Scientific Research Publishing

Advances in Microbiology, 2019, 9, 541-555
http://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Effects of Different Broth Enrichment upon Phage Magnetoelastic Biosensor for Fast Detecting Low *Salmonella* Counts on Problematic Produce

I-Hsuan Chen[1*], Yuzhe Liu[1], Songtao Du[1], Jiajia Hu[2], Fengen Wang[3], Shin Horikawa[1], Tung-Shi Huang[4], Bryan A. Chin[1]

[1]Material Research and Education Center, Auburn University, Auburn, AL, USA
[2]Institute of Quality Standard and Testing Technology for Agro-Products, Shandong Academy of Agricultural Sciences, Jinan, China
[3]Jiangsu Key Laboratory of Materials Surface Technology, School of Materials Science and Engineering, Changzhou University, Changzhou, China
[4]Department of Poultry Science, Auburn University, Auburn, AL, USA
Email: *chenih1@auburn.edu, huangtu@auburn.edu

How to cite this paper: Chen, I.-H., Liu, Y.Z., Du, S.T., Hu, J.J., Wang, F.G., Horikawa, S., Huang, T.-S. and Chin, B.A. (2019) Effects of Different Broth Enrichment upon Phage Magnetoelastic Biosensor for Fast Detecting Low *Salmonella* Counts on Problematic Produce. *Advances in Microbiology*, **9**, 541-555.
https://doi.org/10.4236/aim.2019.96033

Received: June 3, 2019
Accepted: June 23, 2019
Published: June 26, 2019

Abstract

According to the FDA Bacteriological Analytical Manual (BAM) for *Salmonella* identification in produce, two pre-enrichment steps with 48 hours of incubation are the golden procedures. Lactose broth is recommended for the first pre-enrichment step medium for leafy greens, and the universal pre-enrichment (UP) broth is for tomatoes. However, the suggested broths were evaluated to have the maximum performance using the culture-dependent methods, and may not be applied to other methods, such as biosensor detection platform. A wireless bacteriophage magnetoelastic (ME) biosensor has been recently developed for real-time or rapid detection of food-borne pathogens in various foods. This affinity-based biosensor utilizes a phage oligonucleotide as the probe to capture target bacteria. In this study, the efficiencies of different pre-enrichment media for early detection of low *Salmonella* on spinach leaves and tomatoes use ME biosensors to shorten detection time. Four broths of modified peptone water, Lennox broth (LB), lactose broth, and UP broth were selected in this study. Various pre-enrichment times for ME biosensor detection were investigated. After spiking 4 cfu/g *Salmonella* on the tomatoes surfaces, the phage biosensor was able to detect *Salmonella* within 5 hours of pre-enrichment comparing to 24 hours in the FDA procedures. For *Salmonella* spiked spinach leaves, the same medium showed *Salmonella* positive within 7 hours. This study demonstrated that LB

broth is the best medium to shorten pre-enrichment time to pass *Salmonella* number detection thresholds for ME biosensor detection in spinach and tomatoes when comparing to FDA procedures.

Keywords

Biosensors, Fresh Produce, Pre-Enrichment, FDA Procedure, *Salmonella* Detection

1. Introduction

With the increased consumptions of fresh produce, foodborne illnesses related to contaminated produce become a huge food safety concern to public. According to the Center for Disease Control and Prevention (CDC), contaminated produce causes 46% of foodborne illness and 23% of foodborne illness-related deaths [1]. Salmonellosis is one of the major food-illnesses in the outbreaks of produce. From 1973 to 2018, *Salmonella* sp. was associated with outbreaks in alfalfa sprouts, melons, apple/orange juices, leafy greens, tomatoes, cucumbers, precut celery and mixed fruits [2] [3] [4]. On April 30th-July 2nd, 2018, CDC reported multistate outbreaks of *Salmonella* Adelaide infections linked to pre-cut melon supplied by the Caito Foods, LLC with 77 people infected and 36 people hospitalized [4]. From harvesting in farms to the dining table, food safety of fresh produce needs to be inspected and monitored, before it can reach the retail stores and ultimately, consumption by individuals.

The current Bacteriological Analytical Manual 8th edition (BAM) from U.S. The Food and Drug Administration (FDA) [5] for the detection of *Salmonella* in produce requires several steps before providing results. The FDA standard testing procedures for identification of *Salmonella* in foods are classified by food types. The standard steps for detection of *Salmonella* in leafy greens samples include: sample preparation procedures, 1st pre-enrichment step with lactose broth for 24 hours, 2nd pre-enrichment step with Rappaport-Vassiliadis (RV) medium and Tetrathionate (TT) broth for 24 hours, and isolation of *Salmonella* from selective media such as Xylose lysine desoxycholate (XLD) agar or Lysine iron agar (LIA) for another 24 hours (**Figure 1(a)**). After the 72 hours of *Salmonella* identification steps, it takes up to another 3 days to perform other serotyping tests to confirm the *Salmonella enterica* serotypes in the contaminated samples [6]. According to the descripted procedures, this standard method is very laborious and time consuming.

