A Portable Impedance Aptasensing System for Rapid Detection of *Salmonella* Typhimurium in Poultry Products

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Abstract

A portable impedance aptasensing system for the rapid and sensitive detection of *Salmonella* Typhimurium in poultry was developed using an interdigitated microarray electrode (IDME). The aptasensing system consisted of a gold IDME, a signal acquisitive interface, and a laptop computer with LabVIEW software. The IDME was first functionalized with 20 mM 16-mercaptohexadecanoic acid followed by surface immobilization with NH_2 conjugated *S*. Typhimurium aptamer. Poly (ethylene glycol) methyl ether thiol (0.1mg/mL in PBS) was used for surface blocking following aptamer immobilization. The IDME was then left to rest for 48 h at room temperature to be ready for use in bacterial tests. After sample preparation, 50µL of the sample containing *S*. Typhimurium was dropped onto the IDME's surface which allowed the immobilized aptamer to capture the *Salmonella* cells. The impedance change caused by the capture of the bacterial cells was measured in the presence of a redox probe and recorded using a laptop with LabVIEW software. The results showed that there was a linear relationship with a correlation coefficient of 0.95 between the impedance change and the log value of *S*. Typhimurium in concentrations of 1.14×10^1 to 1.14×10^5 CFU/50 µL in pure culture samples. The detection time was under 1 hr. The aptamer concentration used for the surface immobilization of the IDME was optimized using a QCM electrode and determined to be 10 mM in PBS. The developed portable impedance aptasensor had a limit of detection (LOD) of 7.39% or 1.14×10^1 CFU/50µL and was highly specific to *S*. Typhimurium when tested against five non-target bacterial. This aptasensor has the potential to shorten detection time, lower detection costs, and improve sensitivity for in-field detection of pathogens.

Introduction

Each year, *Salmonella* Typhimurium causes an estimated 19,000 hospitalizations and 380 deaths. It is considered one of the most dangerous foodborne pathogens and a major threat to human health. *S*. Typhimurium is typically transmitted to people through the consumption of food products such as poultry, meat, and eggs. Traditional methods that depend on microbiological methods for detection of *Salmonella* are time consuming and labor intensive since they require multiple steps for enrichment and growth of the bacteria Due to this drawbacks, there is an urgent need for the development of a rapid and reliable method to detect *Salmonella* in food products.

Objectives

The objectives of this project were:

- To design and fabricate an impedance aptasensor to detect *S*. Typhimurium
- To determine the specificity of the aptasensor for *S*. Typhimurium;
- To evaluate the aptasensor for rapid detection of *S*. Typhimurium in poultry products

Materials and Methods

Bacteria

- *C. jejuni,* ATCC 11168
- *E. coli* O157:H7, ATCC 43888
- *E. coli* K12, ATCC 29425
- *L. innocua*, ATCC 33090
- *L. monocytogenes*, ATCC 43251
- *S*. Typhimurium ATTC 14028

Reagents

- 16-mercaptohexadecanoic acid (MHDA), 20mM
- EDC/NHS, 75mM/30mM, v/v 1:1
- NH_2 - *Salmonella* Typhimurium aptamer
- $[Fe(CN)_6]^{3-/4-}$ as redox probe
- Poly (ethylene glycol) methyl ether thiol (PEG), 0.1mg/mL in PBS

Surface modification of the IDME

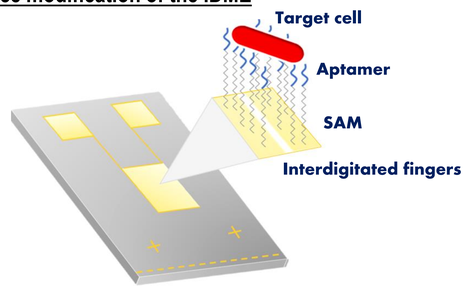

Fig. 1. Surface modification of IDME

Principle of the Aptasensor

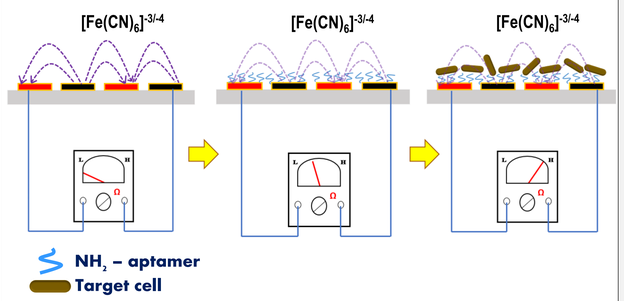

Fig. 2. Principle of the immunosensor: (a) bare IDME; (b) IDME immobilized antibody; and (c) IDME with captured bacterial cells

Setup of the aptasensing system

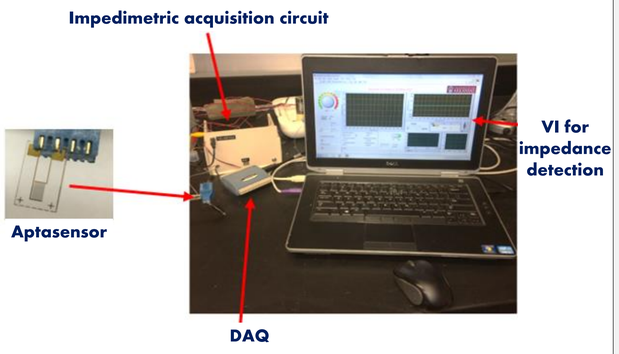

Fig. 3. Setup of the portable aptasensing system

Materials and Methods (Cont.)

