Journal of Microbiological Methods 95 (2013) 162–166

Contents lists available at ScienceDirect

Journal of Microbiological Methods

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

Identification of *Salmonella* Typhimurium-specific DNA aptamers developed using whole-cell SELEX and FACS analysis

Jihea Moon, Giyoung Kim *, Sangdae Lee, Saetbyeol Park

Department of Agricultural Engineering, National Academy of Agricultural Sciences, Suwon 441-707, South Korea

ARTICLE INFO

Article history:
Received 14 May 2013
Received in revised form 5 August 2013
Accepted 8 August 2013
Available online 24 August 2013

Keywords:
Salmonella Typhimurium
ssDNA
Aptamer
Bacteria-base SELEX
FACS analysis

ABSTRACT

Conventional methods for detection of infective organisms, such as *Salmonella*, are complicated and require multiple steps, and the need for rapid detection has increased. Biosensors show great potential for rapid detection of pathogens. In turn, aptamers have great potential for biosensor assay development, given their small size, ease of synthesis and labeling, lack of immunogenicity, a lower cost of production than antibodies, and high target specificity. In this study, ssDNA aptamers specific to *Salmonella* Typhimurium were obtained by a whole bacterium-based systematic evolution of ligands by exponential enrichment (SELEX) procedure and applied to probing *S.* Typhimurium. After 10 rounds of selection with *S.* Typhimurium as the target and *Salmonella* Enteritidis, *Escherichia coli* and *Staphylococcus aureus* as counter targets, the highly enriched oligonucleic acid pool was sorted using flow cytometry. In total, 12 aptamer candidates from different families were sequenced and grouped. Fluorescent analysis demonstrated that aptamer C4 had particularly high binding affinity and selectivity; this aptamer was then further characterized.

1. Introduction

Salmonella infection causes severe illness in the elderly, infants, and those with compromised immune systems. The major symptoms are fever, abdominal pain, nausea, vomiting, and diarrhea (Moon et al., 2009; Kim et al., 2009). *Salmonella* outbreaks have been associated with raw or undercooked eggs and poultry; however, recently these outbreaks have resulted from various plant foods, like fresh-cut vegetables and fruits (Beuchat, 1998). *Salmonella* has been recognized as a major cause of food poisoning and is related to common and large-scale food-borne outbreaks (Velge et al., 2005). In Korea, *Salmonella* caused 9.1% of foodborne illnesses recorded during 2009−2011 (7.45% in 2009, 9.96% in 2010, and 9.9% in 2011) (Lee, 2012). In the USA, 40,000 cases of salmonellosis are reported annually (Lampel et al., 2012).

Methods for detection and control of these organisms are well established. Microbiological testing has played an important role in verifying the presence of *Salmonella*. However, conventional detection methods are complicated and require multiple steps, so that the need for rapid detection of *Salmonella* has increased (Joshi et al., 2009). In this context, biosensors show great potential for rapid detection of pathogens (Kim et al., 2009). Biosensors utilize various biological reactions at the surface of a physical sensor and involve immobilizing bio-ligands, such as enzymes, antibodies, cells, and nucleic acids (Torres-Chavolla and Alocilja, 2009). Although biosensors directly monitor these bio-reactions in real-time, their sensitivity and specificity depend on the affinity of the ligands for pathogen capture (Joshi et al., 2009; Kim et al., 2011). Antibodies have limited application, as it is difficult to modify the immune system, and it is hard to control the stability in different environments (Cibiel et al., 2011).

