



Bacterial assessment of phage magnetoelastic sensors for *Salmonella enterica* Typhimurium detection in chicken meat



I-Hsuan Chen ^{a,*}, Shin Horikawa ^a, Kayla Bryant ^b, Rebecca Riggs ^b, Bryan A. Chin ^a, James M. Barbaree ^b

^a Materials Engineering Program, Department of Mechanical Engineering, Auburn University, Auburn, AL 36849, USA

^b Department of Biological Sciences, Auburn University, AL 36849, USA

ARTICLE INFO

Article history:

Received 19 February 2016

Received in revised form

3 June 2016

Accepted 1 July 2016

Available online 4 July 2016

Keywords:

Salmonella detection

Phage

Biosensors

Raw chicken

Bacterial detection

ABSTRACT

Salmonella is one of the most common pathogens associated with foodborne illness in chickens. Food outbreaks from this pathogen haven't declined in the past 15 years according to the data from Centers for Disease Control and Prevention. It is our goal to improve food safety monitoring in this area by developing a real time *Salmonella* detection sensor on food surfaces. Previously, we demonstrated the use of phage C4-22 immobilized onto a rapid magnetoelastic (ME) biosensor for use as a front-line detection ligand to detect all *Salmonella enterica* serotypes in Tris Buffer Saline (TBS). In this study, by using fluorescent imaging, the phage peptide binding to *Salmonella enterica* serotype Typhimurium cells is again confirmed. Moreover, we constructed two detection models to evaluate the detection of *Salmonella* on/in chicken meat using the phage coated ME sensors.

In the chicken surface detection method, phage C4-22 sensors demonstrated more than 12 times higher *Salmonella* binding capacity than the control sensors with no phage for the *Salmonella* spiked at the concentration of 7.86×10^5 cfu/mm². In the second model, phage sensors were placed at different depths inside the chicken breast (0.1 cm; 0.5 cm; 1.0 cm below the meat surface) after surface inoculation of *Salmonella*. The second detection system showed that 23.27%–33% of the inoculated *Salmonella* cells absorbed inside the chicken breast fillets below 0.1 cm of the surface.

The data for direct detection on chicken showed that phage C4-22 ME biosensors bind ultimately when there are high concentrations of *Salmonella* on the chicken surface. The results also suggest that the phage sensors can detect *Salmonella* effectively when the bacterial contaminants are absorbed into the chicken, and are not detectable by the surface detection method.

© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Every year, *Salmonella* is estimated to cause one million illnesses in the United States, with 19,000 hospitalization and 380 deaths (CDC, 2015b). Food outbreaks from this pathogen haven't declined in the past 15 years according to the data from Centers for Disease Control and Prevention (CDC, 2015a). On July 1, 2015, The Food Safety and Inspection Services in the United State Department of Agriculture (USDA-FSIS) issued a public health alert due to concerns about illnesses caused by *Salmonella* that may be associated with raw, frozen, breaded and pre-browned, stuffed chicken products. In 2012, a study reported that *Salmonella* spp. still are

prevalent at a high rate in raw chicken (>50%) despite the efforts to improve the hygiene in poultry processing plants (Abbassi-ghozzi et al., 2012).

Conventional microbiological methods and real time PCR based methods are commonly used to monitor *Salmonella* contamination in raw chicken. The conventional culture methods usually require days to confirm the contaminated target. While real time PCR may provide faster detection, it usually requires 16–18 h of pre-enrichment growth, short enrichment steps, or chicken rinses steps (Bailey & Cosby, 2003; Park et al., 2008). Besides, the sensitivity and specificity of PCR methods in detection of *Salmonella* contaminated poultry are limited and varied because of the non-standard methods for sample preparation, differences in enrichment protocols (Myint, Johson, Tablante, & Heckert, 2006), and inhibitors in the samples. A study showed different sample preparation methods can affect *Salmonella* detection results in chicken

* Corresponding author.

E-mail address: chenih1@auburn.edu (I.-H. Chen).

samples (Kanki et al., 2009.) Their results showed *Salmonella* positive data varied from 21.7% to 48% in the same lot of chicken prepared by two sample preparation methods in PCR detections. Therefore, it would be ideal to have a robust, real-time, and direct *Salmonella* detection method on chicken with no sample preparation methods and pre-enrichment steps.

The phage-based magnetoelastic (ME) biosensors have been successfully shown to detect various pathogens, including *Salmonella*, on food surfaces with high sensitivity and specificity (Li et al., 2010). Recently, a new platform of phage-based ME biosensors using a surface-scanning detector has been employed for the direct detection of *Salmonella* on various produce surfaces in real-time with no need of sample preparation (Chai et al., 2013). In addition, the direct detection of this new wireless phage biosensor platform coupled with a frequency monitoring device can be performed within 2–10 min (Horikawa et al., 2015.) The detection limit of these phage sensors is 500 cfu/mm² of *Salmonella* on tomato surfaces. This sensing method was selected as a finalist in the FDA 2014 Food Safety Challenge of *Salmonella* detection on fruits and vegetables (FDA Food Safety Challenge, 2014).

