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Fiber optic and light scattering sensors: Complimentary approaches to rapid detection of *Salmonella enterica* in food samples

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ABSTRACT

Salmonella-related foodborne infections present a major public health problem worldwide despite more stringent regulations. *Salmonella enterica* serovars Enteritidis and Typhimurium are the two most frequent causes of poultry related outbreaks; therefore, their rapid and accurate detection would improve *Salmonella* control at the farm, processing plant, and at retail. In this study, we investigated if a fiber optic immunosensor and light scattering sensor, BARDOT (**ba**cterial **r**apid **d**etection using **o**ptical scattering **t**echnology) could facilitate the detection of these two serovars in naturally contaminated poultry ($n = 50$). The fiber optic sensor with a detection limit of 10^3 CFU/ml identified *S. enterica* in selective enrichment broth in less than 12 h. The colonies (1.0 ± 0.2 mm) produced by plating the enriched samples on selective XLT4 agar for 13–15 h were scanned using BARDOT and *S. enterica* was identified after matching individual colony scatter patterns to the scatter image library with a sample-to-answer time of about 24 h. Both sensors identified 4 positive samples (8%), which corresponded to the results of the USDA-FSIS protocol, PCR, and lateral flow immunoassays. The colony scatter patterns identified all natural isolates as *S.* Enteritidis, which was further verified by serovar-specific PCR. The sensors used individually or in combination demonstrate potential for accurate and rapid detection of *S. enterica* in poultry.

1. Introduction

Salmonellosis, a disease caused by *Salmonella* species, is a major public health concern worldwide due to high morbidity rate and significant economic impact. Non-typhoidal salmonellosis is the leading cause of human food poisoning and gastrointestinal diseases. The Centers for Disease Control and Prevention (CDC) have reported a total of 1491 salmonellosis-related outbreaks between 1998 and 2008; among them, 1193 were caused by a single serotype (Jackson, Griffin, Cole, Walsh, & Chai, 2013). In the United States, about 1.2 million cases of salmonellosis are reported annually resulting in 378 deaths (Scallan et al., 2011) with an estimated economic loss exceeding $4.4 billion (Scharff, 2012). Among different *Salmonella* serovars, *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) and *S. enterica* serovar Typhimurium (*S.* Typhimurium) are the two most frequently reported serovars implicated in foodborne outbreaks worldwide. According to the National Enteric Disease Surveillance: *Salmonella* Annual Report, 2011 (CDC, 2011), *S.* Typhimurium and *S.* Enteritidis are responsible for 41.5% and 60% of the total *Salmonella*-related outbreaks in the USA and worldwide, respectively (Hendriksen et al., 2011). Poultry products are the most frequently identified reservoir for *S. enterica* serovars (Betancor et al., 2010; Vandeplas, Dauphin, Beckers, Thonart, & Thewis, 2010). However, products containing raw eggs, unpasteurized milk, beef, nuts, sprouted seeds, fruits, and unpasteurized fruit juices are also found to be contaminated with *S. enterica* (Callejón et al., 2015; CDC, 2012; Zweifel & Stephan, 2012).

Conventional culture-based methods employ standard USDA-FSIS or FDA protocols for *Salmonella* isolation and identification, which require approximately 5 days to obtain results (Taskila,

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Tuomola, & Ojamo, 2012; USDA-FSIS, 2013). Polymerase chain reaction (PCR)-based tests have been proven useful for *S. enterica* detection (Lofstrom, Krause, Josefsen, Hansen, & Hoorfar, 2009; Rodriguez-Lazaro et al., 2014). However, they are highly susceptible to matrix-associated inhibitors, may not differentiate live from dead bacteria (Hedman & Radstrom, 2013; Trombley Hall, McKay Zovanyi, Christensen, Koehler, & Devins Minogue, 2013), and do not allow whole cell recovery. Therefore, alternative methodologies, especially the ones that allow the recovery of the target pathogen for further epidemiological, molecular, and physiological (pathogenesis, antibiogram, response to sanitizers) investigations should be explored. The use of emerging novel techniques would provide highly specific, rapid, and sensitive results to assess food contamination with *S. enterica* (Bhunia, 2014). Furthermore, combined application of two or more detection technologies would yield greater accuracy and reduce false results. False-negative results can lead to foodborne outbreaks and human illnesses, while false-positive results may cause product rejection and significant economic losses, leading to overall erosion of confidence in a specific test as well as consumer's trust in a food brand. Therefore, in this study, we explored a possibility of using two sensing platforms, a fiber optic immunosensor and laser light scattering sensor as complimentary approaches for the detection and identification of *S. enterica* in artificially inoculated and naturally contaminated samples.

The fiber optic immunosensor is a sandwich immunoassay, which is based on capturing and detecting target pathogens on a polystyrene waveguide employing pathogen-specific fluorophor-labeled biorecognition molecules, including antibodies, aptamers, and receptor proteins (Bhunia, Nanduri, Bae, & Hirleman, 2010; Ohk, Koo, Sen, Yamamoto, & Bhunia, 2010; Sharma & Mutharasan, 2013). This sensor can be applied directly to the samples that are pre-enriched or selectively enriched in culture media, and the results can be obtained within 8–24 h depending on the type of pathogens and assay sensitivity (Bhunia et al., 2010; Sharma & Mutharasan, 2013). The method provides quantitative detection of the target pathogen, because signal intensity is proportional to pathogen concentration (Taitt, Anderson, & Ligler, 2005), and has been successfully applied for identification of many foodborne pathogens (Bosch, Sanchez, Rojas, & Ojeda, 2007; Leung, Shankar, & Mutharasan, 2007), including *S. enterica* (Kramer & Lim, 2004; Valadez, Lana, Tu, Morgan, & Bhunia, 2009).

