Development of on-site bacteriophage-based magnetoelastic biosensor method for *Salmonella* detection on fresh produce
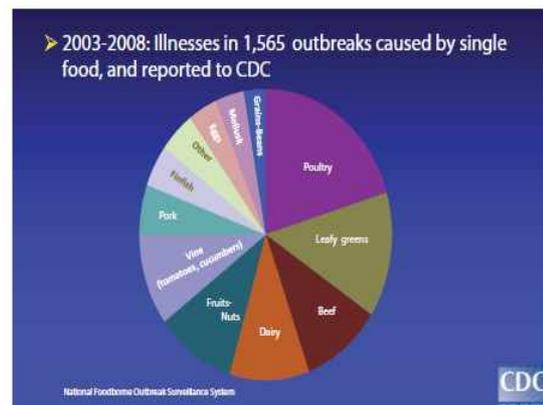
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From 1996 to 2008, eighty-two foodborne illness outbreaks was associated with the consumption of a wide variety of fresh produce. (Beuchat 2002; Lynch et al. 2009). *Salmonella* was one of main causes for the fresh produce-associated outbreaks (Lynch et al. 2009). Contamination of fresh produce with *Salmonella* may occur anywhere along the route from farm to table due to the presence of tainted soil, manure, fouled irrigation water, feces of rodents or ruminants, unsanitary workers, and improper storage and handling (Beuchat and Rye 1997; Guo et al. 2002). Furthermore, currently increasing imports from other country requires systematic inspection plan in-field and entire food supply chain along with a sensitive and reliable on-site detection method. Therefore, a free-standing, phage-based magnetoelastic (ME) biosensor has been developed as a novel wireless system for real-time and on-site detection method (Li et al. 2010; Park et al. 2012).

Recent studies have demonstrated new direct method for *Salmonella* detection on the fresh surfaces using the ME biosensors. In their studies, the sensors were directly placed on food surfaces, where binding of the

sensors with target bacteria occurred, followed by measurement of the sensors. In addition, the ME biosensors could detect *Salmonella* on the surface of a fresh produce without any washing procedures and sample preparation (Fig. 1). Furthermore, minimizing the biosensor size down to 1 mm length enhanced the sensitivity and cost-effectiveness of ME biosensor method. These recent studies suggest the potential applications of ME biosensor to on-site detection of pathogens. For the valid evaluation of the ME biosensor, it was compared with other widely recognized alternative detection methods such as qPCR. The purpose of this study was to demonstrate the detection of *Salmonella* on the surface of fresh produce on-site and to evaluate the ME biosensor method by comparison with qPCR method. Finally, the practicability and applicability of the ME biosensor method was compared with qPCR assay for use as an on-site and in-field detection method.

1.1 Observation of surface morphology of fresh produce

The surface morphologies of tomatoes and spinach observed by the SEM are illustrated in Fig. 2. Since the spinach had two exposed surfaces for the placement of sensors, they are called adaxial (top) (Fig. 2B) or abaxial (bottom) surfaces (Fig. 2C). As expected, the surface morphology of spinach was obviously different from that of tomato. The tomato had ridges and depressions while the spinach had valleys and peaks. In addition, peaks found on the adaxial surface of spinach were rougher and deeper than the peaks on the abaxial surface. Although both surfaces of the spinach had stomata (arrow bar in Fig. 2C), the abaxial surface had more stomata and some stomata on the abaxial surface were wider than the stomata on the adaxial surface. This result agreed with other studies in that the abaxial surface of spinach usually has significantly more stomata than the adaxial surface.
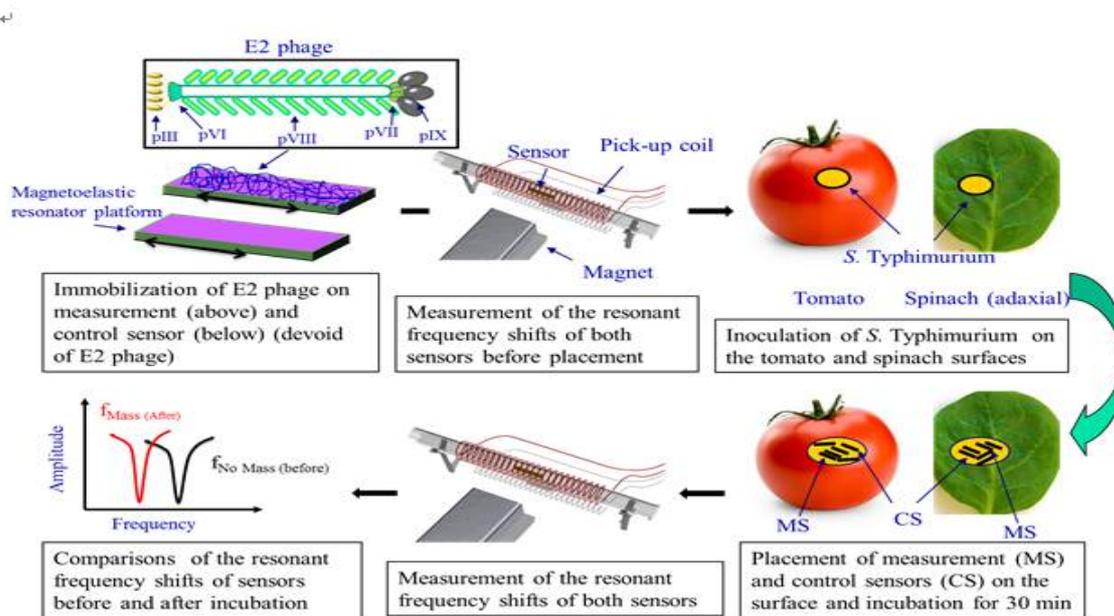

