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The analytical comparison of phage-based magnetoelastic biosensor with TaqMan-based quantitative PCR method to detect *Salmonella* Typhimurium on cantaloupes

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ABSTRACT

A phage-based magnetoelastic (ME) biosensor method for the detection of *Salmonella* Typhimurium was compared with a TaqMan-based quantitative real-time PCR (qPCR) method on cantaloupes. The selectivity of a qPCR primer set was investigated and different DNA extraction methods were compared. The limits of detection (LOD) of both detection methods were determined by serial inoculation of *S.* Typhimurium suspensions on cantaloupe surfaces. The repeatability of both detection methods was determined over three-day periods. Among 74 bacteria tested, only *S.* Typhimurium exhibited positive amplification, and other bacterial strains did not show any positive amplification. The DNeasy tissue kit showed the best performance for DNA purification due to the significantly superior threshold cycle (Ct) values, compared with either direct applications of qPCR without any extraction procedure or after employing a simple boiling method for extraction. The LOD and repeatability of qPCR and ME biosensor methods for *S.* Typhimurium detection were determined to be 1.35 ± 0.07 and 2.47 ± 0.50 log CFU/2 mm^2 surface of cantaloupe, and 2.41 and 6.28%, respectively. The results demonstrate that the ME biosensor method is one of promising and practical on-site detection methods for *S.* Typhimurium on fresh produce.

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1. Introduction

From 1996 to 2008, eighty-two foodborne illness outbreaks occurred that were associated with the consumption of a wide variety of fresh produce including lettuce, alfalfa, artichokes, bean sprouts, cabbage, cilantro, parsley, spinach, watermelon, tomatoes and cantaloupes (FDA, 2011; Park & Oh, 2012). The foodborne illness outbreaks caused by the consumption of fresh produce have been associated with *Salmonella*, *Shigella*, *Escherichia coli* O157:H7, *Campylobacter*, *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus* and *Clostridium botulinum* (Beuchat, 2002; Heaton & Jones, 2008). Among those, *Salmonella* was one of main causes for the fresh produce-associated foodborne illness outbreaks (Heaton & Jones, 2008). Since contamination of fresh produce with *Salmonella* may occur anywhere along the route from farm to table (Beuchat & Ryu, 1997), new *Salmonella* detection methods require the capability to identify *Salmonella* rapidly in-the-field and on-site at any locations along the food chains.

A free-standing, phage-based magnetoelastic (ME) biosensor has been developed as a novel wireless system for real-time and on-site detection of *Salmonella* Typhimurium in foods (Li et al., 2010). An ME biosensor method is composed of an ME resonator platform and genetically engineered filamentous E2 phage for the specific recognition of *S.* Typhimurium. The ME resonator platform allows to undergo its vibration under an applied alternating magnetic field and the initial resonant frequency of the ME platform immobilized with the filamentous E2 phage can be measured by a wireless pickup coil. Then, the measured ME biosensor is incubated for the binding with the *S.* Typhimurium in food. The binding of the *S.* Typhimurium with the E2 phage increases the mass of the ME biosensor and results in a decrease in the resonator's resonant frequency. This change in resonant frequency is directly proportional to the number of pathogens bound to the ME biosensors, therefore, the bacteria can be identified and quantified from the resonant frequency shifts of the ME biosensors (Grimes, Roy, Rani, & Cai, 2011; Park, Wikle, Chai, Horikawa & Chin, 2012). Recent studies (Li et al., 2010; Park, Li, & Chin, 2013) have demonstrated that *S.* Typhimurium on the surface of food can be successfully detected by directly placing the 1 mm-sized biosensors on the

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contaminated surface. The direct placement of biosensors on the surface of food decreases the redundant washing procedures so that the total detection time can be decreased to several minutes. 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.

To evaluate the ME biosensor method as a new rapid detection method, the performance of ME biosensor method should be compared with other methods that widely accepted detection methods such as quantitative real-time PCR (qPCR). A qPCR has showed excellent sensitivity, selectivity, and precise for effective identification and quantification of *Salmonella*. In addition, the qPCR has recognized as the widely accepted method for bacterial contamination identification in the laboratory (Noble & Weisberg, 2005; Postollec, Falentin, Pavan, Combrisson, & Sohier, 2011). The introduction of fluorogenic labelled probes (TaqMan® and Scorpion®) to qPCR also has enhanced the sensitivity and accuracy of qPCR, because the probe has a unique structure specifically designed to be hybridized to the target sequence (Elizaquível & Aznar, 2008).

