cells with no aptamers) was quantified. The fluorescently labeled ssDNA library was used as a control for nonspecific binding in each experiment. Binding curves were created to estimate K_d values by varying the aptamer concentration (0−150 nM) with a fixed number of cells (10^8 cfu/mL). GraphPad Prism 5.0 software was used to fit a nonlinear regression curve from which K_d values were estimated. All cultures used for flow cytometric analysis were harvested in stationary phase to minimize differences in the expression of cell surface molecules.

■ RESULTS AND DISCUSSION

Aptamer Selection. The whole-bacterium SELEX process is outlined in Scheme 1. Nine rounds of selection were performed to select for aptamers that recognize *V. parahemolyticus*. Prior to incubation with the target cells, the double-stranded DNA aptamer pool was rendered single-stranded by heat denaturation. Following incubation of the ssDNA library with *V. parahemolyticus* cells, the majority of the DNA library/

Scheme 1. Schematic Representation of the Aptamer Selection Procedure

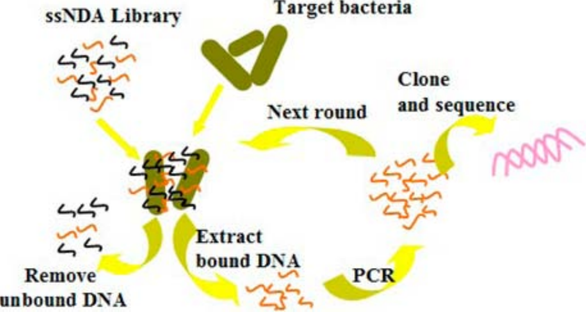


Table 1. Tested Aptamer Sequences with (87 nt) and without (40 nt) Primers[a]

name	sequence
A1	5′FAM-TAGAGATATGACAGCGGGGAAGGTTAAGAGGCGCTAGGAG-3′
A1P	5′FAM- ATA GGA GTC ACG ACG ACC AGA A TAGAGATATGACAGCGGG GAAGGTTAAGAGGCGCTAGGAG TAT GTG CGT CTA CCT CTT GAC TA A T-3′
A3	5′FAM- TCTAAAAATGGGCAAAGAAACAGTGACTCGTTGAGATACT-3′
A3P	5′FAM- ATA GGA GTC ACG ACG ACC AGA A TCTAAAAATGGGCAAAGA AACAGTGACTCGTTGAGATACT TAT GTG CGT CTA CCT CTT GAC TA A T-3′
A17P	5′FAM- ATA GGA GTC ACG ACG ACC AGA A AGGCCGGCCCCTCTGAACTAGATGCAGGGGAGGCGAGCGA TAT GTG CGT CTA CCT CTT GAC TAA T-3′
A18P	5′FAM- ATA GGA GTC ACG ACG ACC AGA A GCGGGCAGCTCCACCGG TAGGCTCCGAGTCATCACGGTCG TAT GTG CGT CTA CCT CTT GAC TA A T-3′
A21P	5′FAM-ATA GGA GTC ACG ACG ACC AGA A TTTTGACGAAGTCAGTGC GTCGAAGCGCAAGCATTACAGA TAT GTG CGT CTA CCT CTT GAC TA A T-3′

[a]Primer sequences are underlined.