Since 2001, FDA started to include rapid methods, such as some antibody based methods or DNA based real-time polymer-chain reaction (RT-PCR) assays for quick detections of foodborne pathogens in BAM [7]. These methods can serve as alternative procedures to detect *Salmonella* in the pre-enrichment samples with good sensitivities and fast screening results. However, some of

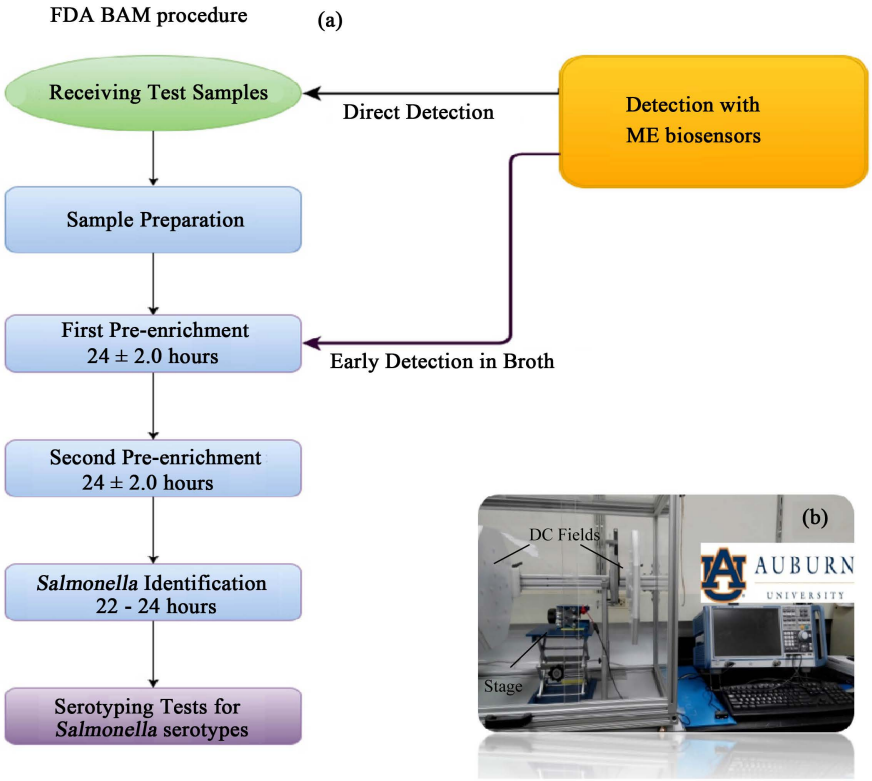

Figure 1. (a) The illustration of FDA BAM procedures (8th edition) for *Salmonella* detection in food and the applicability of phage ME biosensors into FDA methods; (b) Prototype of detection device for phage ME biosensors with adjustable working stage, DC magnetic fields, and a network analyzer for data analysis.

those methods also encounter problems in certain produce types. For example, chlorophyll from damaged leaves in the leafy samples will be released in the pre-enrichment steps and inhibit the PCR reaction. This usually results in the decreasing of sensitivity and failure to detect low counts of *Salmonella* DNA [7] in leafy samples. As for tomatoes, endogenous polyphenol and a low pH range (pH 4.0 - 4.4) are factors that may affect and interfere with antibody methods and PCR detections, especially when smaller *Salmonella* populations are present in the samples [8] [9]. While following FDA guidelines for detecting *Salmonella* in problematic produce types (such as tomatoes and leafy greens), one only would have choices of new techniques from the laborious BAM pre-enrichment steps or rapid PCR methods which may sacrifice the detection sensitivities. In this case, other rapid, more sensitive, and reliable methods are urgently needed for *Salmonella* detection on those problematic produce samples [10].

Using bacteriophage, instead of antibodies or aptamers, as bio-recognition element on the sensor is a recent developed technique [11] [12] [13]. The magnetoelastic (ME) biosensors consist of a freestanding striped-shaped ME resonator coated with engineered phage oligonucleotide probes that specifically binds with the pathogens of interest [14]. When a time-varying magnetic field is applied (**Figure 1(b)**), the ME biosensors can be placed into mechanical reson-

ance by magnetostriction. Upon the attachment of the target pathogen toward on the phage immobilized sensor, a resonant frequency shift of the biosensor is recorded through a network analyzer and gives a positive detection signal.

The phage ME biosensors are successfully shown to be rapidly detected *Salmonella* on produce surfaces with *Salmonella* detection limit at 500 cfu/mm^2 on the surfaces of tomatoes, apples, shell eggs, watermelons, and spinach leaves [14] [15] [16]. These biosensors can also detect the bacteria in the liquid format [17] [18] [19] and on raw chicken meat [20]. With the new designed wireless platform, it speeds up the biosensor detection time to less than 2 minutes [16]. Not to mention, the cost of the phage ME sensor was as low as $0.001 per sensor [14]. Combining the rapid detection time with the high sensitivity of detecting low *Salmonella* cells using bacteriophage probes, the ME biosensors is truly applicable to detect *Salmonella* in FDA-BAM pre-enrichment process and shorten the detection time. In this study, we evaluated the use of the wireless phage ME biosensors by studying the *Salmonella* detection relative effectiveness in four different media as an outline of 1st pre-enrichment step in FDA's *Salmonella* identification procedure. Four media used in this study were lactose broth, modified peptone water (MPW), universal pre-enrichment (UP) broth, and Lennox broth (LB). Lactose broth, MPW, and UP broth are the recommended pre-enrichment media for FDA-BAM and USDA-FSIS protocols [5] [21], while LB broth is a common medium used in the most labs for growing Enterobacteriaceae. The shortest incubation time for ME biosensor detection was also investigated. This study is an extension of our previous paper of Wang *et al*., 2017 [22]. In this paper, two problematic produce, tomatoes and spinach were studies; microbiological data was shown to explain details of suitable pre-enrichment broth selection in the exploratory of four types of media in early detection of *Salmonella*.

2. Materials and Methods

2.1. Preparation of *Salmonella* Typhimurium Solution

Salmonella enterica Typhimurium (ATCC 13311) was used in this study. *Salmonella* Typhimurium was grown from a single colony in Lennox Broth (LB broth) overnight in a shaking incubator at 37˚C, at a speed of 200 rpm. Overnight bacterial cultures were centrifuged at 5500 rpm for 10 min at 4˚C and re-suspended in PBS twice. The bacterial populations were then adjusted to an OD of 600_{nm}, which equates to 1.0 in PBS. *Salmonella* suspensions were then further diluted to 10^3 cfu/mL.

