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SHORT COMMUNICATION

Screening and preliminary application of a DNA aptamer for rapid detection of Salmonella O8

Guoqing Liu · Xiaofeng Yu · Feng Xue · Wei Chen · Yongkan Ye · Xiaojiao Yang · Yingqi Lian · Yi Yan · Kai Zong

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Abstract We report on a rapid method for the detection of Salmonella O8. It does not require an enrichment step but rather uses an aptamer as a probe that was selected by system evolution of ligands by exponential enrichment (SELEX) assay. Firstly, aptamer against Salmonella O8 was selected from a 78 bp random DNA library that was prepared in-vitro. The binding ability of the aptamers to target bacterium was examined by aptamer-linked immobilized sorbent assay. A high affinity aptamer was successfully selected from the initial random DNA pool, and its secondary structure was also investigated. Next, this high affinity aptamer B10 was used to recognize Salmonella O8 via fluorescence microscopy. The selected aptamer has a high specificity and high affinity against its target. We believe that the resulting fluorescence in-situ labeling assay is a potentially useful alternative in rapid screening and detection of foodborne pathogens.

Keywords Salmonella O8 · Aptamer · SELEX · Rapid detection

G. Liu · W. Chen (✉) · Y. Ye · X. Yang · Y. Lian
School of Biotechnology and Food Engineering,
Hefei University of Technology,
Hefei 230009, People's Republic of China
e-mail: chenweishnu@hfut.edu.cn

X. Yu · Y. Yan · K. Zong (✉)
Anhui Entry-exit Inspection and Quarantine Bureau,
Hefei 230022, People's Republic of China
e-mail: zongkai2002@126.com

F. Xue
Jiangsu Entry-exit Inspection and Quarantine Bureau,
Nanjing 200002, People's Republic of China

Introduction

Food borne pathogen contaminations are worldwide problems and have induced serious food safety issues including the toxic cucumber in German and contaminated eggs in America. Salmonellae are one of the most important food borne pathogens leading to millions cases of enteric diseases, hospitalizations and deaths annually. The majority of infections are associated with ingestion of bacterium contaminated plant or animal origin foods. In 2010, the Salmonella contamination of eggs and tropical fruits in California (USA) had induced more than 200 people infections. Under these conditions, rapid and accurate identification of Salmonella subspecies is of great importance for preventing and monitoring of enteric diseases caused by Salmonella. Numerous new methods such as PCR-based techniques, immunology techniques, metabology techniques and protein fingerprint techniques have been developed for bacterium detection [1, 2]. However, all these food borne pathogens detection methods generally go through several steps including (a) colony isolation on selective media, (b) use of biochemical tests and (c) serotyping using antibodies against specific bacterial antigens, which greatly increase the assay time and cost. In certain cases, the identity of a particular bacterium would take several days [3], which could not meet the current requirements of rapid detections. New detection method using piezoelectric method has been reported and realize the detection in 5 h, which is still a comparable long time and not suitable for on-site detection [4]. Following, research about using laser-induced fluorescence microchip electrophoresis for bacterium detection has been reported, which could achieve the specific detection in less than 150 s [5]. However, the specific microchip and setup are needed for the detection, disturbing the widely application of this method. Therefore, development of new

approaches for specific bacterium detections with high accuracy, speed and simplicity is of great importance in different fields especially for food safety detections [6, 7]. Herein, in this research, the Salmonella O8 was adopted as the model for detections.

As we all know that some traditional rapid detection methods are based on the immunoreactions between antibody and antigen. However, the preparation of antibody is a long term work and the restore and application conditions of antibody are very strict. Comparatively, nucleic acid probes have attracted many attentions for its easy-preparation and stability. Aptamer is one kind of specific nucleic acid probes. Usually aptamers are ssDNA or ssRNA oligonucleotides that can bind to a wide range of non-nucleic acid targets with high affinity and specificity [8–10], which can form stable and specific complexes with desired targets [11–14]. Through an in vitro process named SELEX (systematic evolution of ligands by exponential enrichment), it is an oligonucleotide-based combinatorial library approach that has been extensively used to isolate high-affinity aptamers for a wide variety of targets. There are several potential advantages over traditional antibodies. Aptamers are chemically stable, readily synthesized, and can be chemically modified and labeled more easily [15, 16]. Currently, so many methods have been developed using aptamer as probe to recognize target analyte [17–21]. Our group has also developed some aptamer-based rapid detection methods for toxin detections [12, 22]. However, reports about detection method using aptamer as probes are rarely although there have been many research about screening aptamers against different bacterium [23–25]. Specifically, it is found that screening aptamers against the Salmonella O8 is rarely reported.

