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Aptasensors for quantitative detection of *Salmonella Typhimurium*

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ABSTRACT

Salmonella is one of the most frequent causes of food borne infectious disease. Among nearly 2500 documented serotypes are reported, *Salmonella Typhimurium* is the number one serotype associated with salmonellosis worldwide. Many different methods have been developed for the detection and quantification of *S. typhimurium*. Most of these assays are usually expensive, time consuming and require difficult sample preparation steps. Therefore, it is necessary to develop rapid, robust, cost-effective and sensitive alternative detection methods. In the last years, aptasensors, used for detection of *S. typhimurium* in different samples. In this review, recent advances and applications of aptasensors for the detection and quantification of *S. typhimurium* in details have been summarized.

Contents

Introduction

Bacterial infection is responsible of many serious and fatal diseases. The diagnosis of pathogenic bacteria is an essential urge of the clinical microbiology. Among all the food-borne pathogens, Salmonella is one of the most frequent causes of food borne infectious disease, especially gastroenteritis [50]. Nearly 2500 documented serotypes are reported, *Salmonella Typhimurium* is the number one serotype associated with salmonellosis worldwide [34]. *S. typhimurium*, a Gram-negative bacteria pathogen, transmitted primarily through the consumption of raw or uncooked eggs, vegetables, fruits, and poultry [14,52] and causes of food-borne illness in human and animal hosts worldwide [7]. The presence of this organism in food can be a serious threat to public health. Also lipopolysaccharides (LPS) as endotoxins are integral components of the outer membrane of all Gram-negative bacteria such as *S. typhimurium*. LPS leads to septic shock [15], pyrogenic

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reaction [54], hypotension, diarrhea and vascular blood clotting [42], therefore detection of LPS is also crucial for medical and food security. So sensitive and quantification methods for *S. typhimurium* detection are critical in food quality and security. The routine detection methods for *S. typhimurium* are culture-based methods, amplification-based assays, real-time polymerase chain reaction (PCR) [57,68,87] and immunoassays such as enzyme-linked assays, chemiluminescent immunoassays, and fluorescence immunoassay [13,49,83,84]. These methods have some limitations such as requiring specialized instrumentation and highly trained personnel as well as some methods are time consuming with false negative results. Therefore, there is a continuous need for a rapid, sensitive and reliable assay for *S. typhimurium* detection. Biosensors are analytical devices with two main functional components, target recognition and signal transduction, which can sense and quantity targets by changing the recognition of targets into a physically detectable signal, such as electronic, magnetic or optical signals. Aptamer is single-stranded nucleic acid (DNA or RNA) with high affinity and a significant conformation upon binding with a wide range of targets. Typically, aptamer sequences are selected through an in vitro Systematic Evolution of Ligands by EXponential enrichment (SELEX) [11]. Aptamer-based biosensors, known as aptasensors, have been developed as potential methods for bacterial recognition. Furthermore, aptamers can be easily improved with different markers in order to either increase their stability or labeling them with fluorophores as well as quenchers to facilitate the production of biosensors [12]. Aptasensors, facilitate detection of pathogens without any need to tedious procedures such as multiple incubations and washings [43]. Up till now, various aptamers have been identified which targeted many bacteria, such as *Staphylococcus aureus* [9], *Lactobacillus acidophilus* [36], *Vibrio parahaemolyticus* [21], *Salmonella Typhimurium* ([22], *Campylobacter jejuni* [30], *Group A Streptococcus* [35], *Listeria monocytogenes* [19], *Mycobacterium tuberculosis* [10] *and Shigella dyse*nteriae [20]. Aptamers can potentially be functional to diagnosis of pathogenic bacteria because of specificity and high affinity to their target, rapid and reproducible synthesis, and convenient modifications [25]. In this review, we will focus on recent advances in aptamer based biosensors developed for *S. typhimurium* as the most important serotype of this genus.

Optical aptamer-based biosensors

Optical aptasensors are the combination of aptamer as the recognition part with different optical analytical techniques as the signal transductions. Optical analysis methods are extensively used in the constructions of aptasensors due to their high sensitivity, rapid response and simple use. There are five main classes of optical aptasensores including colorimetry, chemiluminescence (CL), surface-enhanced Raman scattering (SERS), surface plasmon resonance (SPR), and fluorescence [33,63]. In this section, we explained all optical aptasensors for *S. typhimurium* based on detection techniques such as SERS, fluorescence and colorimetry.