2.2. Spiking of *Salmonella* on Spinach and Tomatoes and Pre-Enrichment in Different Broths

Packages of triple washed spinach leaves were purchased from local supermarkets in Auburn, AL. The leaves, with a total weight of 125 grams, were collected and rinsed with filtered water in a sterilized beaker. Under a biosafety cabinet,

the leaves were sprayed with 70% EtOH and air dried under the cabinet. Each batch of spinach leaves (now 25 grams/ batch) was sterilely picked up, placed inside a sterile plastic tote bag, sprayed with a fresh *Salmonella* Typhimurium suspension (total 100 cfu) with a sterile adjustable sprayer (Spray Anywhere, Fisher Scientific, Pittsburgh, PA.) The bag of leafy greens (25 grams) with the final *Salmonella* suspension of 4 cfu/g was then mixed manually for 10 min to be homogenized in the biosafety cabinet.

Three different pre-enrichment broths were used in spinach study. They are lactose broth, LB, and MPW. Each broth (50 ml), was added into the bag and mixed well. The sterile bag was folded loosely to ensure oxygen aeration for *Salmonella* growth. The pre-enrichment solution was held in room temperature for 1 hour to stabilize the pH and then incubated at 37˚C for 5, 7, and 22 hours. At each incubation time, the pre-enrichment solution was centrifuged at 10,000 rpm for 10 minutes twice. The centrifuged pellet was re-suspended to a 1.0 mL final volume using filter sterilized water.

Boxes of Campari tomatoes were also purchased from local supermarkets in Auburn, AL. Two Campari tomatoes were collected as a group. Each group of tomatoes was then weighted, rinsed, and cleaned with 70% EtOH as previously described. Tomatoes were placed in a sterile plastic bag and sprayed with *Salmonella* Typhimurium suspension at (4 cfu/g) using a sterile adjustable Sprayer. After the spiking of *Salmonella* on tomatoes, the three pre-enrichment broths were used. They are UP broth, LB broth, and MPW broth. Each broth, at a ratio of 1:2 (tomatoes: broth, w/v), was added into the bag with contaminated tomatoes and mixed well. The sterile bag was folded loosely to ensure oxygen aeration for *Salmonella* growth, and the rest of pre-enrichment steps were performed the same as in the previous paragraph.

2.3. Preparation of Phage ME Biosensors

Magnetoelastic (ME) biosensors were fabricated by a commercially available Metaglas 2826MB ribbon (Metaglas, Inc). Further detailed descriptions of sensor fabrication, dicing, and final treatment processes are included in Horikawa *et al*., 2015 [16]. The ME sensors used in this study had a final layer coated with Au (gold), and were cut into a strip shape at the size of 1 mm × 0.2 mm × 0.028 mm. Phages are bound to the gold-coated sensor layer due to physical adsorption. Phage E2 was an fd-tet filamentous phage and was selected through Phage Display method. This phage was used as bio-recognition elements in this study for specific binding to *Salmonella* Typhimurium in produce samples. The detail bio-panning procedures, sensitivity tests, and specificity of this phage were described in Sorokulova at al., 2005 [23]. Phage sensors were prepared by coating the ME sensor with 1×10^{11} virons of phage solution in TBS for one hour at room temperature on a rotator. The phage coated sensor was then washed three times with TBS. Bovine Serum Albumin (BSA) at 0.1% was used as a blocking reagent for preventing non-specific binding. The phage sensor was put in 0.1%

BSA solution for 1 hour at room temperature on a rotator. Sensors coated with 0.1% BSA (without phages) served as negative controls in the frequency measurement experiments.

2.4. Frequency Measurement by Phage ME Biosensors and Statistics

The final 1.0 mL solution from pre-enriched spinach leaves or tomato samples (Section 2.2) was further concentrated down to 330 μL in filtered water by centrifugation. After the phage ME biosensor was incubated with *Salmonella* suspensions for one hour at room temperatures, each sensor was washed with 1× TBS three times and taken out for frequency measurements and data analysis. The procedures for frequency measurements were the same as described in Wang *et al*., 2017 [22]. Each batch of inoculated produce was tested by 8 sensors and the experiment was repeated twice. Three non-phage sensors with BSA only served as controls in each group.

Overall, data collected from frequency measurements was analyzed by an on-tailed unpaired student's t-test with $p < 0.05$ and $\alpha = 0.05$ (as described in Wang *et al*., 2017 [22]). The degree of dissimilarity between control and measurement sensors was calculated and the shortest incubation time was decided for sensor detection. Confidence level of difference equated to (1 − p value) × 100%.

2.5. *Salmonella* Numbers on the Sensors and *Salmonella* Capture Rates in Broth

XLD agar were used in this section of experiments. Frist, the 10 μL of the final 1.0 mL bacterial suspension concentrated from each test group (various media types and incubation times) was diluted and plated on XLD agar. The resulted plates were incubated at 37˚C for 16 - 18 h for total *Salmonella* counts in pre-enrichment (as Total *Salmonella Counts*). The rest of final 1mL bacterial suspension was then incubated with a phage coated sensor in a 1.5 mL micro-tube for 1 hour at room temperature with gentle rocking. The sensor was washed three times by 1 × TBS. *Salmonella* cells on the sensor were eluted by 0.1 M Glycine buffer (pH 2.2) for 10 min and neutralized by adding 1M Tris-HCl (pH 9.1). The neutralized bacterial suspension was diluted with PBS and plated on XLD agar plates for recorded as *Salmonella* number on sensor. The *Salmonella* capture rate was calculated by *Salmonella* capture rate in broth = (*Salmonella* number on one sensor/Total *Salmonella* Counts in broth) × 100%.