Aptamers have great potential for biosensor assay development, given their small size, ease of synthesis and labeling, lack of immunogenicity, a lower cost of production than antibodies, and high target specificity (Tombelli et al., 2005). In particular, whole-cell systematic evolution of ligands by exponential enrichment (SELEX), can be used to create aptamers that can bind to live bacteria (Mao et al., 2009). This methodology is composed of multiple steps, followed as: 1) screening random oligonucleotide bound with the target, 2) multiple separating and exponential amplifying the binding oligonucleotide, and 3) cloning and sequencing of the final specific binding molecules, identified as the best sequences. These specific nucleic acid sequences are called aptamers and can bind to targets with high affinity and specificity (Torres-Chavolla and Alocilja, 2009). These aptamers hold great potential for use as probes for the development of biosensors for *Salmonella* detection. A few reports on the use of aptamers for the detection of *Salmonella* have been published in recent years (Pan et al., 2005; Joshi et al., 2009; Hyeon et al., 2012). However, single-stranded DNA (ssDNA) aptamers that identify specific proteins extracted from *Salmonella*, rather than the whole cell, were reported (Pan et al., 2005; Joshi et al., 2009). Therefore, in order to find ligands with a higher affinity for the target than that of antibodies, the SELEX method was adopted.

This study aimed to design and validate the whole-cell SELEX method targeting *Salmonella* Typhimurium, including estimation of its selectivity

* Corresponding author. Tel.: +82 32 290 1899; fax: +82 32 290 1900.
E-mail address: giyoung@korea.kr (G. Kim).

0167-7012/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.mimet.2013.08.005

and sensitivity when preceded by counter-SELEX, flow cytometry, and fluoro-photometric analysis.

2. Methods and materials

2.1. Bacterial strains

Stock cultures of *S.* Typhimurium were obtained from the Korean Collection of Type Cultures (KCTC, South Korea). Cells were grown overnight at 35 °C in brain heart infusion medium (Difco, NJ, USA). Cells were harvested by centrifugation, washed 3 times in phosphate buffered saline (PBS), and finally suspended in 100 μl of PBS prior to use in experiments.

2.2. Preparation of DNA library

The DNA template was synthesized as a single-stranded DNA containing 40 random nucleotides 5′-CGGATGCGAATTCCCTAATACGACT CACTATAGGGCGT-N_{40}-GGTGGATCCATATTCCTACTCG-3′. The initial DNA library was prepared by PCR amplification with Pyrobest polymerase (Takara, Japan).

2.3. SELEX procedures

Aptamers were screened using a modification of Liu's methods (Liu et al., 2012). In the initial selection rounds, 1.0 μg DNA library dissolved in 50 μl PBS (pH 7.4, Sigma, MO, USA) was denatured by heating at 95 °C for 10 min and then placed on ice for 10 min. The denatured ssDNA library was incubated with 100 μl of 1.0×10^9 cfu/ml *S.* Typhimurium, with shaking (500 rpm) at room temperature for 45 min. Unbound ssDNA was removed by centrifugation at 5,000 × *g* for 10 min. Then, the bound ssDNA was eluted by heating bacteria–aptamer complexes at 95 °C for 10 min in 500 μl of sterile ddH_2O. After centrifugation, the supernatant was used as an aptamer candidate template for amplification by PCR (30 cycles each of 30 s at 98 °C, 30 s at 68 °C, 15 s at 72 °C, followed by 5 min at 72 °C). Electrophoresis on 2.0% agarose gel was used to confirm the purity and verify the size of the PCR products. All PCR products were purified using Ultracel (Millipore, MA, USA). After verification of the band on the gel, the target band was carefully cut out and extracted using a MinElute gel extraction kit (Qiagen, Germany). These extraction methods were also used in the subsequent rounds of selection.

After 5 selection-rounds, the selected ssDNA pool was mixed with 100 μl of 1.0×10^8 cfu/ml *S.* Typhimurium to allow binding. To acquire aptamers with high affinity and specificity, we used 6-rounds of counter-SELEX against *Escherichia coli*, *Salmonella* Enteritidis, and *Staphylococcus aureus*.

