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Surface Plasmon Resonance Aptamer Biosensor for Discriminating Pathogenic Bacteria *Vibrio parahaemolyticus*

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In this paper, whole-bacteria SELEX (WB-SELEX) strategy was adopted to isolate specific aptamers against *Vibrio parahaemolyticus*. Round selection for *V. parahaemolyticus* was conducted 11 rounds, including two negative selection rounds. It was determined through real-time PCR amplification and post-SELEX experiment. The selected aptmers had high binding property and specificity to *V. parahaemolyticus*. Of 28 aptamers tested, VPCA-apta#1 had the highest binding affinity compared to other aptamer candidates obtained. To detect *V. parahaemolyticus*, aptamer based SPR biosensor platform was constructed and pathogenic bacteria sensing was conducted in two steps. The first step was to construct 5′-biotinylated VPCA-apta#1 binding probe. The second step was to incubate *V. parahaemolyticus* and test microbes in functionalized SA sensor chip in parallel. Our platform showed significant activity for detecting and discriminating *V. parahaemolyticus* from other enteric species such as *Escherichia coli*, *Listeria monocytogenes*, *Sigella sonnei*, and *Vibrio fischeri*. This is the first report on the use of whole-SELEX to isolate DNA aptamers specific for *V. parahaemolyticus*. We demonstrated the feasibility of using aptamer platform for the detection of *V. parahaemolyticus* in various food supplies. It might be used in multiple points of care for diagnosing Vibriosis.

Keywords: Pathogenic Bacteria, *Vibrio parahaemolyticus*, Vibriosis, Aptamer Biosensor, Whole Cell-SELEX, DNA Aptamer.

1. INTRODUCTION

Vibrio parahaemolyticus is a natural inhabitant of marine waters. Fresh seafood, particularly mussels, is often contaminated with this pathogen. *V. parahaemolyticus* is a facultative, anaerobic, Gram-negative, flagellated, halophilic, and asporogenous bacterium. It inhabits marine and estuarine environment.[1] This pathogen is also an important enteropathogen that causes acute human gastroenteritis throughout the world.[2] Therefore, a rapid, simple, and specific detection method is urgently needed for this pathogen. In this study, we carried out SELEX (Systematic evolution of ligands by exponential enrichment) process to obtain DNA aptamers with high affinity and specificity against *V. parahaemolyticus*. Aptamers are short single-stranded RNA or DNA molecules that can bind to a wide range of target molecules (such as metal ions, small molecules, drugs, peptides, and proteins) with high affinity and specificity. In recent years, whole-bacterium SELEX or complete target SELEX has emerged as an alternative to the

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more traditional SELEX approach applied to crude or purified extracellular surface targets.[3,4] This aptamer selection technology has been applied in group A *Streptococcus*, *Lactobacillus acidophilus*, *Mycobacterium tuberculosis*, *Staphylococcus aureus*, and *Campylobacter jejuni*. These bacteria can be grown in suspension, thereby allowing for simple separation by centrifugation. A serial of DNA aptamers against target live bacteria including *Listeria monocytogenesis*, *Salmonella typhimulium*, and *Shigella sonnei* have been isolated by our research group. Aptamer based detection methods have been also developed for the detection of live bacteria.[5–8] DNA aptamers for *V. parahaemolyticus* were used to SPR-based biosensor in this study. Specific aptamers are considered as recognizing element for target bacteria instead of antibody used in *surface plasmon resonance* (SPR) sensor platform. They are predisposed for SPR applications due to their stability and small size compared to antibodies. Our SPR-aptamer sensor offers a convenient and specific platform for diagnosing *Vibrio* infection with great potential for pathogen detection. Nanomaterials used in sensor development include a long list of nanostructured systems, the improvement of these systems and the increase of specificity are clearly associated with a decrease in size of the components, which can lead to obtaining more rapid action, almost in real time.[9–12]

