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

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Background

- *Salmonella* Typhimurium is a pathogenic Gram-negative, rod-shaped bacterium (Sheikhzadeh,2016).
- People infected with *Salmonella* develop salmonellosis. The symptoms for salmonellosis include diarrhea, fever, and abdominal cramps, which can last between four to seven days (CDC, 2012).
- In the US, Salmonellosis causes an estimated 1.2 million cases each year, resulting in 23,000 hospitalization and 450 deaths. (CDC, 2012).
- *Salmonella* is usually transmitted to humans by consuming contaminated meat and poultry products (Tao et al., 2017). If meat and poultry products are not cooked the appropriate minimum internal temperatures, *Salmonella* is able to survive the heat treatment (USDA, 2013).

Fig.1 *Salmonella* cells, copyright 2014 Discovery Scientific Solutions*

https://www.cdc.gov/salmonella/typhimurium-01-13/
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Conventional detection methods

Culture and colony based methods:

- Culture based methods are the most reliable and accurate techniques for detection of foodborne pathogens (Velusamy et al., 2010).
- These methods have been used for the detection of a broad range of pathogens such as: *L. monocytogenes, S. aureus, Salmonella, Coliforms, E. coli, C. jejuni, and Y. Enterocolitica* (Artault et al., 2001; Deboer and Beumer, 1999; Stephan et al., 2003; Aycicek et al., 2004; Weagan, 2008)
- Depend on microbiological techniques, such as pre-enrichment steps, cultivation of bacteria, and validation of suspicious colonies (Velusamy et al., 2010). However, some bacterial strains can become dormant during cultivation leading to underestimating the number of cells present in a sample or failure to isolate the target cells from contaminated samples (Porter et al., 2005).
- The major disadvantages to these methods are that they are labor-intensive and time-consuming. It can take 2-3 days for preliminary results and 7-10 days for confirmation (Velusamy et al., 2010).

Velusamy, V., Arshak, K., Korostynska, O., Oliwa, K., & Adley, C. (2010). An overview of foodborne pathogen detection: In the perspective of biosensors. Biotechnology advances, 28(2), 232-254.

Rapid detection methods

I. **Polymerase chain reaction (PCR)**

- PCR is able to detect a single target cell by detecting a single copy of the target's DNA sequence (Velusamy et al., 2010).
- Allows for simultaneous detection of multiple organisms (multiplex PCR) and continuous monitoring of target organism's behavior in response to its environment (real-time PCR) (Zhao et al., 2014).
- It can take 5 – 24 h to obtain results (excluding enrichment) depending on PCR method used (Lazcka et al., 2007)

II. **Immunological based methods**

- Enzyme-Linked Immunosorbent Assay (ELISA) is one of the most widely used immunological assays for rapid foodborne pathogen detection (Zhao et al., 2014).
- Immunomagnetic separation assay (IMS) uses Immunomagnetic beads (IMBs) to capture target.

III. **Biosensors**

- Allows for real-time detection, portability, and multi-pathogen detection for both infield and lab analysis
- With real-time detection, corrective measures can be taken to prevent further contamination of foods and stop consumption of foods (Zhao et al., 2014)

Zhao, X., Lin, C. W., Wang, J., & Oh, D. H. (2014). Advances in rapid detection methods for foodborne pathogens. J. Microbiol. Biotechnol, 24(3), 297-312.
Lazcka, O., Del Campo, F. J., & Munoz, F. X. (2007). Pathogen detection: A perspective of traditional methods and biosensors. *Biosensors and bioelectronics*, *22*(7), 1205-1217.

Biosensors for *Salmonella* detection

- Several portable biosensor for in-field detection have been developed, including an SPR-based immunosensor able to detect *S*. Typhimurium in a range of 10^7 – 10^9 CFU/ml within an hour (Nguyen et al., 2016).
- An amperometric immunosensor was developed for the detection of S. Typhimurium in milk samples. The biosensor had a limit of detection (LOD) of 10 CFU/mL and a detection time of 125 minutes (Alexander et al., 2018).
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Nguyen, H. H., Yi, S. Y., Woubit, A., & Kim, M. (2016). A Portable Surface Plasmon Resonance Biosensor for Rapid Detection of Salmonella typhimurium. Applied Science and Convergence Technology, 25(3), 61-65.
Alexandre, D. L., Melo, A. M. A., Furtado, R. F., Borges, M. F., Figueiredo, E. A. T., Biswas, A., ... & Alves, C. R. (2018). A Rapid and Specific Biosensor for Salmonella Typhimurium Detection in Milk. *Food and Bioprocess Technology*, *11*(4), 748-756.

Objectives

- The overall goal of this research is to develop and demonstrate a portable aptasensor for the rapid detection of *Salmonella* Typhimurium in poultry products.