2.4. Flow cytometric analysis (FACS; fluorescence-activated cell sorting)

To monitor the enrichment of aptamers in the selected ssDNA pool, *S.* Typhimurium was prepared with 1.0×10^9 cfu/ml in 100 μl of PBS. This analysis was used to assess the binding of the individual selected ssDNA to *Salmonella* cells. The selected DNA was fluorescently labeled via PCR amplification with 5′-FAM (fluorescein)- and 3′-FAM-modified primers. The dsDNA was denatured to ssDNA by heat and ice shock. Binding assays were then carried out by incubating 1.0 μg of fluorescently labeled aptamer candidates with 10^8 cells for 45 min in PBS and then washing the cells once in PBS buffer. Thereafter, cells were resuspended in 100 μl of PBS for flow cytometry analysis. This method has been called to fluorescence-activated cell sorting (FACS) analysis for measuring cell-assorted fluorescence (Herzenberg et al., 2006). Forward scatter, side scatter, and fluorescence intensity were measured, and gated fluorescence intensity above background was quantified. Aptamer candidates bound to *S.* Typhimurium were sorted by FACS and were then amplified with primers. These amplified aptamer candidates were cloned and sequenced at Macrogen (Seoul, Korea). The aptamer sequences were analyzed using a commercial genetic analysis program (CLC Workbench 6, Aarhus, Denmark).

2.5. Aptamer binding assay

All binding assays using FAM-labeled aptamers were analyzed using an Infinite M1000 spectrophotometer (TECAN, Switzerland). The binding and selective affinity of aptamer groups (1.0 μmole) for *S.* Typhimurium among different bacterial populations (*S.* Typhimurium, *E. coli* and *S. aureus*; 5×10^7 cfu cells each) were assessed. Aptamer candidates labeled with FAM were incubated with different bacteria in 500 μl PBS for 45 min at room temperature. After incubation, bacteria–aptamer complexes were washed twice with 500 μl PBS. Finally, the pellet of FAM-ssDNA bound to bacteria was resuspended in 100 μl PBS and transferred to 96-well black microplates (Corning, USA). Fluorescence was measured at 494 nm excitation, and emission from fluorescently labeled analytes was monitored at 510 nm. Optimal binding affinity of different concentrations (0.1, 0.5, 1.0, 5.0, and 10.0 μmole) of a high-affinity FAM-labeled aptamer C4 for *S.* Typhimurium (1×10^7 cfu) was also assessed.

3. Results and discussions

3.1. Whole-cell SELEX for evolution of S. Typhimurium-specific aptamers

Our study of the use of SELEX-based aptamers against live *Salmonella* cells was characterized by 5 important steps. First, the ssDNA library was incubated with live *Salmonella* cells at room temperature to allow binding between aptamers and live cells. Second, only ssDNA bound to cells was separated by centrifugation, allowing separation from non-bound ssDNA. Third, ssDNA released from cell surfaces was amplified by incubating of fractions eluted after washing. In this process, ssDNA was concentrated by PCR-Ultracel, because the concentration of the ssDNA is too low to allow amplification for the next selection. Finally, ssDNAs were sorted, cloned, sequenced, and then individually characterized according to their binding affinity for *Salmonella* cells by flow cytometry analysis. In total, 12 aptamer candidates were sequenced after 10 rounds of selection and 6 rounds of counter-selection (Table 1).

This study was based on a whole cell-SELEX approach, in which live cells are used to select aptamers (Chen et al., 2007; Cibiel et al., 2011). Generally, the success rate of whole cell-SELEX is almost 50% (Mayer et al., 2010). Because the cell surface carries a net negative charge, DNA polyanion is bind to cells with difficult, due to charge repulsion (Sefah et al., 2010). To solve this problem, the selection process in our study included two important processes. One was the PCR amplification process after elution of ssDNA from cells. During the 1st round of selection, the target band (100 bp) could not be detected visually; however, it could be detected after the 10× concentration by Ultracel. Therefore,

Table 1
Screened aptamer sequences.