2. RESULTS AND DISCUSSION

2.1. Whole Bacteria Cell SELEX for Isolation of *V. parahaemolyticus* Specific DNA Aptamers

The randomized oligonucleotide aptamer library containing about 10^{16} diverse sequences was initially introduced by incubating with live *V. parahaemolyticus* cells. In each round of selection against *V. parahaemolyticus*, the concentration of bound DNA species was increased to further drive the selection toward a high-affinity and high-specificity aptamer pool. The initial oligonucleotide library consists of 40 nt randomized region (N_{40}). Primer binding sites were chemically synthesized. After 11 rounds of SELEX, nanodrop spectrophotometer analysis was performed to monitor and assess the binding affinity of aptamer sub-pools to *V. parahaemolyticus*. To eliminate non-specific and weak bound DNA aptamers, we incorporated two times of negative-selection rounds using *E.coli* and *Bacillus subtillus* cells. As the SELEX process was progressed, non-specific and/or weak binding aptamer candidates were efficiently excluded using negative counter-partner *E. coli* in the 4th round and *Bacillus subtilis* in the 6th round. This indicated that the selected cell-specific aptamers could recognize targets of *V. parahaemolyticus* expressed outside the cells. Therefore, they could be used as high affinity binding probes in a detection platform. To monitor if DNA aptamers against *V. parahaemolyticus* cells would be enriched during the SELEX process, the amount of recovered ssDNA from each selection round was analyzed. Results are shown in Figure 1. The eluted ssDNA showed the highest concentration at the 8th selection round, indicating that ssDNA aptamer candidates could be successfully enriched for whole live bacteria cells by the SELEX process. SsDNA concentration can also decrease in a negative selection round due to the removal of unbound ssDNA. Therefore, the 8th selection round was optimal for isolating appropriate aptamer candidates.

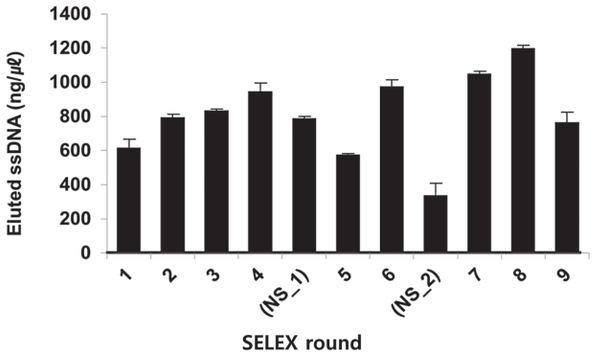
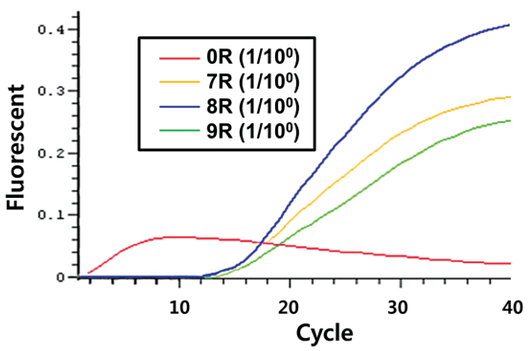


Figure 1. Concentration of eluted aptamers during the SELEX processes. The ssDNA of each round was determined. Recovery ratio was calculated as the amount of output DNA after the SELEX process to that of the input DNA. Negative round (N. R.) was performed after round 4 and round 6 to prevent enrichment of nonspecific binding aptamers for *V. parahaemolyticus.*

2.2. Analysis of Optimum Enrichment State for Isolating Aptamers

The pattern of retrieval concentration showed an overall tendency of increase as the selection round progressed.



Selection round	ssDNA Conc.	Dilution rate	Ct value	Efficiency (%)
Round 7	1004.2 ng/μL	1 X 10^0	8.73	93.81
Round 8	1196.6 ng/μL	1 X 10^0	7.68	98.15
Round 9	680.1 ng/μL	1 X 10^0	7.95	92.98

Figure 2. Monitoring of the optimum enrichment status for isolating aptamers. PCR efficiency and $C(t)$ values were determined using MJ opticon monitor analysis software version 3.1 (Bio-Rad, USA).