Flowchart of the assay

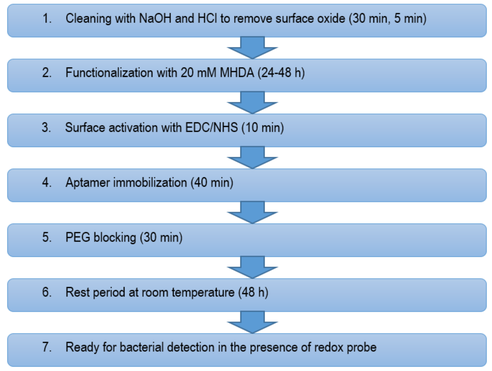

Fig. 4. Flowchart of the assay

Results

Captured *S*. Typhimurium cells on the surface of the IDME

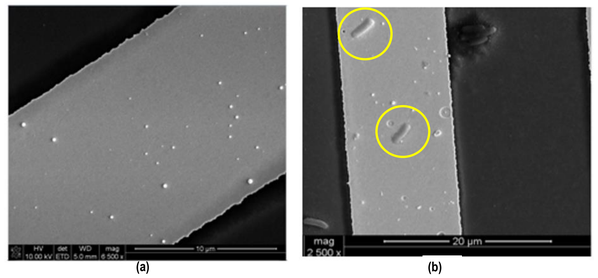

Fig. 5. (a) SEM image of functionalized IDME; (b) SEM image of bound *Salmonella* cells

Detection of *S*. Typhimurium

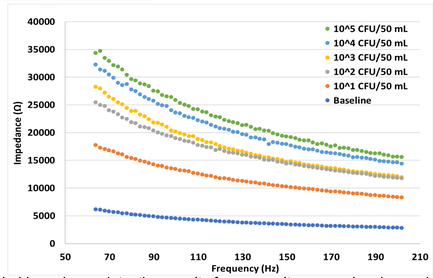

Fig. 6. Recorded impedance detection results for pure culture samples dropped onto IDME surface

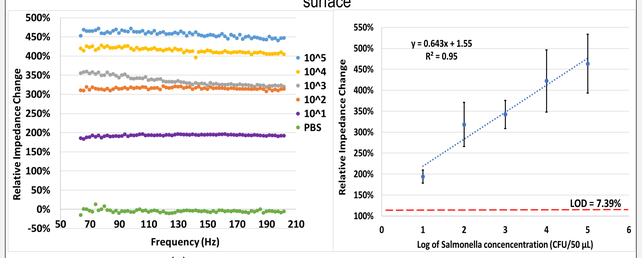

Fig. 7. (a) Relative impedance change of negative control (PBS) and concentrations of S. Typhimurium in pure culture, 10^1 to10^5 CFU/50 μL^1 and (b) relationship between the logarithmic value of *S*. Typhimurium concentrations and relative impedance change at 101 Hz

Results (Cont.)

Specificity of the immunosensor

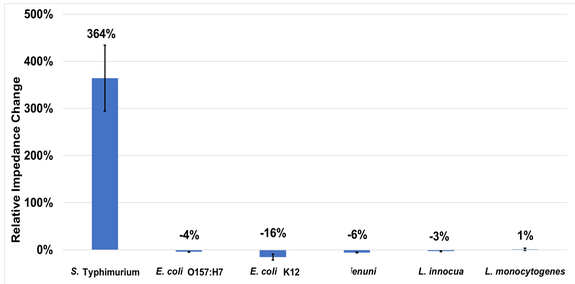

Fig. 8. Specificity tests of five non-target bacteria compared to *S*. Typhimurium at concentrations of 10^5 CFU/50 µL

Optimization of the aptamer concentration using QCM electrode

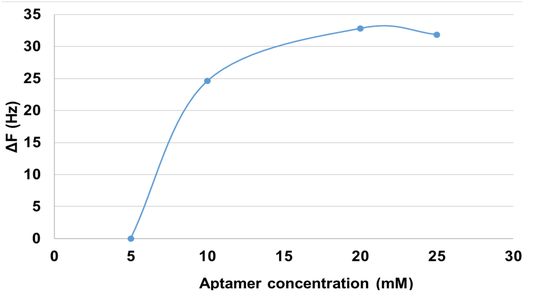

Fig. 9. Change in frequency in response to aptamer concentration and number of *S*. Typhimurium cells captured

Conclusions

- The results showed that there was a linear relationship with a correlation coefficient of 0.95 between the impedance change and log values of *S*. Typhimurium in a range of concentrations of 1.14×10^1 to 1.14×10^5 CFU/50 µL in pure culture samples.
- The aptasensor showed a high specificity for *S*. Typhimurium with an average relative impedance change of 364% compared to the -16% to 1% relative impedance change of five non-target bacteria at concentrations of 10^5 CFU/50µL.
- The aptamer concentration used for the surface immobilization of the IDME was optimized using a QCM electrode and determined to be 10 mM in PBS.
- The developed impedance aptasensor has the potential to increase the sensitivity of detection, shorten detection time, lower costs per test, and allow for portability for in-field detection of *Salmonella* Typhimurium.
- The on-going research is focused on detection of S. Typhimurium in poultry products using a flow cell imbedded with the aptamer immobilized IDME.

Acknowledgements

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