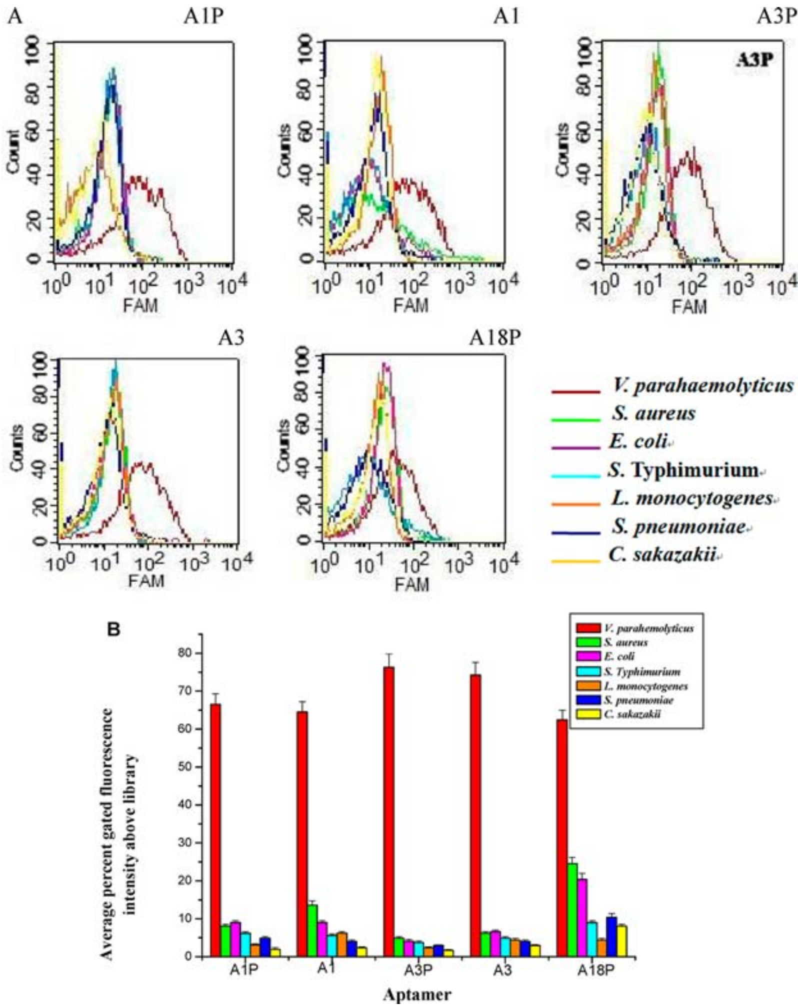


Figure 1. Characterization of the specificity of individual aptamers for *V. parahemolyticus*. Selected aptamer sequences preferentially bind to *V. parahemolyticus* over other species of bacteria. The 5′-FAM-labeled individual aptamers were incubated with 10^8 bacteria at 37 °C for 45 min. (A) Flow cytometric analysis of aptamer binding to bacteria. Differently colored curves represent different species of bacteria. (B) Histogram values of the percent gated fluorescence intensity above library background for individual aptamers.

aptamer pool remained unbound and in the supernatant; only a small portion of the aptamer pool bound to the target cells. The amplification products of the heat-eluted cell-bound aptamer (CA) fraction represented the DNA sequences that were tightly bound to the cells. The negative control did not yield detectable PCR products of the expected aptamer size. The observation of a single 87-bp band on the gel following 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 of the CA fraction were used in each subsequent round of selection following purification.

The aptamer pools from the ninth round of SELEX displayed the highest affinity for the target cells when screened by flow cytometry. The aptamer pools were cloned and sequenced, and a total of 26 sequences were obtained. These sequences were subsequently divided into nine families on the basis of the

homology of the DNA sequence and the similarity of the secondary structure. Analysis of all sequences, both with and without primers, revealed minimal sequence repetition with many sequences containing high GC content, which is indicative of secondary structure formation. Sequences were chosen for further screening on the basis of not only their repetitiveness but also on their predicted secondary structure and free energy of formation.

Determination of Specificity and Affinity. Fluorescently labeled aptamer sequences were incubated with *V. parahemolyticus* and were analyzed by flow cytometry. Aptamer sequences from the ninth round of SELEX appeared to have the greatest affinity for *V. parahemolyticus*. A total of seven sequences from this pool displayed a gated fluorescence intensity that was more than 50% greater than that of a randomized library control. These sequences are presented in Table 1. Sequences A3 and A3P (with primers) possessed the greatest binding affinity with values for gated fluorescence above background of approximately 75%.

Sequences A1 and A3 form hairpins both in the absence and in the presence of the primer sequences. The affinity of these two aptamers for *V. parahemolyticus* changed minimally upon the inclusion or the exclusion of primers in the sequence; the gated fluorescence intensity above background for both A3P and A3 was 75%; for A1P, 67%; for A1, 64%; and for A18P, 62% (Figure 1A). These results suggest that the 40-nt variable region of the aptamer sequence, not the 20-nt primers flanking either end, is probably responsible for the high-affinity binding to the target.

Fluorescently labeled aptamer sequences A1, A1P, A3, A3P, and A18P were tested against a variety of other bacteria, including *L. monocytogenes*, *E. coli*, *S. typhimurium*, *S. aureus*, *C. sakazakii*, and *S. pneumoniae*. All of these aptamer sequences showed preferential binding to *V. parahemolyticus* over the other types of bacteria. Aptamer A18P, which seemed to bind weakly to *S. aureus* and *E. coli*, demonstrated the lowest specificity. However, the affinity of A18P for these bacteria was low compared to its affinity for *V. parahemolyticus*. The percent gated fluorescence intensity above the library background was 20% for A18P incubated with *S. aureus* and *E. coli* compared to 62% when incubated with *V. parahemolyticus* (Figure 1A,B). The aptamer A3P demonstrates greatly reduced binding to *S. aureus* and *E. coli* compared to *V. parahemolyticus*, thereby resulting in a percent gated fluorescence intensity above the library background of 4%. In contrast, the same sequence binding to *V. parahemolyticus* yielded a gated fluorescence above background value of 77% (Figure 1A,B). It can therefore be concluded that the tested aptamer sequences are specific for *V. parahemolyticus*.

