

Direct detection of *Salmonella* Typhimurium on rough, non-flat surfaces of spinach leaves using micron-scale phage-based magnetoelastic biosensors

Shin Horikawa^a, Yating Chai^a, Kiril A. Vaglenov^b, James M. Barbaree^b, Bryan A. Chin^a

^aMaterials Research and Education Center, Auburn University, Auburn, AL 36849

^bDepartment of Biological Sciences, Auburn University, Auburn, AL 36849

Attempts to detect *Salmonella* Typhimurium directly on fresh produce (e.g., tomatoes and shell eggs) have been recently made by using phage-based magnetoelastic (ME) biosensors [1,2]. These biosensors are acoustic-wave, mass-sensitive biosensors that are composed of a freestanding, strip-shaped ME resonator as the wireless signal transducer and the E2 phage [3] as the biomolecular-recognition element that specifically binds with *S. Typhimurium*.

These biosensors can be placed directly on a produce surface due to their freestanding, wireless nature. When the biosensor and *S. Typhimurium* cells, if present on the produce surface, come into contact with each other, the E2 phage binds the cells to the biosensor, thereby increasing the total mass of the biosensor. This change in mass causes a corresponding decrease in the biosensor's resonant frequency. This methodology allows rapid bacterial detection because it eliminates any pre-test sample preparation, including the washing of produce surfaces in the preparation of pathogen-containing liquid samples, followed by their purification, concentration, and/or enrichment.

In this investigation, the above methodology was employed to detect *S. Typhimurium* on fresh spinach leaves. As can be seen in Fig. 1, spinach leaves, in general, possess complex surface topography, which is likely to affect the degree of physical contact between biosensors and *S. Typhimurium* cells on the leaf surfaces. Hence, to reduce such an effect, micron-scale ME biosensors ($150\ \mu\text{m} \times 30\ \mu\text{m} \times 4\ \mu\text{m}$) were fabricated and used.

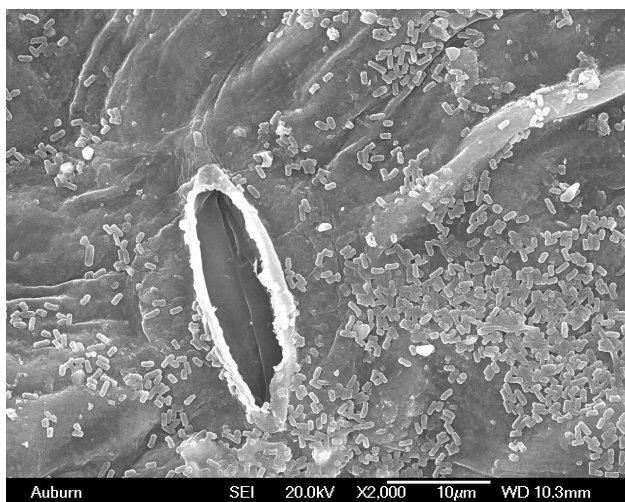


Figure 1. *Salmonella* Typhimurium cells on a spinach leaf surface with complex topography. A stoma can be seen in the figure.

The experimental procedures are schematically illustrated in Fig. 2. Both measurement (with phage) and control (without phage) sensors with a pre-determined resonant frequency were placed on wet spinach leaves inoculated with various concentrations of *S. Typhimurium*. After 25 min to allow for binding, the biosensors were collected with a bar magnet, and measurement of their final resonant frequency was completed within 20 min. The total test time was, hence, roughly 45 min.

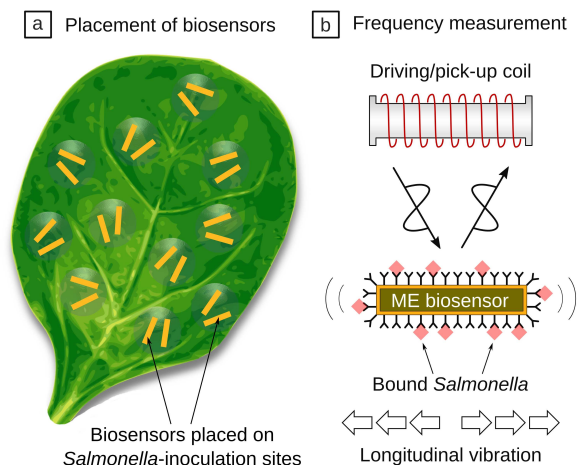


Figure 2. Schematic illustration of the experimental procedures.

The dose-response relationship is shown in Figure 3. Resonant frequency changes for the measurement sensors (solid circles) were found to be largely dependent on the surface density of *S. Typhimurium*. By contrast, the control sensors (hollow squares) showed much smaller responses, indicating that selective binding of *S. Typhimurium* on the measurement sensors occurred. The limit of detection (LOD) was found to be 4.8×10^2 cells/mm².

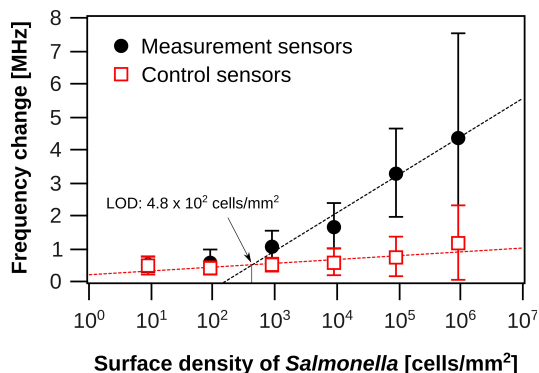


Figure 3. Dose-response plot. Ten measurement and control sensors each were used for each concentration.

References

- Li, S., Li, Y., Chen, H., Horikawa, S., Shen, W., Simonian, A., Chin, B.A., 2010. Biosensors and Bioelectronics 26, 1313 – 1319.
- Chai, Y. Li, S., Horikawa, S., Park, M.-K., Vodyanoy, V., Chin, B. A., 2012. Journal of Food Protection 75, 631 – 636.
- Sorokulova, I.B., Olsen, E.V., Chen, I.H., Fiebor, B., Barbaree, J.M., Vodyanoy, V.J., Chin, B.A., Petrenko, V.A., 2005. Journal of Microbiological Methods 63, 55 – 72.