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A Rapid and Automated Fiber Optic–Based Biosensor Assay for the Detection of *Salmonella* in Spent Irrigation Water Used in the Sprouting of Sprout Seeds

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ABSTRACT

Recent outbreaks of foodborne illness have been linked to the consumption of contaminated sprouts. The spent irrigation water used to irrigate sprouts can carry many microorganisms, including pathogenic strains of *Escherichia coli* and *Salmonella enterica*. These pathogens are believed to originate from the seeds. The U.S. Food and Drug Administration recommends that sprout producers conduct microbiological testing of spent irrigation water from each production lot at least 48 h after seeds have germinated. Microbial analysis for the detection of *Salmonella* is labor-intensive and takes days to complete. A rapid and automated fiber-optic biosensor assay for the detection of *Salmonella* in sprout rinse water was developed in this study. Alfalfa seeds contaminated with various concentrations of *Salmonella* Typhimurium were sprouted. The spent irrigation water was assayed 67 h after alfalfa seed germination with the RAPTOR (Research International, Monroe, Wash.), an automated fiber optic–based detector. *Salmonella* Typhimurium could be positively identified in spent irrigation water when seeds were contaminated with 50 CFU/g. Viable *Salmonella* Typhimurium cells were also recovered from the waveguides after the assay. This biosensor assay system has the potential to be directly connected to water lines within the sprout-processing facility and to operate automatically, requiring manual labor only for preventative maintenance. Therefore, the presence of *Salmonella* Typhimurium in spent irrigation water could be continuously and rapidly detected 3 to 5 days before the completion of the sprouting process.

Raw seed sprouts are perceived as a healthy and beneficial food. However, recent outbreaks of *Salmonella, Bacillus cereus,* and *Escherichia coli* O157:H7 infections in the United States and abroad have been linked to the consumption of raw sprouts *(16, 22, 23, 26).* The U.S. Food and Drug Administration (FDA) has issued health warnings regarding the consumption of sprouts stating that persons in high-risk categories (i.e., children, elderly people, and immunocompromised people) and people who wish to reduce their risk of foodborne illness should not eat raw or lightly cooked sprouts *(2, 3).* The evidence points to sprout seeds as the source of the contamination *(13).* Soaking sprout seeds in a 20,000-mg/liter (ppm) calcium hypochlorite solution for 15 min is the current approved chemical seed treatment *(2),* but no FDA-approved treatment eliminates all bacteria from sprout seeds *(10, 23).* When even small numbers of pathogenic bacteria are present on sprout seeds, the bacteria multiply exponentially to infectious doses because of the microbiologically favorable warm, moist, and nutrient-rich conditions encountered during the sprouting process *(9, 22).*

The bacterial counts for the spent irrigation water used in the sprouting process have been shown to be within ca. 1 log of the bacterial counts found for the sprouts *(8, 9).* The spent irrigation water used to irrigate sprouts can act as a carrier of many microorganisms, including pathogenic strains of *E. coli* and *Salmonella enterica.* Sprout producers have been advised by the FDA to include microbiological testing of spent irrigation water at least 48 h after seeds have germinated as part of an overall strategy to enhance the safety of sprouts *(2).* Microbial analysis for the detection of specific microorganisms is labor-intensive and takes many days to complete *(1).* Therefore, there is a need for a rapid and automated assay for the detection of potential pathogens in spent sprout irrigation water.

Biosensors represent a unique technology with much potential to meet the need for rapid, sensitive, and versatile microbial detection systems. Fiber-optic evanescent-wave biosensors have been used to detect a wide variety of molecules, including the fraction 1 antigen of *Yersinia pestis (4), Clostridium botulinum* toxin A *(17),* pseudexin and ricin toxin *(18),* trinitrotoluene *(2, 12, 20, 21),* PCR–amplified DNA *(14),* staphylococcal enterotoxin *(24),* and D-dimer *(19).* In addition, fiber-optic evanescent-wave biosensors have been used to detect the human pathogens *Listeria monocytogenes (25)* and *E. coli* O157:H7 *(5–7).*