Estimation of Binding Dissociation Constants (K_d) of High-Affinity Aptamer Sequences. Figure 2 shows a representative binding saturation curve from the flow cytometric analysis of the fluorescently labeled A3P aptamer binding to 10^8 *V. parahemolyticus* cells. Estimates from a nonlinear regression curve fit to the data revealed a K_d value of 16.88 ± 1.92 nM. Binding of a fluorescently labeled randomized oligonucleotide library to *V. parahemolyticus* cells was used as a negative control. All tested aptamer sequences exhibited saturation binding kinetics.

Table 2 summarizes the estimated K_d values and the predicted secondary structures of the aptamers with the greatest selectivity for *V. parahemolyticus*. Sequences A1P and A3P demonstrated the greatest affinity and selectivity for the

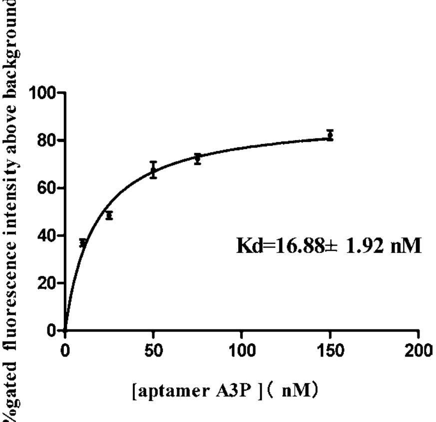
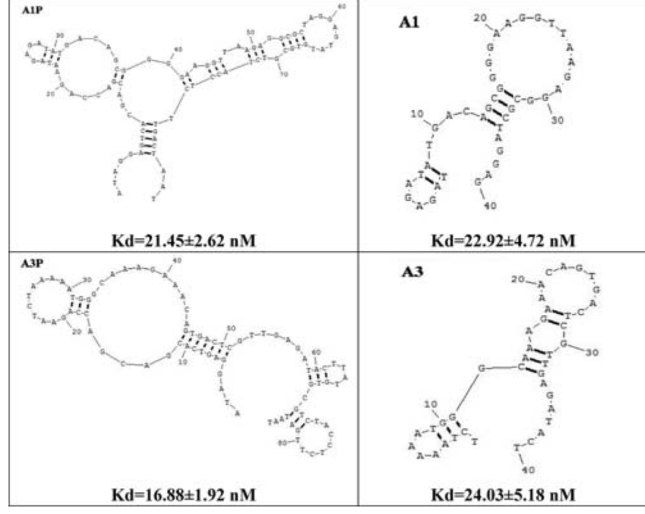


Figure 2. Binding saturation curve of aptamer A3P to *V. parahemolyticus*. A nonlinear regression curve was fit to the data using GraphPad Prism 5.0. Similar experiments were conducted for all high-affinity aptamer sequences, and the estimated K_d values are summarized in Table 2.

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



target cells with K_d values of approximately 20 nM (21.45 ± 2.62 nM for A1P and 16.88 ± 1.92 nM for A3P). Sequence A18P is a less desirable aptamer, due to its high cross-reactivity with *S. aureus* and *E. coli* (Figure 1).

In conclusion, this study is the first report of the use of whole-bacterium SELEX to identify DNA aptamers that are specific for *V. parahemolyticus*. On the basis of the results of our study, we anticipate that the DNA aptamer A3P may be used to capture *V. parahemolyticus* and allow for its detection, even when applied to a complex sample matrix, such as food. Utilizing the sequences reported in this study, aptamers can be linked to magnetic nanoparticles to capture and concentrate bacteria with the help of a magnetic field. Moreover, aptamers can be chemically modified and conjugated to highly sensitive detection probes (e.g., fluorescent and enzymatic probes), demonstrating great potential in food-safety control. These applications are currently under investigation in our laboratory. This study provides a proof-of-concept that aptamers targeting *V. parahemolyticus* can be isolated using a whole-bacterium

dx.doi.org/10.1021/jf300395z | J. Agric. Food Chem. 2012, 60, 4034−4038

SELEX process and that these aptamers demonstrate the required binding affinity for preanalytical sample processing and detection methods.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +86 510 85917023. Fax: +86 510 85917023. E-mail: wangzp@jiangnan.edu.cn.

Funding

This work was partly supported by Science and Technology Supporting Project of Jiangsu Province (BE2011621, BE2010679), National S&T support program of China (2012BAK08B01), Mérieux Research Grant Program, Research Fund for the Doctoral Program of Higher Education (20110093110002), and Program for New Century Excellent Talents in University (NCET-11-0663).

