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DNA aptamer-based colorimetric detection platform for *Salmonella* Enteritidis

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ABSTRACT

Food safety is a major issue to protect public health and a key challenge is to find detection methods for identification of hazards in food. Food borne infections affects millions of people each year and among pathogens, *Salmonella* Enteritidis is most widely found bacteria causing food borne diseases. Therefore, simple, rapid, and specific detection methods are needed for food safety. In this study, we demonstrated the selection of DNA aptamers with high affinity and specificity against *S.* Enteritidis via Cell Systematic Evolution of Ligands by Exponential Enrichment (Cell-SELEX) and development of sandwich type aptamer-based colorimetric platforms for its detection. Two highly specific aptamers, crn-1 and crn-2, were developed through 12 rounds of selection with K_d of 0.971 μM and 0.309 μM, respectively. Both aptamers were used to construct sandwich type capillary detection platforms. With the detection limit of 10^3 CFU/mL, crn-1 and crn-2 based platforms detected target bacteria specifically based on color change. This platform is also suitable for detection of S. Enteritidis in complex food matrix. Thus, this is the first to demonstrate use of *Salmonella* aptamers for development of the colorimetric aptamer-based detection platform in its identification and detection with naked eye in point-of-care.

1. Introduction

Salmonella is a gram-negative and rod-shaped bacillus which shares same characteristics with other members of *Enterobacteriaceae* family. It was firstly isolated from the abdominal lymph nodes and the spleen in 1879, yet named as *Salmonella* after its isolation from pigs having cholera in 1884 (Kim et al., 2004). Since 1880s, the incidences of *Salmonella* outbreaks have been closely observed and now it is known as one of the most common causative agents of food poisoning worldwide. Salmonellosis, a disease caused by *Salmonella*, is a food poisoning illness which develops diarrhea, fever and abdominal cramps within 8–72 h after consumption of contaminated foods (Kim et al., 2009; Moon et al., 2009). Recent studies have reported that there are over 2500 serotypes of *Salmonella;* among which, *Salmonella enterica* serovar Enteritidis and *Salmonella enterica* serovar Typhimurium represent the most widely found serovars causing salmonellosis (Andino and Hanning, 2015; CDC, 2007, 2013)

The Center for Disease Control and Prevention (CDC) estimates that the consumption of contaminated poultry, egg, milk, beef, and raw foods causes approximately 1.2 million of *Salmonella* infection in the United States annually (Scallan et al., 2011). According to the data of World Health Organization (WHO), approximately 16 million cases of typhoid fever, 1.3 billion cases of gastroenteritis, and 3 million deaths resulted from *Salmonella* infections have been reported worldwide, annually. “Reports of Salmonella Outbreak Investigations from 2015” published by CDC indicates that there had been 9 outbreaks of *Salmonella* in different kinds of food such as pork, chicken, nut butter and cucumber, and among them, three *S.* Enteritidis outbreaks reported in chicken entrees and live poultry (CDC, 2015).

Most commonly used detection technique of the *Salmonella* is the culture method on plates which involves sampling, pre-enrichment on common media and selective enrichment on specific media. Food and Drug Administration (FDA) published *Salmonella* culture method for laboratories which is a 5-step method lasting for 4 days (Lee et al., 2015). Due to being time consuming and inaccuracy, faster screening techniques have been used as an alternative. So far, PCR-based techniques targeted the amplification of invasion protein gene (*invA*) (Rahn et al., 1992; Ferretti et al., 2001; Malorny et al., 2003) major

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fimbrial subunit encoding gene (*fimA*) (Doran et al., 1994), virulence gene (*spv*) (Lampel et al., 1996), and invasion gene transcriptional activator (*hilA*) (Guo et al., 2000; Pathmanathan et al., 2003). Monoclonal and polyclonal antibody-based techniques were also studied for *Salmonella* detection; however, since both techniques require pre-sampling for enrichment of bacteria in samples and have higher detection limits, the development of new detection methods have become very important in terms of preventing life threatening complications and spread of infections. Recent studies have focused on the use of aptamers as capturing or sensing elements in the construction of biosensors against food pathogens.

Aptamers are single stranded DNA or RNA molecules that bind targets with high affinity and selectivity. Being different from base pairing between complementary sequences, higher binding affinity of aptamer is related to specific folding at binding conditions (Proske et al., 2005). They were discovered by three different groups spontaneously and independently in 1990 (Robertson and Joyce, 1990; Tuerk and Gold, 1990; Ellington and Szostak, 1990). They are short oligonucleotides with generally ≤ 100-mer long and selected for a wide variety of molecules such as ions, amino acids, proteins, viruses and even whole pathogenic bacteria, plant or animal cells (Sefah et al., 2010; Bayrac et al., 2011; Davydova et al., 2016). They are selected in vitro by a method called SELEX. A typical SELEX involves iterative cycles of selection process, each of which is composed of basically four stages; incubation of DNA/RNA library with target, removal of unbound sequences, elution of bound sequences and finally amplification of eluted sequences for the next round of selection process. Due to their higher selectivity, affinity and stability, aptamers have been applied as novel detecting agents to different platforms for diagnoses of certain diseases (Ozalp et al., 2015; Derkus et al., 2016) and also for food pathogens.