The usefulness of the fiber-optic evanescent-wave biosensor detection system has increased dramatically with the manufacture of an automated portable device, the RAPTOR (Research International, Monroe, Wash.). The RAPTOR uses a 635-nm laser diode to provide the excitation light that is launched into the proximal end of a waveguide, an injection molded optical polystyrene fiber. A coupon containing four fiber-optic waveguides is easily inserted into

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the RAPTOR prior to testing. Fluorescent molecules within ca. 100 to 1,000 nm of the waveguide surface are excited by the evanescent field, and a portion of their emission energy recouples into the fiber. A photodiode allows the quantitation of the collected emission light at wavelengths of >650 nm. The fiber-optic biosensor assay is based on a sandwich immunoassay that uses antibodies or other molecules to capture and detect the target pathogen. The captured target analyte is tagged with cyanine 5 (Cy5)–labeled antibody. Emission from the Cy5 is recorded in picoamperes (pA). The data are expressed as increases in fluorescence that are proportional in rate and magnitude to the target pathogen concentration. Sample preparation is typically minimal, since particulate matter does not interfere with the assay performance. The coupon can be reused for up to 40 different assays as long as the pathogen(s) under interrogation is not detected. In addition, upon completion of the detection assay, if the pathogen of interest has been positively identified, it can potentially be recovered by incubating the fiber-optic waveguide in enrichment broth and subsequently plating it onto a selective differential medium *(11)*.

A rapid (20-min) automated fiber optic–based biosensor assay for the detection of *S. enterica* Typhimurium in spent sprout irrigation water was developed in this study. *Salmonella* Typhimurium was then recovered from the waveguides in the coupon, and isolated colonies were grown on xylose lysine desoxycholate (XLD) medium. This automated detection system could potentially be set up to run automatically in-line in the spent irrigation water piping system for the continuous detection of pathogens in the spent irrigation water.

MATERIALS AND METHODS

Bacteria. *S. enterica* Typhimurium ATCC 49416, *Citrobacter freundii* ATCC 8090, *Klebsiella pneumoniae* ATCC 13883, *E. coli* ATCC 29417, and *E. coli* ATCC 23724 were obtained from the American Type Culture Collection (Manassas, Va.). *Shigella flexneri* ATCC 12022 and *Yersinia enterocolitica* ATCC 23715 were purchased from Hardy Diagnostics (Santa Maria, Calif.). *E. coli* O157:H7 was obtained from Dr. Harvey George (Massachusetts Department of Public Health State Laboratory Institute, Jamaica Plain, Mass.). Cultures were maintained on tryptic soy agar (TSA; Remel, Lenexa, Kans.) plates at 4°C. Cultures for assays and viable counts were grown on TSA plates (Becton Dickinson, Cockeysville, Md.) for 18 h in a 37°C incubator and serially diluted in sterile 0.01 M phosphate-buffered saline (PBS, pH 7.4) for use in experiments.

Preparation of spent alfalfa sprout irrigation water. Alfalfa sprout seeds were purchased from The Sprout House (Forest Hills, N.Y.). With a straight teasing needle, the bottoms of disposable 57-mm aluminum weigh dishes were perforated with 100 holes (1 mm each). One-gram seed samples were then placed onto each dish. Each dish was then placed in a 400-ml beaker, and the 1-g seed samples were soaked with 20 ml of deionized water at 24°C for 6 h. The seeds in each dish were then drained, washed with additional deionized water, and drained again. To contaminate seeds with *Salmonella* Typhimurium, aliquots of 100 μl of PBS or serial dilutions of *Salmonella* Typhimurium in PBS were added to each seed sample. The beakers were covered with cheesecloth and put in a cardboard box (to maintain darkness) at 24°C. The beakers were incubated for up to 5 days after seed germination. During the sprouting process, deionized water was added to the bottoms of the beakers in 1-ml aliquots once or twice a day to maintain high humidity. Samples of spent irrigation water were collected from the bottoms of the beakers. After *Salmonella* Typhimurium had grown in the spent irrigation water, viable cell counts for the spent irrigation water collected at the bottoms of the beakers were obtained. Viable cells were plated on BBL XLD agar (Becton Dickinson) and incubated at 37°C for 18 to 24 h. Colonies growing on the XLD agar were tested for slide agglutination with BiosPacific (Emeryville, Calif.) *Salmonella* Typhimurium monoclonal antibody.