Herein, in this research, we firstly adopted a whole bacteria SELEX strategy to get the ssDNA aptamer against Salmonella O8 with high affinity and specificity, which was named B 10 in our selection process. The dissociation constant of the selected aptamer was calculated to be low nanomolar level. Furthermore, the aptamer based fluorescence in situ labeling (FISL) assay was applied for Salmonella O8 detection in short time. Results indicated that the selected aptamer and the developed aptamer based FISL method could be a potential powerful method for rapid and on-site Salmonella O8 detections.

Experimental

Materials and reagents

All culture medium for different bacterium were bought from Beijing Huamei Bioscience Technology Ltd. (http://www.huameisk.cn). The ssDNA library, the biotinylated primers for PCR amplification and Digoxin-labeled aptamer with high performance liquid chromatography (HPLC) grade purity were all synthesized by Sangon Biotech. (Shanghai, http://www.sangon.com) Co., Ltd. The agarose gel extraction Kit and pGEM-T vector were also purchased from Sangon Biotech. (Shanghai, http://www.sangon.com) Co., Ltd. Bovine serum albumin (BSA) was purchased from Beijing Biodee Biotechnology Ltd. (http://www.biodee.net/). Other normal chemicals were bought from Sinopharm Chemical Reagent Co. Ltd (http://www.crc-bj.com/). All chemicals were used directly as received without further purification.

Apparatus

All PCR amplifications were carried out by Eppendorf Mastercycle ep384. Optima L-100XP centrifuge was used for all purification treatments. Beckman Paradigm plate reader was used for ALISA analysis. Rapid fluorescent detection was carried out by the Carl Zeiss upright metallurgical Microscope Axio Imager A2m (German).

Experimental procedures

Salmonella O8, E. coli O86:K61 and S. choleraesuis were maintained in nutrition agar and incubated at 37 °C for 24 h prior to harvest while in the log growth phase. The treatment of bacterium was according to the reference with little variations [1]. Typically, to prepare the targets for the selection and the counter-selection, all these bacteria were inactivated by bathing in a 6 % formaldehyde/NaCl solution at 62.5 °C for 1 h, and then centrifuged at 6,000 rpm, 4 °C for 10 min. The sediment of bacteria was washed with 0.9 % NaCl for three times, and re-suspended in selection buffer (50 mM Tris–HCl pH 7.4, 5 mM KCl, 100 mM NaCl, and 1 mM $MgCl_2$) and preserved at 4 °C. The ssDNA library consisted of a central randomized sequence of 35 nucleotides (nt) flanked by two primer hybridization sites (5′-GGG AGC TCA GAA TAA ACG CTC AA-35nt-CGA CAT GAG GCC CGG ATC-3′), Digoxin-labeled5′-primer (5′-Digoxin–GGG AGC TCA GAA TAA ACG CTC AA-3′) and a triple biotinylated (trB) labeled 3′-primer (5-trB-GAT CCG GGC CTC ATG TCG AA-3) were used to amplify double-labeled DNA molecules with conventional PCR procedure.

SELEX procedures

Aptamers were screened by the method described previously with slight modifications [26–28]. In the initial selection rounds, the10 μg ssDNA library dissolved in 500 μL pH 7.2 phosphate buffered saline (PBS) was denatured by heating at 95 °C for 5 min and then placed on ice for 10 min. The denatured ssDNA library was incubated with 30μL

$1.5*10^8$ cfu Salmonella O8 with mild shaking at 37 °C for 30 min. Bovine serum albumin (BSA) was added into the selection system to reduce the background binding. Unbound ssDNAs were washed away with 1 mL selection buffer (containing 0.2 % BSA) by centrifugation at 10,000 g, 4 °C for 10 min, and the bound ssDNAs were eluted by heating the bacteria–aptamers complex at 100 °C for 5 min in 200 μL of sterile ddH_2O. After centrifugation, the supernatant was used as a target template for amplification of bound aptamers by PCR with Digoxin- or trB-labeled primers (20–30 cycles of 0.5 min at 94 °C, 0.5 min at 55 °C, 0.5 min at 72 °C, followed by 5 min at 72 °C). Electrophoresis of 3 % agarose gel electrophoresis was used to confirm the purity and amplified length of PCR products. All PCR products were purified using a gel extraction kit. After 11-rounds selections, the selected ssDNA pool was PCR-amplified using unlabeled primers. The PCR products were cloned into pGEM-T vector and transformed into E. coli. Individual cultured colonies were picked out randomly and their inserts were sequenced by Invitrogen, Guangzhou, China. The aptamer sequences were analyzed by Clustal X software and the secondary structure was predicted by RNA structure v3.50.