Each test group had duplicate plates. Each experimental parameter (various broth types and incubation times) was tested by four sensors and the whole experiment was repeated twice.

3. Results and Discussions

According to FDA BAM "Chapter 5-*Salmonella*" [5], different pre-enrichment media should be used for different food types. Lactose broth is the recommend-

ed broth to use in the first pre-enrichment step of *Salmonella* detection leafy samples. However, in the study of Wang *et al*., 2015 [24], it was found that some other broths appeared to be more effective in PCR assays than the current BAM suggested broths. It may be because the BAM suggested broths was evaluated to have the maximum results in the culture-dependent methods. Broths recommended by BAM may not provide the best results in other type of assays, such as DNA-based methods or phage-based biosensor detection platforms. Incubation techniques meant to raise the number of target microorganisms above detection thresholds were also addressed in the paper. This is applicable especially in food samples contaminated with low *Salmonella* counts, which are needed to raise target bacterial numbers for detection. Therefore, there is a need to study a suitable enrichment broth to be used on each detection platform and the minimum incubation time for the detection. The aim of this study was to find a better broth for phage ME biosensors platform to be used in the pre-enrichment condition with the shortest incubation time to detect low *Salmonella* spiked spinach leaves or tomato samples.

3.1. Frequency Shift Measurements in Artificially Spiked Spinach and Tomatoes Samples

Table 1 shows the data of the frequency shift of biosensors for *Salmonella* detection on spiked spinach leaves in MPW, Lac broth, and LB broth for 7 and 22 hours pre-enrichment times. In this study, a one-tailed student t test with p value smaller than 0.05 ($\alpha = 0.05$) was used. The confidence level of differences (CLD) of 95% or more was needed to achieve the significant *Salmonella* positive signal from the differences of frequency shifts between measurements and control sensors. According to Table 1, after 7 hours of incubation, the value of CLD

Table 1. Frequency measurements of phage ME biosensor platform for *Salmonella* detection in artificially spiked spinach leaves in MPW, Lac, and LB broths for 7 hours, and 22 hours.

Broth Type	Frequency Measurements of Biosensors	7 h	7 h-STDV	22 h	22 h-STDV
Sp-MPW	Measurement (KHz)[a]	0.558	0.083368	2.161	0.027153
	Control (KHz)[b]	0.233	0.039	0.280	0.033394
	Confidence Level of Difference (%)[c]	88.57		99.34	
Sp-Lac	Measurement (KHz)[a]	0.648	0.057	2.519	0.138
	Control (KHz)[b]	0.354	0.039	0.038	0.024
	Confident Level of Difference (%)[c]	91.27		97.09	
Sp-LB	Measurement (KHz)[a]	0.756	0.047	2.247	0.412
	Control (KHz)[b]	0.246	0.008	0.058	0.001
	Confident Level of Difference (%)[c]	96.76		96.47	

Note: [a]-frequency shifts (kHz) of measurement sensors; [b]-frequency shifts (kHz) of control sensors; [c]-confident level of difference (%).

of both MPW and Lac broths did not reach or exceed 95% and was lower than the CLD of 96.76% in LB broth. This data showed that LB broth is the only broth among the three to effectively yield *Salmonella* positive signals in artificially spiked spinach at a 7 hours incubation period. After 22 hours of incubation, all three broths produced significant *Salmonella* positive signals in frequency shifts with high CLD values exceed 95% (Table 1).

As for tomatoes samples, the detail study of resonant frequency changes in three broths (MPW, UP, and LB) after 5, 7, and 22 hours of pre-enrichment are presented in Table 2. By analyzing the data with a CLD above 95%, tomato samples in LB broth demonstrated significant differences in frequency shift of measurement sensors in compared to controls as early as 5 hours of incubation time. However, tomato samples in MPW and UP after 5 hours and 7 hours pre-enrichment did not show any significant frequency differences between measurements and control sensors. After an incubation time of 22 hours, significant frequency differences were observed in all three broths.

In order for easy understanding, CLD values, measured by frequency shift (Table 1 and Table 2) and passed 95% significant differences between the measurement and control sensors in various broths, are expressed as *Salmonella* positive signals. Otherwise, the CLD values lower than 95% were categorized as *Salmonella* negative signals. Table 3 and Table 4 are the summary tables of *Salmonella* detection signals of artificially spiked spinach leaves tomatoes in four different broths for 7 and 22 hours of incubation using phage ME biosensor platform. This summary data clearly demonstrated that LB is the best pre-enrichment medium to be use for early detection of 5-7 hours incubation for detection low *Salmonella* contaminations in both tomato and spinach samples.

Table 2. Frequency measurements of phage ME biosensor platform for *Salmonella* detection in artificially spiked tomatoes in MPW, UP, and LB broths for 5 hours, 7 hours, and 22 hours of pre-enrichment.

Broth Type	Frequency Measurements of Biosensor	5 h	5 h-STDV	7 h	7 h-STDV	22 h	22 h-STDV
Tom-MPW	Measurement (KHz)[a]	0.167	0.010	0.300	0.034	2.549	0.101
	Control (KHz)[b]	0.100	0.027	0.189	0.036	0.299	0.070
	Confidence Level of Difference (%)[c]	76.18		86.67		97.98	
Tom-UP	Measurement (KHz)[a]	0.323	0.069	0.648	0.057	2.782	0.113
	Control (KHz)[b]	0.103	0.020	0.323	0.048	0.303	0.049
	Confidence Level of Difference (%)[c]	86.16		92.10		97.95	
Tom-LB	Measurement (KHz)[a]	0.575	0.024	0.721	0.065	1.324	0.076
	Control (KHz)[b]	0.84	0.059	0.104	0.035	0.188	0.027
	Confidence Level of Difference (%)[c]	98.54		98.01		98.27	

Note: [a]-frequency shifts (kHz) of measurement sensors; [b]-frequency shifts (kHz) of control sensors; [c]-confident level of difference (%).