- The specific objectives of this research are:
  i. To design and fabricate an aptasensor to detect *S.* Typhimurium
  ii. To determine the specificity of the aptasensor for *S*. Typhimurium
  iii. To evaluate the aptasensor for rapid detection of S. Typhimurium in poultry products

System Setup

A Portable Immunosensing System for Rapid Detection of *Salmonella* Typhimurium

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- Published in Sensors journal (vol. 17, page 1973) in 2017.
- System included a laptop with LabVIEW software, a data acquisition card (DAQ), and antibody immobilized electrode.
- Determined that the best frequency range was 63-201 Hz

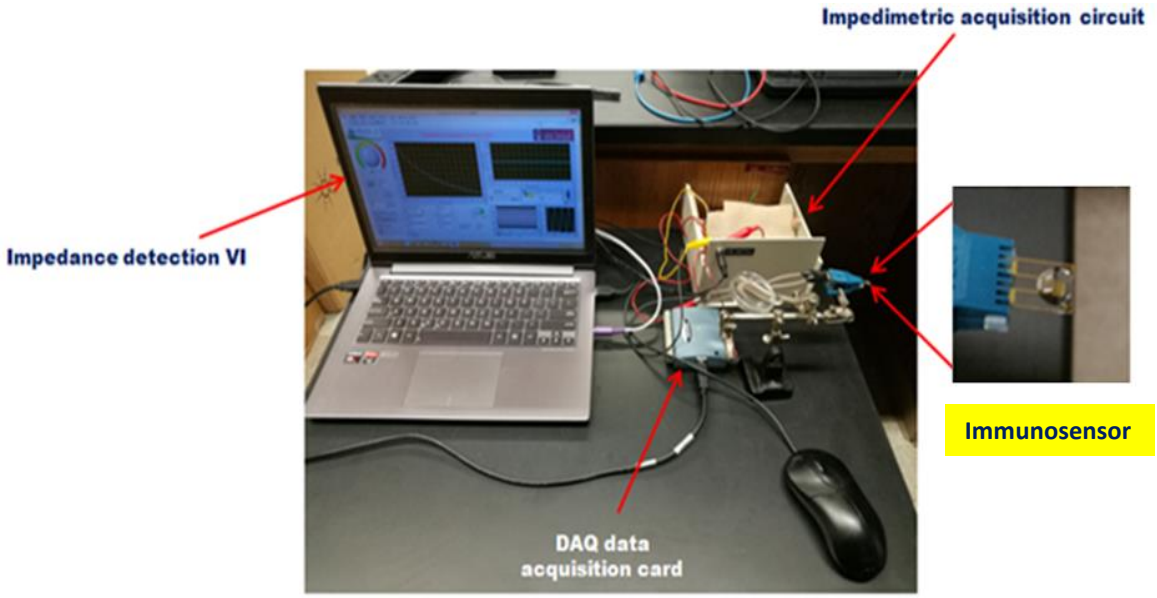

Fig. 2 Set up of the system (Wen et al., 2017)*

Wen, T., Wang, R., Sotero, A., & Li, Y. (2017). A Portable Impedance Immunosensing System for Rapid Detection of *Salmonella* Typhimurium. Sensors, 17(9), 1973.

Why aptamers?

- Why aptamers instead of antibodies?
  - Higher target affinity in some cases
  - Aptamer are selected using in vitro, systematic evolution of ligands by exponential enrichment (SELEX), whereas antibodies are produced in vivo biological systems
    - Uniform production
    - Cheaper to produce
  - Aptamers are highly stable, and therefore have unlimited shelf life if stored in the proper conditions
  - Aptamer-based biosensors have the potential to be recyclable since aptamers can undergo reversible changes in conformation based on temperature or salt-concentration.