To acquire the aptamers with high affinity and specificity, we progressively increased the selective pressure by increasing the number of washes (from two to five) and by decreasing the incubation time (from 60 to 20 min). The counter-selection against E. coli and S. choleraesuis was introduced in the 5th and the 8th rounds, respectively. To ensure elimination of non-specific ssDNAs against the polypropylene-partitioning matrix of the reaction tubes, tubes were blocked with 1 % BSA-PBS.

The affinity of the selected ssDNA aptamer cocktail we selected was determined by ALISA. Reaction tubes were washed with PBS pH 7.2 and blocked with Superblock for 30 min and dried at room temperature. When ssDNA: Salmonella O8 equaled to 50pmol: $2*10^8 cfu{\cdot}mL^{-1}$ which was incubated in 200 μL pH 7.2 PBS (0.2 % BSA). Following the mixture was denatured by heating at 95 °C for 5 min and then cooled on ice for 10 min, and mild shaken at 37 °C for 30 min. Afterwards the mixture was centrifuged at 8,000 g, 4 °C for 5 min. After centrifugation, the supernatant was removed and reaction tubes were washed with 250 μL pH 7.2 PBS for three times, then anti-Digoxin-alkaline phosphatase at a dilution of 1:5000 was added to the Reaction tubes and allowed to bind at 37 °C for 30 min, centrifugation at 8,000 g, 4 °C for 5 min. After the supernatant was removed, another 250 μL pH 7.2 PBS was added. After three times washes with PBS, excess enzyme was almost removed. Finally, the membrane discs were developed with BCIP/NTB-BLUE (Sigma). OD_{405} values were determined using a microplate reader from BIO-TEK. All the experiments were performed in triplicate.

For FISL analysis, the selected aptamer modified with FAM was used as fluorescent probe. Aptamer probe dissolved in PBS was denatured by heating at 95 °C for 5 min and then on ice for 10 min. A loop of an isolated colony was suspended in 500 μL pH 7.2 PBS and 10 μL of the suspension was spread onto a glass slide. The smear was air dried and fixed with acetone for 5 min. Twenty-five microliters of the aptamer probe was added onto the smears. Coverslips were added, and the slides were placed in a humidity chamber and incubated at 37 °C for 30 min, followed by a stringent wash with pre-warmed pH 7.2 PBS. The slides were then mounted in 70 % glycerol in PBS after distilled water wash. The hybridized smears were further examined with the Carl Zeiss upright metallurgical Microscope Axio Imager A2m (German). Bacteria were identified on the basis of bright green fluorescence and morphology. All slides were examined by two independent investigators blinded to the laboratory identification of the specimens.

Results and discussion

In aptamer research, whole-cell target detection is a promising area. One of its principal advantages is the ability to target specific cell types without having previous knowledge of the membrane molecules or structural changes related to that cell type [24, 29]. To date, most of the isolated aptamers against microorganisms have been selected for clinical applications. Aptamer application to detect environmental and foodborne pathogens is a promising area of research. Detection, identification and quantification of food borne pathogens are crucial for public food health protection. In order to develop the rapid detection method of Salmonella O8, choose the optimal recognition aptamer is of great importance. Firstly, whole-bacterium SELEX

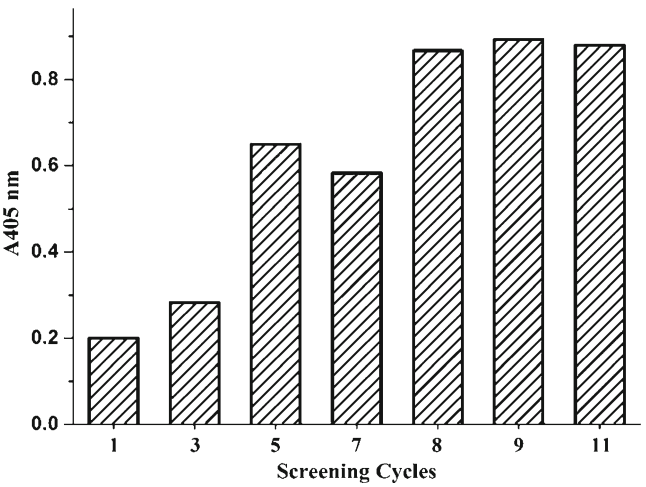

Fig. 1 Results from screening of the sequenced Salmonella O8 candidate aptamer clones