Antibodies and labeling. Lyophilized affinity-purified goat antibody to common structural antigen-1 (CSA-1) of *Salmonella* Typhimurium was purchased from Kirkegaard & Perry Laboratories Inc. (KPL, Gaithersburg, Md.). Monoclonal antibody clone A6530228P to *Salmonella* Typhimurium was purchased from BiosPacific. Antibodies were rehydrated or diluted, labeled with Cy5 or biotin, and purified as described by DeMarco et al. *(7)*.

ELISA. Both the KPL and the BiosPacific antibodies were evaluated for specificity by enzyme-linked immunosorbent assay (ELISA). Volumes of all reactants (100 μl) were added to duplicate wells of 96-well microplates (Nunc MaxiSorp, Nalge Nunc International, Rochester, N.Y.). Serial dilutions of pure cultures in PBS were coated on wells for incubation for 18 h at 4°C. All further incubations were carried out at 24°C. Plates were washed one time with PBST (0.1 M PBS, 0.1% Tween 20 [pH 7.4]) and blocked with blocking buffer (2 mg of casein per ml and 2 mg of bovine serum albumin per ml in PBS). Plates were washed again, and then KPL or BiosPacific antibody diluted to 10 μg/ml in blocking buffer was incubated in wells for 30 min. Wells were washed three times with PBST, and horseradish peroxidase–labeled secondary antibody, anti-goat immunoglobulin G (IgG), or anti-mouse IgG diluted 1:500 in blocking buffer was added and incubated for 30 min. Wells were washed three times with PBST, and QuantaBlue substrate (Pierce Biotechnology, Rockford, Ill.) was added and incubated for 15 min. QuantaBlue stop solution was then added, and fluorescence was measured (in relative fluorescence units [RFU]) at 325 nm excitation and 420 nm emission with a Spectra Max Gemini XS fluorometer (Molecular Devices, Sunnyvale, Calif.). All ELISAs were performed at least twice. The KPL or BiosPacific antibody was designated cross-reactive when its fluorescence measurement was at least twofold higher than the that of the negative control (PBS, no adsorbed cells).

Instrument and waveguide preparation. The RAPTOR is a portable automatic fiber-optic biosensor. Polystyrene waveguides (4.5 cm long; Research International) used with the RAPTOR were sonicated for 30 s in an isopropanol bath. Waveguides were rinsed with deionized water; the distal tip of the waveguide was dipped in black paint to provide a light dump. After the paint dried, waveguides were added to glass capillary tube–incubation chambers and were incubated at 4°C for 18 to 22 h with streptavidin (Sigma Chemical Co., St. Louis, Mo.) at 100 μg/ml. Waveguides were rinsed with PBST, added to clean glass capillary tube–incubation chambers, and incubated with 0.1 ml of biotinylated antibody (100 μg/ml) in PBS (capture antibody) at 24°C for 30 min. The capture antibody solution was replaced with fresh solution and incubated for an additional 30 min. Four waveguides coated with capture antibody were then glued into each RAPTOR coupon. The coupon was sealed with sealing tape, and a coupon

