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Isolation of DNA Aptamers for Enteropathogenic *Escherichia coli* (EPEC) Detection using Bacterial-SELEX Approach

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ABSTRACT

Enteropathogenic *Escherichia coli* (EPEC) is a Gram-negative pathogenic bacterium that causes diarrheal disease, especially in infants and children. Aptamers are short chain oligonucleotides that have high affinity, specificity, and selectivity to their targets, which have potential to be developed as a method for diagnosing pathogens. In this study, aptamer was isolated through the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) method using whole cells bacteria (Bacterial-SELEX) for recognizing pathogenic *E. coli* EPEC K1.1 which was isolated from children with diarrhea in Indonesia. Ten rounds of bacterial-SELEX procedure were conducted with modification conditions by using Top10, DH5a *E. coli* cells, *Listeria monocytogenes*, and *Lactobacillus plantarum* S34 as counter-selections. The selection process was started with a pool of ssDNA random library consisting of a random base with 40-nucleotides long flanked with fixed primers sequence for aptamer amplification purpose. Short single-stranded DNA amplification was done by symmetric and asymmetric PCR. The highly enriched oligonucleotide pools (pooled 8, 9, and 10) were cloned and the resulting ssDNA aptamers were identified by Sanger DNA sequencing. Finally, twelve aptamers with unique sequences and various secondary structures including G-quadruplex sequence motif within aptamers were obtained as candidates specific aptamer for detection and capturing of EPEC K1.1.

1. Introduction

The World Health Organization (WHO) reports that infectious diseases caused by microorganisms are the leading cause of death worldwide. This is due to an increasingly serious level of environmental pollution, a rapidly growing population and the emergence of antimicrobial resistance. Infectious diseases caused by microorganisms such as viruses or bacteria become a serious public health problem throughout the world and cause significant economic losses. Therefore, in maintaining food safety and the environment and also public health, effectiveness in microbial detection plays an important role (Zhang *et al.* 2015).

Enteropathogenic *Escherichia coli* (EPEC) is one of the Gram-negative pathogenic bacteria that cause diarrhea, especially in infants and children (Ochoa and Contreras 2011). Diarrhea is one of the leading causes of death in children, reported to be around 2 million deaths every year worldwide (Bryce *et al.* 2005). In developing countries such as Indonesia, diarrhea is an endemic disease with the potential to cause major outbreaks, due to its high morbidity and mortality. Budiarti (1998) reported that EPEC bacteria had been successfully isolated from the feces of children with diarrhea in several regions in Indonesia, where EPEC bacteria with Serotype O142 occupied the highest percentage in Depok and Ciamis, West Java. Moreover, EPEC K1.1 has been reported to be resistant to several antibiotics including ampicillin, cefixime, cotrimiksazole, nalidixic acid, ciprofloxacin, and chloramphenicol (Eghdami and Islami 2014).

The conventional pathogen detection methods commonly used are culture and colony counting methods, biochemical tests, immunology-based

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methods, polymerase chain reaction (PCR) and so on. These methods are time-consuming and require complicated procedures, depending on analytical reagents and expensive instruments (Zhang *et al.* 2015). Therefore, more effective and faster methods for detecting specific microbes need to be developed.

Biological detection agents currently being developed are known as aptamers, short chain oligonucleotides with high affinity, specificity, and selectivity in recognizing their targets. Aptamers can recognize various molecular targets including proteins, small molecules, ions, toxic molecules, and even whole cells. Aptamers are single-strand nucleic acid molecules (DNA or RNA) selected *in vitro* from a random library of synthetic oligonucleotides by a method called Systematic Evolution of Ligands by Exponential Enrichment (SELEX), which involves several repeated selection cycles. The SELEX method has 4 important stages including incubation, selection, elution, and amplification (Ellington and Szostak 1990; Song *et al.* 2008).

Aptamers have high potential as an alternative to antibody-based detectors, which for years have always been used to detect molecular markers in cases of food contamination and viral outbreaks, also for forensic and diagnostic purposes (Ilgu and Nilsen-hamilton 2016). When compared to antibodies, aptamers have many advantages including their molecules that are more stable and can be chemically modified to improve their performance and stability. In addition, production costs of aptamers are lower with higher reproducibility (Marton *et al.* 2016; Song *et al.* 2012).