Table 1 Sequences of selected aptamers for Salmonella

Families	Aptamers	Sequences	ΔG
Family1	E06	TGATCCGGGCCTCATGTCGAACCCACACCCCACAACCACCCAGCCCCAGCCCGCTATTGAGCGTTTATTCTGAGCTCCCA	−6.6
	F04	TGATCCGGGCCTCATGTCGAACACAACACCCAGCCAACGACCACAACTCCAACTCATTGAGCGTTTATTCTGAGCTCCCA	−5.0
	C10	TGATCCGGGCCTCATGTCGAAACCACAACCCAACAACAAGAACCACAAAGAGCCCCTTGAGCGTTTATTCTGAGCTCCCA	−3.9
Family2	B06	TGATCCGGGCCTCATGTCGAACACACACAGAACCACAACCACTAAACACGAGGGCCTTGAGCGTTTATTCTGAGCTCCCA	−6.8
	B10	GATCCGGGCCTCATGTCGAACACCCCCCAACTAAAACAACAAAACACCACCGCCATTGAGCGTTTATTCTGAGCTCCCA	−2.6
Family3	C04	TGATCCGGGCCTCATGTCGAACCAACAAGGCAGAAAGAAACCGACAAACCGCACTGTTGAGCGTTTATTCTGAGCTCCCA	−7.2
	F05	TGATCCGGGCCTCATGTCGAACCAACAAGGCAGAAAGAAACCGACAAACCGCACTGTTGAGCGTTTATTCTGAGCTCCCA	−7.2
Family4	B05	TGATCCGGGCCTCATGTCGAACCGAACGACTCAAAGATCAAGCCAAGCCACGCCCGTTGAGCGTTTATTCTGAGCTCCCA	−7.7
	G05	TGATCCGGGCCTCATGTCGAACCAACCCAACACCAAAGAGACCACCACCACACGAGTTGAGCGTTTATTCTGAGCTCCCA	−3.7
Family5	D04	TGATCCGGGCCTCATGTCGAACACGAGCCACCAACGCACCAAAACCCGTCCTCCACTTGAGCGTTTATTCTGAGCTCCCA	−9.0
	E04	TGATCCGGGCCTCATGTCGAAGGCACGCGCACCAAAACACAAACTCCCCCCGCACCTTGAGCGTTTATTCTGAGCTCCCA	−9.4
	G06	TGATCCGGGCCTCATGTCGAAGGGCCACCTAACCACCTCTGCCAATACCCCCCGCGTTGAGCGTTTATTCTGAGCTCCCA	−8.3
Family6	C05	TGATCCGGGCCTCATGTCGAAGGGAAGTCATGGCATGGATGCGCTCCCCGCCCACCTTGAGCGTTTATTCTGAGCTCCCA	−13.3
	C06	TGATCCGGGCCTCATGTCGAAGGGAAGTCATGGCATGGATGCGCTCCCCGCCCACCTTGAGCGTTTATTCTGAGCTCCCA	−13.3
Family7	H05	TGATCCGGGCCTCATGTCGAACGGCGCAGTGACACGGTAGGGAGTCGTGCCGCGGCTTGAGCGTTTATTCTGAGCTCCCA	−12.8
Family8	H06	TGGGAGCTCAGAATAAACGCTCAAGGTGGGCGGGCGGCGTTGCTGTTCTTTTGCTGGTGTTCGACATGAGGCCCGGATCA	−9.1
	F06	TGGGAGCTCAGAATAAACGCTCAAGAGGCGGCCTGTTCGCTTGTTGTGGGTCCGCTTGGTTCGACATGAGGCCCGGATCA	−10.1
Family9	A05	TGGGAGCTCAGAATAAACGCTCAAGGGCACTGGGTGGTGATGTTTTGTTTGTCTGGCGGTTCGACATGAGGCCCGGATCA	−19.3
	D06	TGGGAGCTCAGAATAAACGCTCAAGGGGGGGGTGGAGGGGTATGCAGTTTCGGTGGCCGTTCGACATGAGGCCCGGATCA	−5.8