Bacteriophages have been used as promising molecular recognition probes immobilized on various sensor platforms for detection, such as ME sensors and acoustic wave biosensors (Nanduri et al., 2007;; Olsen et al., 2006). Previously, we have developed a high affinity phage oligopeptide probe C4-22 for detection of all *Salmonella enterica* serovars verified using an Enzyme-linked immunosorbent assays (ELISA) procedure, and demonstrated with ME sensors in a Tris Buffer Saline (TBS) model (Chen, Vaglenov, Chai, Chin, & Barbaree, 2014). Our previous data showed that these phage C4-22 ME biosensors had a 30 times higher binding capacity to test for *Salmonella* in TBS, when compared to the control sensors. In this study, we further evaluated this phage C4-22 ME biosensor for direct detecting *Salmonella* on the surfaces of chicken breast fillets and inside the chicken meat. In order to focus on studying the performance and effectiveness of phage probes capturing *Salmonella* on contaminated chicken samples, a microbiological method was used to analyze and calculate the percentages of phage sensors captured *Salmonella* on chicken. For reference purposes, phage ME sensors were put under the real-time frequency monitoring device for *Salmonella* detection on chicken samples using frequency measuring procedures in produce studies (Horikawa et al., 2015.)

2. Materials and methods

2.1. Binding of fluorescent labeled phages to *Salmonella enterica* Typhimurium

Phage labeling procedures were followed with modifications using the manual of Alexa Fluor 488 Succinimidyl Ester (NHS ester) Dye (Life Technologies, Carlsbad, CA.) In the first step, a polyethylene glycol (PEG)/ NaCl precipitation was performed. The solution of phage C4-22 at 2×10^{12} vir/ml was added by 20% w/v of PEG 8000 with 2.5 M NaCl (Kim et al., 2015.) The resulting sample was inverted 100 times and stored at 4 °C overnight. After microcentrifugation at 14,000 rpm for 15 min, the supernatant was discarded and the phage pellet was re-suspended in 0.1 M sodium bicarbonate solution (pH 8.3). Five microliters of Alexa-fluo 488 dye at concentration of 10 mg/ml was added to 200 μ l of phage solution. The tube was wrapped with foil and put on the rotator for 1 h at room temperature. Next, another PEG/ NaCl precipitation was performed to precipitate the labeled phages and followed by microcentrifugation. After discarding the supernatant, the pellet of fluorescent labeled phage was re-suspended in 100 μ l PBS. The Degree of Labeling (DOL) was calculated according to the manual.

After calculation, the fluorescent labeled phages prepared from above procedures were in the suitable labeling range suggested by manual and can be used for experiments (not over labeled or under labeled).

The Alexa Fluor 488 labeled phages were diluted to 10^{11} and 10^{10} vir/ml in PBS. *Salmonella enterica* Typhimurium AMES (ATCC 29631) was obtained from the laboratory of Dr. Stuart B. Price in Department of Pathobiology, Auburn University, AL. A solution of *S. enterica* Typhimurium (5×10^8 cfu/ml) was mixed with the equal volume of the labeled phage solution. The tube with the phage-*Salmonella* mixture was wrapped with foil and placed on the rotator for 1 h at room temperature for binding to occur. The solution was then centrifuged at $6000 \times g$ for 10 min, and the pellet washed twice with PBS. A Cytoviva fluorescent microscope (Cytoviva Dual Mode Fluorescent System, Cytoviva Inc., Auburn, AL 36832) was used for further analysis of the phage-*Salmonella* mixture. The photo data were recorded under full light illumination and the fluorescein isothiocyanate filter (FITC/ Excitation at 490 nm and Emission at 520 nm) with a total magnification of $100\times$.

2.2. *Salmonella* preparation on chicken

Packages of fresh boneless and skinless chicken breast fillets were purchased randomly from three local supermarkets in Auburn, AL. The packages of fresh chicken filets were used and stored in the refrigerator for two days from the date opened for experiments. Once the package of chicken filet was opened, the package was covered and sealed by a sterile plastic bag. Each piece of chicken breast was aseptically taken out from the package by a sterile twister and placed on a sterile Petri Dish under a biosafety cabinet without rinsing. Before inoculation of *Salmonella* on chicken, each inoculation spot was checked by swab test for the existence of *Salmonella* after plating on Brilliant Green Agar (BGA) (BD/Difco, Sparks, MD 21152, USA) and Xylose Lysine Deoxycholate plates (XLD) (Hardy Diagnostics, Santa Maria, CA 93445, USA) for an overnight incubation at 37 °C. If the inoculation spot showed *Salmonella* positive after overnight incubation, the data obtained from the spot was discarded.