Several studies have been carried out successfully in isolating single-strand DNA (ssDNA) aptamers that bind specifically to *Escherichia coli*, including Kim *et al.* (2013), against fecal *E. coli* isolates; Marton *et al.* (2016) in Brazil, against *E. coli* ATCC 25922, a common type of *E. coli* which has no virulence activity. Furthermore, Amraee *et al.* (2017) successfully selected specific aptamers that were able to detect *E. coli* O157 which is a pathogenic bacterium that causes foodborne disease. Meanwhile, in this study, we have isolated ssDNA aptamers through the bacterial-SELEX method against EPEC K1.1, an endemic *Escherichia coli* pathogenic bacterium in Indonesia. Furthermore, we exhibited the melting curve analysis of ssDNA aptamers, also diversity in terms of secondary structure and G-quadruplex within aptamer sequences, to provide information for its application as a diagnostic tool for EPEC detection.

2. Materials and Methods

2.1. Bacterial Strains and Culture

The bacterial strain EPEC K1.1 as the target was cultured in the selective medium Eosin Methylene Blue Agar (EMBA) to ascertain the bacterial isolates used. EPEC K1.1 colonies that grow on EMBA will be metallic green. Subsequently, a single EPEC K1.1 colony was cultured on Luria-Bertani (LB) medium at 37°C until it reached an OD_{600} of 0.3 (equivalent to~10^8 CFU/ml). The culture was washed twice using PBS and then dissolved using a selection buffer (PBS containing 1.4 mM $MgCl_2$).

The following bacterial strains were used in the SELEX process: Top10, DH5a *E. coli* strains, *Listeria monocytogenes*, and *Lactobacillus plantarum* S34 as counter-selections. All strains were cultured on Luria-Bertani (LB) medium at 37°C except for *Lactobacillus plantarum* S34, which were cultured on DeMan, Rogosa and Sharpe (MRS) medium. All bacterial strains were obtained from the collection of the Research Center for Genetic Engineering, National Research and Innovation Agency (BRIN), Cibinong, Bogor.

2.2. Random DNA Library and Primers

The single-stranded DNA (ssDNA) random library used here consists of a random base with 40-nucleotides (N40) long flanked with fixed primer sequences at the 3' and 5' ends for aptamer amplification purpose: (5'–CCGGAATTCCTAATACGACTC–N40–TATTGAAAACGCGGCCGCGG–3'), represents a collection of 81-mer nucleotides (Wang *et al.* 2013). This random library was modified in terms of N number to reduce the structure complexity during PCR amplification.

2.3. Bacterial-SELEX Procedure

The ssDNA library was dissolved in buffer selection and denatured for 5 minutes at 95°C. After that, the aptamer library was cooled for 15 minutes at 25°C and incubated with 1 ml of 10^7 colony forming

units (CFU)/ml EPEC K1.1 for 45 minutes at room temperature and constantly agitated at 220 rpm. The bacterial suspension was then centrifuged at 8000 × g for 6 minutes. Discard the supernatant, followed by washing the pellets using 1 ml buffer selection to remove the unbound ssDNA sequences. Then, the bacterial cells were centrifuged again at 8000 × g for 6 minutes. The pellet containing bound ssDNA sequences were eluted by adding 100 µl of sterile H_2O and heating at 95°C for 5 minutes. Subsequently, the aptamer-bacterial complex was centrifuged at 12,000 rpm for 10 minutes at 20°C. The ssDNA aptamer in the supernatant were amplified using symmetric PCR to get dsDNA amplicon followed by asymmetric PCR to get ssDNA amplicon. Optimization of symmetric PCR cycles was performed with variations of 4, 6, 8, 10, 12, 14, 16, 18, and 20 cycles, while optimization of primer ratios in asymmetric PCR was performed with forward: reverse 20:2, 20:1, and 20:0.1. PCR was performed using NZYTaq DNA Polymerase PCR kit, with 5 minutes of initial denaturation at 95°C, 45 seconds of denaturation at 94°C, 45 seconds of annealing at 64°C and 45 seconds of elongation at 72°C. Finally, ds/ssDNA was purified using QIAquick® Gel Extraction Kit (Qiagen) and was ready for the next round of selection. A total of ten rounds of selection and amplification were performed. This bacterial–SELEX method was performed according to previous reports (Marton *et al.* 2016) with a few modifications. A total of 10 SELEX cycles were carried out to increase the specificity of the aptamer with various conditions in certain cycles. Bacteria-SELEX for counter selection was carried out in cycles 4, 5, and 6. In this study, the variation of selection conditions of the SELEX process (Table 1) was modified from the research conducted by Yu *et al.* (2017).