BARDOT (bacterial rapid detection using optical scattering technology) is a laser-based forward light scattering sensor that utilizes a 635-nm red diode laser beam to capture scatter signatures of individual ~1-mm diameter colonies for real-time interrogation on the plate (Bhunia, 2011). The method is used for direct capturing of phenotypic characteristics of bacterial colonies obtained by classical microbiological methods to provide non-destructive, high-throughput analysis and real-time detection of *S. enterica* on agar plates without any labeling reagents or probes (Bae, Aroonnual, Bhunia, Robinson, & Hirleman, 2009; Banada et al., 2009). Recently, BARDOT has been validated to detect *S. enterica* serovars with high accuracy (>90%) using the colony scatter pattern library consisting of the top 20 *S. enterica* serovars of human origin (Singh et al., 2014). It can also classify the top 2 serovars (Enteritidis and Typhimurium) with high accuracy (>85%) three times faster than a conventional culture-based method (Singh et al., 2014). Furthermore, BARDOT was successfully applied to detect Shiga-toxigenic *Escherichia coli* (Tang et al., 2014), *Listeria monocytogenes* (Banada et al., 2007; Banada et al., 2009), *Vibrio* spp. (Huff et al., 2012), *Bacillus* spp. (Singh et al., 2015), and *Campylobacter* spp. (He et al., 2015) with classification accuracy of 90–99%.

In order to employ novel biosensor platforms to help with pathogen control at the farm, processing plant, and retail levels, the sensors need to be thoroughly evaluated using naturally contaminated samples. In our previous studies, it was reported that a fiber optic sensor (Valadez et al., 2009) and the light scattering sensor, BARDOT (Singh et al., 2014) can be used for the detection of *S. enterica* serovars. Because the fiber optic sensor detects pathogen-specific molecules, it is label-dependent, while BARDOT provides label-free detection based on the colony scatter image library. Therefore, our goal for this study was to demonstrate that both sensors could be used as complimentary approaches for early detection of *S. enterica* in naturally contaminated chicken carcasses after a brief enrichment step.

The critical points to consider for improving food safety are the reduction of foodborne salmonellosis through mitigating the passage of infectious agents in food supply chain, including pre-harvest and post-harvest operations, via efficient on-farm hygienic practices and food manufacturing; and accurate sample testing at each step during the farm-to-fork food supply continuum (Doyle & Erickson, 2012). Biosensors such as BARDOT have great potential to be used as high throughput screening tools to test samples without the need for labeling reagents or time-consuming isolation of a target microorganism (Bhunia, 2014). In order to facilitate potential application of this technology for on-farm food testing, it needs to be thoroughly evaluated for the performance in naturally contaminated samples. Therefore, the objective of this study was to investigate simultaneous implementation of two complimentary optical sensors for specific and accurate detection of *S. enterica* in artificially inoculated meat or naturally contaminated chicken carcass rinse samples from poultry processing plants in less than 24 h.

2. Materials and methods

2.1. Bacterial cultures and growth media

Ten strains of each *S enterica* serovars Enteritidis (PT1, PT4, PT6, PT7, PT8, PT13, PT13a, PT14, PT21 and PT28) and Typhimurium (NOS1, NOS2, NOS3, NOS3, NOS4, NOS5, NOS6, NOS7, NOS8, NOS20 and NOS24), and miscellaneous cultures (*L. monocytogenes*, *Klebsiella* spp., *Citrobacter* spp., *Proteus* spp., *Enterobacter aerogenes*, *E. coli*, and *Staphylococcus aureus*) were used in the experiments (Table S1). The cultures were grown from −80°C-frozen glycerol stocks by inoculation into brain heart infusion broth (BHI) and incubation at 37 °C overnight. To develop a light-scatter image library, selective agars xylose lysine deoxycholate (XLD; Acumedia, Neogen, Lansing, MI) and xylose lysine tergitol-4 (XLT4, Becton Dickinson, Sparks, MD, USA) were used. Buffered peptone water (BPW) and Rappaport-Vassiliadis (RV) broths (Acumedia) were used for pre-enrichment and selective enrichment, respectively.

2.2. Light scattering sensor-based detection

All laboratory cultures were grown for 16–18 h in BHI broth, serially (ten-fold) diluted in 20 mM phosphate buffered saline (PBS), pH 7.4, plated onto XLD and XLT4 agars, and incubated at 37 °C for 10–12 h and 13–15 h, respectively, or until colony diameter reached 1.0 ± 0.2 mm. Plates were screened using a prototype BARDOT machine, reported previously (Banada et al., 2009; Singh et al., 2015) and the colony scatter patterns were matched against the *S. enterica* scatter image libraries generated in our previous study (Singh et al., 2014). Initially, scatter patterns were compared with the CDC classified top 20 *Salmonella* serovars and non-*Salmonella* libraries, to determine if the isolates belonged to the genus *Salmonella*. Next, only *Salmonella*-positive scatter patterns were matched against the top 8 or top 2 *Salmonella* serovar libraries reported earlier (Singh et al., 2014) to identify if the