SERS aptasensor

Application of SERS has been reported in many different fields, such as the environmental monitoring [6,53], biomedical field [37,41], and food quality assurance [1,48]. Also some researchers used SERS-based assays to detection of bacterial cells [5,73]. There have been some reports of using a SERS sensing platform based on an aptamer for the sensitive and rapid detection of *S. typhimurium*. In one method the Au@Ag core/shell NPs exploited as the enhanced substrate for SERS and apt1 was immobilized on the surface of Au@Ag core/shellNPs. ROX-modified apt2 attached with the target in the same way as Au@Ag-apt1 and the Raman signal intensity was generated from it [18]. In the presence of the *S. typhimurium*, Au@Ag-apt1 recognized and attached to *S. typhimurium*. Following the addition of ROX-modified apt2, Au@Ag-apt 1-target-apt 2-ROX sandwich-like complexes organized based on the high specificity and affinity of aptamer and *S. typhimurium*. More *S. typhimurium* was added, the surface loading of bound ROX-apt 2 increased, resulting in increased SERS intensity. By monitoring the SERS signal increase along with changes in concentration, highly sensitive quantification of *S. typhimurium* could be realized. The linear relationship between the SERS intensity and the *S. typhimurium* concentration was in the range from 15 to 1.5×10^6 cfu/ml. The detection limit was found to be 15 cfu/mL the linear regression equation is defined as $Y = 592.54x - 768.98$, and the corresponding correlation coefficient was 0.996. This technique can potentially be used for the rapid and sensitive detection of other pathogenic bacteria for food safety assurance. Common SERS-enhanced substrates are silver nanoparticles, gold nanoparticles and other heavy metal nanomaterials [8,38,60]. A SERS-aptasensor, using nanoparticles was established for the quantitative detection of *S. typhimurium* and *S. aureus* simultaneously with the detection limit of 35 cfu/mL for *S. aureus* and 15 cfu/mL for *S. typhimurium* by Zhang and coworkers [86]. In this platform, gold nanoparticles (GNPs) modified with Raman molecules (Mercaptobenzoic acid (MBA) and 5, 5′-Dithiobis (2-nitrobenzoic acid) (DNTP)) and aptamers were applied as the signal probe. Thiolated aptamers of *S. typhimurium* and *S. aureus* are as the capture probe with MBA and DNTP respectively, fixed on Fe3O4 magnetic gold nanoparticles (MGNPs). In this platform the Raman intensified spectrum demonstrated the quantification of *S. typhimurium* and *S. aureus*.

Fluorescence-based aptasensors

Among aptamer-based assays, fluorescence detection methods are attractive, to some extent because of their high sensitivity, feasibility of quantification and potential for high-throughput analysis [51]. Labeled and label-free fluorescence aptasensors are two main approaches for these aptasensors [33]. Most current fluorescent aptamer-based methods require fluorophore or fluorescent nanoparticle labeling of the aptamer molecules [2,45,46]. These labeled fluorescent approaches are laborious, time-consuming and expensive. Therefore, it is essential to develop convenient and label-free approaches for pathogenic bacteria detection. The fluorescence of PicoGreen, SYBR Green dye and AccuBlue reagent themselves are very weak; however, the dye molecule becomes highly fluorescent on binding to dsDNA, but when it binds to ssDNA no considerable fluorescence change can be observed. A universal fluorescent aptasensor with two models, “signal on” and “signal off,” based on AccuBlue dye with unmodified aptamers to detect *S. typhimurium* was introduced by Duan et al. [25]. In the presence of target, aptamer bound to its target, causing the cDNA to separate from the dsDNA duplex and leading to low fluorescence intensity as a result of the release of AccuBlue. The intercalation time of AccuBlue into the aptamer/cDNA duplex distinctly influenced the fluoresence signal. This aptamer based sensor showed a linear range from 50 to 10^6 cfu/mL with a low LOD of 25 cfu/mL. Benefitting AccuBlue and elimination of DNA labeling this sensor can be adapted for detection of wide variety of other pathogens [25].

Quantum dots (QDs), as efficient fluorescence nanomaterials and flow cytometry, as a high throughput biophysical analytical technique were served for quantitative detection of *Vibrio parahaemolyticus* and *S. typhimurium*. In this DNA aptasensor quantum dots (QDs) as a fluorescence marker and aptamers as the molecular recognition element and fluorescence carrier used to detect these