Name	Sequence
C1	5′-GCGTGCGTCGGAGCCAGGATGCGAGGTCTGTAGGTCTGCGGGCGG-3′
C2	5′-ACGGCGGGCGAGTTGACGGCGTAATCGTGCTGCCGCGTG-3′
C3	5′-GCGGGCGTGGCTCAGAGTGGGGGTCGGTCAGTCTTGTGCGCG-3′
C4	5′-ACGGGCGTGGGGGCAATGCCTGCTTGTAGGCTTCCCCTGTGCGCG-3′
C5	5′-GCGTGCGGACGCCTGCGTGGCTGAGGCTTCGGTCTGCGCCG-3′
C6	5′-CGTGCGGCGGGCAGGATGGGATGCTGTAGGTCTGCGGGCGCGCG-3′
C7	5′-CGTGCGGAGCCAGGATGGGAGGTCTGTAGGTCTGCGGGGCGCG-3′
C8	5′-GCGTGGGACGGGTACCGGGCGTGTGGCGTGCCTGCCGCG-3′
C9	5′-ACGTGCGGGCCGGATGGGAGCTCTGTAGGTTGCGGGCGCGG-3′
CA1	5′-GCGTGCGGGGGCCAGGATGCGA-3′
CA2	5′-GGTCTGTAGGTCTGCGGCGCGCG-3′
CA3	5′-GCGTGCGGGGGCCAGGATGCGAGGTCTGTAGGTCTGCGGCGCGCG-3′

we employed PCR-Ultracel concentration after the PCR amplification process. The other important step was the use of centrifugation to separate ssDNA bound to bacteria from unbound ssDNA. In the process of whole-cell SELEX, there are very effective and economically feasible approaches to selectively separate bound ssDNA from unbound ssDNA using centrifugation (Mayer et al., 2010; Sefah et al., 2010; Hamula et al., 2011). This separation of ssDNA bound to live bacteria from unbound ssDNA is the crucial point of selection.

In this study, this separation was made by centrifugation, and heat treatment was then used to elute ssDNA from bacteria.

In total, 10 rounds of SELEX and 6 rounds of counter-SELEX were performed. All of DNA library that was not bound to *Salmonella* cells was removed in this repeated selection process by centrifugation, leaving only ssDNA on the bacteria. Therefore, the PCR products amplified from the ssDNA heat-eluted from the cells was of too low in concentration to demonstrate a target band upon gel electrophoresis. However, Ultracel concentration process after PCR amplification was more effective than simple PCR for the whole-cell SELEX (Fig. 1). Observation of a target band on the gel after each round of selection suggested that aptamer candidates could bind *S.* Typhimurium. Thus, in this study, all rounds of SELEX and counter-SELEX were performed using Ultracel concentration. Moreover, no amplified DNA was detected in the negative control, which consisted of cells that underwent incubation, washing, and heat elution, but without addition of the ssDNA library.

3.2. Screening of aptamer candidates by FACS analysis

Flow cytometry is a crucial method for selecting target aptamers specifically bound to cells, by sorting, counting, and fluorescence detection (Cibiel et al., 2011). Because the whole-cell SELEX process could select nucleic acids with good affinity but with poor specificity, FACS was used to screen aptamers that are specific to the target (Hamula et al., 2011). FACS thus allowed detection of aptamers with both high affinity and specificity for the target.

Once the selection process had been completed, we evaluated the specificity of the aptamer candidates for *S.* Typhimurium using FACS and a FAM-labeled primer. The control was the same ssDNA with a general primer lacking FAM. The negative control was *S.* Typhimurium to which no ssDNA had been added. Compared with these control groups, the selected ssDNAs labeled with FAM demonstrated good binding affinity to *S.* Typhimurium (Fig. 2). The positive and negative control groups did not show any increase of fluorescence for all counts compared to the negative control. However, the test groups (FAM-ssDNA with *S.* Typhimurium) showed an average fluorescence increase. According to other studies, this increase is due to binding of aptamers