The first use of aptamers as recognition elements in biosensors was reported for the detection of human neutrophil elastase (Davis et al., 1996), and since then, many aptasensor studies have been reported in literature. Some of these included aptamer-based optical biosensors (Stojanovic et al., 2001; Wang et al., 2005; Zhang et al., 2010; Feng et al., 2011), electrochemical biosensors (Zhou et al., 2010; Radi, 2011; Xie et al., 2014), mass-sensitive biosensors (Wang et al., 2008; Min et al., 2008; Qiao et al., 2016) and colorimetric biosensors (Chang et al., 2014, 2016; Wang et al., 2015; Huo et al., 2016). Aptamer-based colorimetric recognition of food pathogens has provided accurate, simple and visual way of detection for the prevention of food poisoning, thus, food safety. So far, colorimetric aptasensors have been reported for *Staphylococcus aureus* (Yuan et al., 2014; Chang et al., 2016), *Escherichia coli* O157:H7 (Wu et al., 2012a, 2012b), *Vibrio parahemolyticus* (Wu et al., 2015) and *Salmonella* Typhimurium (Wu et al., 2012a; Park et al., 2015).

In the present study, we described the selection of DNA aptamers to *S.* Enteritidis using cell-SELEX and demonstrated the development of aptamer-based colorimetric detection platform for this pathogen for the first time. The selection process was monitored by fluorescence aptamer binding assay and melting curve assay. Two aptamers were generated at the end of twelve rounds and they were characterized in accordance with their affinity and selectivity by two different methods, Enzyme-Linked Aptamer Assay **(**ELAA) and Isothermal titration calorimeter (ITC). These methods gave promising results for the evaluation of potential use of aptamers as sensing elements in the construction of biosensor. Aptamers were used for the construction of capillary-based detection platform against *S.* Enteritidis with low detection limit. This platform was shown also applicable for the detection of pathogen in food matrix without sample's extraction and purification. By providing simple detection way with naked eye, this platform provides applicability for direct use in the field. To the best our knowledge, this was the first time that aptamer-based platforms were constructed for the detection of *Salmonella* Enteritidis, colorimetrically.

2. Materials and methods

2.1. Bacterial strains

Target organism for aptamer selection, *Salmonella* Enteritidis, was obtained from the Faculty of Veterinary Medicine, Ankara University (Ankara, Turkey). *S.* Typhimurium (ATCC 29630) and *Escherichia coli* (ATCC 25922) were obtained from American Type Culture Collection (Manassas, VA). The standard laboratory strain of *Staphylococcus aureus* (ATCC 8324) was purchased from the Public Health Institution of Turkey (Ankara, Turkey). The culture conditions and preparations of these cells were explained in Supplementary material.

2.2. DNA library and primers

A 80 nucleotides single-stranded DNA (ssDNA) library comprising a 42 nucleotides randomized region flanked by 19 nucleotides constant primer binding regions (5′-AAGGGCTGGCTGGGATGGA-N_{42}-TCACTCCACGGACCCCACT-3′) was synthesized with ABI 3400 DNA synthesizer (Applied Biosystem Inc., Foster City, CA). Based on the standard primer considerations, FAM-labeled forward primer and biotin-labeled reverse primer were designed as (5′-FAM-AAGGGCTGGCTGGGATGGA-3′) and (5′-Bio-AGTGGGGTCCGTGGA GTGA-3), respectively. They were purchased from Integrated DNA Technologies Inc. (Coralville, IA).

2.3. Cell-SELEX

Whole cell-based selection was performed for generation of DNA aptamer against *S.* Enteritidis. Negative selection with *E. coli* was applied first at the sixth round of Cell-SELEX and repeated for each subsequence positive round till end of selection. Progress of SELEX was monitored via fluorescent aptamer binding assay and melting curve analysis of DNA pools. The ssDNA pool eluted at 12th round of SELEX was sequenced with Ion torrent semiconductor sequencing technology (Life technologies). Sequence results were analyzed with multiple sequence alignment tools; MAFFT (Multiple Alignment using Fast Fourier Transform) (Katoh and Standley, 2013), Clustal, MView (EMBL-EBI) and possible minimum free energy structures of candidate aptamer sequences were drawn by Nucleic Acid Package (NUPACK, www.nupack.org) (Dirks et al., 2007). The characterization of candidate aptamers in terms of specificity and affinity was studied via Enzyme-Linked Aptamer Assay **(**ELAA) and Isothermal titration calorimeter (ITC), respectively. The whole selection process was shown in Scheme 1. For detailed information about the selection and characterization process, see Supplementary material.

2.4. Construction of aptamer-based sandwich type capillary detection platform

Glass capillaries were washed with washing solution (10% (w/v) sodium hydroxide in 60% (v/v) ethanol solution) for 2 h. After washing with distilled water twice they were incubated with poly-L-lysine solution (17% (v/v) in 0.1X PBS) for 60 min at room temperature. Coated capillaries were dried by spinning at 1000 rpm for 5 min and stored at 4 °C for at least 15 days before experiment. The cross-linker solution, N-epsilon-Maleimidocaproyl-oxysulfosuccinimide ester (Sulfo-EMCS) was prepared fresh before each experiment in 10 mM PBS (pH: 7.2). Meanwhile, thiol modified aptamers were denatured at 95 °C for 5 min and instantly cooled down on ice. Capture solution containing 2 mM of Sulfo-EMCS, 0.1 mM of tris(2-carboxyethyl)phosphine (TCEP) and 10 μM of crn-1-SH or crn-2-SH was prepared in 1X of PBS and incubated at room temperature for 30 min. After pre-incubation, capture solution was applied into poly-L-lysine coated capillaries and they were incubation at room temperature for 30 min.

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