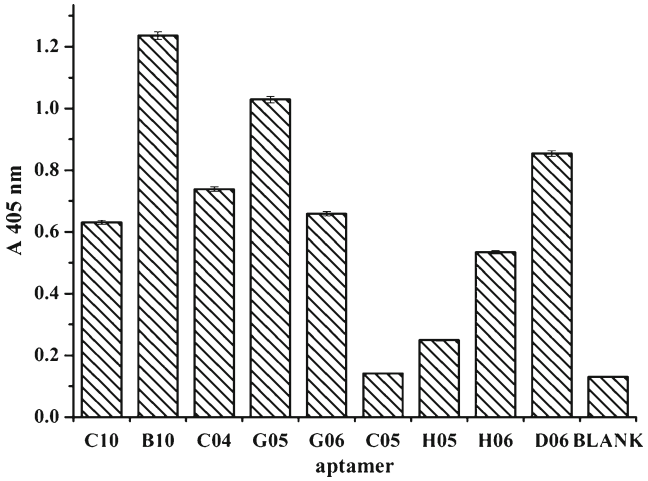

Fig. 2 Binding assay of aptamers against Salmonella O8

strategy was adopted to select the aptamer against Salmonella O8 with high selectivity and affinity. The ALISA was applied to monitor the select results of each cycle. As show in Fig. 1, it is easy to observe that the absorption intensity at 405 nm is gradually increased with the increase of selection round for aptamers. Till round 8, the intensity is coming to the constant value, indicating the binding ability of the selection pools reached a plateau after 8 rounds of selection. In order to further confirm the high affinity of the selected aptamer, the 11th round aptamer was adopted and cloned into E. coli DH 5α using the pGEM-T vector system. Following, twenty-two individual clones were picked out and sequenced and multiple sequence alignments results revealed that several groups of sequences were modestly enriched. According to the sequenced results and homology of the DNA sequence, the majority of sequences could be classified into nine families as shown in Table 1.

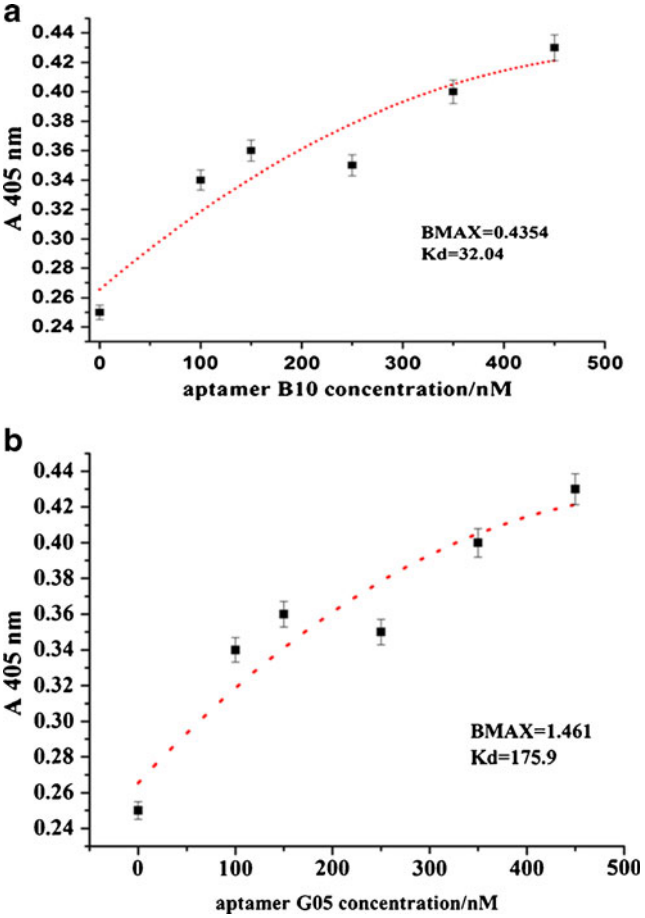

Fig. 4 Binding affinities of aptamer B10 (**a**) G05 (**b**) against Salmonella O8

The binding ability of the selected aptamers was further chosen from each family based on the minimal free energy principle. Afterwards, the optimized sequence of the aptamer were synthesized and modified with FAM at 5′ end to label the Salmonella O8 using ALISA. From the