Table 3. *Salmonella* detection signals of artificially spiked spinach leaves in MPW, Lac, and LB broths for 7 and 22 hours of incubation using phage ME biosensor platform.

Test Group	7 h	22 h
Spinach-MPW	−	+
Spinach-Lactose	−	+
Spinach-LB	+	+

Note: Negative *Salmonella* detection signal (-) represented CLD is under 95%; positive signal (+) represented CLD equals or larger than 95%. Eight sensors were used for each test condition and the experiment was repeated twice.

Table 4. *Salmonella* detection signals of artificially spiked tomatoes in MPW, UP, and LB broth for 5, 7, and 22 hours incubation using phage ME biosensor platform.

Test Group	5 h	7 h	22 h
Tomatoes-MPW	-	-	+
Tomatoes-UP	-	-	+
Tomatoes-LB	+	+	+

[1]Note: Negative *Salmonella* detection signal (−) represented CLD is under 95%; positive signal (+) represented CLD equals or larger than 95%. Eight sensors were used for each test condition and the experiment was repeated twice.

3.2. *Salmonella* Numbers on the Phage ME Biosensor in Contaminated Spinach and Tomato Samples

In order to understand the detail performances of phage biosensors in each broth, the number of *Salmonella* on the sensor in all three broths for 7 and 22 hour incubation times were evaluated. The data are shown in **Figure 2** for spinach samples and **Figure 3** for tomato samples. According to the report of Li *et al*., 2010 [14], the *Salmonella* detection limit of phage ME biosensor was 500 cfu/mm^2 as determined by the direct detection of *Salmonella* on the tomatoes surfaces. Their study of phage biosensor direct detection of *Salmonella* was conducted by air-dried *Salmonella* cells on the surface of tomatoes. Therefore, the detection unit was expressed as cfu/mm^2. In this study, the same *Salmonella* amount was used for set up the detection threshold of *Salmonella*. Since this study was performed in the liquid detection format, the unit was expressed as cfu/sensor. After a 7 hour incubation period, the phage biosensor was able to capture 937 cfu/sensor of *Salmonella* with spinach samples in LB, which passed the detection threshold of *Salmonella* counts (present as a red line in **Figure 2**). In MPW and Lac broth enrichment, *Salmonella* numbers did not exceed 500 cfu/sensor. Therefore, these two broths failed to pass the detection threshold and also didn't show *Salmonella* positive signals in frequency tests. At 22 hours incubation, all three broths were able to demonstrate a high *Salmonella* number of 10^4 - 10^5 cfu/sensor (**Figure 2**), which triggered the *Salmonella* positive signal in biosensor frequency detections with high CLD values. With the early detection of 7 hours incubation time in the 1st pre-enrichment step of FDA procedures, LB appeared to be the best among the three media for spinach samples to use in *Salmonella* detection by ME biosensors.

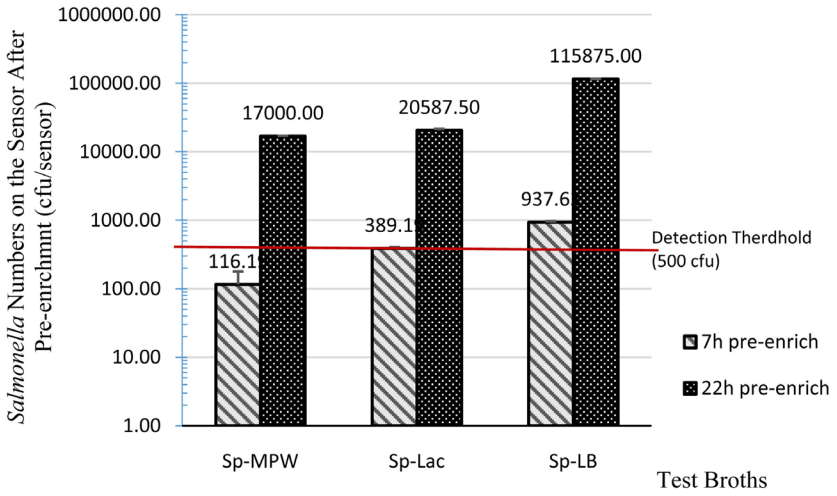

Figure 2. *Salmonella* numbers on the sensor in three broths after 7 h and 22 h incubation of artificially spiked spinach leaves at low *Salmonella* population (4 cfu/ gram). The red line represents the *Salmonella* detection threshold (500 cfu/sensor) of the phage ME biosensor detection platform.