Lennox Broth (LB broth) containing *Salmonella enterica* Typhimurium AMES was grown in a shaking incubator at 200 rpm and 37 °C overnight. *Salmonella* cultures were centrifuged at 5500 rpm for 10 min at 4 °C, and re-suspended in PBS twice. The bacterial concentrations were then adjusted to an OD of 600, which equals to 1.0 in PBS. Solutions of *Salmonella* at five concentrations of 7.86×10^3 , 3.93×10^4 , 7.86×10^4 , 3.93×10^5 , and 7.86×10^5 cfu/mm² were inoculated on the surface of chicken breast. The spiked spot was about 4.5 mm in diameter. Immediately after *Salmonella*-spiking, the spiked chicken was air-dried for 15 min under the biosafety cabinet. Then, the air-dried chicken was used for microbiological analysis by International Organization for Standardization (ISO) method (ISO 6579:2002) and for direct detection method of using biosensors (Section 2.3).

The ISO protocol consists of a pre-enrichment of 25 g *Salmonella* spiked chicken samples in 225 ml of buffered peptone water (BPW) (Hardy Diagnostics/ Criterion, Santa Maria, CA, USA) at 36 °C for 20 h. Then, the procedure is continued by putting 0.1 ml of pre-enrichment samples into 9.9 ml of selective enrichments in Rappaport–Vassiliadis Soy Broth (Hardy Diagnostics) at 41.5 °C, and 1 ml of pre-enrichment into 9.0 ml of Muller-Kauffmann Tetrathionate broth (TT) (Hardy Diagnostics) at 36 °C for 24 h, separately. A loopful (10 μ l) of each selective enrichment broth was transferred onto XLD plates and BGA plates for final identification of *Salmonella* in the spiked samples.

2.3. Surface detection of *Salmonella* on chicken meat

Magnetoelastic (ME) biosensors were obtained from the laboratory of Dr. Bryan A. Chin in the Materials Engineering Program, Auburn University, AL. The detail descriptions of sensor fabrication, dicing, and final treatment processes were present in [Chen et al., 2014](#). The ME sensors were cut into strip shapes at the size of 4 mm × 0.8 mm × 0.028 mm and coated with a final layer of Au (gold). The phages were bound to the gold-coated sensor layer due to hydrophobic binding, weak hydrogen bonding, van der Waals forces, and covalent binding between the gold surface and cysteine residues in the minor coat protein of the phage ([Nanduri et al., 2007](#)).

Phage C4-22 was selected through Phage Display method. The detail biopanning procedures, sensitivity tests, and specificity of this phage were described in [Chen et al. \(2014\)](#). Phage sensors were prepared by coating with 1×10^{10} virions of phage C4-22 in TBS for an hour at room temperature, and washed three times with TBS. BSA (0.1%), which was used as a blocking reagent after phage coating on the sensor. Sensors coated with 0.1% BSA only served as controls. At each *Salmonella* concentration group, three phage sensors were placed on three individually spiked spots on the same chicken for *Salmonella* detection. The experiments were done three times using different pieces of chicken samples. After direct detection on air-dried chicken, cells of *Salmonella enterica* Typhimurium captured by each sensor were eluted with 0.1 M Glycine (pH 2.2) to break phage-*Salmonella* binding and then neutralized with 1 M Tris-HCl (pH 9.1.) The numbers of *Salmonella* were determined using a standard aerobic plate count method with TSA and BGA plates. The *Salmonella* captured effectiveness by the biosensors was calculated as: The percent *Salmonella* binding on biosensors = $(Ac/Ci) \times (\text{the Elution Factors}) \times 100\%$. Where Ac is the average *Salmonella* cell counts (triplicates) eluted from one Sensor and Ci is the input of *Salmonella* concentration on the sensors. Each *Salmonella*-spiked concentration was used in three sensor experiments to calculate the means ± standard deviations among each test group.

2.4. Real-time frequency measurements of biosensors after direct detection on chicken

Phage ME sensors and control sensors were prepared according to the description in Section 2.3. Three concentrations of *Salmonella* solutions at 7.86×10^3 , 7.86×10^4 , and 7.86×10^5 cfu/mm² were used to spike on the surface of chicken breasts. The procedures of preparing *Salmonella* contaminated chicken samples were described as in Section 2.2. On the same piece of chicken filet, three sensors were placed on three individually spiked spots at each *Salmonella* concentration. The experiments were done twice using different pieces of chicken breast samples. After placing the sensor directly on the spiked spot for 2 min, the sensor was put under the surface scanning coil for wireless frequency measurements. The sensor placements, sensor contacting time with food, and frequency monitoring procedures were followed by the procedures described in [Horikawa et al., 2015](#).