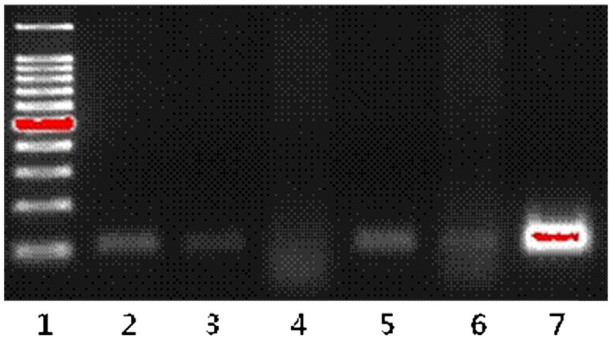

Fig. 1. PCR-amplified nucleic acid fractions of initial DNA library, after the first and fifth rounds of SELEX. Lane 1 is the DNA ladder (100 bp). Lanes 2 and 3 are the initial DNA library. Lanes 4 and 6 are the first and fifth rounds of aptamer pool (the template, the supernatant collected containing the heat-eluted cell aptamer faction, of PCR concentration was 100 and 10 μg/ml). Lane 5 (1st round) and Lane 7 (5th round) are the results of amplification by Ultracell.

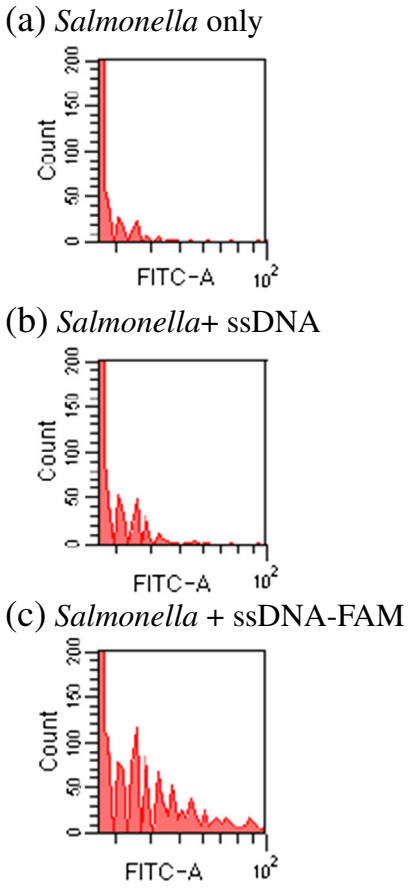

Fig. 2. Flow cytometry assay to monitor the binding of selected aptamer candidates with *S.* Typhimurium, comparing the background binding of selected DNA aptamers with and without FAM. For *S.* Typhimurium, there was an increase in binding capacity of the selected DNA with FAM, whereas there was little change for the selected DNA only.

to the cell surface with good affinity (Hamula et al., 2008; Dwivedi et al., 2010). Therefore, these findings of this study indicate that aptamer candidates can specifically bind to some component that exists on the surface of *S.* Typhimurium.

Aptamer candidates bound to *Salmonella* cells were sorted during FACS. The sorted aptamer candidates were amplified by PCR and were then cloned and sequenced. Based on sequence homology, the majority of the sequences could be classified into 12 groups (Table 1).

3.3. The binding affinity and selectivity of aptamer C4 for S. Typhimurium

Different analysis methods are used for determination of binding affinity of aptamers to cells, such as aptamer linked immobilized sorbent (ALISA) assay, FACS analysis, electrophoretic mobility shift (EMS) assay, and slide-binding assays (Hamula et al., 2008; Joshi et al., 2009; Dwivedi et al., 2010; Liu et al., 2012). Some studies have reported detection of the binding affinity of aptamers by fluorometry (Lee et al., 2012; Wang et al., 2011). In the present study, a fluorescent spectrophotometer was used given its ease of use, rapidity, and sensitivity (Houston and Kodadek, 1994).