Principle of the aptasensor

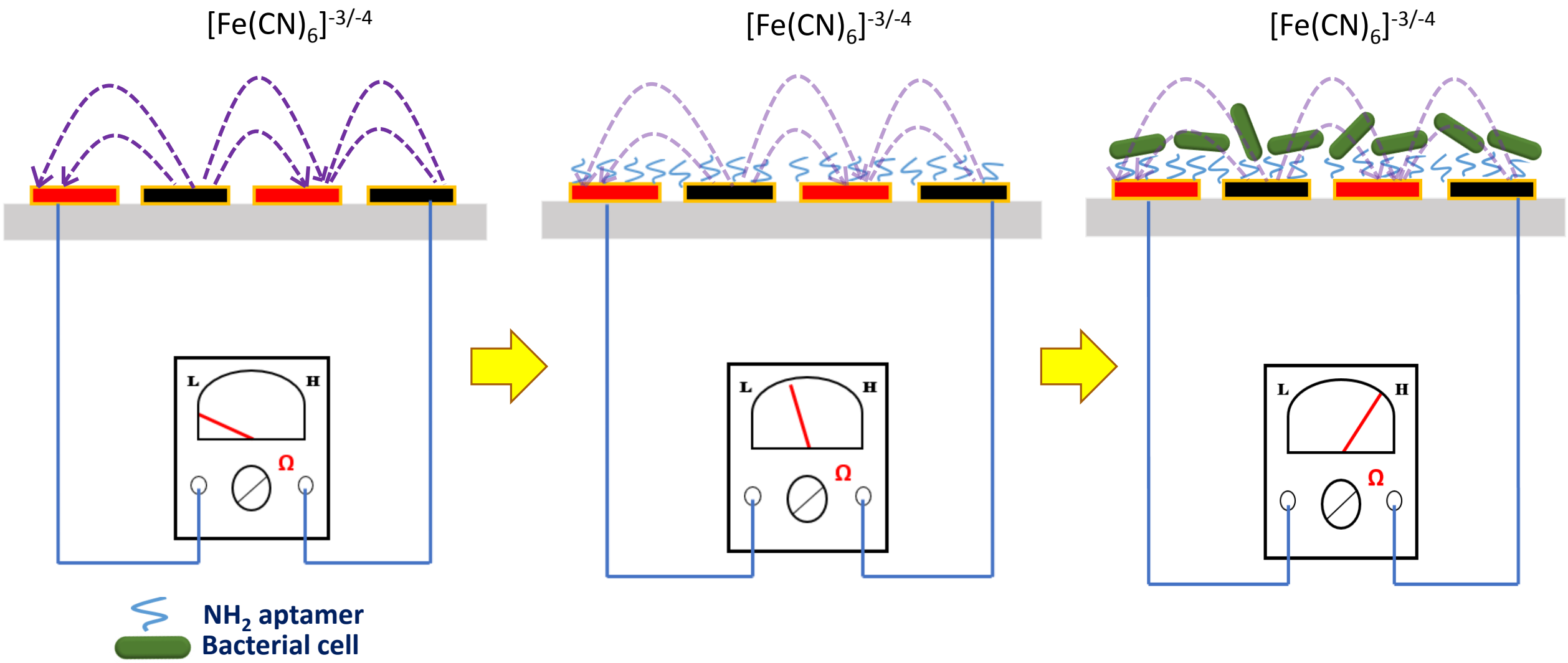

Fig. 3 Principle of the aptasensor

Materials

Reagents

- 16-mecaptohexadecanoic acid 20 mM **(MHDA)**
- N-(3-dimehylaminoporpyl)-N'-ethylcarbodiimide hydrochloride, N-hydroxysuccinimide solution, 75 mM/30 mM, v/v, 1:1 **(EDC/NHS)**
- NH_2-aptamer (Integrated DNA Technologies)
- Poly (ethylene glycol) methyl ether thiol, 0.1 mg/ml in PBS **(PEG)**
- Redox probe, $[Fe(CN)_6]^{3-/4-}$