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Yeung, P. S.; Boor, K. J. Epidemiology, pathogenesis, and prevention of foodborne *Vibrio parahaemolyticus* infections. *Foodborne Pathog. Dis.* **2004**, *1*, 74−88.

(2) DePaola, A.; Nordstrom, J. L.; Bowers, J. C.; Wells, J. G.; Cook, D. W. Seasonal abundance of total and pathogenic *Vibrio parahaemolyticus* in Alabama oysters. *Appl. Environ. Microbiol.* **2003**, *69*, 1521−1526.

(3) Lozano-Leon, A.; Torres, J.; Osorio, C. R.; Martinez-Urtaza, J. Identification of TDH-positive *Vibrio parahaemolyticus* from an outbreak associated with raw oyster consumption in Spain. *FEMS Microbiol. Lett.* **2003**, *226*, 281−284.

(4) Kiiyukia, C.; Venkateswaran, K.; Navarro, M.; Nakano, H.; Kawakami, H.; Hashimoto, H. Seasonal distribution of *Vibrio parahaemolyticus* serotypes along the oyster beds in Hiroshima coast. *J. Fac. Appl. Biol. Sci.* **1989**, *28*, 49−61.

(5) Yano, Y.; Kaneniwa, M.; Satomi, M. Occurrence and density of *Vibrio parahaemolyticus* in live edible crustaceans from markets in China. *J. Food Protect.* **2006**, *69*, 2742−2746.

(6) Rippey, S. R. Infections diseases associated with molluscan shellfish consumption. *Clin. Microbiol. Rev.* **1994**, *7*, 419−425.

(7) Wrzesinski, J.; Ciesiolka, J. Characterization of structure and metal ions specificity of CO^{2+}-binding RNA aptamers. *Biochemistry* **2005**, *44*, 6257−6268.

(8) Lorsch, J. R.; Szostak, J. W. In vitro selection of RNA aptamers specific for cyanocobalamin. *Biochemistry* **1994**, *33*, 973−982.

(9) Famulok, M. Oligonucleotide aptamers that recognize small molecules. *Curr. Opin. Struct. Biol.* **1999**, *9*, 324−329.

(10) Weiss, S.; Proske, D.; Neumann, M.; Groschup, M.; Kretzschmar, H.; Famulok, M.; Winnacker, E. RNA aptamers specifically interact with the prion protein PrP. *J. Virol.* **1997**, *71*, 8790−8797.

(11) Bock, L. C.; Griffin, L. C.; Latham, J. A.; Vermaas, E. H.; Toole, J. J. Selection of single-stranded DNA molecules that bind and inhibit human thrombin. *Nature.* **1992**, *355*, 564−566.

(12) Tuerk., C.; Gold, L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science* **1990**, *249*, 505−510.

(13) Ellington, A. D.; Szostak, J. W. In vitro selection of RNA that bind specific ligands. *Nature* **1990**, *346*, 818−822.

(14) Osborne, S. E.; Matsumura, I.; Ellington, A. D. Aptamers as therapeutic and diagnostic reagents: Problems and prospects. *Curr. Opin. Chem. Biol.* **1997**, *1*, 5−9.

(15) Famulok, M.; Hartig, J. S.; Mayer, G. Functional aptamers and aptazymes in biotechnology, diagnostics, and therapy. *Chem. Rev.* **2007**, *107*, 3715−3743.

(16) Camille, L. A.; Hamula, X.; Chris, L.; Li, X. F. DNA aptamers binding to multiple prevalent M-types of *Streptococcus pyogenes*. *Anal. Chem.* **2011**, *83*, 3640−3647.

(17) Hamula, C. L. A.; Zhang, H.; Guan, L. L.; Li, X. F.; Le, X. C. Selection of aptamers against live bacterial cells. *Anal. Chem.* **2008**, *80*, 7812−7819.

(18) Chen, F.; Zhou, J.; Luo, F.; Mohammed, A. B.; Zhang, X. L. Aptamer from whole-bacterium SELEX as new therapeutic reagent against virulent *Mycobacterium tuberculosis*. *Biochem. Biophys. Res. Commun.* **2007**, *357*, 743−748.

(19) Cao, X.; Li, S.; Chen, L.; Ding, H.; Xu, H.; Huang, Y.; Li, J.; Liu, N.; Cao, W.; Zhu, Y.; Shen, B.; Shao, N. S. Combining use of a panel of ssDNA aptamers in the detection of *Staphylococcus aureus*. *Nucleic Acids Res.* **2009**, *37*, 4621−4628.

(20) Dwivedi, H. P.; Smiley, R. D.; Jaykus, L. A. Selection and characterization of DNA aptamers with binding selectivity to *Campylobacter jejuni* using whole-cell SELEX. *Appl. Microbiol. Biot.* **2010**, *87*, 2323−2334.