2.5. Inside meat detection of *Salmonella* on chicken meat

During the surface detection experiments, it was found that the spiked *Salmonella* solution was quickly absorbed by the chicken meat. Therefore, in the second method, phage sensors were placed at different depths of 0.1 cm, 0.5 cm, and 1.0 cm inside the chicken filet. Each chicken breast with more than 1.0 cm thickness was preselected, and placed aseptically onto a sterile Petri Dish under the biosafety hood. Solutions of *Salmonella enterica* Typhimurium

at three concentrations (7.89×10^3 cfu/mm², 7.89×10^4 cfu/mm², and 7.89×10^5 cfu/mm²) were separately spiked on the surface of chicken breast and allowed to dry for 15 min. Phage sensors preparation and cells captured calculations were the same as mentioned in Section 2.3.

2.6. Statistical analysis

Data collected for surface detection of *S. enterica* Typhimurium on chicken breasts were analyzed by ANOVA, and regression calculations were determined using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA). The α value was 0.05.

3. Results and discussion

3.1. Binding of fluorescent labeled phage to *Salmonella enterica* serotype Typhimurium

Engineered phages that have properties of having a wide range of functional groups on its surfaces can be selected through phage display methods to have specificity and sensitivity toward a certain target. This type of selected phage has been used to show that it can serve as an analytic ligand on biosensors ([Zhu, White, Suter, & Fan, 2008](#)) and other assay platforms, like ELISAs and colorimetric Lateral Flow assays (LFAs) ([Kim et al., 2015](#)).

Moreover, phages labeled with fluorophores provide ways to track or direct visualize the interaction of the fluorescent phage toward its target. Therefore, the procedure of fluorescent labeling of phages is essential. There are two methods for phage labeling: direct fluorophores labeling on phages or labeling on anti-phage antibodies ([Abbineni, Safiejko-Mroczka, & Mao, 2010](#).) Here, we chose to use a PEG precipitation process instead of dialyzing the phage into the sodium bicarbonate solution (pH 8.3), and labeling the phage directly with commercial fluorophores ([Lin et al., 2011; Kim et al., 2015](#)). PEG precipitation method is a simple procedure without having steps of buffers changing in every 4–8 h. Besides, it is easier to retain the original phage volume for labeling, when there are always risks to not withdraw all liquid from dialysis caste in the dialyzed method. After the calculations of the DOL provided by the manual, the sensitivity and stability of the labeled phages prepared from both methods didn't show differences (data not shown.)

Our results here showed successful fluorescent images of labeled phage C4-22 binding with *Salmonella* cells. Microscope images in [Fig. 1](#) – showed *Salmonella* only without phage as control under regular light illumination (no filter.) Images of the binding of Alexa-Fluor 488 labeled phage to *Salmonella* cells are shown in [Fig. 1-2 and 1-3](#), under the magnification of 100× with the FITC filter. In [Fig. 1-2 and 1-3](#), the fluorescent labeled phages were prepared with a one log differences at the concentrations of 1×10^{10} and 1×10^{11} vir/ml. In the setting for the higher concentration of phage binding to *Salmonella* in [Fig. 1-3](#), the higher intensity of green fluorescence appeared on the *Salmonella* cell surfaces than in [Fig. 1-2](#). These data give us clear visualized characteristics of the phage probe C4-22 binding to *S. enterica* Typhimurium cells.

3.2. Surface detection of *Salmonella* on chicken breast filets

In the surface detection study of chicken, the ISO method of “Isolation of *Salmonella* spp. from food and animal feces” (ISO 6579:2002, 2010 version) was used to confirm positive results of *Salmonella* artificially spiked on the chicken breasts. This conventional culture method took more than 72 h to identify the presence of *Salmonella* which is causing a lot of labor time. Conversely, the

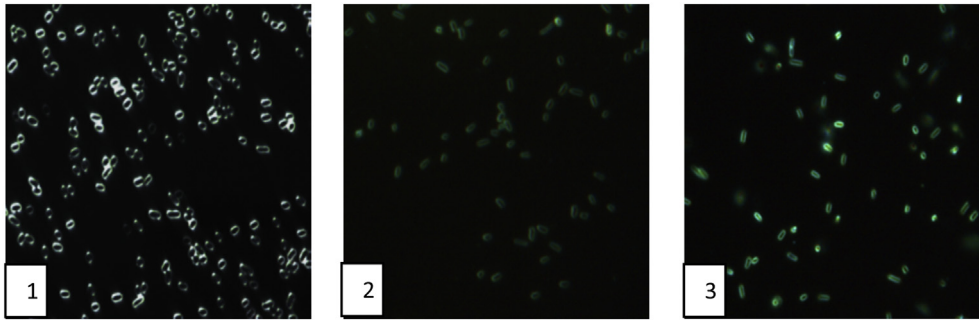


Fig. 1. Images of fluorescent labeled phage C4-22 binding to *Salmonella enterica* Typhimurium cells. (1) *Salmonella* only without C4-22 phages under CytoViva Fluorescent Microscope (regular light; 100×) (2) fluorescent labeled phages (1×10^{10} virions; FITC filter; 100×) and *Salmonella* (3) fluorescent labeled phages (1×10^{11} virions) with *Salmonella*.