Our previous study reported the direct experimental comparisons of the ME biosensor method with a TaqMan-based quantitative PCR method (qPCR) in vegetables such as tomatoes (Park, Park, Wikle, & Chin, 2012) and spinaches (Park, Park, Wikle, & Chin, 2013). Fruits and vegetables have diverse surface morphologies due to the roughness and curvature of fresh produce. Our preliminary study showed that the surface morphologies of cantaloupe surface were completely different from the surface of other vegetables. However, little information is known regarding the efficacy of ME biosensors on the different surfaces of fruits. Therefore, the comparison of the ME biosensors with the qPCR method will be performed on different fresh produce in order to obtain fully valid evaluation of the ME biosensor method. Since our filamentous E2 phage in ME biosensor method is selected from a landscape f8/8 phage library and specific to *S.* Typhimurium (Olsen, Sorokulova, Chen, Barbaree, & Vodyanoy, 2006; Petrenko & Sorokulova, 2004; Sorokulova et al., 2005), the specific primer for *S.* Typhimurium in qPCR is needed for valid comparison of both methods. Although Park et al. (2008) already designed a primer set specific to *S.* Typhimurium, there were still limited information and recognition of the primer set. Therefore, objectives of this study were to 1) demonstrate the selectivity of the qPCR primer set against *S.* Typhimurium, 2) establish the TaqMan-based qPCR method for *S.* Typhimurium detection on fresh produce, and 3) compare the ME biosensor method with TaqMan-based qPCR method on cantaloupes.

2. Materials and methods

2.1. Bacterial strains

Salmonella Typhimurium (ATCC 13311) and 74 bacterial strains listed in Table 2 were provided by Dr. Barbaree's laboratory in the Department of Biological Sciences at Auburn University, Auburn, AL, USA.

2.2. Spot inoculation

Cantaloupes (*Cucumis melo var. cantalupensis*) were purchased from a local grocery store (Auburn, AL, USA.). Only cantaloupes free of visible defects such as bruises, cuts, and abrasions were washed with sodium hypochlorite solution (5%) (Sigma–Aldrich Co., St. Louis, MO, USA) to remove or reduce all possible contaminants. After washing with sterilized distilled water (DW) five times, squares (25 mm^2) were drawn on the top of the smooth rind of cantaloupe with a permanent marker using a sterile paper template. Prior to inoculations, the culture of *S.* Typhimurium (10 log CFU/ml) was subsequently diluted with sterilized filtered water for use as the culture suspension. Ten microlitres of *S.* Typhimurium suspension at designed concentrations were inoculated on the marked square. The inoculated cantaloupes were placed in a glass container under laminar air flow for 90 min to allow the attachment *S.* Typhimurium to the surface of the cantaloupes.

Table 1
Primers and probes used in this study.

Target gene	Primer name	Sequence (5′–3′)	*Tm* (°C)	Reference
STM4497	Sal-F	GCG CAC CTC AAC ATC TTT C	54.4	Park et al. (2008)
	Sal-R	CGG TCA AAT AAC CCA CGT TCA	55.4	
	Sal-Probe	6FAM ATC ATC GTC GAC ATG CMGBNFQ		

2.3. qPCR method

The qPCR primer set (based on the STM4497 gene) was synthesized by IDT (Integrated DNA Technologies Inc., Coralville, IA, USA) and the *Salmonella*-probe was purchased from Applied Biosystems (Foster City, CA, USA) (Table 1). Primers and probe concentration were optimized using the 7300 Real-Time PCR System (Applied Biosystems). A reaction volume of 25 μl of PCR mixture contained 12.5 μl of TaqMan universal PCR master mix (Applied Biosystems), 10 μm each of primers and probe, filtered distilled water and 5 μl of extracted DNA. The thermal cycling condition was: 2 min at 50 °C, 10 min at 95 °C, 50 cycles of 15 s at 95 °C, and 1 min at 60 °C. All of the experiments were performed with three replicates of each purified DNA, with positive and negative controls. Detection and quantification of amplified DNA was expressed as threshold cycle (Ct) values. The Ct value is defined as the point at which probe fluorescence rises appreciably above the background.

2.4. ME biosensor method

Magnetoelastic (ME) strip-shaped resonator platforms (1×2 mm^2) were fabricated from METGLAS® 2826 MB ribbon (Honeywell Inc., Melville, NY, USA) following the procedures given in a previous study (Park, Wikle et al., 2012). E2 phage suspension [1.0×10^{12} vir/ml in Tris-Buffered Saline (TBS, pH 7.4)] provided by Dr. Barbaree's laboratory was mixed with the equal amounts of TBS buffer to adjust the concentration of E2 phage. Each ME platform was placed in an Eppendorf tube containing 300 μl of diluted E2 phage suspension and incubated on a rotary shaker (8 rpm) at 22 °C for 1 h. The ME resonator platforms were then washed three times with the TBS buffer and twice with sterilized distilled water in order to remove any unbound phage and salt debris. After washing, any unbound area of the ME platform was blocked with 300 μl of 1% bovine serum albumin (BSA, Sigma–Aldrich Co., St. Louis, MO, USA) at 22 °C for 1 h. Finally, the ME resonator platform was washed three times with sterilized DW and allowed to air dry for use as a measurement sensor. Control sensors were prepared using the same procedure, except for the elimination of the E2 phage immobilization step. Prior to placement of both measurement and control sensors on an inoculated surface, the initial resonant frequency of both types of sensors were measured with an HP 8751A network analyzer combined with an *S*-parameter test set.

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