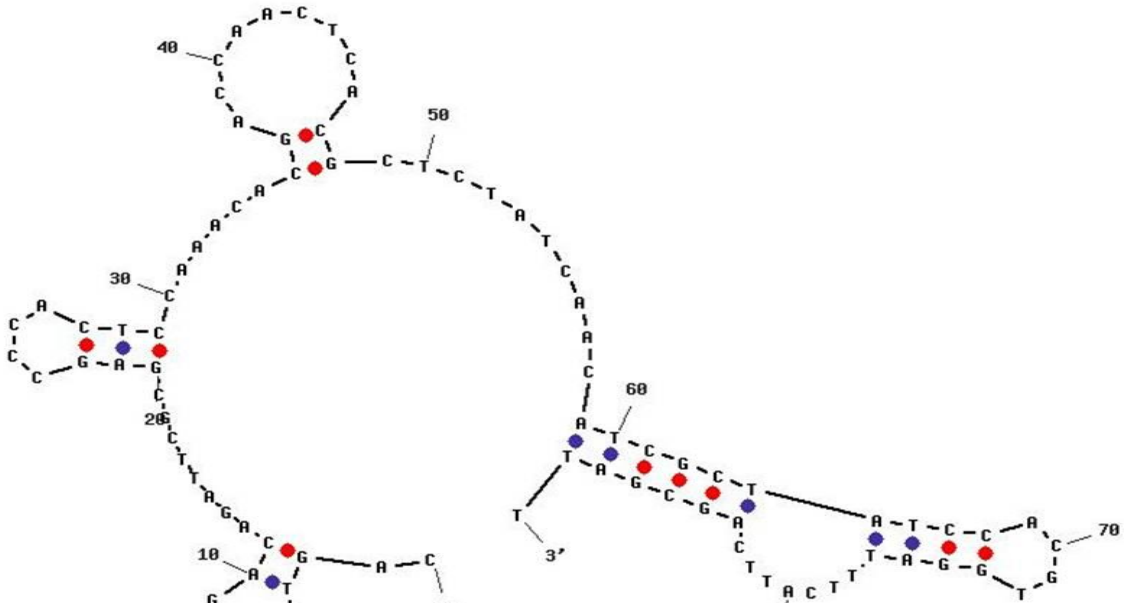

Fig. 4 Predicted secondary structure of the aptamer (Wang et al., 2017)*

Wang, L., Wang, R., Chen, F., Jiang, T., Wang, H., Slavik, M., ... & Li, Y. (2017). QCM-based aptamer selection and detection of *Salmonella* Typhimurium. Food chemistry, 221, 776-782.

Materials

Bacteria

- *Campylobacter jejuni* (ATCC 11168)
- *Escherichia coli* O157:H7 (ATCC 43888)
- *Escherichia coli* K12 (ATCC 29425)
- *Listeria monocytogenes* (ATCC 43251)
- *Listeria innocua* (ATCC 33090)
- *Salmonella* Typhimurium (ATCC 14028)

Electrode

- Interdigitated array microelectrode, IDME (15 µm digit width, 3 mm digit length, 15 µm inter-digit space)

Culture Preparation

- Cultures were grown in BHI broth for 18 h.
- Heat-killed using boiling water bath for 30 min.
- Non-selective, trypticase soy agar (TSA, EM Science) and selective agars were used for colony counting to obtain CFU/mL.
  - XLT_4 (Remel)
  - MacConkey sorbitol agar (Remel)
  - Modfied Oxford medium (Oxoid)
- Colonies were counted after 24 h incubation period.

Methods

1. Electrode cleaning with 1 M **NaOH** (30 min) and 1 M **HCl** (5 min) to remove surface oxide.
2. Functionalization of electrode surface with **MHDA** to form self-assembled monolayer (24 - 48 h).
3. Electrode surface activation with **EDC/NHS** (10 min).
4. Aptamer immobilization on electrode surface with 50 µL **NH_2-aptamer** (40 min).
5. Background noise blocking with 50 µl **PEG** (30 min).
6. Rest period at room temperature (48 h).
7. Bacteria detection (40 min).

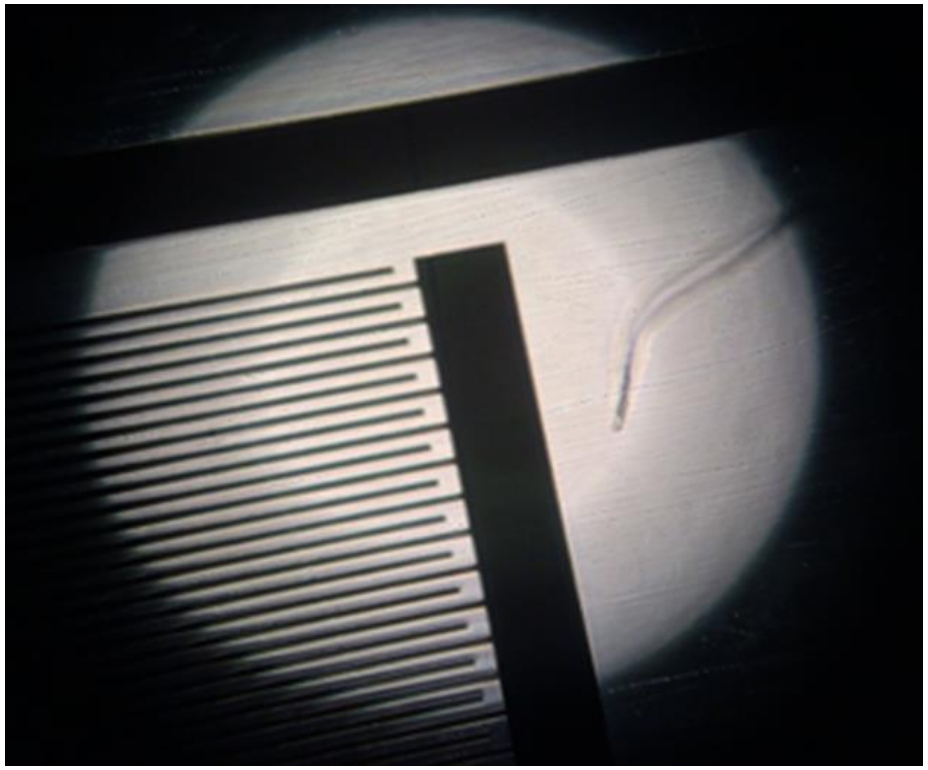

Fig. 5 Interdigitated array microelectrode

(Wen et al., 2017)*

Wen, T., Wang, R., Sotero, A., & Li, Y. (2017). A Portable Impedance Immunosensing System for Rapid Detection of *Salmonella* Typhimurium. *Sensors*, 17(9), 1973.