Fig. 1. Scheme used for the direction of *Salmonella* on a tomato and spinach surface using a phage-based ME biosensor method
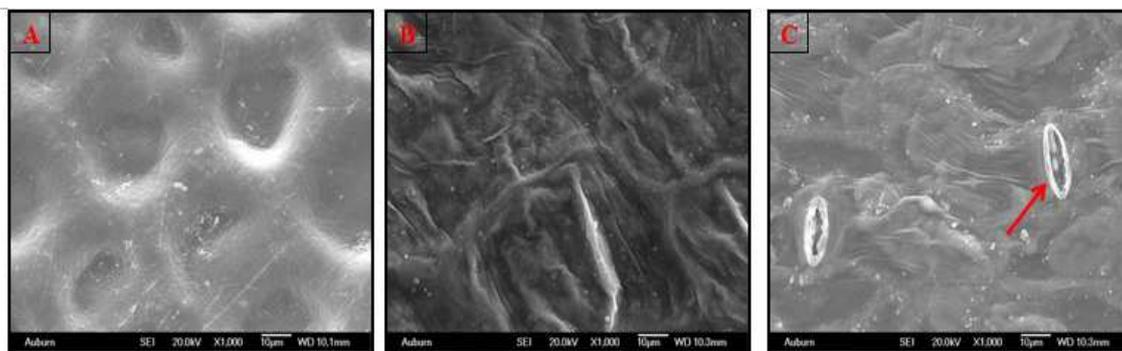
Fig. 2. SEM images of the surface of (A) tomato (unwashed), (B) the adaxial (top) and (C) the abaxial (bottom) surface of spinach (unwashed). An arrow bar indicates stomata in spinach leaves.

1.2 Attachment and distribution of *Salmonella* on the surface of fresh produce

The diverse surface morphologies of fresh produce could affect the attachment and colonization of bacterial cells on the produce surfaces. The selective attachment of bacterial cells on produce surfaces was presumably derived from the roughness and curvature of the surface morphologies and chemical constituents present on the surface. Thus, the attachment and distribution of *Salmonella* on different surfaces were observed by an SEM (Fig. 3A-F). The bacteria spread across the spinach and tomato surfaces. However, the distribution of the bacteria was not uniform, especially when the concentration of *Salmonella* was low. SEM observations of both the tomato and spinach surfaces showed that the bacteria favored attachment and distribution on exposed areas such as ridges and peaks rather than the depressions (Fig. 3A, 3B). Although the size (approximately 10 μm in length) of the stomata in spinach leaves are much bigger than the size of *Salmonella* (2 × 0.5 μm^2), the stomata, if present, didn't trap or harbor the cells or affect the attachment of cells significantly. As the cell concentration increased, the distribution of *Salmonella* over the tomato and

spinach surfaces became more uniform (Fig 3C, 3D), finally covering the whole inoculated area (Fig 3E, 3F).