2.4. Melting Curve Analysis

Aptamer specificity analysis was performed using the Tm Shift Method. All symmetric PCR purification products from each SELEX cycle were used in this process. A total of 3 µl dsDNA were mixed with 5 µl SYBR® Green Realtime PCR Master Mix (Toyobo) and 0.4 µM of each primer respectively. Overall, the temperature is gradually increased from 60°C to 95°C at a step size of 0.7°C/min on the CFX96 Real-Time PCR system (Bio-Rad). Changes in fluorescence intensity were monitored continuously and melting peaks were calculated using Cycler software (Bio-Rad).

Table 1. Bacterial-SELEX condition for isolating the DNA aptamers in this study

SELEX cycle	Target (cells/ml)	Aptamer (ng/µl)	Competitor	Binding time (min)	Washes (time, number, and volume)	PCR modification	
						Symmetric	Asymmetric
1	10^8 EPEC K1.1	0.84	-	45	3 min, 1 x, 500 µl	Cycle optimization	Primer ratio optimization
2	10^8 EPEC K1.1	1.68	0.1 µg/µl salmon sperm DNA	45	3 min, 1 x, 500 µl	Template: 2 µl	F:R = 20:1
3	10^8 EPEC K1.1	1.68	0.1 µg/µl salmon sperm DNA, BSA 0.05 µg/µl	45	3 min, 1 x, 500 µl	Template: 2 µl	F:R = 20:1
4	10^7	1.68	*Listeria monocytogenes*	45	3 min, 5 x, 100 µl	Template: SN, Vol: 2 µl	Template: 2 µl, 4 µl
5	10^7	1.68	*Lactobacillus plantarum* S34	45	3 min, 5 x, 100 µl	Template: SN, Vol: 2 µl	Template: 2 µl, 4 µl
6	10^7	1.68	10^4 *E. coli* TOP10+ 10^4 *E. coli* DH5α	45	3 min, 5 x, 100 µl	Template: SN, Vol: 2 µl	Template: 2 µl, 4 µl
7	10^7 EPEC K1.1	1.68	0.2 µg/µl salmon sperm DNA, BSA 0.1 µg/µl	30	10 min, 1 x, 500 µl	Template: 2 µl	Template: 2 µl, 4 µl
8	10^7 EPEC K1.1	1.68	0.2 µg/µl salmon sperm DNA, BSA 0.1 µg/µl	30	10 min, 1 x, 500 µl	Template: 2 µl	Template: 2 µl, 4 µl
9	10^7 EPEC K1.1	1.68	0.2 µg/µl salmon sperm DNA	30	10 min, 1 x, 500 µl	Template: 2 µl	Template: 2 ul, 4 uL
10	10^7 EPEC K1.1	1.68	BSA 0.1 µg/µl	30	10 min, 1 x, 500 µl	Template: 2 µl	Template: 2 µl, 4 µl

2.5. Identification of Specific Aptamer Sequences

PCR products obtained after 8, 9, and 10 SELEX rounds were cloned using TOPO® TA Cloning® Kit (Invitrogen), with pGEM-T Easy vector (Promega) and transformed into *E. coli* TOP10 bacteria by using a heat shock method. Positive colonies by blue-white selection were picked and plasmid DNA was isolated using a PrestoTM Mini Plasmid Kit (Geneaid). Positive clones with aptamer insert then sequenced by Sanger DNA sequencing.

2.6. Aptamer Secondary Structure Analysis

The secondary structures of aptamers were predicted through in silico analysis using the Mfold software program (Zuker 2003). Moreover, G-quadruplex secondary structures prediction was performed by QGRS Mapper software program (Kikin *et al.* 2006).

3. Results

3.1. Symmetric and Asymmetric PCR Optimization

Optimization of symmetric and asymmetric PCR was performed in two different SELEX phases, (a) Pre-SELEX and (b) after the First SELEX. Symmetric PCR was conducted with cycle optimization and asymmetric PCR was conducted with primer forward and reverse optimization. The optimization results of the two PCR conditions were shown in Figure 1. The results of symmetric PCR cycles optimization for dsDNA aptamers amplification at pre-SELEX and after the first SELEX round are 10 and 20 cycles, respectively. These were indicated by the appearance of the single PCR band at 81 bp (Figure 1). Meanwhile, Asymmetric PCR optimization aims to obtain ssDNA aptamer amplification through a variation of the forward: reverse (F:R) primers ratio. The best