use of the phage ME biosensor coupled with the frequency monitoring device took only 2–10 min for *Salmonella* detection on food surfaces, and it was not necessary to perform any sample preparation. Captured *Salmonella* by sensors were analyzed through plate count methods. The evaluation of *Salmonella* captured by phage C4-22 ME biosensors on spiked chickens is depicted in Fig. 2. Phage C4-22 sensors showed a 12 times higher *Salmonella* binding capacity than the control sensors at the *Salmonella* spiked concentration of 7.86×10^5 cfu/mm². In overall spiked concentrations, the phage sensors demonstrated a significantly higher binding ability towards *Salmonella* than the control sensors ($P < 0.05$; $r = 0.976$.) The percent of *Salmonella* captured by sensors increased (Fig. 2), while the spiked concentrations increased. There was a dose response relationship between spiked concentrations and *Salmonella* captured by phage sensors (Fig. 3). This microbiological data presented the applicability of using phage ME biosensors for front-line foodborne pathogen detections on raw chicken products in poultry processing plant or outbreak investigations.

The frequency measurements of phage sensors and control sensors for direct *Salmonella* detection on spiked chicken were also conducted. In Fig. 4, it is clearly shown that the resonant frequency shifts in phage sensor groups were higher than the control sensor groups at all three *Salmonella* spiked concentrations. Only 0.065% of *Salmonella* (Fig. 2) can be detected by the plate count methods at *Salmonella* spiked concentration of 7.85×10^3 cfu/mm² on chicken, ME biosensors can still detect *Salmonella* positives at the same spiked concentration by real-time measuring the mass changes on

sensors using surface-scanning coil device (Fig. 4). The frequency shifts data provides the demonstration of rapid and effective frequency monitoring abilities using the phage ME sensors for direct *Salmonella* detection on real food surfaces.

According to Li et al. (2010) and Horikawa et al. (2015), the *Salmonella* detection limits of phage ME biosensors on tomato

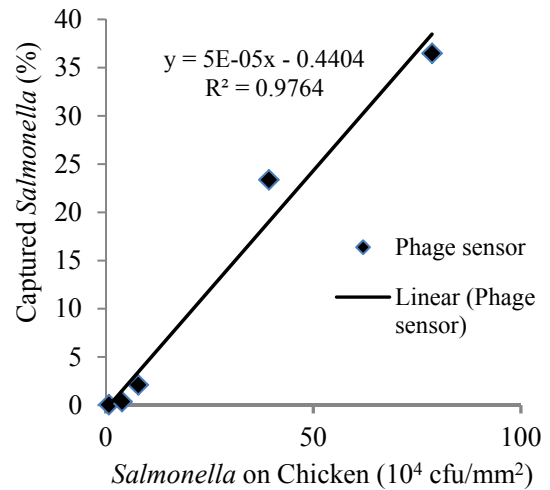


Fig. 3. Linear regression graph of the dose response relationship between spiked concentrations and *Salmonella* captured by phage sensors.

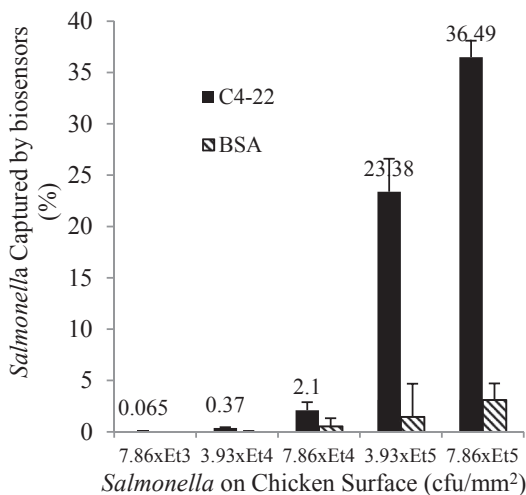


Fig. 2. Percent *Salmonella* captured on chicken surfaces by phage coated ME biosensors.