1.3 Detection of *Salmonella* on the surface of fresh produce using ME biosensor method

Fig. 4 shows the responses of three sets of sensors after the exposure of sensors to *Salmonella* on the surface of a tomato and spinach. As the concentration of the *Salmonella* inoculant increased, the resonant frequency shift increased proportionally. While measurement sensors showed much larger resonant frequency shifts for the larger concentrations of *Salmonella* inoculated, there were no significant differences in the resonant frequency shifts of the control sensors despite the increase in *Salmonella* concentration ($P > 0.05$). Fig. 4 also shows the linearity and detection limit of the ME biosensors detecting *Salmonella* on tomato and spinach surfaces. Tomato surfaces showed a good linear relationship in measurement sensor with a correlation coefficient (R^2) of 0.955 and a generated slope of 1389. Both surfaces of spinach showed similar results in measurement sensor with an R^2 of 0.962 and 0.980 and a generated slope of 1200 and 1327 for the adaxial and abaxial surfaces, respectively. There was
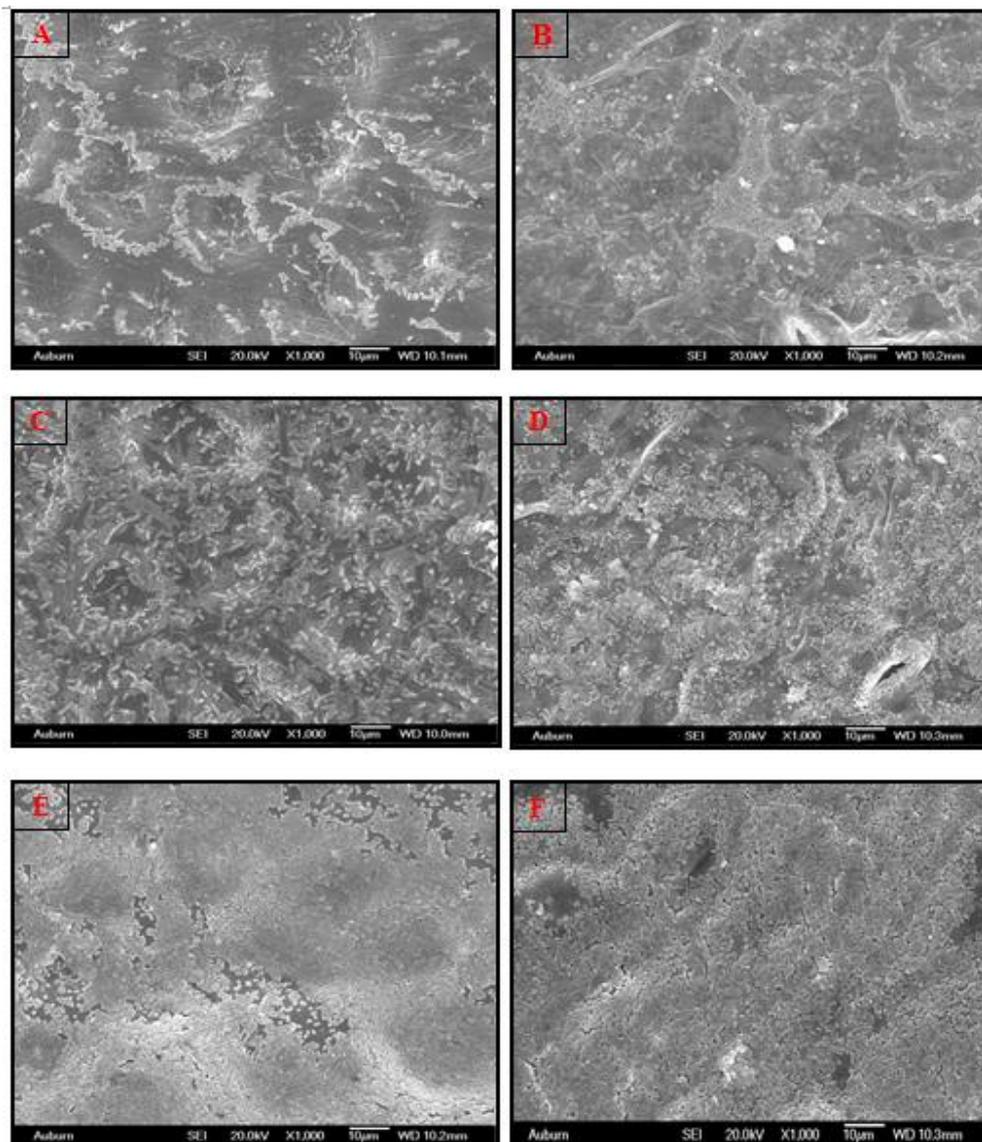
Fig. 3. SEM images of *Salmonella* on the tomato surfaces with inoculation of (A) 10^4 CFU/cm^2, (C) 10^6 CFU/cm^2, and (E)10^8 CFU/cm^2, and on the (B) abaxial surface of spinach with inoculation of 10^4 CFU/cm^2, and on the adaxial surface of spinach with inoculation of (D) 10^6 CFU/cm^2 and (F) 10^8 CFU/cm^2. The bars indicate 10 μm.