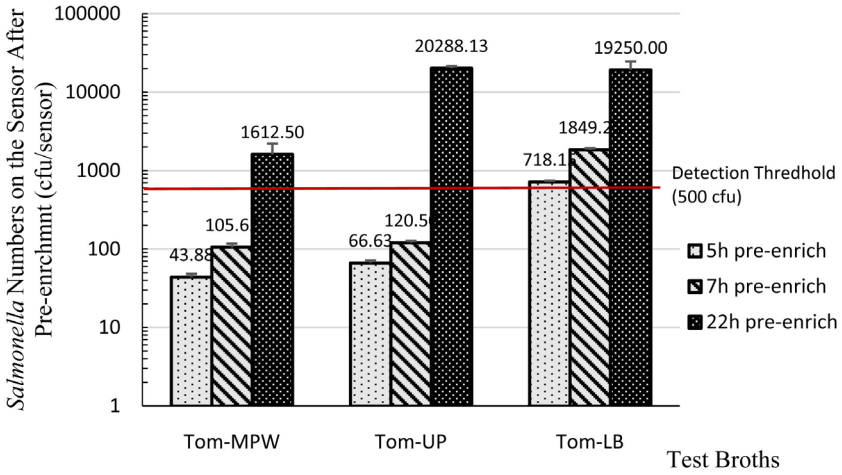

Figure 3. *Salmonella* numbers on the sensor in MPW, UP, and LB broth after 5 h, 7 h, and 22 h incubation of artificially spiked spinach leaves at low *Salmonella* population (4 cfu/ gram). The red line represents the *Salmonella* detection threshold (500 cfu/sensor) of the phage ME biosensor detection platform.

The microbiological analysis of contaminated tomato samples in MPW, UP, and LB broths after 5, 7, and 22 hours of pre-enrichment time are shown in **Figure 3**. According to **Figure 3**, LB broth was the only broth in the three, which is able to capture enough *Salmonella* cells (719 cfu/sensor) and passed the biosensor detection threshold in the fifth hour of the early incubation stage. MPW and UP did not show enough *Salmonella* numbers on the sensor until 22 hours of incubation time. This data may indicate that MPW and UP didn't promote *Salmonella* growth in tomato samples after short hours of incubation. As the same concept mentioned previously in the study of Wang et al, in 2015 [24], MPW and UP may not be the suitable broth for quick incubation of phage ME biosen-

sors for detecting *Salmonella* in tomatoes.

3.3. *Salmonella* Capture Rate Study

When cross referencing the microbiological data of *Salmonella* numbers on the sensors and the data of frequency shifts, it yields interesting findings. In the LB broth with both 7 and 22 hours of incubation time, the CLD values in tomatoes (Table 3) were higher than the values in spinach samples (Table 1). However, the *Salmonella* number on the sensors was lower in tomatoes (Figure 3) than the number in spinach (Figure 2). The frequency measurements and microbiological data of LB in spinach and tomatoes samples seemed to contradictory to each other. Therefore, it is curious to know whether the affinity actions of the phages to *Salmonella* was still performed normally in the same broths, but different produce types. In the report of Qiang *et al*., in 2017 [25], proteins and components in different blocking buffers would inhibit the affinity of phage probes to its target and promote the non-specific binding in ELISA assays. To answer this question, *Salmonella* capture rates of the phage biosensors were studies. The *Salmonella* capture rate was calculated by the percentages of *Salmonella* numbers captured by phages on the sensor and divided by the total *Salmonella* growth in each test broth with 7 hours of incubation period. The data truly revealed the fruit types played as a factor to affect the phage affinity to capture target pathogens in LB. Figure 4 shows that in 7 hours of pre-enrichment of LB, 9.606% of *Salmonella* was captured by phages on the biosensors in tomatoes samples, while the capture rate was only 8.57% in spinach. Apparently, substances in spinach samples (such as chlorophyll or flavonoids) may slightly inhibit phage affinity to *Salmonella.* More studies are needed to seek a conclusive statement about this finding. Besides, in this set of data, LB again demonstrated the highest capture rates among all broths, while MPW showed the lowest *Salmonella* capture ability for phage biosensors to detect *Salmonella* in two tested produce samples.

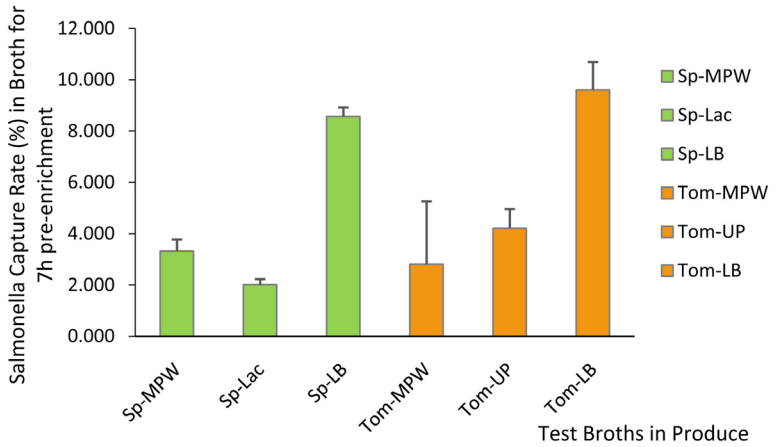

Figure 4. *Salmonella* captured percentages on the sensor in MPW, Lac, UP, and LB broth after 7 h incubation of artificially spiked tomatoes and spinach leaves at low *Salmonella* population (4 cfu/ gram).

Overall, the phage ME biosensors were able to detect *Salmonella* in the LB broth within 5 hours of incubation in tomatoes samples and within 7 hours in low *Salmonella* spiked spinach samples. MPW may not be a suitable short time pre-enrichment medium for detecting *Salmonella* in both produce samples using phage ME biosensor. The comparison of the phage ME biosensors to FDA BAM culture method and qRT-PCR method are summarized in Table 5. The detection limit of *Salmonella* by phage ME sensor method may not be as low as two other methods, but the pre-enrichment time is tremendously decreased. This may be compensated by extending the pre-enrichment time less than one hour to reach the same detection limit. The cost of the ME sensor method is also much lower than the two other methods. Our data also demonstrated that it is possible to implant phage ME biosensors into FDA BAM methods for screening and shortening the *Salmonella* pre-enrichment times and then perform the BAM culture or PCR methods.