The selected ssDNA was separated into 12 groups and the binding affinities of the selected aptamers to *S.* Typhimurium (5×10^7 cells each) were compared. Group C4 showed the highest florescence, followed by group SA-2, while comparatively lower affinity was shown by aptamer C7 and SA-3 (Fig. 3). After analysis of the binding affinity, we compared the selective intensity of aptamers C4, C9, SA-1, and SA-2 for *S.* Typhimurium, *E. coli*, and *S. aureus* (5×10^7 cells each) (Fig. 4). Aptamer SA-1 has shown minimal binding affinity to *S. aureus*, and C4 and SA-2 have shown minimal binding affinity

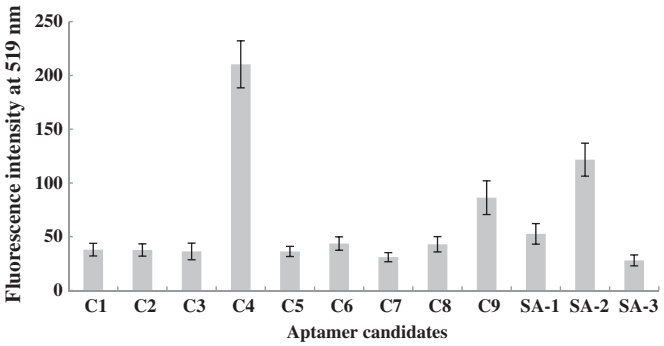

Fig. 3. The fluorescence intensity at 519 nm of aptamer candidates bound to *S.* Typhimurium. All aptamers were fluorescently labeled with 5′-FAM. Each sample contained 1.0 μmole of aptamer and 10^7 cells (error bars are standard error from triplicate analyses).

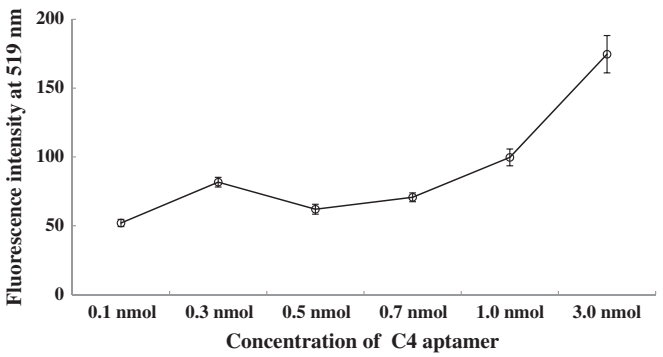

Fig. 5. Binding characteristics of aptamer C4 as determined via fluorescence spectrophotometer. Various concentrations of aptamer C4 were incubated with 10^7 *S. typhimurium* cells in PBS for 45 min and analyzed via fluorescence spectrophotometer under the same conditions as in Fig. 3 (error bars are standard error from triplicate analyses).

to *E. coli*. However, binding affinities of SA-1 to both target and non-target bacteria were low. In the case of C4 and SA-2, strong specific binding to *S.* Typhimurium was observed. Especially, the increase in the fluorescence intensity of C4 was less than 30% when the fluorescent aptamer pools were tested against *E. coli* and *S. aureus*. This suggests that the binding between aptamer C4 and *S.* Typhimurium is target specific. However, we did not attempt to measure the binding forces and perform other binding assays, such as test of capture efficiency, ALISA assay, and FISL assay (Joshi et al., 2009; Liu et al., 2012). Further studies are underway to confirm the binding forces and evaluate *S.* Typhimurium capturing ability of aptamer C4.

3.4. Concentration of aptamer C4

On the basis of specificity screening of selected aptamers, aptamer C4 was selected for further characterization by fluorescence detection (Hamula et al., 2008). *S.* Typhimurium cells (1×10^7 cfu) were treated with increasing concentrations of FAM-aptamer C4 and analyzed by fluorescence spectrophotometer. As the concentration of aptamer C4 was increased, there was an increase in the total fluorescent intensity. The FAM intensities of C4 for *Salmonella* were similar for the concentrations of 0.3–1.0 nmol, while a significant fluorescent intensity increase was observed at 3.0 nmol of C4 (Fig. 5). Previous studies on aptamers selected using whole-cell SELEX have reported binding affinities for more than 50 nmol (Dwivedi et al., 2010; Liu et al., 2012). In the further study, binding analyses with the fluorescence detection will be performed for wider concentration ranges (0 up to 100 nmol).