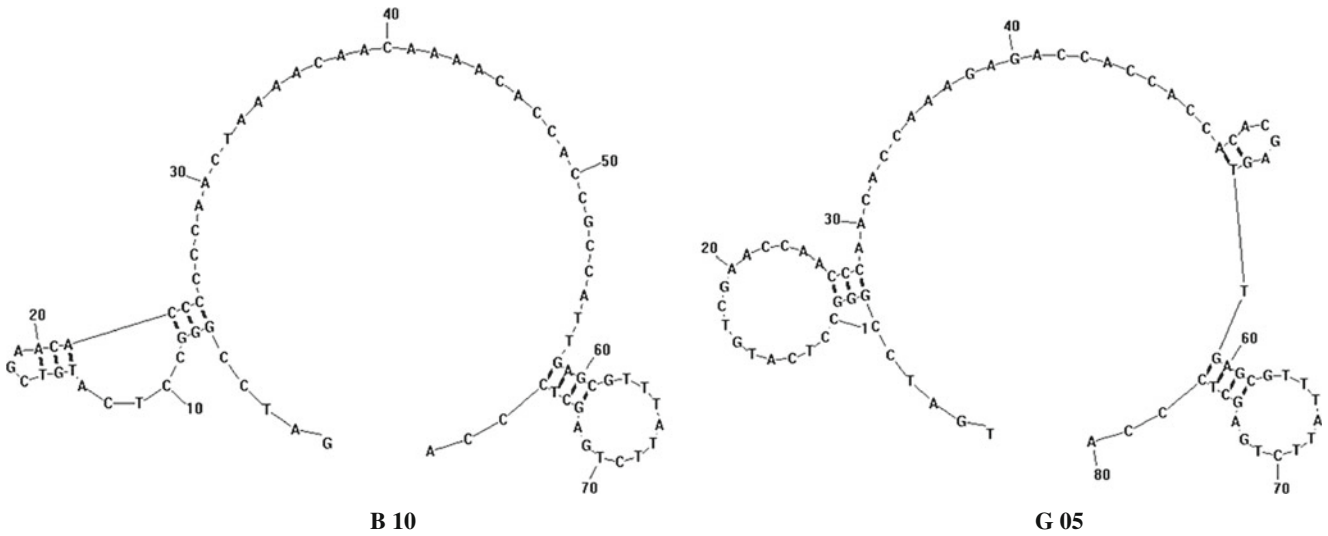

Fig. 3 The secondary structure model of aptamers B10 and G05

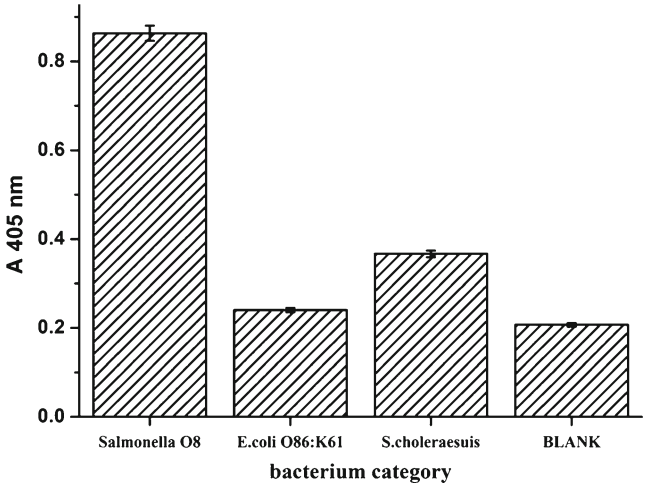

Fig. 5 Specificity of aptamer B10 binding to Salmonella O8 and counter selection bacteria

ALISA results shown in Fig. 2, it is demonstrated that, among nine candidate aptamers, two of them (B10 and G05) show the strongest binding affinity with Salmonella O8 compared with the control and other candidate aptamers. In order to further investigate these two selected aptamers, we analyzed the secondary structures of each aptamer by the classic RNA structure software v3.5. From the predicted secondary structure, we find that the aptamer-B 10 could form two stem-loop branches on a larger central loop while the aptamer-G05 form three stem-loop branches off from the larger central loop (Fig. 3).

The binding affinity of aptamer B10 and G 05 was further evaluated by measuring the dissociation constant K_d based on ALISA. From the detected results shown in Fig. 4, the fit curve could be developed in the one site binding model (hyperbola) by the GraphPad Prism Software v5.0, which gives the smallest error with respect to the experimental data. Through the calculation, the dissociation constant K_d and ΔG between B 10 and Salmonella O8 is 32.04 nM and -2.6 kJ·mol^{-1}, respectively. The dissociation constant K_d and ΔG between G 05 and Salmonella O8 is 175.9 nM and -3.7 kJ·mol^{-1}, respectively. From these results, it is easy to find out that the bind affinity of aptamer B 10 is stronger than that of aptamer G 05. Therefore, aptamer B 10 was selected as the optimal aptamer probe against Salmonella for following fluorescent detections.

The aptamer B 10 was used as probe in the ALISA. As shown in Fig. 5, the absorption intensity of Salmonella O8 is higher than that of other control bacterium and blank group, indicating the excellent specificity of the screened aptamer B 10. The specificity of Salmonella O8 detection was further carried out by the direct observation with fluorescent microscopy. Aptamer B 10 was modified with digoxigenin for direct fluorescent observation after combining with target bacteria. Through the direct observation with fluorescent microscopy, we found that the digoxigenin-aptamer could bind to the target Salmonella O8 with high specificity, showing obvious fluorescent signal (Fig. 6c). Comparatively, there is no fluorescent signal in the blank group (Fig. 6d) while there is a little fluorescent signals in the control group of E. coli O86:K61 and S. choleraesuis (Fig. 6a and b), which may be caused by the non-specific adsorption. These results also demonstrated the high specificity and affinity of the selected aptamer B10 against target Salmonella O8. These indicated that the selected aptamer B 10 could be a potential powerful probe for Salmonella O 8 detections and