4. Conclusion

Phage ME biosensors have been demonstrated as a powerful and rapid *Salmonella* detection platform in contaminated fresh produce and liquid systems. In this study, the phage ME biosensor showed to have a great potential application as an early detection method in FDA BAM pre-enrichment procedures for *Salmonella* detection in the problematic produce. For *Salmonella* detection in spinach leaves and whole tomatoes, the proposed phage biosensor platform was able to reduce the detection time from 72 hours to 5 - 7 hours. FDA recommended pre-enrichment broths for detecting *Salmonella* in spinach and tomato samples didn't demonstrate the maximum results in phage ME biosensor detection, as compared to the LB broth. By using LB broth as an alternative pre-enrichment medium along with the phage ME biosensor method, the detection time can be reduced to as short as 5 hours in tomatoes samples and 7 hours in spinach samples.

Table 5. Comparisons of phage-Me biosensors with LB to two FDA-BAM methods for *Salmonella* detection in produce.

	Phage-ME Biosensor with LB broth	FDA-BAM Cultured Method [24] [26]	FDA-BAM Fast qRT-PCR Method [27]
Detection Limit of *Salmonella*	4 cfu/g	1 cfu/25 g or 0.1 - 0.14 cfu/g	2 - 10 cfu/25 g
1st pre-enrichment	5 - 7 h	24 h	24 h
2nd pre-enrichment	-	24 h	-
Detection time	<2 min	Up to 72 h if only use serological methods	1 - 2 h
Cost	$0.001 USD per sensor with 8 hours labor fee	Media; 72 hours labor fee	Taq-Man kit ($2.00 USD/reaction); 26 hours labor fee

Acknowledgements

This work was supported by the Specially Crop Research Initiative Competitive Grant No. USDA-2011-51181-30642A and Auburn University Detection and Food Safety Center (AUDFS). We would like to thank Eric Ching-Feng Au and Harrison Michael for their assistance in this study.

Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Painter, J.A., Hoekstra, R.M., Ayers, T., Tauxe, R.V., Braden, C.R., Angulo, F.J. and Griffin, P.M. (2013) Attribution of Foodborne Illnesses, Hospitalizations, and Deaths to Food Commodities by Using Outbreak Data, United States, 1998-2008. *Emerging Infectious Diseases*, **19**, 407-415. https://doi.org/10.3201/eid1903.111866

[2] Jacobsen, C.S. and Bech, T.B. (2012) Soil Survival of Salmonella and Transfer to Freshwater and to Fresh Produce. *Food Research International*, **45**, 557-566. https://doi.org/10.1016/j.foodres.2011.07.026

[3] Sivapalasingam, S., Friedman, C.R., Cohen, L. and Tauxe, R.V. (2004) Fresh Produce: A Growing Cause of Outbreaks of Foodborne Illness in the United States, 1973 through 1997. *Journal of Food Protection*, **67**, 2342-2352. https://doi.org/10.4315/0362-028X-67.10.2342

[4] Center for Disease Control and Prevention (CDC). https://www.cdc.gov/salmonella/adelaide-06-18/index.html

[5] FDA Bacteriological Analytical Manual, Chapter 5 for *Salmonella*, Updated 08/2016. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm

[6] Bell, R.L., Jarvis, K.G., Ottesen, A.R. and McFarland, M.A. (2016) Brown E.W. Recent and Emerging Innovations in *Salmonella* Detection: A Food and Environmental Perspective. *Microbial Biotech*, **9**, 279-292. https://doi.org/10.1111/1751-7915.12359

[7] FDA Bacteriological Analytical Manual, Appendix 1. BAM: Rapid Methods for Detecting Foodborne Pathogens. http://www.fda.gov/downloads/Food/FoodScienceResearch/UCM244777.pdf

[8] Guo, X., Chen, J., Beuchat, L.R. and Brackett, R.E. (2000) PCR Detection of *Salmonella enterica* Serotype Montevideo in and on Raw Tomatoes Using Primers Derived from hilA. *Applied and Environmental Microbiology*, **66**, 5248-5252. https://doi.org/10.1128/AEM.66.12.5248-5252.2000

[9] Ogunjimi, A.A. and Choudary, P.V. (1999) Adsorption of Endogenous Polyphenols Relieves the Inhibition by Fruit Juices and Fresh Produce of Immuno-PCR Detection of *Escherichia coli* O157:H7. *FEMS Immunology and Medical Microbiology*, **23**, 213-220. https://doi.org/10.1016/S0928-8244(98)00138-2

[10] Guntupalli, R., Hu, J., Lakshmanan, R.S., Huang, T.S., Barbaree, J.M. and Chin, B.A. (2007) A Magnetoelastic Resonance Biosensor Immobilized with Polyclonal Antibody for the Detection of *Salmonella typhimurium*. *Biosensors and Bioelectronics*, **22**, 1474-1479. https://doi.org/10.1016/j.bios.2006.06.037

[11] Nanduri, V., Sorokulova, I.B., Samoylov, A.M., Simonian, A.L., Petrenko, V.A. and

Vodyanoy, V. (2007) Phage as a Molecular Recognition Element in Biosensors Immobilized by Physical Adsorption. *Biosensors and Bioelectronics*, **22**, 986-992. https://doi.org/10.1016/j.bios.2006.03.025

[12] Zourob, M. and Ripp, S. (2007) Bacteriophage-Based Biosensors. In: Zourob, M., Ed., *Recognition Receptors in Biosensors*, Springer, New York, 415-448. https://doi.org/10.1007/978-1-4419-0919-0_11

[13] Cinti, S., Volpe, G., Piermarini, S., Delibato, E. and Palleschi, G. (2017) Electrochemical Biosensors for Rapid Detection of Foodborne *Salmonella*: A Critical Overview. *Sensors*, **17**, 1910. https://doi.org/10.3390/s17081910