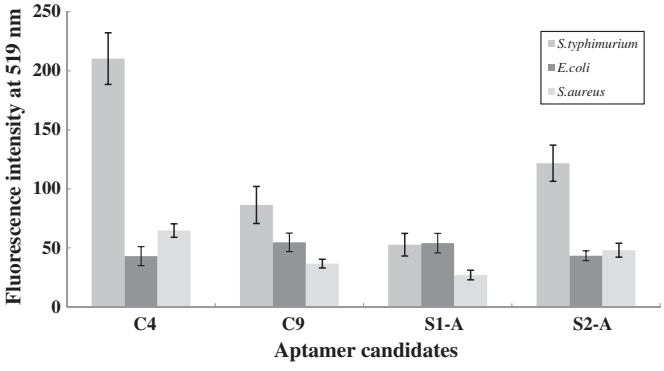

Fig. 4. The fluorescence intensity of aptamer candidates bound to *S.* Typhimurium, *E. coli* and *S. aureus*. All aptamers were fluorescently labeled with 5′-FAM. Each sample contained 1.0 μmole of aptamer and 10^7 cells (error bars are standard error from triplicate analyses).

4. Conclusion

In this study, aptamer against *S.* Typhimurium was selected through the whole-cell SELEX technique. Whole-cell SELEX is considered to be a useful aptamer selection tool, as aptamers derived in this way can target whole live bacteria. Even though these methods are limited and challenging, the whole-cell SELEX strategy can yield aptamers with high affinity and specificity (Chen et al., 2007). We modified the SELEX process to increase the success rate of whole-cell SELEX by adding the Ultracel concentration at PCR amplification process, and centrifugation at separating bound ssDNA to target bacteria from unbound ssDNA. The present results suggest that aptamer C4 could bind to the surface of live *S.* Typhimurium.

In conclusion, our modified strategy has improved the development of aptamers against bacteria. Furthermore, aptamer C4 could be a potential ligand for capturing *S.* Typhimurium, and be useful in development of easy, rapid, and sensitive methodology for pathogen detection.

Acknowledgement

This work was supported by a grant from the Agenda Program (PJ008440), Rural Development Administration, Republic of Korea.

References

Beuchat, L.R., 1998. Surface decontamination of fruits and vegetables eaten raw: A review. Food Safety Unit, World Health Organization. WHO/FSF/FOS/98.2.
Chen, F., Zhou, Z., Luo, F., Mohammed, A.B., Zhang, X.L., 2007. Aptamer from whole-bacterium SELEX as new therapy reagent against virulent Mycobacterium tuberculosis. Biochem. Biophys. Res. Commun. 357, 743–748.
Cibiel, A., Dupont, D.M., Ducongé, F., 2011. Methods to identify aptamers against cell surface biomarkers. Pharmaceuticals 4, 1216–1235.
Dwivedi, H.P., Smiley, R.D., Jaykus, L.A., 2010. Selection and characterization of DNA aptamers with binding selectivity to *Campylobacter jejuni* using whole-cell SELEX. Appl. Microbiol. Biotechnol. 87, 2323–2334.
Hamula, C.L.A., Zhang, H., Guan, L.L., Li, X.F., Le, X.C., 2008. Selection of aptamers against live bacterial cells. Anal. Chem. 80, 7812–7819.
Hamula, C.L.A., Zhang, H., Li, F., Wang, Z., Le, X.C., Li, X.F., 2011. Selection and analytical applications of aptamers binding microbial pathogens. Trends Anal. Chem. 30, 1587–1597.
Herzenberg, L.A., Tung, J., Moore, W.A., Herzenberg, L.A., Park, D.R., 2006. Interpreting flow cytometry data: a guide for the perplexed. Nat. Immunol. 7, 681–685.
Houston, P., Kodadek, T., 1994. Spectrophotometric assay for enzyme-mediated unwinding of double-stranded DNA. Proc. Natl. Acad. Sci. 91, 5471–5474.
Hyeon, J.Y., Chon, J.W., Choi, I.S., Park, C., Kim, D.E., Seo, K.H., 2012. Development of RNA aptamers for detection of *Salmonella* Enteritidis. J. Microbiol. Methods 89, 79–82.
Joshi, R., Janagama, H., Dwivedi, H.P., Senthil Kumar, T.M.A., Jaykus, L.A., 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.
Kim, G., Moon, J.H., Hahm, B.K., Morgan, M., Bhunia, A., Om, A.S., 2009. Rapid detection of *Salmonella* Enteritidis in pork samples with impedimetric biosensor: effect of electrode spacing on sensitivity. Food Sci. Biotechnol. 18, 89–94.