Data Analysis

Due to the fabrication and individual quality of each IDME, the baseline tended to drift. The relative impedance change was used to compare the baseline results of each IDME. The relative impedance change was calculated using equation 1.

$$Z_R = \frac{Z_T - Z_B}{Z_B} * 100\% \qquad (1)$$

Where,

Z_R = relative impedance change, %

Z_T = impedance values caused by target detection, Ω

Z_B = impedance value of the baseline, Ω

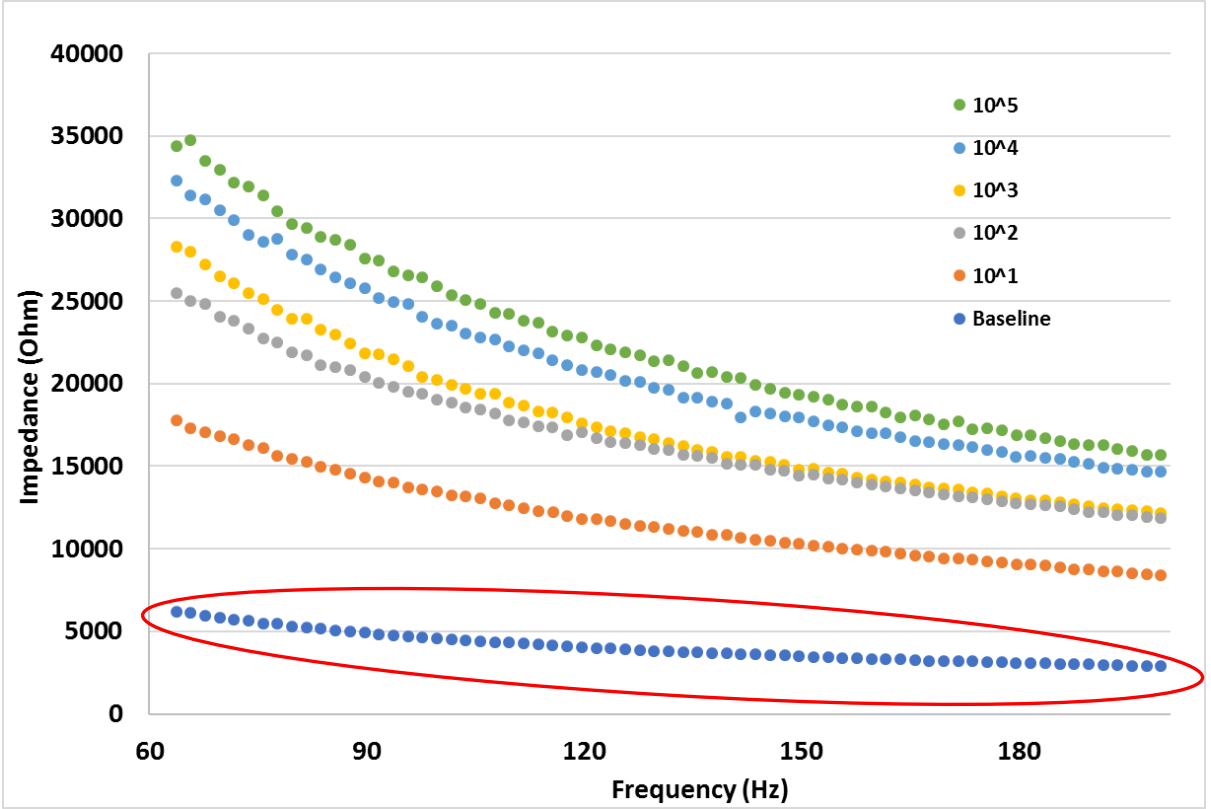

Fig. 6 Graph of the raw data showing a baseline

Results

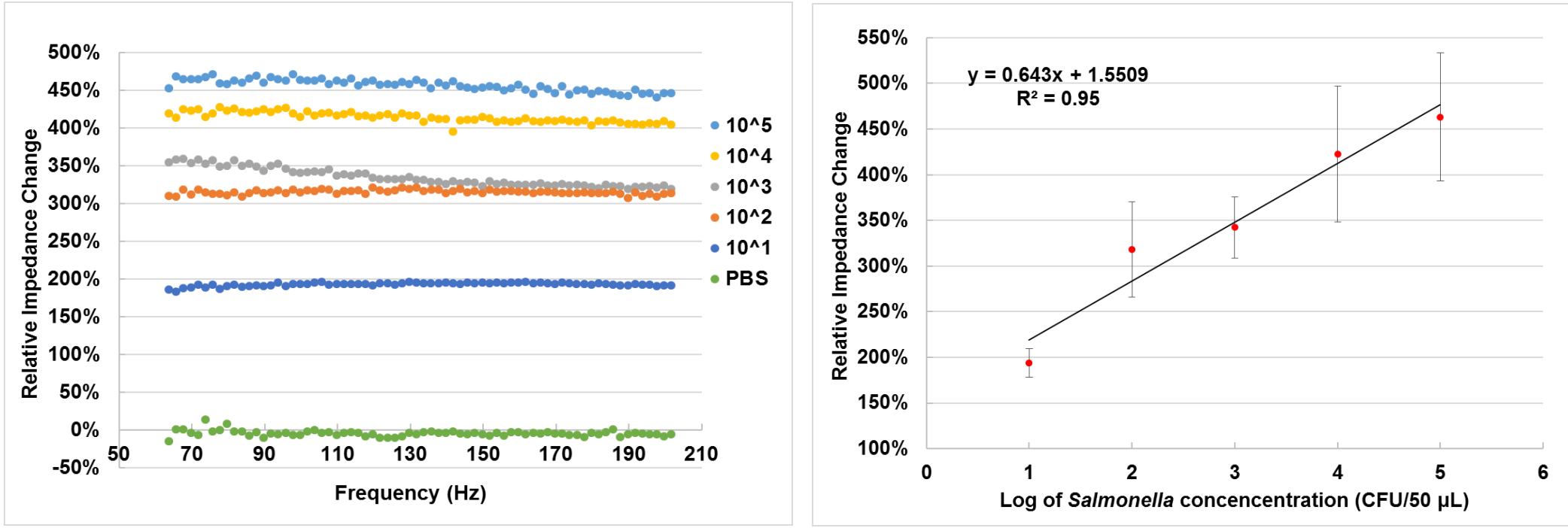

Fig. 7 (a) Relative impedance change of *S.* Typhimurium in concentrations ranging from 1.14×10^{1} to 1.14×10^{5} CFU/50 µL in pure culture samples; (b) linear relationship between the logarithmic value of the *S.* Typhimurium concentrations and the relative impedance change at frequency to 101 Hz. The mean and standard error bars were determined using two replication.