[14] Li, S., Li, Y., Chen, I.H., Horikawa, S., Shen, W., Simonian, A. and Chin, B.A. (2010) Direct Detection of *Salmonella typhimurium* on Fresh Produce Using Phage-Based Magnetoelastic Biosensors. *Biosensors and Bioelectronics*, **26**, 1313-1319. https://doi.org/10.1016/j.bios.2010.07.029

[15] Chai, Y., Wikle, H.C., Wang, Z., Horikawa, S., Best, S., Cheng, Z., Dyer, D.F. and Chin, B.A. (2013) Design of a Surface-Scanning Coil Detector for Direct Bacteria Detection on Food Surfaces Using a Magnetoelastic Biosensor. *Journal of Applied Physics*, **114**, Article ID: 104504. https://doi.org/10.1063/1.4821025

[16] Horikawa, S., Chai, Y., Wikle, H.C., Suh, S., Barbaree, J.M. and Chin, B.A. (2015) Direct Detection of *Salmonella* on Fresh Produce. *ECS Transactions*, **69**, 25-31. https://doi.org/10.1149/06938.0025ecst

[17] Lakshmanan, R.S., Guntupalli, R., Hu, J., Kim, D.J., Petrenko, V.A., Barbaree, J.M. and Chin, B.A. (2007) Phage Immobilized Magnetoelastic Sensor for the Detection of *Salmonella typhimurium*. *Journal of Microbiological Methods*, **71**, 55-60. https://doi.org/10.1016/j.mimet.2007.07.012

[18] Huang, S., Yang, H., Lakshmanan, R.S., Johnson, M.L., Wan, J., Chen, I.-H., Wikle, H.C., Petrenko, V.A., Barbaree, J.M. and Chin, B.A. (2009) Sequential Detection of *Salmonella typhimurium* and *Bacillus anthracis* Spore Using Magnetoelastic Biosensors. *Biosensors and Bioelectronics*, **24**, 1730-1736. https://doi.org/10.1016/j.bios.2008.09.006

[19] Du, S., Horikawa, S., Liu, Y., Chai, Y., Hu, J., Wang, F. and Chin, B.A. (2015) Rapid Detection of Pathogens by a 3D Biomolecular Filter and Automated Biosensor Measurement System. *ECS Transactions*, **69**, 17-24. https://doi.org/10.1149/06938.0017ecst

[20] Chen, I.-H., Horikawa, S., Bryant, K., Riggs, R., Chin, B.A. and Barbaree, J.M. (2017) Bacterial Assessment of Phage Magnetoelastic Biosensor for *Salmonella enterica* Typhimurium Detection in Chicken Meat. *Food Control*, **71**, 273-278. https://doi.org/10.1016/j.foodcont.2016.07.003

[21] Rose, B.E. (1998) Isolation and Identification of Salmonella from Meat, Poultry and Egg Products. In: Dey, B.P. and Lattuada, C.P., Eds., *USDA/FSIS Microbiology Laboratory Guidebook*, 3rd Edition, U.S. Department of Agriculture, Food Safety and Inspection Services, Washington DC, 4.1-4.14.

[22] Wang, F., Horikawa, S., Hu, J., Wikle, H.C., Chen, I.-H., Du, S., Liu, Y. and Chin, A.B. (2017) Detection of *Salmonella typhimurium* on Spinach Using Phage-Based Magnetoelastic Biosensors. *Sensors*, **17**, pii: E386. https://doi.org/10.3390/s17020386

[23] Sorokulova, I.B., Olsen, E.V., Chen, I.-H., Fiebor, B., Barbaree, J.M., Vodyanoy, J.V., Chin, B.A. and Petrenko, V.A. (2005) Landscape Phage Probes for *Salmonella typhimurium*. *Journal of Microbiological Methods*, **63**, 55-72. https://doi.org/10.1016/j.mimet.2005.02.019

[24] Wang, H., Gill, V.S., Cheng, C.M., Gonzalez-Escalona, N., Irvin, K.A., Zheng, J., Bell, R.L., Jacobson, A.P. and Hammack, T.S. (2015) Evaluation and Comparison of Rapid Methods for the Detection of *Salmonella* in Naturally Contaminated Pine Nuts Using Different Pre-Enrichment Media. *Food Microbiology*, **46**, 58-65. https://doi.org/10.1016/j.fm.2014.06.028

[25] Qiang, X., Sun, K., Xing, L., Xu, Y., Wang, H., Zhou, Z.P., Zhang, J., Zhang, F., Caliskan, B., Wang, M. and Qiu, Z. (2017) Discovery of a Polystyrene Binding Peptide Isolated from Phage Display Library and Its Application in Peptide Immobilization. *Scientific Reports*, **7**, Article No. 2673. https://doi.org/10.1038/s41598-017-02891-x

[26] Jacobson, A.P., Wang, H., Gill, V.S., Duvall, R., Arce, G., Chirtel, S. and Hammock, T.S. (2017) Relative Effectiveness of Selected Preenrichment Media for the Detection of Salmonella from Leafy Green Produce and Herbs. *Food Microbiology*, **63**, 123-128. https://doi.org/10.1016/j.fm.2016.11.006

[27] González-Escalona, N., Hammack, T.S., Russell, M., Jacobson, A.P., De Jesús, A.J., Brown, E.W. and Lampel, K.A. (2009) Detection of Live Salmonella sp. Cells in Produce by a TaqMan-Based Quantitative Reverse Transcriptase Real-Time PCR Targeting invA mRNA. *Applied and Environmental Microbiology*, **75**, 3714-3720. https://doi.org/10.1128/AEM.02686-08