It reached the highest at the 8th round. After that, it was decreased. This implies that cell specific aptamers might have been enriched and saturated after the SELEX processes.[13–15] To further determine whether there was any difference in binding ability between rounds, real-time PCR (RT-PCR) was used to determine the optimal status of DNA aptamer enrichment. Potential aptamer pools collected from the initial round (0 R), the 7th, 8th, and the 9th round were again incubated with *V. parahaemolyticus* cells (1.2×10^8 cfu/ml) in parallel for 1 h. After three times of washing, bound DNA was recollected. The recovered each DNA sample was amplified using an optimized RT-PCR cycle and the fluorescence signal was monitored by MiniOpticon™ Real-time PCR fluorescence signal detection system (Bio-Rad, USA). PCR experiment was independently performed in triplicates. Data were analyzed after obtaining the average $C(t)$ values. Results are shown in Figure 2. The sample obtained from the 8th round SELEX showed a lower $C(t)$ value (7.68) compared to that of other samples [$C(t) = 8.73$ for the 7th round and 7.95 for the 9th round]. Based on these RT-PCR experiments, it was clear that the sample collected from the 8th round had higher amounts of DNA aptamers for *V. parahaemolyticus* cells. In order to isolate potential aptamer candidates that could recognize whole live *V. parahaemolyticus*, the DNA aptamers obtained from the 8th selection round were cloned and 28 clones (VPCA_Apta#1 to VPCA_Apta#28) were sequenced followed by multiple sequence alignment using ClustalX software program (Table I).

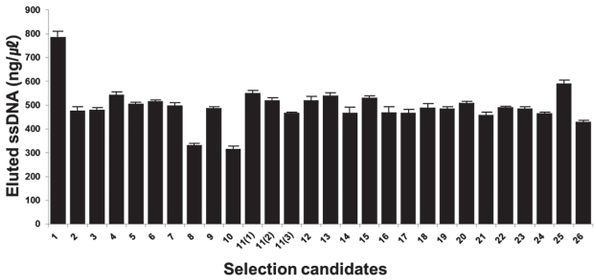


Figure 3. Characterization of binding aptamers. Of the 28 aptamer candidates analyzed by post-SELEX method as described in Materials and Method, 4.2 SELEX procedure. After incubating with *V. parahaemolyticus* cells, aptamers were retrieved. The concentration (ng/μl) of each aptamer was determined in triplicates.

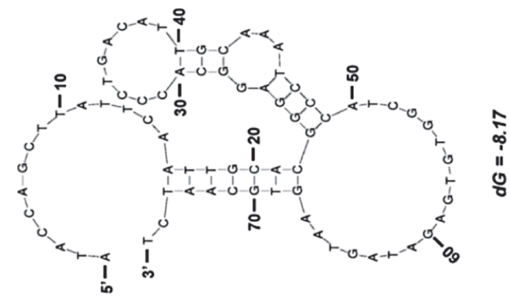
2.3. Binding Test of Aptamer Candidates by Post-SELEX

The binding value for each apamer was determined by analyzing its concentration after post-SELEX experiment. Results are shown in Figure 3. Briefly, 180 pmol of each aptamer was incubated with *V. parahaemolyticus* cells (4×10^8 cfu/ml) at 4 °C for 1 h in 200 μL. After washing, each aptamer-cell complex was precipitated.

Table I. List of sequences of the isolated aptamer candidates.