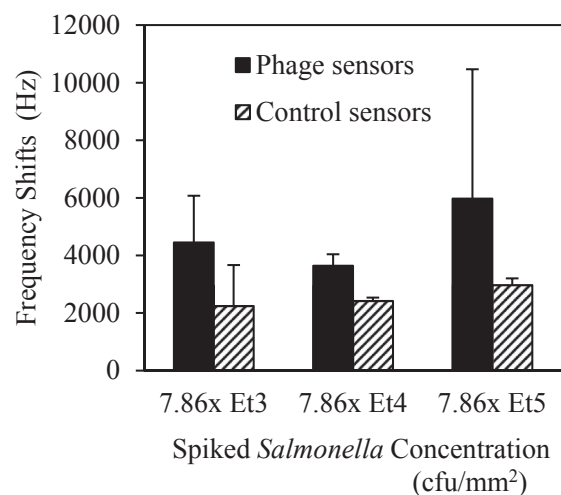


Fig. 4. Frequency measurements of ME biosensors for detection *Salmonella* on the surface of chicken breasts.

surfaces was 500 *Salmonella* cfu/mm² using frequency monitoring. It was noticed that the detection limits of this chicken surface study by plate count methods appeared to be 7.85×10^3 cfu/mm² (Fig. 2), which is a higher *Salmonella* concentration compared to previous findings. There are two possible factors which are contributing the higher numbers of the detection limit. The first factor is using a glycine solution to elute *Salmonella* from phage sensors. This acid wash step could kill or damage some *Salmonella* cells in the elution liquid and affected the plate count results.

Another possible influencing factor would be the different surface characteristics of tomatoes and chicken. It is understood that the surfaces of tomatoes and chicken breasts are physically totally different. The muscle from the poultry meat is approximately 75% water, 20% protein, and 5% representing a combination of fat, carbohydrate and minerals (Food Safety and Inspection Service, 1996). The fat in meats is found both between muscles and within muscles. As for the surfaces of tomatoes, it has found to consist of cutin, cuticle waxes, polysaccharides, and phenols (López-Casado, Matas, Domínguez, Cuartero, & Heredia, 2007; Mintz-Oron et al., 2008). The differences of surface structure and topology may affect the detection limits.

In addition, according to our previous data of whole cell ELISA tests (Chen et al., 2014), phage C4-22 showed higher binding affinity to *Salmonella enterica* serotypes in O-antigen D group than in *Salmonella* O-antigen B and C groups. The detection limit of using the phage C4-22 sensors on a food samples contaminated in mix *Salmonella* populations should be further studied. During the experiments, we also observed that the spiked *Salmonella* solution was quickly absorbed into chicken meat. This discovery led us to conduct another model for detecting *Salmonella* inside the chicken breast fillets.

3.3. Inside meat detection of *Salmonella* on chicken meat

Fig. 5 illustrates the use of the phage ME biosensor to detect *Salmonella* inside chicken meat. The phage sensors detected *Salmonella* at three different depths inside the chicken breast. After collecting more than 30 chicken breast fillets randomly from local grocery stores, the thickness of the chicken breasts were between 1.0 and 2.0 cm (data not shown). Due to this finding, we chose to detect *Salmonella* inside chicken breasts at a depth up to 1 cm, which should be at the center of the thickest chicken breast purchased from the retail package. In Fig. 6, the results showed when *Salmonella* was spiked at a concentration of 7.86×10^5 cfu/mm², phage ME biosensors detected more than 30% of the inoculated *Salmonella* cells absorbed inside the meat below 0.1 cm of the chicken surface. Twenty three percent of *Salmonella* was detected

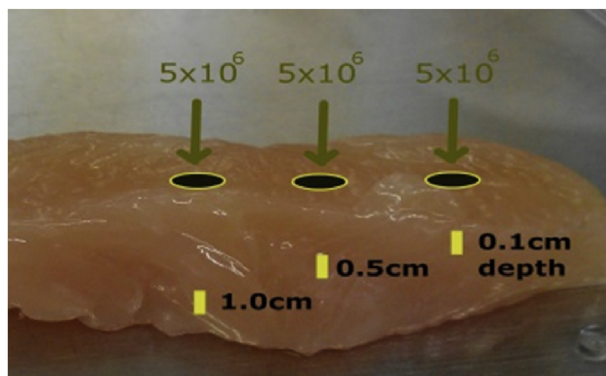


Fig. 5. *Salmonella* detection inside the chicken breast by phage ME biosensors. Each sensor is depicted as a yellow square.