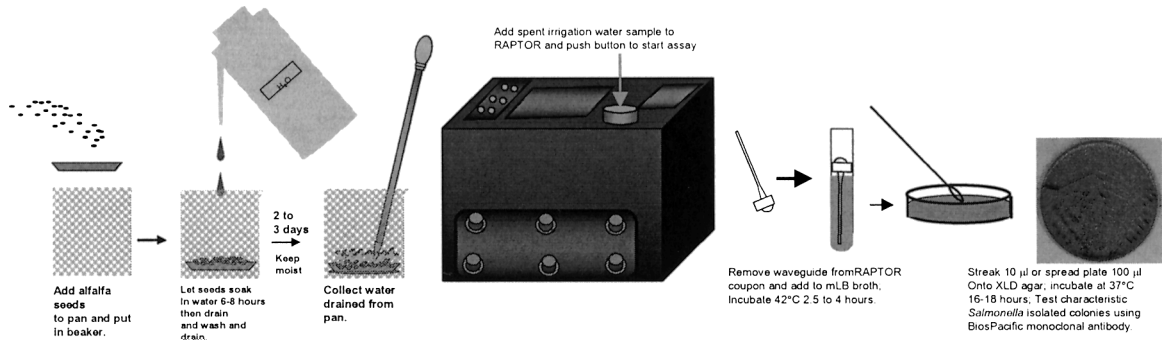

FIGURE 1. *Flow chart of entire procedure used in experiments.*

label was added. The RAPTOR was programmed to run automatically with a baseline recipe for each baseline reading and a sample recipe for each sample reading. The baseline recipe consisted of the following steps: the four waveguides were rinsed with PBST, the waveguides were incubated with reagent (Cy5-labeled antibody) for 5 min, the reagent was returned to the holding vessel, and the waveguides were rinsed twice with PBST. The 635-nm diode laser was then activated, and the emission signal was recorded after 5 s. The sample recipe consisted of incubating the four waveguides with a 1-ml sample for 10 min, rinsing the four waveguides with PBST, incubating the waveguides with reagent for 5 min, returning the reagent to the holding vessel, and rinsing the waveguides twice with PBST. The 635-nm diode laser was then activated, and the emission signal was recorded after 5 s. All automated assays were carried out at 24°C.

Baseline readings. Six baseline readings were taken. Reagent (detection antibody) consisted of Cy5-labeled antibody at 10 μg/ml in detection buffer (casein at 2 mg/ml and bovine serum albumin at 2 mg/ml in PBS). The emission value for each baseline reading was subtracted from the subsequent baseline reading; the calculated value was designated the ΔpA previous signal for baselines 2 through 6. The detection limit was calculated as the mean ΔpA previous signal for baselines 2 through 6 (mean baseline value) plus three times the standard deviation (SD).

Biosensor sensitivity assay. A 1-ml sample containing buffer or spent sprout irrigation water was added to the sample chamber of the RAPTOR, and the ''run assay'' button was pushed. The RAPTOR automatically ran a sample recipe as described above. When the first sample was tested, the last baseline reading was subtracted from the sample reading. When additional samples were tested, each previous sample reading was subtracted from the next sample reading. These calculated values for the samples were designated the ΔpA previous signals. The detection limit was subtracted from the ΔpA previous signal for each sample to obtain the value for the ΔpA previous signal minus the detection limit. If the value for the ΔpA previous signal minus the detection limit was larger than the detection limit, the sample was considered positive for *Salmonella* Typhimurium. If the value for the ΔpA previous signal minus the detection limit was less than or equal to the detection limit, the sample was considered negative for *Salmonella* Typhimurium. Four waveguides were tested in each assay, and all assays were performed at least three times.

To reduce variation among waveguides, the ΔpA previous signal value for each assay was normalized to the ΔpA previous signal obtained for 10^8 CFU of *Salmonella* Typhimurium. The ΔpA previous signal minus the detection limit was calculated for all assay readings. The last assay performed for each waveguide included a positive control sample containing 1×10^8 CFU of *Salmonella* Typhimurium per ml. The ΔpA previous signal for the sample containing 1×10^8 CFU of *Salmonella* Typhimurium per ml was arbitrarily adjusted to 100. The ΔpA previous signal values for all of the other samples were normalized to this value by the equation (ΔpA previous signal for sample tested/ΔpA previous signal for positive control) × 100.