Group	Sequence (N40)	Size (bp)	Identical sequences
VPCA_Apta#1	CATACCAAGGCTACCGAGGTTCCAGATATTGGCCGCTATG	40	2
VPCA_Apta#2	CCACGTTTCCACGTATCCTCGGAGCTTGCTTAACCGTACG	40	2
VPCA_Apta#3	TGCGTAGTTCTTTTAACAACCATTCAGCGTATTTTCGTGG	40	2
VPCA_Apta#4	CCCTCGGCGTAACTAGTACCGTTGCACCACCAACGTGATT	40	1
VPCA_Apta#5	CGCAAGGGATTTCGGACCGGGCTTGGTCACACACAGAGCA	40	1
VPCA_Apta#6	CCAGCAGTGGGGCATGGGGGAACGATAAGATTATAAGGGGG	41	1
VPCA_Apta#7	CACGCAAGCAGAAGCCACTCCCCCTGGTCGTACTATTTAG	40	1
VPCA_Apta#8	CACAGGCGTACCACTGCCCCAACGATCCTTTTGGTTACCG	40	1
VPCA_Apta#9	GTCGGAGAGCCTGGCCGTCTGCTCGTAAGTACTATCATAGC	41	1
VPCA_Apta#10	TGGAGAAGCGGCAGTTGATGCCTGGTACCCGTCTGCTACG	40	1
VPCA_Apta#11	CGTAAAGGACGCTGCCATGAATGTGCTATCCAAGCCTGGG	40	1
VPCA_Apta#12	CGTAAAGAACGCTGCCATGAATGTGCTATCCAAGCCTGGG	40	1
VPCA_Apta#13	CGTAAGAACGCTGCCATGAATGTGCTATCCAGGCCTGGG	39	1
VPCA_Apta#14	CAACGGAGGGAAGTTTCCATGTCCGGTACCAAGCGGTTTTTG	42	1
VPCA_Apta#15	GCACGGGAGGCACCCTGACATTGCAAATCCCATCGGTGTG	40	1
VPCA_Apta#16	ACCTATAACAGCCATCATACGAGGTAATCCCCTCCCTCGA	40	1
VPCA_Apta#17	CCAACTGGATCTTACTGAGGAACGGTCCATTAGCACATCG	40	1
VPCA_Apta#18	CGCGACCACATCCTGCGTAAACACATCTCCGCCAGTCCAT	40	1
VPCA_Apta#19	CGCAAGAGCAGCATACAGCACGAACGAGCCATTTACGCCA	40	1
VPCA_Apta#20	CGAAGACCAGACATGGTAGCCACGCATAGTCCAACTTAAG	40	1
VPCA_Apta#21	ACACCACACTAATGCATGCATTCTGTGAGTCACAGTTCA	39	1
VPCA_Apta#22	CACTCAAACCCAAACCATGCAAACTGAACAACGCAACACA	40	1
VPCA_Apta#23	CACCAAACTGCCCAAATTTGAGGACGCCCTATACATGCG	39	1
VPCA_Apta#24	GCCCAATACGTATGTTGATACATGCCCCTTACCCTTTGCG	40	1
VPCA_Apta#25	CACCGTAGTAAGTAAGCAGACGCTAAGGATGTTGCCAGTG	40	1
VPCA_Apta#26	GTGACATGAACTGGTTTTCCACAGCTTAGGATCATGGATG	40	1
VPCA_Apta#27	GGGCATGTGGGCTTGCGATTTCGGTTTGGTGTGGTTGGGG	40	1
VPCA_Apta#28	CCGTCGCTATAACCCTGTCTTTATATATCTTCGCCCACGGG	41	1

Table II. Sequence and structure analysis of VPCA-apta#1, a specific binding aptamer to *V. parahaemolyticus*.

Clone	Selected sequence	Size (bp)	Secondary structure
VPCA-apta #1	ATACCAGCTTATTCAATT CCATGGTCCCTCGTGTTTAT TATGTTGTCTGAACTGGCTG AGATTGCACTTACTATCT	77	

Aptamers were recovered from cells. Since nonspecifically hybridized and weak DNA aptamers could be eliminated by washing three times with 500 μL TE buffer in the post-SELEX step, concentration could be used to determine which aptamer could tightly bind to their target cells. Of the 28 aptamers tested, the concentration of VPCA-apta#1 was significantly higher (798.41 ng/ul) than that of the other aptamers (Fig. 3). Most of the 28 aptamers showed weak binding to *V. parahaemolyticus* cells. However, VPCA-apta#1 aptamer showed tight binding to target cells.

In order to compare the similarity of secondary structures, we used web-based tool Mfold (http://mfold.bioinfo.rpi.edu/cgi-bin/dna-form1.cgi). Results of both sequence and secondary structure analysis for VPCA-apta#1 aptamer are detailed in Table II.