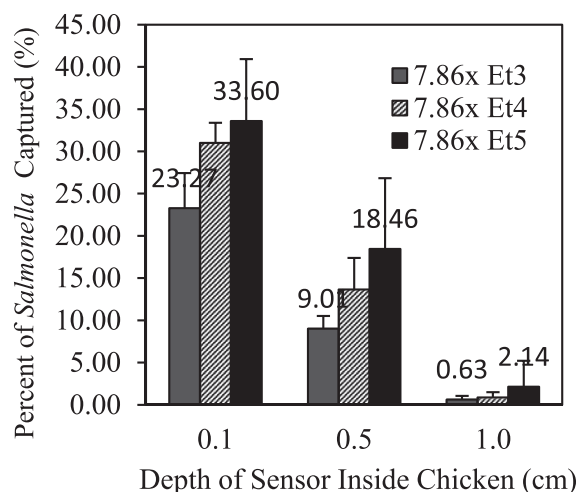


Fig. 6. Percent *Salmonella* captured inside the chicken meat by ME biosensors (data of control sensors were subtracted).

at the depth of 0.5 cm. There was only 2.15% spiked *Salmonella* detected when the phage sensors were at a depth of 1.0 cm in the chicken breast fillet. At the lower *Salmonella* spiked concentration of 7.86×10^3 cfu/mm² and 7.86×10^4 cfu/mm², there were still 23.27%–31% of spiked *Salmonella* detected at the depth of 0.1 cm inside the chicken meat.

Chicken breasts consist mainly of water (77.49 g) in 100 g of chicken meat (de Almeida, Perassolo, Camargo, Bragagnol, & Gross, 2006). Our data indicates that if there is a bacterial contamination on the surface of chicken meat, the pathogens may absorb down to at least 1 cm of the chicken meat (Fig. 6) possibly via the water content inside the meat or the juice inside the package. This inside meat detection method allows us to directly monitor *Salmonella* or other targeted pathogens absorbed into the chicken breasts without any sample preparation. This is the first report to our knowledge about direct sensor detection on the chicken surface and inside the chicken meat.

4. Conclusion

In summary, it is proven that phage C4-22 probes could be effectively used on ME sensor platform to detect low *Salmonella* concentration of 7.86×10^3 cfu/mm² on raw chicken breast fillets. Furthermore, by using the proposed phage sensors coupled with the frequency scanning devices, *Salmonella* detection on raw chicken samples can be easily monitored in real-time on site without sample preparation procedures and pre-enrichment steps. Other than the advantages of shorter response time and simplified steps, the inside meat detection method presented here also enables more detailed monitoring of *Salmonella* contaminated chicken.

Acknowledgment

This work was supported by Auburn University Detection and Food Safety Center (AUDFS) and a USDA grant (USDA-2011-51181-30642A). We would like to thank Elyse V. Johnson from Cytoviva Inc. for her assistance with the fluorescent imaging.

References

- Abbassi-ghozzi, I., Jaouani, A., Hammami, S., Martinez-urtaza, J., Boudabous, A., & Gtari, M. (2012). Molecular analysis and antimicrobial resistance of *Salmonella* isolates recovered from raw meat marketed in the area of "Grand Tunis"