no significant difference in the resonant frequency shifts between tomatoes and both surfaces of spinach when the same concentration of *Salmonella* was inoculated on each surface (P > 0.05). From these curves, the detection limit of the ME biosensor method was mathematically determined. The detection limit is defined as the point of intersection of two linear lines for the measurement and control sensors. Finally, the detection limit for direct measurement using the ME biosensors on fresh produce was determined to be 1.87 log CFU/cm^2 for tomato, 1.72 log CFU/cm^2 for adaxial surface of spinach, and 2.16 log CFU/cm^2 for abaxial

surface of spinach. More importantly, there was no significant difference in the detection limits of tomatoes and spinach (P > 0.05).
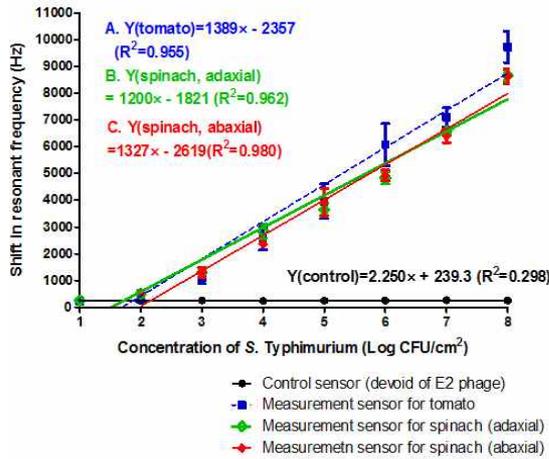

Fig. 4. Standard curves for a 10-fold dilution series of *Salmonella* on (A) tomato, (B) adaxial surface of spinach and (C) abaxial surface of spinach after ME biosensor performance; measurement sensor (N=9); control sensor (devoid of E2 phage) (N=27).

1.4 Evaluation of ME biosensor method by comparison with qPCR

For the direct comparison on both detection methods, 3 log CFU of S. Typhimurium was inoculated with nutrients on the surface of spinach and tomato. As shown in Fig. 5, The quantified concentration of *Salmonella* determined by qPCR method was determined to be 6.60 ± 0.20 log CFU/tomato, which was significantly different from BGS-plate count method (5.33 ± 0.21) (P<0.05). The fact that the quantified concentration determined by qPCR was greater than that obtained from the BGS-plate count method may be assumed due to no differentiate between live and dead cells in qPCR analysis. The quantified concentrations of *Salmonella* by the ME biosensor method (6.28 ± 2.07 log CFU/tomato) was also significantly different from that from BGS-plate count method. However, there was no significant difference between the qPCR and ME biosensor method.
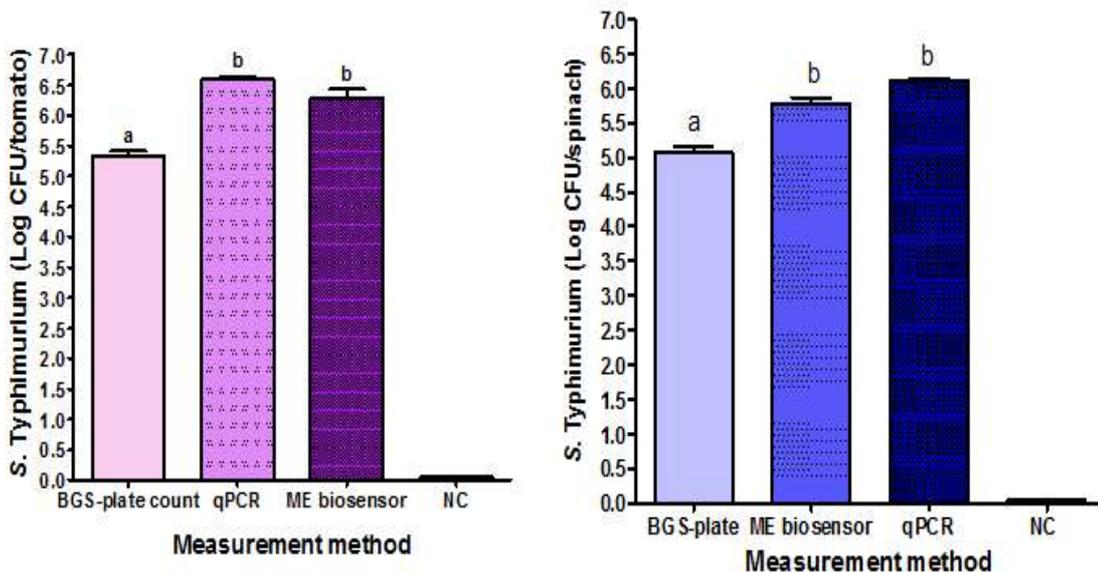

Fig. 5. Quantified concentration of *Salmonella* determined using BGS-plate count, qPCR, and ME biosensor methods. *Salmonella* (3 log CFU) was inoculated on each of tomato surface, except for the negative control (inoculated with DW), prior to incubation for 24 h at 37°C. Different letters (a, b) indicated that there were significant differences between measurement methods at P <0.05.