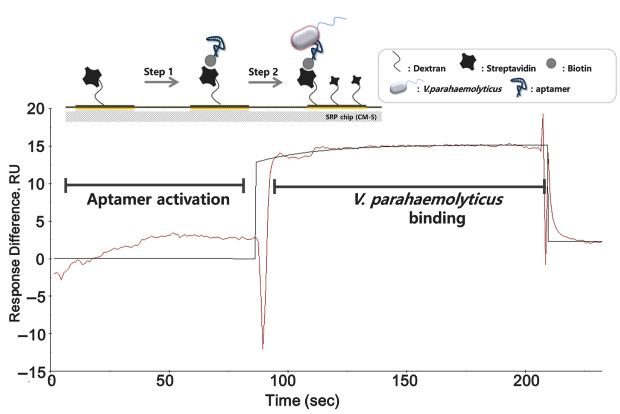


Figure 4. Results of binding affinity test by SPR. Biotinylated VPCA-apta#1 against *V. parahaemolyticus* was analyzed by Surface Plasmon Resonance using a Biacore 3000 Instrument (GE Healthcare, Sweden) at 24 °C. The activation and functionalization of the SA chip (GE Healthcare, Sweden) were carried out according to standard protocol (BIAapplication Handbook, Biacore® AB) during step 1 to 2. Target microbes were introduced to the sensor chip and affinity values were compared (Table IV).

2.4. Detection and Discrimination by Aptamer-Based SPR Biosensor

An aptamer-based SPR biosensor assay was designed to detect and discriminate *V. parahaemolyticus* cells using the selected aptamer VPCA-apta#1. SPR analysis is one of the representative strategies that can offer the advantage of label-free and real-time detection method in pathogen-biosensor platform.[16,17] The principle of the SPR-biosensor strategy is illustrated in Figure 4. Biotinylated VPCA-apta#1 was attached via streptavidin coupling method (step 1 and 2, Fig. 4). Aptamer-based SPR biosensor was then challenged with different test cells (*V. parahaemolyticus*, *E. coli*, *L. monocytogenes*, *S. soneii*, and *V. fischeri*) of 4×10^8 cfu per mL of total cells. We were able to observe significant affinity (K_D value) of this biosensor only for *V. parahaemolyticus* cells as shown in Table III. The detection of *V. parahaemolyticus* by our Aptamer-Based SPR Biosensor platform was clearly distinguishable from the controls, including nutrient broth (LB), buffer solution (TBS), and DW (data not shown).

Many types of *V. parahaemolyticus* detection methods have been developed in the last decade, including immunosorbent assay (ELISA) and polymerase chain reaction (PCR) amplification analysis.[18,19] However, ELISA and PCR-based amplification assay have high risk of obtaining false-positive or false-negative results due to inhibition by cellular component itself and clinical sample matrix.[20,21] Moreover, ELISA requires primary and secondary antibodies. There is considerable cross-reactivity in

Table III. Aptamer based SPR affinity to target microbes.

Target microbe	K_D value (M)
V. parahaemolyticus	$2.04E^{-9} \pm 0.12$
E. coli	$1.44E^{-8} \pm 0.36$
L. monocytogenes	$1.02E^{-8} \pm 0.25$
S. soneii	$8.17E^{-6} \pm 0.37$
V. fischeri	$8.22E^{-6} \pm 0.19$

Table IV. Culture media and growth conditions of bacteria used in this study.

Bacteria	ATCC no.	Culture media	Culture condition
Vibrio parahaemolyticus	17802	Nutrient Agar/Broth with 3% NaCl	37 °C Aerobic conditions
V. fischeri	BAA-1741	*Lactobacillus Selection* Broth.	30 °C Aerobic conditions
Escherichia coli K12	29425	Nutrient Agar/Broth	37 °C Aerobic conditions
Shigella sonnei	25931	Nutrient Agar/Broth	37 °C Aerobic conditions
L. monocytogenes	19115	Brain Heart Infusion	37 °C Aerobic conditions
Bacillus subtilis	6051	Nutrient Agar/Broth	30 °C Aerobic conditions

the use of antibody which can recognize Gram-negative bacteria serotype O antigens since many O groups of *E. coli* are cross-reactive or identical to O regions of *Vibrio parahaemolyticus*.[22–24] In this paper, aptamer based fluorescent biosensor platform was developed and evaluated for its critical discrimination of *V. parahaemolyticus* cells from other enteric pathogens. The biosensor platform consisted of a molecular recognition element VPCA-apta#1. These aptamers were efficiently selected by Whole Bacteria-SELEX (WB-SELEX). A key procedure of SELEX experiment was the recognition of the optimum round in the enrichment process. Here, using post-SELEX procedure and real-time PCR analysis, we obtained a highly efficient selection of aptamers. Count-partner bacteria *E. coli* and *Bacillus subtillis* Gram-negative bacterial were also included in the section process. Significantly low K_D value of the aptamer was found for *V. parahaemolyticus* cells, but not for *E. coli*, *L. monocytogenes*, *S. soneii*, or *Vibrio fischeri*. In particular, the aptamer provided a clear modification mechanism for integrating onto a solid surface within the sensor platform. This allowed specific and unique orientation to detect targets when anchored to the surface. This approach has potential to be used as a direct detection system of pathogens in multiple and portable concepts.[25–27] It might be used in the development of detection method for other pathogens by substituting aptamers as diagnostics and therapeutics.