- Tunisia. *Pathologie Biologie (Paris)*, 60, 49–54.
- Abbineni, G., Safiejko-Mroccka, B., & Mao, C. (2010). Development of an optimized protocol for studying the interaction of filamentous bacteriophage with mammalian cells by fluorescence microscopy. *Microscope Research Technology*, 73(5), 548–554. <http://dx.doi.org/10.1002/jemt.20793>. May.
- Almeida, J. C., Perassolo, M. S., Camargo, J. L., Bragagnol, N., & Gross, J. L. (2006). Fatty acid composition and cholesterol content of beef and chicken meat in Southern Brazil. *Brazilian Journal of Pharmaceutical Science*, 42(1), 109–117. <http://dx.doi.org/10.1590/S1516-93322006000100012>.
- Bailey, J. S., & Cosby, D. E. (2003). Detection of *Salmonella* from chicken rinses and chicken hot dogs with the automated BAX PCR system. *Journal of Food Protection*, 66(11), 2138–2140.
- Center for Disease Control and Prevention (CDC). (2015a). *Salmonella Infection hasn't decline for 15 years*. <http://www.cdc.gov/vitalsigns/FoodSafety/index.html> Accessed 02.05.16.
- Center for Disease Control and Prevention (CDC). (2015b). *Pathogen causing US foodborne illness, hospitalizations, and death, 2000–2008*. <http://www.cdc.gov/foodborneburden/PDFs/pathogens-complete-list-01-12.pdf> Accessed 02.05.16.
- Chai, Y., Wikle, H. C., Wang, Z., Horikawa, S., Best, S., Cheng, Z., et al. (2013). Design of a surface-scanning coil detector for direct bacteria detection on food surfaces using a magnetoelastic biosensor. *Journal of Applied Physics*, 114, 104504. <http://dx.doi.org/10.1063/1.4821025>.
- Chen, I., Vaglenov, K., Chai, Y., Chin, B. A., & Barbaree, J. M. (2014). Use of LPS extract to validate phage oligonucleotide that binds all *Salmonella enterica* serovars. *Advances in Microbiology*, 4, 549–559. <http://dx.doi.org/10.4236/aim.2014.49061>.
- Food Safety and Inspection Service (FSIS). (1996). *Water in Meats: Moisture content of meats and poultry*. <http://dwb.unl.edu/teacher/nsf/c10/c10links/www.fsis.usda.gov/oa/pubs/watmeat.htm> Accessed 02.05.16.
- Horikawa, S., Chai, Y., Wikle, H. C., III, Suh, S. J., Barbaree, J. M., & Chin, B. A. (2015). Direct detection of *Salmonella* on fresh produce. *ECS Transactions*, 69(38), 25–31.
- International Organization for Standardization (ISO). (2002). Laboratory protocol "Isolation of *Salmonella* spp. From food and animal faces." ISO 6579:2002. <https://www.iso.org/obp/ui/#iso:std:iso:6579:ed-4:v1:en> Accessed 02.05.16.
- Kanki, M., Sakata, J., Taguchi, M., Kumeda, Y., Ishibashi, M., Kawai, T., et al. (2009). Effect of sample preparation and bacterial concentration on *Salmonella enterica* detection in poultry meat using culture methods and PCR assaying of preenrichment broths. *Food Microbiology*, 26(1), 1–3. <http://dx.doi.org/10.1016/j.fm.2008.07.010>. Epub 2008 Aug 22.
- Kim, J., Adhikari, M., Dhamane, S., Hagström, A. E. V., Kourentzi, K., Strych, U., et al. (2015). Detection of viruses by counting single fluorescent genetically biotinylated reporter immunophage using a Lateral Flow Assay. *ACS in Applied Materials and Interface*, 7(4), 2891–2898. <http://dx.doi.org/10.1021/am5082556>.
- Li, S., Li, Y., Chen, I. H., Horikawa, S., Shen, W., Simonian, A., et al. (2010). Direct detection of *Salmonella typhimurium* on fresh produce using phage-based magnetoelastic biosensors. *Biosensors and Bioelectronics*, 26, 1313–1319. <http://dx.doi.org/10.1016/j.bios.2010.07.029>.
- Lin, A., Jimenez, J., Derr, J., Pedro, V., Manapat, M. L., Esvelt, K. M., et al. (2011). Inhibition of bacterial conjugation by phage M13 and its protein g3p: Quantitative analysis and model. *PLoS One*, 6(5), e19991.
- López-Casado, G., Matas, A. J., Domínguez, E., Cuartero, J., & Heredia, A. (2007). Biomechanics of isolated tomato (*Solanum lycopersicum* L.) fruit cuticles: The role of the cutin matrix and polysaccharides. *Journal of Experimental Botany*, 58(14), 3875–3883. <http://www.ncbi.nlm.nih.gov/pubmed/17975209>.
- Mintz-Oron, S., Mandel, T., Rogachev, I., Feldberg, L., Lotan, O., Yativ, M., et al. (2008). Gene expression and metabolism in tomato fruit surface tissues. *Plant Physiology*, 147(2), 823–851. <http://dx.doi.org/10.1104/pp.108.116004>.
- Myint, M. S., Johnson, Y. J., Tablante, N. L., & Heckert, R. A. (2006). The effect of pre-enrichment protocol on the sensitivity and specificity of PCR for detection of naturally contaminated *Salmonella* in raw poultry compared to conventional culture. *Food Microbiology*, 23(6), 599–604. Epub 2005 Nov 8.
- Nanduri, V., Sorokulova, I. B., Samoylov, A. M., Simonian, A. L., Petrenko, V. A., & Vodyanov, V. J. (2007). Phage as molecular recognition elements in biosensors immobilized by physical adsorption. *Biosensors and Bioelectronics*, 22, 986–992. <http://dx.doi.org/10.1016/j.bios.2006.03.025>.
- Olsen, E. V., Sorokulova, I. B., Petrenko, V. A., Chen, I.-H., Barbaree, J. M., & Vodyanov, V. J. (2006). Affinity-selected filamentous bacteriophage as a probe for acoustic wave biodetectors of *Salmonella typhimurium*. *Biosensors and Bioelectronics*, 21(8), 1434–1442.
- Park, H.-J., Kim, H.-J., Park, S.-H., Shin, E.-G., Kim, J.-H., & Kim, H.-Y. (2008). Direct and quantitative analysis of *Salmonella enterica* serovar Typhimurium using real-time PCR from artificially contaminated chicken meat. *Journal of Microbiological Biotechnology*, 18(8), 1453–1458.
- U.S. Food and drug administration (FDA). (2014). Food Safety Challenge/Finalist Abstract/Auburn University. <http://www.foodsafetychallenge.com/finalistsabstracts/> Accessed 02/05/2016.
- Zhu, H., White, I. M., Suter, J. D., & Fan, X. (2008). Phage-based label-free biomolecule detection in an opto-fluidic resonator. *Biosensors and Bioelectronics*, 24, 461–466.