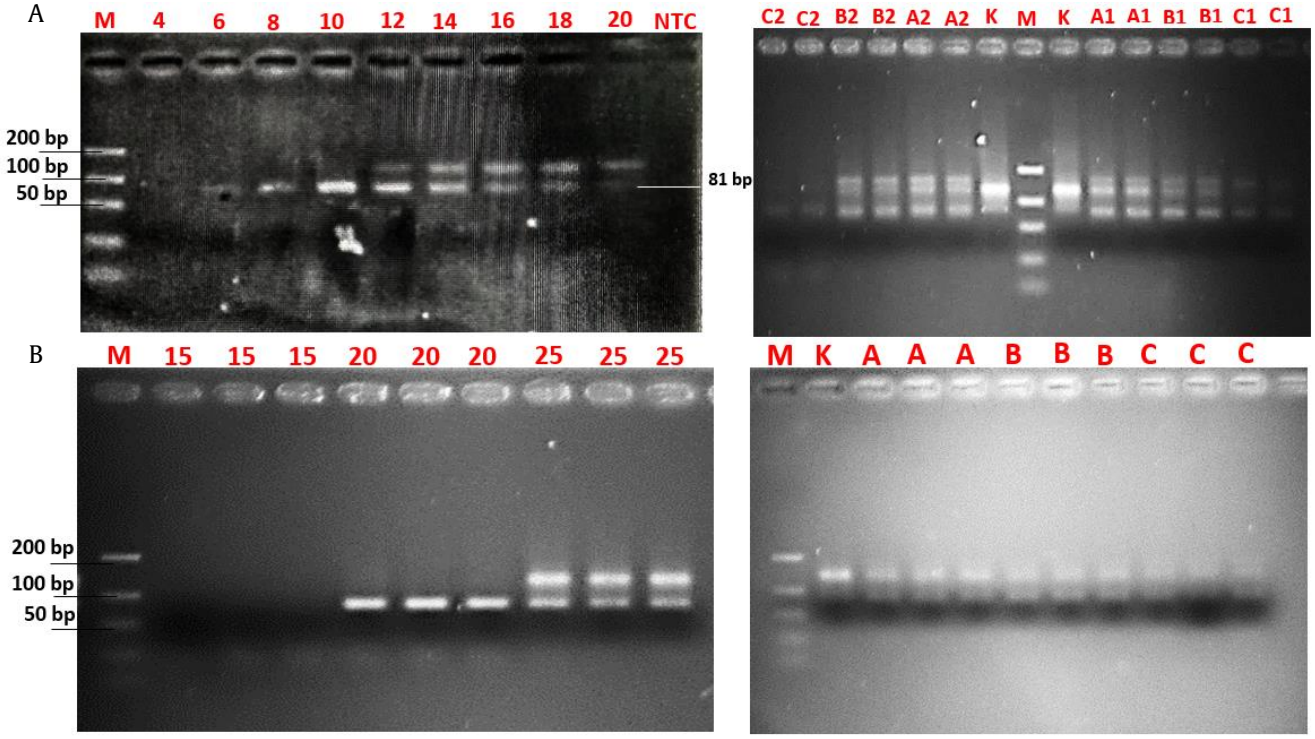

Figure 1. Optimization of symmetric and asymmetric PCR in the pre-SELEX and after SELEX stages. (A) Pre-SELEX, (B) after the First SELEX. Left: Symmetric PCR with cycle optimization (pre-SELEX: 4, 6, 8, 10, 12, 14, 16, 18 and 20; after SELEX: 15, 20, 25); Right: Asymmetric PCR with primer optimization, ((1) F:R ratio and (2) R:F ratio, where A = 20: 2, B = 20:1, C = 20:0.1); F: forward direction of primer, R: Reverse direction of primer, M: DNA ultralow ladder, K: Control (dsDNA)

primers ratio was 20:1 (Figure 1B), shown by the faster migration of the PCR product band compared to dsDNA aptamer, because of its lower molecular weight.

3.2. Melting Curve Analysis of Symmetric PCR Products for Each SELEX Rounds

The aptamer diversity during the isolation process from SELEX 1 to 10 against EPEC K1.1 was analyzed using the melting curve method. Melting curve analysis provides information about the potential of DNA aptamer specifically bound to EPEC K1.1. The sharper Gaussian peak (Melt Peak) indicates a decrease in the diversity of DNA aptamers after each stage of the SELEX cycle. The decrease in DNA aptamer diversity was caused by the separation of ssDNA aptamers that were not bound to EPEC K1.1 and also to ssDNA that were bound to the counter selection bacteria (green line). As shown in Figure 2, after going through the SELEX 1, 2, and 3 stages, it is known that the diversity of DNA aptamers begins to decrease compared to the random library (black line). After that, the melt peaks (red lines) are sharper at 7th, 8th, 9th, and 10th SELEX rounds with Tm (melting temperature) around 80°C, which indicates an increase in the specificity of DNA aptamers to the target.