3. CONCLUSIONS

Whole-Bacteria SELEX (WB-SELEX) strategy was adopted to isolate specific ssDNA aptamers against *Vibrio parahaemolyticus*. The DNA aptamer pool from the 8th selection round were cloned and 28 clones (VPCA_Apta#1 to VPCA_Apta#28) were sequenced followed by multiple sequence alignment. VPCA-apta#1 showed the highest binding affinity. Biotinylated VPCA-apta#1 was attached via streptavidin coupling method and the aptamer-based SPR biosensor was challenged with different test cells (*V. parahaemolyticus*, *E. coli*, *L. monocytogenes*, *S. soneii*, and *V. fischeri*) at 4×10^8 cfu per mL. Our aptamer based SPR biosensor platform exhibited high specificity. This might be due to the formation of secondary and tertiary structure against certain extracellular components of *V. parahaemolyticus*. Our aptamer-Based SPR biosensor platform could clearly distinguish *V. parahaemolyticus* with K_D value of 2.04E-9 ± 0.12M.

4. MATERIALS AND METHODS

4.1. Bacterial Strains and Cell Maintenance

All bacterial strains used in this study are listed in Table IV. They were grown in the described growth conditions. All bacteria were harvested in their logarithmic phase of growth. Harvested bacterial cells were washed. Cell pellets were serially diluted in Tris-buffered saline (TBS, Tris base 10 mmol per L, NaCl 0.85%, pH 8.0). Diluted suspensions (100 μL) were plated onto appropriate agar plates. Bacterial counts were obtained and reported as CFU per mL.

4.2. SELEX Procedure

4.2.1. Initial Random Library

The initial oligonucleotide library consisted of 40 nt randomized region (N40). The following primer binding site was chemically synthesized (Bioneer, South Korea): 5′-ATA CCA GCT TAT TCA ATT-N40-AGA TAG TAA GTG CAA TCT-3′. The random oligonucleotide library was amplified by PCR using forward primer (5′-ATA CCA GCT TAT TCA ATT) and biotinylated reverse primer (5′-AGA TTG CAC TTA CTA TCT-3′). PCR products were purified using QIAquick PCR purification kit (Qiagen, USA). Single-stranded DNA (ssDNA) was generated by removing biotinylated ssDNA using streptavidin agarose resin (Pierce, USA). The resulting ssDNA was confirmed by comparison with double-stranded DNA (PCR product) on 10% polyacrylamide gel containing 0.5X TBE buffer. The ssDNA library (800 pmol) was then heat denatured for 5 min at 85 °C and renatured for 2 h at room temperature (RT) to allow stable structure formation of aptamer.

4.2.2. Whole Cell-SELEX

Subculture of *V. parahaemolyticus* (4×10^8 cfu/ml) was washed with TBS buffer (10 mM Tris-HCl, 0.85% NaCl, pH 8.0). Cell pellet was mixed with structured ssDNA library (800 pmol) in 200 μL nutrient broth and incubated at 4 °C for 1 h with gentle shaking. After the incubation, the mixture was centrifuged and washed with TBS buffer to remove the unbound and weakly bound aptamers. To elute cell-bound aptamers, cells were resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), heated at 85 °C for 5 min, snap-cooled on ice, and centrifuged. The eluted ssDNA in supernatant was recovered by phenol/chloroform extraction followed by

ethanol precipitation. It was then used as template DNA for the next round of selection. The resulting purified aptamers were run on 1% agarose gel to confirm the correct DNA size. To increase the specificity of aptamers candidates, two rounds of negative selection (after the 4th and 6th round) were also performed. The 4th sub-library of ssDNA was incubated with *E. coli* as a counter partner at 4 °C for 1 h in LB broth. The unbound aptamers in the supernatant were collected for the next round of selection. After the 6th round, sub-library was incubated with *Bacillus subtilis* as the second-negative selection round.