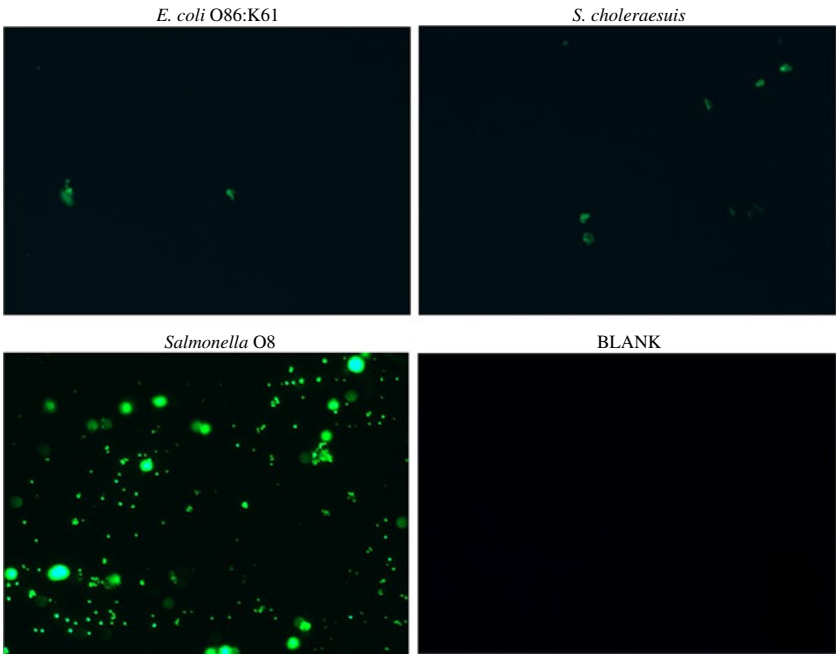

Fig. 6 Fluorescent detection results of aptamer B10 based FISL assay

more useful ultrasensitive and rapid methods could be developed quickly by using this aptamer probe.

Conclusions

In this study, aptamer against Salmonella O8 was selected through the classic in vitro SELEX technique. The aptamer B 10 was obtained from nine families of pre-screened aptamers and had very high selectivity and binding affinity to the target Salmonella O8 with the dissociation constant K_d and ΔG nM 32.04 nM and −2.6 kJ mol^{-1}, respectively. The secondary structure was analyzed, which was benefit for the future recognition mechanism research. Furthermore, a rapid detection method for Salmonella O8 was developed based on the selected aptamer B 10. And the whole procedure of ssDNA aptamer-based detection could be finished in 1.5–2 h. Detection results further indicated the excellent selectivity and bind ability of aptamer B 10. This work provided a good aptamer probe for Salmonella O8 detection and would also contribute greatly to the development of rapid detection methods for bacterium. Further post-SELEX of aptamers with higher affinity and development of other on-site detection methods for Salmonella O8 based on aptamer B 10 are still going on in our lab.