3.3. Identification of Specific Aptamer Sequences

In this study, we cloned and sequenced aptamers that were found in the last 3 pools of the SELEX round to identify the aptamer sequences that specifically detects EPEC K1.1. After 10 rounds of the SELEX process, selected ssDNA were amplified with fixed primer sets to identify their sequences. After the cloning step, ssDNA was isolated from the positive colonies obtained here and then the ssDNA was inserted into the plasmids for sequencing. A total of 12 different ssDNA aptamer sequences were identified from the 8th, 9th and 10th SELEX rounds. Each ssDNA Aptamers sequence found here contains 81 nucleotide bases listed in Table 2.

3.4. Aptamer Secondary Structure Analysis

Analysis through *in silico* method using mfold software to predict secondary structures of aptamer in order to understand the effect of DNA aptamer sequences on target binding. The analysis parameters used here are folding temperature at 25°C, ionic conditions with 137 mM NaCl and 1.4 mM $MgCl_2$, where these parameters are the conditions used in the SELEX process. Prediction results of the secondary structure of 12 ssDNA aptamers with

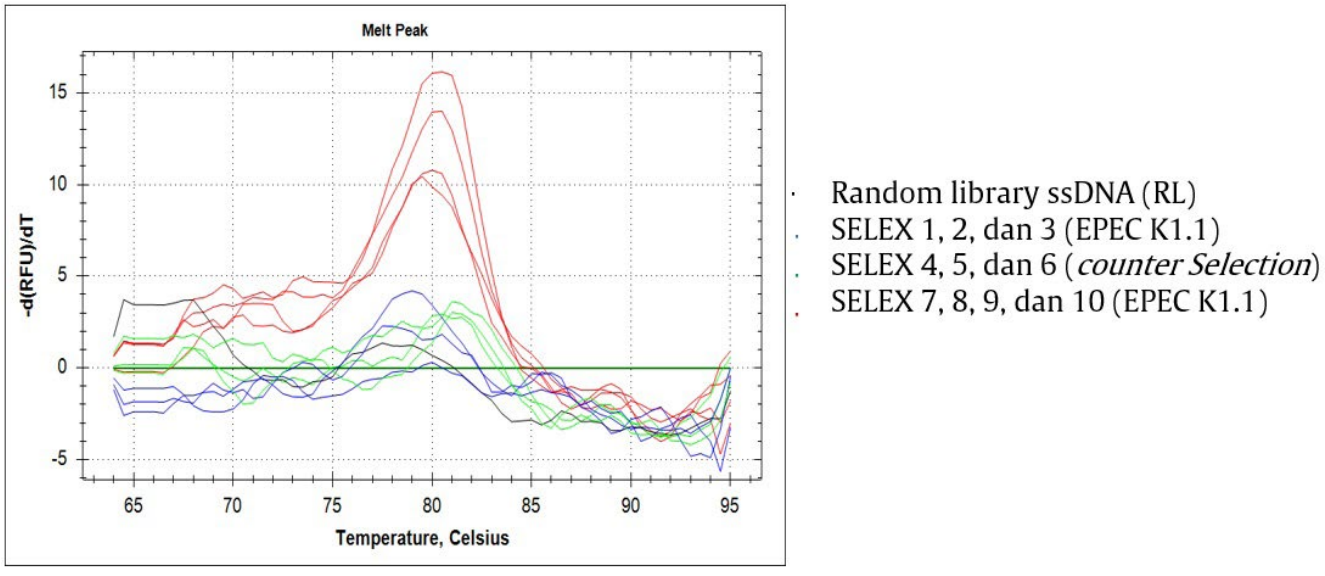

Figure 1. DNA aptamers diversity obtained from each cycle of bacterial-SELEX evaluated by melting curve analysis

Table 2. The collection of DNA aptamers obtained from Bacterial-SELEX in this study