For the application on the surface spinach, the quantified concentration of *Salmonella* using BGS-plate count method was determined to be 5.07 ± 0.25 log CFU/spinach. All 25 spinach leaves showed significant resonant frequency changes of the measurement sensors when performed with ME biosensor method. The average resonant frequency shifts of measurement sensor was 5105 ± 1115 Hz and the quantified concentration of *Salmonella* was determined to be 5.79 ± 0.88 log CFU/spinach using the equation (Y= 1256X - 2163). For the qPCR method, all 25 spinach leaves also showed positive amplification with Ct values ranging between 22.0 and 26.2. The quantified concentration of *Salmonella* was determined to be 6.11 ± 0.26 log CFU/spinach.

The repeatability of the ME biosensor (47.48%) was much worse than that of qPCR (2.84%), and was beyond the range of repeatability (5-7%) recommended by Ivnitski et al (2000). However, when tomatoes with a more uniform distribution of bacteria were used, achieved by inoculating with 8 log CFU/tomato of *Salmonella*, the repeatability of the ME biosensor over a three day period improved to 5.72% (7732 ± 442). Hence, the repeatability of the ME biosensor method is both acceptable range and competitive with qPCR at 1.71% (18.882 ± 0.323).

There are several advantages of the ME biosensor presented in this study compared to qPCR. ME biosensors may be utilized on-site or in-field as well as in the laboratory due in part to the robust stability of E2 phage, even up to 80°C, and the lack of complicated sample preparation procedures. The ME biosensor is a cost-effective detection method due to lack of costly instruments, DNA purification kits, and fluorescence probe and primers. Instead, the ME biosensor method requires only 1 mm-size sensors,

phage for a biorecognition probe, and relatively simple instrumentation for detection. Hence, the level of training or expertise required to perform an analysis using the ME biosensor method is much less than qPCR method. In addition, the ME biosensor method is rapid, requiring only minutes of total detection time with no sample preparation involved whereas qPCR required 2-3 h detection times even after purifying DNA. The direct application of miniscule ME sensors facilitates broad testing of fresh produces by increasing the number of samples analyzed, providing greater assurance that the produce is free of harmful levels of pathogens. These advantages contribute to the ME biosensor method being an effective on-site and in-field detection method.

CONCLUSIONS

In this study, a phage-based ME biosensor was fully developed and evaluated by comparison with a well-recognized qPCR method as an on-site and in-field analysis method for the detection of *Salmonella*. With an advantage of the robust performance of ME biosensors, this study confirmed that a ME biosensor method was competitive and promising as an on-site and in-field detection method for the detection of pathogens.

REFERENCES

1. Beuchat, L.R. (2002). Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes and Infection*, 4,

413-423.

2. Beuchat, L.R., & Ryu, J.H. (1997). Produce handling and processing practices. *Emerging Infectious Diseases*, 3, 439-465.

3. Guo, X., Chen, J., Brackett, R.E., & Beuchat, L.R. (2002). Survival of *Salmonella* on tomatoes stored at high relative humidity, in soil, and on tomatoes in contact with soil. *Journal of Food Protection*, 65(2), 274-279.

4. Li, S., Li, Y., Chen, H., Horikawa, S., Shen, W., Simonian, A., & Chin, B.A. (2010). Direct detection of *Salmonella typhimurium* on fresh produce using phage-based magnetoelastic biosensors. *Biosensors and Bioelectronics*, 26, 1313-1319.

5. Lynch, M.F., Tauxe, R.V., & Hedberg, C.W. (2009). The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiology and Infection*, 137, 307-315.

6. Park, M-K., Wikle, H.C., Chai, Y., Horikawa, S., & Chin, B.A. (2012). The effect of incubation time for *Salmonella* Typhimurium binding to phage-based magnetoelastic biosensors. *Food Control*, 26, 539-545.

7. Ivnitski, D., Abdel-Hamid, I., Atanasov, P., Wilkins, E., Stricker, S. (2000). Application of electrochemical biosensors for detection of food pathogenic bacteria, *Electroanalysis*, 26, 317 - 325.