Results

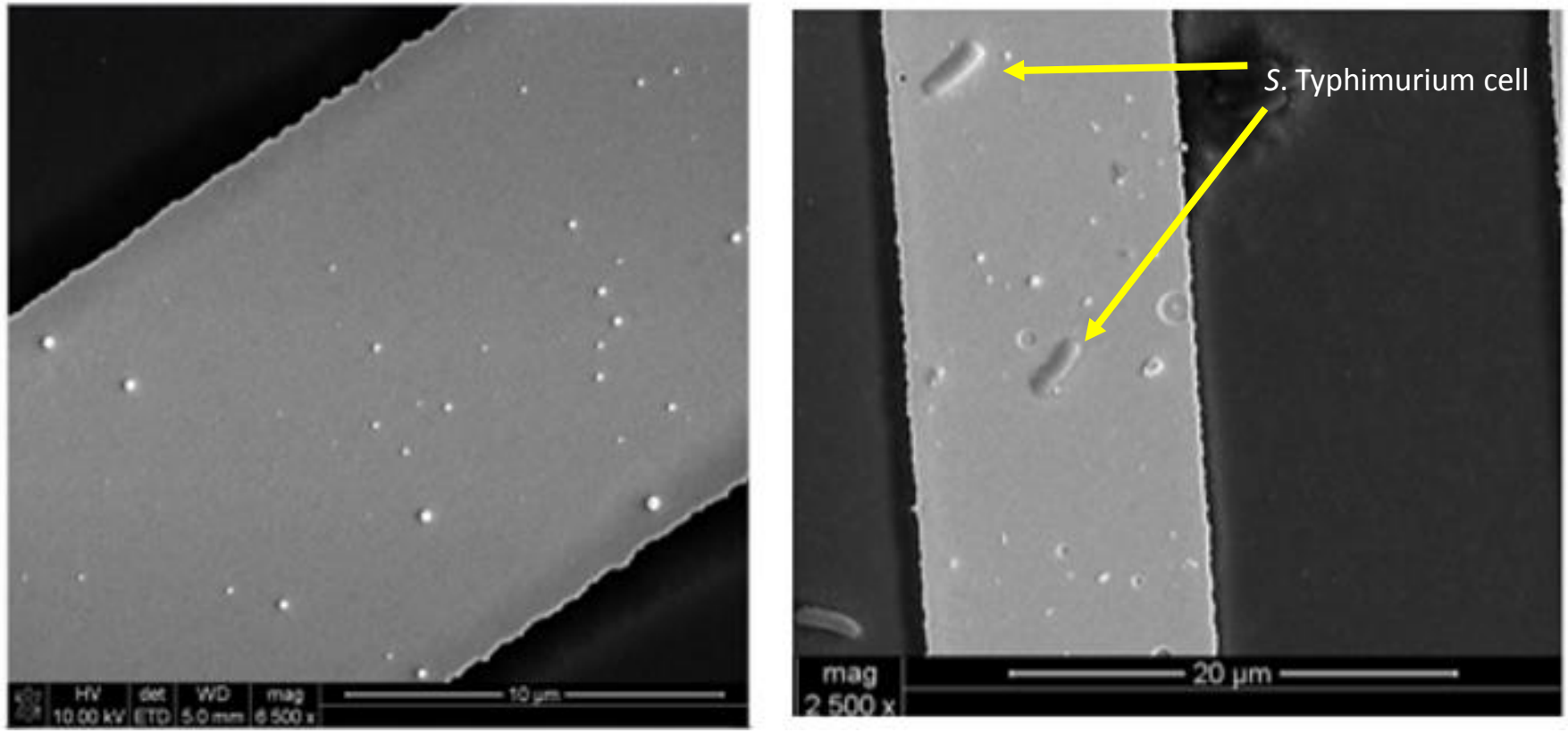

Fig. 8 (a) SEM image functionalized IDME; (b) SEM image of S. Typhimurium cells bound to aptamers immobilized on IDME surface.

Specificity of Developed Aptasensor

Table 1. Relative impedance change for each sample used in specificity tests

Sample	Mean Relative Impedance Change	Interpretation [1]
S. Typhimurium	364%	364%
E. coli O157:H7	-4%	0%
E. coli K12	-16%	0%
C. jenuni	-6%	0%
L. innocua	-3%	0%
L. monocytogenes	1%	0%

[1]A value of 0% was assigned if the sample failed to cause a positive change in signal.