Aptamer	DNA sequence 5→3 (81 bases long)	Quadruplex forming G-Rich sequences
S8-1	CCGGAATTCCTAATACGACTCTGCGGACTGTATCGGTCGGTCGAAAATAGTGAAGT GCTATTGAAACGCGGCCGCGG	0
S8-4	CCGGAATTCCTAATACGACTCACTACGCACGGCGCGAGTAAATCGATCATGGTACT GTGGCTATTGAAAACGCGGCCGCGG	0
S8-7	CCGGAATTCCTAATACGACTCGGTTCCGGTAAGATTAGATCATAACGTATGGCTAG CGCCATATTGAAAACGCGGCCGCGG	0
S9-3	CCGGAATTCCTAATACGACTCGAAAACGTACCACT **GGG**AT**GGG**TTGT**GGG**AG AG**GGG**CCAGGTATTGAAAACGCGGCCGCGG	1
S10-1	CCGGAATTCCTAATACGACTCTATGGAGTTTGTCCGTATGATTACGTGATATCGCG ACGGGTATTGAAAACGCGGCCGCGG	0
S10-2	CCGGAATTCCTAATACGACTCCAGCAAACGCAGTCCAACAGCCGACAAACGGTCT TGAGGCTATTGAAAACGCGGCCGCGG	0
S10-5	CCGGAATTCCTAATACGACTCTACAAAAAGTCGTGGTCCAGTTGCCATGTGTAAA ACTGTGTATTGAAAACGCGGCCGCGG	0
S10-6	CCGGAATTCCTAATACGACTCAACCAGACCACGCGAGAGGGCTCACAGTGAGAC GTGAAGGTATTGAAAACGCGGCCGCGG	0
S10-7	CCGGAATTCCTAATACGACTCTGGTGGGTAAAGACACCATACTGATAGTTACAAG GATGTTTATTGAAAACGCGGCCGCGG	0
S10-8	CCGGAATTCCTAATACGACTCGCAACTTCAGTTCAGCAAGGTGCCGGCCACGCGA CGGTCCTATTGAAAACGCGGCCGCGG	0
S10-10	CCGGAATTCCTAATACGACTCATCCAGCAGATGTGCGCG**GG**TT**GG**T**GG**G**GG**A AC**GG**TG**GG**ATATTGAAAACGC**GG**CCGC**GG**	2
S10-15	CCGGAATTCCTAATACGACTCGTAGCACTATA**GG**CAGCACGAATAT**GG**CCGTC **GG**AGTT**GG**TATTGAAAACGCGGCCGCGG	1

low Gibbs free energy (ΔG) are shown in Figure 3. Analysis of each aptamer shows differences in the secondary structure, which shows a typical stem-loop structure. Furthermore, the G-quadruplex sequence motif within aptamers was predicted by the QGRS Mapper software program. Among the 12 ssDNA aptamers, the G-quadruplex sequence motifs are found in the S9-3, S10-10 and S10-15 aptamer sequences, with G-scores of 40, 20 and 10 (2 motifs), and 13 respectively (Table 2). G-groups that form the G-quadruplex structure contain at least two G's, where larger G-groups produce higher G-scores.

4. Discussion

Aptamers produced through SELEX technology have better characteristics in terms of sensitivity, specificity, and stability, when compared to antibodies. Aptamers are becoming a promising technology as chemical antibodies for rapid diagnosis, which has attracted many researchers around the world to apply them as part of the biosensor component for the detection of various strains of *E. coli* (Zhao *et al.* 2018). In this research we used a whole cell bacterial-SELEX method to select ssDNA aptamers against live EPEC K1.1 cells. The advantage of this method is that we don't need detailed information about the target to start the selection. In addition, this selection procedure was applied under physiological conditions and native conformation of target cells (Amraee *et al.* 2017). Through the whole-cell SELEX method, the selected aptamers have high specificity and affinity for bacterial surface molecules and live bacterial targets (Moon *et al.* 2015). In this study, we modified the variation of selection conditions (Table 1). The SELEX method can be modified in various ways to improve the specificity of aptamer and SELEX efficiency (Kong and Byun 2013).

Symmetric and asymmetric PCR optimization was carried out to obtain the optimal PCR profile for amplification of target aptamer in the next SELEX rounds. Ideally, the optimization process needs to be performed on every SELEX round to get optimum conditions in producing good quality dsDNA and ssDNA aptamers (Citartan *et al.* 2012; Heiat *et al.* 2017). Optimization of symmetric PCR cycles after going through the SELEX process showed the most optimum Aptamer dsDNA amplification product after 20 PCR cycles (Figure 1B). Symmetric PCR cycle differences shown in the pre-SELEX