4.3. Real-Time PCR Analysis

To have optimal selection round, eluted ssDNA concentration from each selection round was quantified by measuring the absorbance value at wavelength of 260 nm using a nanodrop spectrophotometer (Thermo Scientific, USA). The DNA aptamer pool from the initial round, the 7th, the 8th, and the 9th selection round were prepared at the same concentration (360 pmole) and incubated with target cells (1.2×10^8 cfu/ml) in parallel for 1 h. After three times of washing, bound ssDNA was retrieved from the cell-pool complexes. Serial dilutions of each samples [1× (no dilution), 10×, and 100× dilution] were prepared and analyzed by real-time PCR using a MiniOpticon™ Real-time PCR fluorescence signal detection system (Bio-Rad, USA). For PCR amplification, 20 μL of reaction mixture contained 10 μL of 1X iQ SYBR Green Supermix (Bio-Rad, USA), 8.8 μL of PCR water, 0.1 μL of 0.1 mM of forward primer, 0.1 μL of 0.1 mM of reverse primer, and ten-fold serial dilution of each template DNA (1 μL). PCR parameters were: taq activation (3 min at 94 °C); 40 cycles of PCR at 94 °C for 30 sec, 52 °C for 30 sec, and 72 °C for 15 sec; followed by 5 min of extension at 72 °C. All sample analyses were performed in triplicates. Amplification efficiency and threshold cycle (Ct) analysis were performed using MJ opticon monitor analysis software version 3.1 (Bio-Rad, USA). The DNA pool of optimal round (8th round) was cloned into T-blunt vector and transformed to *E. coli* DH5α (Solgent, South Korea). Individual white colonies were picked and cultured for plasmid DNA purification. Purified plasmid DNAs were sequenced and analyzed using ClustalX sequence alignment program.

4.4. Post-SELEX Binding Affinity Assessment

To further confirm the binding affinities of aptamer candidates against *V. parahaemolyticus*, post-SELEX step was carried out. A total of 28 aptamer candidates at the same concentration (180 pmole) were prepared and incubated with target cells (1×10^8 cfu/ml) in parallel. After post-SELEX, each aptamer candidate was analyzed by real-time PCR as described above. The amounts of amplicons were analyzed using nanodrop spectrophotometer (Bio-Rad, USA) and their concentrations were compared to each other. Secondary structures of the final selected *V. parahaemolyticus* cell binding aptamers were predicted with free energy minimization algorithm using Mfold web server (http://mfold.bioinfo.rpi.edu/cgi-bin/dna-form1.cgi).

4.5. Sensitivity Test of *V. parahaemolyticus* Binding Aptamers by Aptamer-Based SPR Biosensor Assay

The binding affinities of the isolated aptamers against *V. parahaemolyticus* were analyzed by Surface Plasmon Resonance using a Biacore 3000 Instrument (GE Healthcare, Sweden) at 24 °C. Streptavidin immobilized sensor chip SA (GE, for Biacore 3000) was used to test heterodimeric binding between aptamer and target cells. 5′-biotin-modified DNA aptamers for *V. parahaemolyticus* (VPCA-apta#1) were immobilized by passing 500 nM aptamers for 10 min at 10 μL per min on SA chip. The SA chip was pre-activated with 1 mL of HBS-EP buffer (GE Healthcare, UK) at a flow rate of 10 μL/min for 10 min. The SA chip was pre-activated with 1 mL of the running buffer at a flow rate of 10 μL/min for 10 min. The 5′-biotinylated aptamer sequence was immobilized onto the chip as recommended by manufacturer's manual. To monitor the interaction, BIACORE instrument was used. All procedures were automatically implemented to create repetitive cycles of sample injection (90 μL injection samples at a flow rate of 10 μL per min) and regeneration (1 M NaCl, 50 mM NaOH) according to the instruction guidelines of BIACORE. Binding interaction was analyzed by subtracting the response unit of the blank flow cell from the response unit of the selected DNA for flow cell (BIA evaluation program, version 4.1).

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