Recovery of *Salmonella* Typhimurium from waveguides after completion of biosensor sensitivity assay. After the biosensor sensitivity assay had been completed, *Salmonella* Typhimurium was recovered from the waveguide as described by Kramer et al. *(11)* with the following modifications. Sterile tweezers were used to remove each waveguide from the coupon and to add the waveguide to 2.5 ml of enrichment broth in a sterile culture tube (12 by 75 mm). Enrichment broth consisted of modified Luria-Bertani (mLB; 1.0% tryptone, 1.0% casamino acids, 0.5% yeast extract, 1.0% NaCl). The enrichment broth was incubated at 42°C for 4 h. In some cases, the enrichment broth was incubated for only 2.5 h. After incubation, 10 or 100 μl of enrichment broth was streaked for isolation or spread onto XLD agar plates and incubated for 16 to 18 h at 37°C. At least three isolated colonies growing on each XLD agar plate were tested for slide agglutination with the use of the BiosPacific *Salmonella* Typhimurium monoclonal antibody. A flow chart of the entire procedure is presented in Figure 1. Duplicate samples were tested in each recovery assay.

RESULTS

Biosensor sensitivity assay. The KPL CSA-1 antibody was known to cross-react with other members of *Enterobacteriaceae* (described by KPL); therefore, the BiosPacific monoclonal antibody was tested for its usefulness as a detection antibody in the RAPTOR assay. Biosensor sensitivity assays were first performed with samples of *Salmonella* Typhimurium in buffer (PBS). When the ''run assay'' button of the RAPTOR was pushed, each 1-ml sample that had been added to the sample chamber was interrogated by four waveguides located in the RAPTOR coupon. The BiosPacific antibody performed as well as or better than the KPL CSA-1 antibody as a detection antibody in the biosensor assay (Fig. 2). Samples of *Salmonella* Typhimurium in buffer could be positively detected when the concentration was $\geq 10^5$ CFU/ml.

The KPL biotinylated CSA-1 *Salmonella* Typhimurium antibody was used for capture and the Cy5-labeled BiosPacific *Salmonella* Typhimurium monoclonal antibody was used for detection in all assays for the detection of *Sal-*

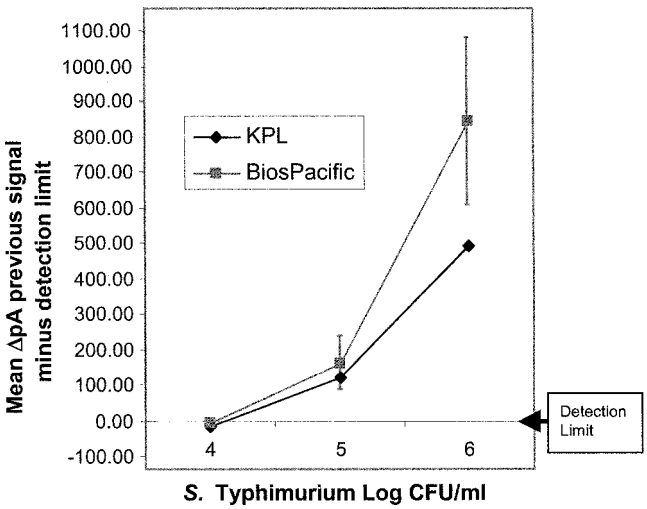

FIGURE 2. *Use of KPL CSA-1* Salmonella *Typhimurium antibody or BiosPacific* Salmonella *Typhimurium monoclonal antibody as a detection molecule in the biosensor assay.* Salmonella *Typhimurium resuspended in PBS was automatically injected into the RAPTOR coupon containing streptavidin and biotinylated KPL CSA-1 coated waveguides. The detection antibody (at 10 μg/ml) consisted of Cy-5-labeled KPL CSA-1* Salmonella *Typhimurium antibody or Cy-5-labeled BiosPacific* Salmonella *Typhimurium monoclonal antibody. The mean ΔpA previous signal minus the detection limit was calculated as described in "Materials and Methods." Error bars represent ±1 SD for the mean of four waveguides.*