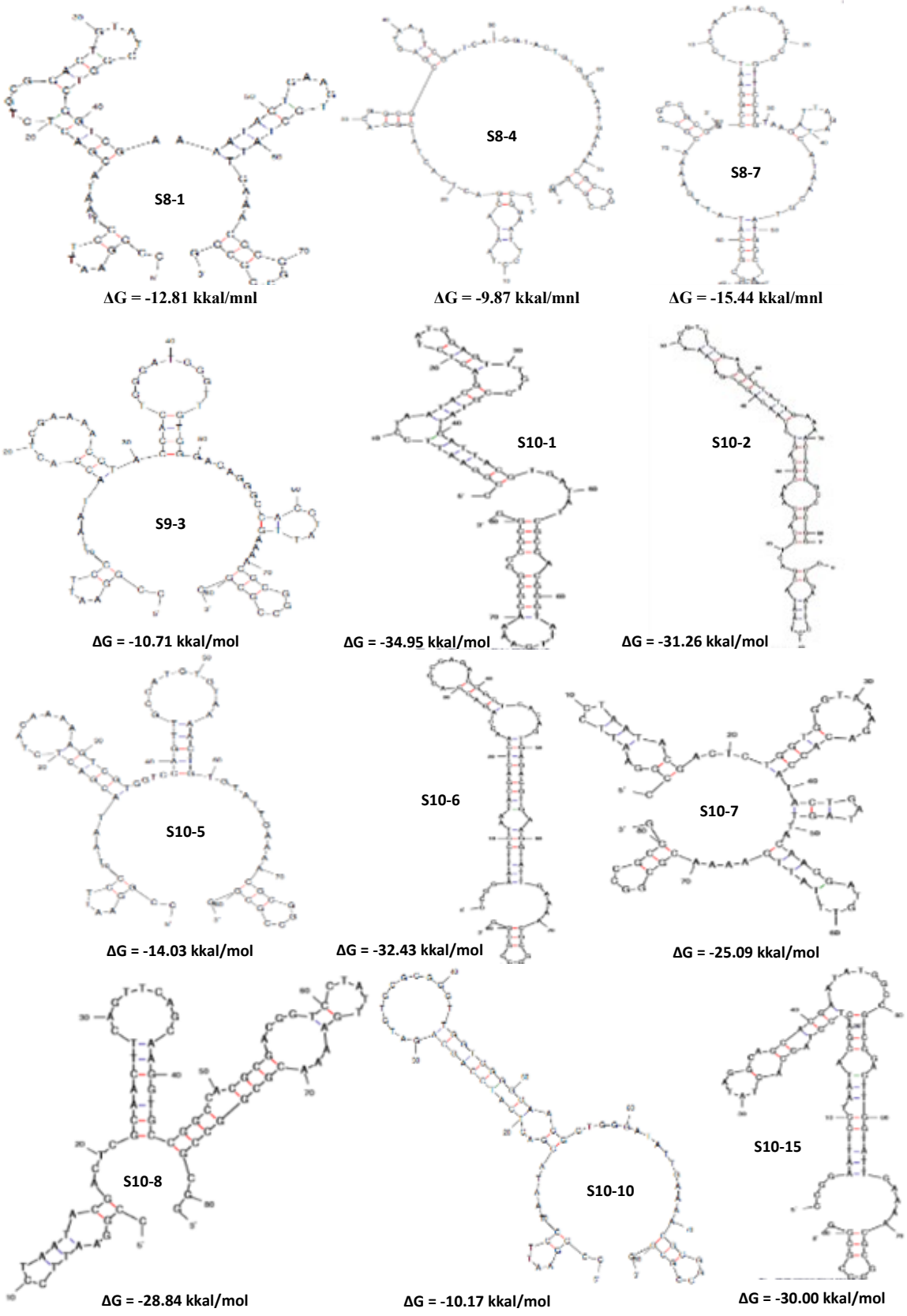

Figure 3. Secondary structure of the isolated DNA aptamers after binding to EPEC K.1.1 in bacterial-SELEX

and after the first SELEX round are due to the reduced concentration of the DNA template after the SELEX cycle. The DNA template used after each SELEX round was ssDNA that binds to the target bacterium (EPEC K1.1), while non-binding ssDNA has been removed during the washing process. DNA template concentration can affect the optimal number of PCR cycles (He *et al.* 2013). In addition, the number of PCR cycles was optimized to avoid over-amplification, which can be caused by mis-annealing in PCR products (Lavu *et al.* 2016). Meanwhile, asymmetric PCR is carried out specifically to increase ssDNA target, reduce primer dimers and non-specific amplification (Lavu *et al.* 2016). The results obtained in this study are in accordance with Citartan *et al.* (2012) which successfully produced maximum ssDNA with primers ratio of 20:1 (F:R) in 30 PCR cycles.