Kim, G., Yang, G., Park, S.B., Kim, Y.H., Lee, K.J., Son, J.Y., Kim, H.J., Lee, S.R., 2011. Rapid detection kit for *Salmonella* Typhimurium. J. Biosyst. Eng. 36, 140–146.
Lampel, K.A., Al-Khaldi, S., Cahill, S.M., 2012. *Salmonella spp.* In: Bad Bug Book, Foodborne Pathogenic Microorganisms and Natural Toxins, Second Edition. Food and Drug Administration, pp. 9–13.
Lee, S.Y., 2012. Food Poisoning statistics. Korea Food & Drug Administration.
Lee, Y.J., Han, S.R., Maeng, J.S., Cho, Y.J., Lee, S.W., 2012. In vitro selection of *Escherichia coli* O157:H7-specific RNA aptamer. Biochem. Biophys. Res. Commun. 417, 414–420.
Liu, G., Yu, X., Xue, F., Chen, W., Ye, Y., Yang, X., Lian, Y., Yan, Y., Zong, K., 2012. Screening and preliminary application of a DNA aptamer for rapid detection of *Salmonella* O8. Microchim. Acta 178, 237–244.
Mao, X., Phillips, J.A., Xu, H., Tan, W., Zeng, L., Liu, G., 2009. Aptamer-nanoparticle strip biosensor for rapid and sensitive detection of cancer cells. Anal. Chem. 81, 10013.
Mayer, G., Ahmed, M.S.L., Dolf, A., Endl, E., Knolle, P.A., Famulok, M., 2010. Fluorescence-activated cell sorting for aptamer SELEX with cell mixtures. Nat. Protoc. 5, 1993–2004.
Moon, J.H., Shin, H.A., Rha, Y.A., Om, A.S., 2009. The intrinsic antimicrobial activity of bamboo salt against *Salmonella* Enteritidis. Mol. Cell. Toxicol. 5, 323–327.
Pan, Q., Zhang, X.L., Wu, H.Y., He, P.W., Wan, F., Zhang, M.S., Hu, J.M., Xia, B., Wu, J., 2005. Aptamers that preferentially bind type IVB pili and inhibit human monocytic-cell invasion by *Salmonella enterica* serovar Typhi. Antimicrob. Agents Chemother. 49, 4052–4060.
Sefah, K., Shangguan, D., Xiong, X., O'Donoghue, M.B., Tan, W., 2010. Development of DNA aptamers using Cell-SELEX. Nat. Protoc. 5, 1169–1185.
Tombelli, S., Minunni, M., Mascini, M., 2005. Piezoelectric biosensors: strategies for coupling nucleic acids to piezoelectric devices. Methods 37, 48–56.
Torres-Chavolla, E., Alocilja, E.C., 2009. Aptasensors for detection of microbial and viral pathogens. Biosens. Bioelectron. 24, 3175–3182.
Velge, P., Cloeckaert, A., Barrow, P., 2005. Emergence of Salmonella epidemics: the problems related to *Salmonella enterica* serotype Enteritidis and multiple antibiotic resistance in other major serotypes. Vet. Res. 36, 267–288.
Wang, K.Y., Zeng, Y.L., Yang, X.Y., Li, W.B., Lan, X.P., 2011. Utility of aptamer-fluorescence in situ hybridization for rapid detection of *Pseudomonas aeruginosa*. Eur. J. Clin. Microbiol. 30, 273–278.