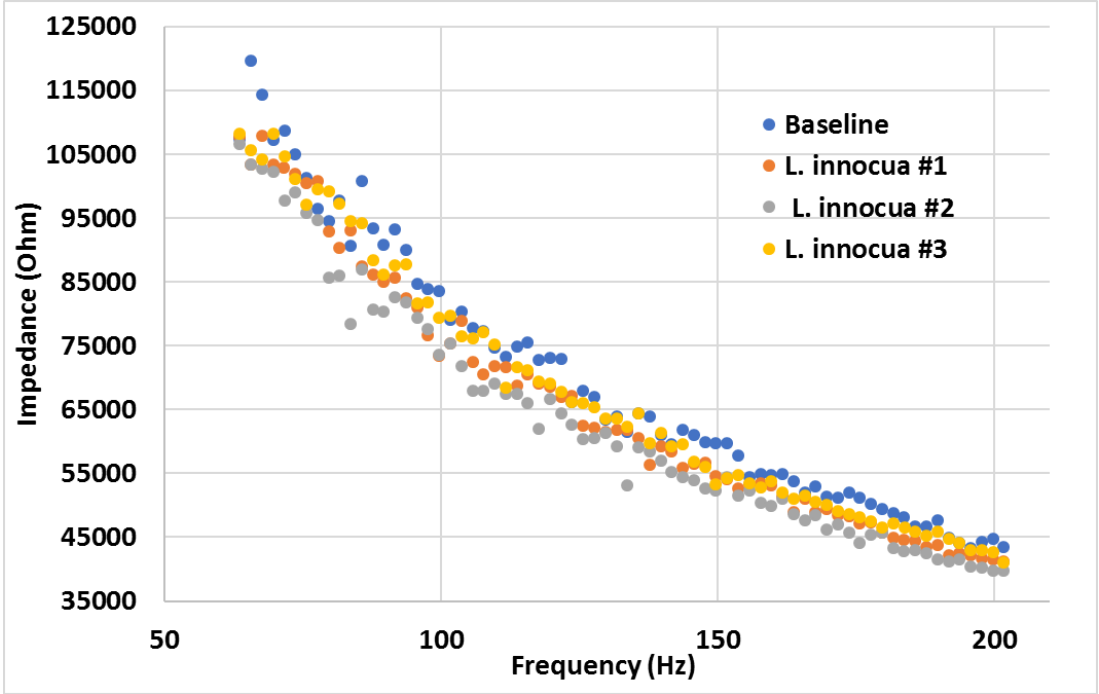

Fig. 9 Impedance curve generated in the test with *L. innocua*

Specificity of the Developed Aptasensor

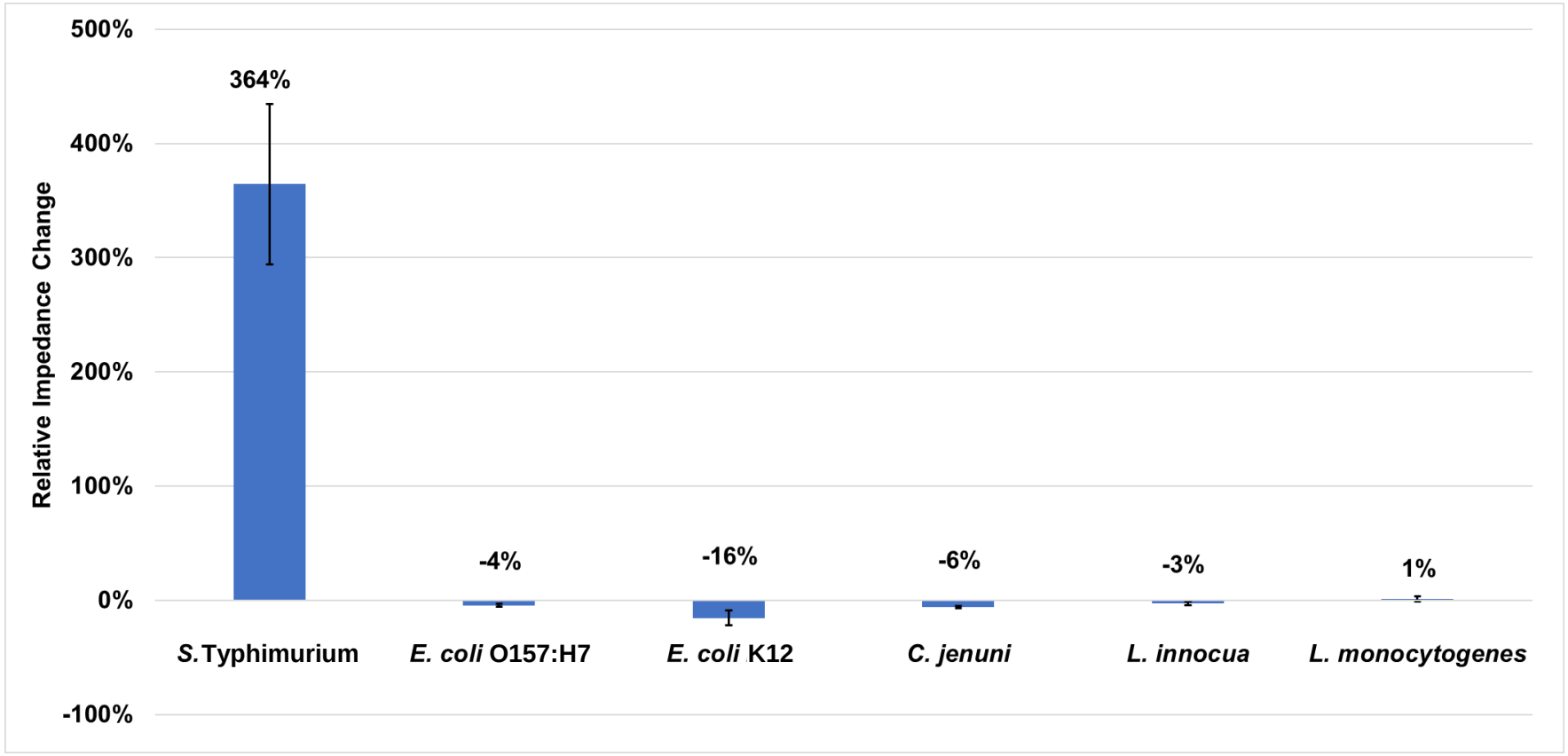

Fig. 10 Specificity results of the five non-target bacteria compared to *S.* Typhimurium in 10^5 (CFU/ 50µL)

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 from 1.41×10^{1} to 1.41×10^{5} CFU $(50\ \mu L)^{-1}$ of pure culture samples.
- The aptasensor also showed a high specificity for *S*. Typhimurium with an average relative impedance change of 463% compared to 0% - 1% relative impedance change of non-target bacteria (*C. jejuni, E. coli* K12, *E. coli* O157:H7, *L. innocua, and L. monocytogenes*) at a concentration of 10^{5} CFU/50 µL.
- 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 pathogens.

On-going Research

- Detection of *S*. Typhimurium in chicken rinse water
- Detection of *S*. Typhimurium in pure culture with IDME imbedded flow cell
- Detection of *S*. Typhimurium in chicken rinse water with IDME imbedded flow cell

Acknowledgments

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Thank you for your time!

Questions?