Melting curve analysis was performed on symmetric PCR products to evaluate the ssDNA generations for each SELEX round (Kouhpayeh *et al.* 2017). The principle of melting curve method is denaturation of PCR products that have been labeled with SYBR Green I. When the temperature of instrument approaches the melting point of PCR product, DNA strands begin to separate and release dyes resulting in decreased fluorescent intensity (Mencin *et al.* 2014). The diversity of DNA aptamers in each SELEX round was determined according to the formation and shifting of the gaussian curve (melt peak). The gaussian curves that shifted to the right on the graph, followed by sharpening of the gaussian peak indicated a decrease in DNA aptamers diversity after going through each SELEX round. The melting peak which is characterized by the Tm value (melting temperature) indicates the identity of the selected ssDNA aptamers (Kouhpayeh *et al.* 2017).

The SELEX process was performed in 10 rounds to increase the specificity of the aptamer. The repetitive SELEX process (around 6-12 rounds) aims to reduce diversity while increasing the specificity of the aptamer pools that bind to the target ligand (Song *et al.* 2008). This iterative selection process ensures that the nucleic acid obtained has a higher affinity for its target (Kong and Byun 2013). During SELEX rounds, four counter selection processes were applied to enhance the selectivity of aptamers to the target cells using other bacterial species (Top10, DH5a *E. coli* strains, *Listeria monocytogenes*, and *Lactobacillus plantarum* S34) after the 4th, 5th, and 6th rounds of selection (Table 1). Although the Gaussian peak of the bound aptamers at counter selection of our study SELEX exhibited similarity with that of bound aptamers obtained from final SELEX, we argue that the selected final aptamers were due to the bias in SELEX process. Rather, the similarity of the pooled aptamer sequences such as their GC content between these two SELEX conditions might be linked to this event. In practical terms, repeated positive selections with strengthening other conditions such as reducing the number of target cells and prolong the washing incubation time might help increasing the specificity of the final aptamers. Moreover, the counter-SELEX process is used to increase the efficiency of aptamer selection. This process has a pre-clearing step that effectively eliminates non-specific aptamers, which provides a significant improvement in aptamer selection (Kong and Byun 2013).

Formation of the secondary structure of each DNA aptamer occurs spontaneously as indicated by Gibbs free energy (ΔG) which is negative. In the mfold software, aptamers fold into unique secondary structures approximated based on the Watson-Crick base pairing. The selected structures are the most thermodynamically stable, which is indicated by the minimum free energy of the fold (Jeddi and Saiz 2017). The different composition and position of the nucleotide bases in each ssDNA aptamer obtained here, determine the formation of aptamer secondary structures (Figure 3). Differences in the nucleotide base constituents cause aptamer to have the ability to form intramolecular bonds through hydrogen bonds and affect the formation of various secondary structures such as stem, loop, bugle, pseudoknot, triplexes, g-quadruplex, and hairpin (Tan *et al.* 2016). The stem-loop structure of aptamer is important for binding targets in general and also for improving aptamer stability (Mencin *et al.* 2014; Ninomiya *et al.* 2013). Furthermore, the loop structure usually has a conservative motive that plays a role in the aptamer interaction with the target ligand.

The last *in silico* test is using Quadruplex forming G-Rich Sequences (QGRS) Mapper software program that can analyze putative G-quadruplex forming motifs in nucleotide sequences (Kikin *et al.* 2006). G-quadruplex (G4) is a 3D structure consisting of tetrads of G's (guanine) linked by Hoogsteen-type hydrogen bonds. The number of tetrads found in quadruplex is determined by the size of the G-group (Platella *et al.* 2017). The QGRS Mapper program uses a scoring system to predict the existence of quadruplex forming G-Rich sequences in nucleotide sequences. G-scores greater than zero indicated the possibility of G-quadruplex formation, where higher G-scores represent better quadruplex-forming candidates (Jeddi and Saiz 2017). G-quadruplex DNA Aptamers have been reported to have strong and specific binding for various targets,

especially proteins; because of their thermodynamically and chemically stable structure. The development of aptamer technology has revealed the potential of G-quadruplex structure as a promising molecular tool in targeting biologically important ligands and potentially become a diagnostic and therapeutic alternative to antibodies (Roxo *et al.* 2019; Tucker *et al.* 2012).

In conclusion, we report on the selection of ssDNA aptamers having binding specificity to pathogenic *E. coli.* Twelve aptamers with unique sequences were successfully isolated after 10 rounds of selection through the whole bacterial cell SELEX method against EPEC K1.1. Various secondary structures and G-quadruplex sequence motifs within aptamers were obtained, also the result of a melting curve analysis of ssDNA aptamers provide information for its application as a diagnostic tool. Therefore, these aptamers can be used as candidate aptamers specifically to detect and capture the pathogenic *E. coli* that causes diarrhea, with further validation studies to determine the best candidate aptamers.

Conflicts of Interest

The authors declare that there is no conflict of interest in this research.

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