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References

1. Wang KY, Zeng YL, Yang XY, Li WB, Lan XP (2011) Utility of aptamer-fluorescence in situ hybridization for rapid detection of *Pseudomonas aeruginosa*. Eur J Clin Microbiol Infect Dis 30:273–278
2. Wang HY, Zhang CS, Xing D (2011) Simultaneous detection of Salmonella enterica, Escherichia coli O157:H7, and Listeria monocytogenes using oscillatory-flow multiplex PCR. Microchim Acta 173:503–512
3. Mueen A, Joseph H, Smith KL (2003) Development of a PCR-based assay to detect Shiga toxin-producing *Escherichia coli, Listeria monocytogenes* and *Salmonella* in milk. Food Microbiol 20:345–350
4. Plata MR, Contento AM, Rios A (2011) Simplified determination of bacterial contamination by Escherichia coli using a flow injection system with piezoelectric detection. Microchim Acta 172:447–454
5. Cheng S, Wang ZF, Ge SL, Wang H, He PG, Fang YZ, Wang QJ (2012) Rapid separation of four probiotic bacteria in mixed samples using microchip electrophoresis with laser-induced fluorescence detection. Microchim Acta 176:295–301
6. Valdes MG, Gonzalez ACV, Calzon JAG, Diaz-Garcia ME (2009) Analytical nanotechnology for food analysis. Microchim Acta 166:1–19
7. Joshi R, Janagama H, Dwivedi HP, Senthil Kumar TM, Jaykus LA, Schefers J, Sreevatsan S (2009) Selection, characterization, and application of DNA aptamers for the capture and detection of Salmonella enterica serovars. Mol Cell Probes 23:20–28
8. Jayasena SD (1999) Aptamers: An emerging class of molecules that rival antibodies in diagnostics. Clin Chem 45(9):1628–1650
9. Tuerk C, Gold L (1990) Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science 249:505–510
10. Ellington A, Szostak JW (1990) In vitro selection of RNA molecules that bind specific ligands. Nature 346:818–822
11. Kong RM, Zhang XB, Zhang LL, Jin XY, Huan SY, Shen GL, Yu RQ (2009) An ultrasensitive electrochemical "turn-on" label-free biosensor for Hg^{2+} with AuNP-functionalized reporter DNA as a signal amplifier. Chem Commun 37:5633–5635
12. Kuang H, Chen W, Xu DH, Xu LG, Zhu YY, Liu LQ, Chu HQ, Peng CF, Xu CL, Zhu SF (2010) Fabricated aptamer based electrochemical signal-off sensor of Ochratoxin A. Biosens Bioelectron 26:710–716
13. Wilson DS, Szostak JW (1999) In vitro selection of functional nucleic acids. Annu Rev Biochem 68:611–647
14. Ho HA, Leclerc M (2004) Optical sensors based on hybrid aptamer/conjugated polymer complexes. J Am Chem Soc 126:1384–1387
15. Ngundi MM, Kulagina NV, Anderson GP, Taitt CR (2006) Nonantibody-based recognition: alternative molecules for detection of pathogens. Expert Rev Proteomics 3:511–524
16. Schmidt KS, Borkowski S, Kurreck J, Stephens AW, Bald R, Hecht M, Friebe M, Dinkelborg L, Erdmann VA (2004) Application of locked nucleic acids to improve aptamer in vivo stability and targeting function. Nucleic Acids Res 32(19):5757–5765
17. Zhang HX, Jiang BY, Xiang Y, Zhang YY, Chai YQ, Yuan Y (2011) Aptamer/quantum dot-based simultaneous electrochemical detection of multiple small molecules. Analytica Chimica Acta 688:99–103
18. Srinivas RL, Chapin SC, Doyle PS (2011) Aptamer Functionalized microgel particles for protein detection. Anal Chem 83:9138–9145
19. Wang YH, Bao L, Liu ZH, Pang DW (2011) Aptamer biosensor based on fluorescence resonance energy transfer from upcoverting phosphors to carbon nanoparticles for thrombin detection in human plasma. Anal Chem 83:8130–8137
20. Yan SY, Huang R, Zhou YY, Zhang M, Deng MG, Wang XL, Weng XC, Zhou X (2011) Aptamer-based turn-on fluorescent four-branched quaternary ammonium pyrazine probe for selective thrombin detection. Chem Comm 47:1273–1275
21. Nasa S, Koichi A, Koji S, Kazunori I (2010) Selection of DNA aptamer against prostate specific antigen using a genetic algorithm and application to sensing. Biosens Bioelectron 26:1386–1391
22. Wang LB, Ma WW, Chen W, Liu LQ, Ma W, Zhu YY, Xu LG, Kuang H, Xu CL (2011) An aptamer-based chromatographic strip assay for sensitive toxin semi-quantitative detection. 26:3059–3062
23. Torres-Chavolla E, Alocilja EC (2009) Aptasensors for detection of microbial and viral pathogens. Biosens Bioelectron 24:3175–3182
24. Hamula CLA, Guthrie JW, Zhang HQ, Li XF, Le XC (2006) Selection and analytical applications of aptamers. TrAC-Trend Anal Chem 25:681–691
25. Chen F, Zhou J, Luo F, Mohammed AB, Zhang XL (2007) Aptamer from whole-bacterium SELEX as new therapeutic reagent against virulent Mycobacterium tuberculosis. Biochem Biophys Res Commun 357:743–748

26. Tombelli S, Minunni M, Mascini M (2007) Aptamers-based assays for diagnostics, environmental and food analysis. Biomol Eng 24:191–200
27. Henning U, Margaret HM, Maria JMA, Walter C (2002) In vitro selection of RNA aptamers that bind to cell adhesion receptors of trypanosome cruzi and inhibit cell invasion. J Biol Chem 277:20756–20762
28. John GB, Johnathan LK (1999) In vitro selection of DNA aptamers to anthrax spores with electrochemiluminescence detection. Biosens Bioelectron 14:457–464
29. Daniels DA, Chen H, Hicke BJ, Swiderek KM, Gold L (2003) A tenascin-C aptamer identified by tumor cell SELEX: Systematic evolution of ligands by exponential enrichment. Proc Natl Acad Sci USA 100:15416–15421