monella Typhimurium in spent sprout irrigation water. Initially, seeds that had not been inoculated with *Salmonella* Typhimurium were germinated, and the spent irrigation water produced during the sprouting process was collected. The spent irrigation water was then inoculated with *Salmonella* Typhimurium, and these *Salmonella* Typhimurium–seeded samples were tested with the RAPTOR. The results obtained were compared with the results of biosensor assays of *Salmonella* Typhimurium–seeded buffer (Fig. 3A). The results were represented as the ΔpA previous signal minus the detection limit and were calculated as described in "Materials and Methods." The variation in ΔpA previous signal between individual waveguides was eliminated by normalization (Fig. 3B); no significant difference in sensitivity between the spent irrigation water and the buffer samples was observed. Samples of *Salmonella* Typhimurium in buffer or in spent sprout irrigation water could be positively detected when the concentration of *Salmonella* Typhimurium was $\geq 10^5$ CFU/ml.

Biosensor assay for spent irrigation water collected during sprouting of *Salmonella* Typhimurium–inoculated sprout seeds. Sprout seeds that had been inoculated with *Salmonella* Typhimurium were germinated and kept moist so that seeds could sprout. The sprout irrigation water was collected 19, 43, or 67 h after the germination of seeds and was then tested with the RAPTOR. The values were normalized because the nonnormalized values for each individual waveguide were highly variable. For example, the ΔpA previous signal minus the detection limit for the positive control PBS sample containing 1×10^8 CFU of *Salmonella* Typhimurium per ml fell between 600 and 8,000. The detection of *Salmonella* Typhimurium in spent sprout irrigation water collected 19, 43, and 67 h after seed germination is summarized in Table 1.

The assay did not detect the background bacterial flora indigenous to spent sprout irrigation water. No positive detection occurred when the biosensor assay was used to test uninoculated seeds, even though the spent irrigation water recovered during the sprouting of uninoculated seeds contained high levels (10^5 to 10^6 CFU/ml) of non–*Salmonella* Typhimurium bacteria (data not shown). *Salmonella* Typhimurium could be detected in spent sprout irrigation wa-

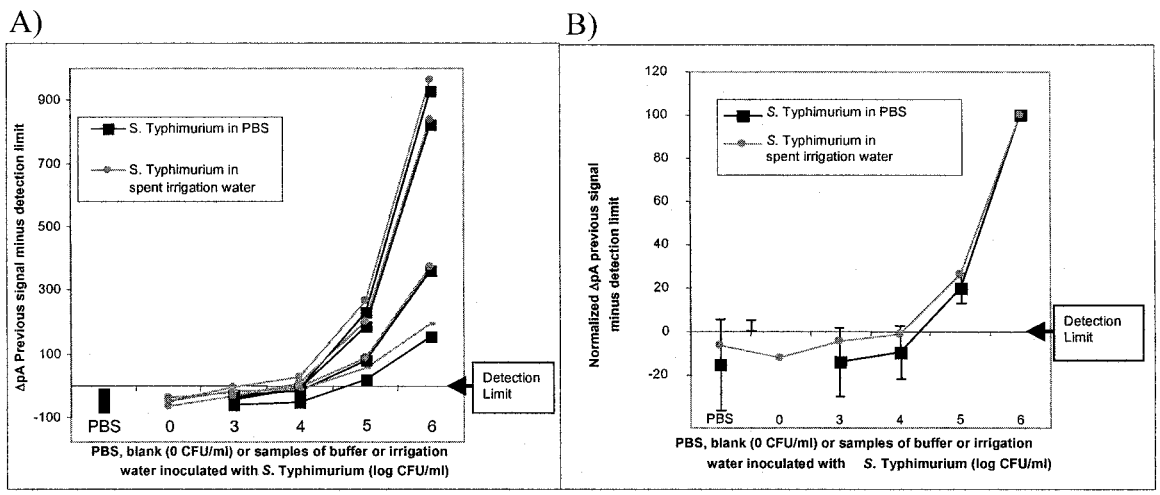

FIGURE 3. *Comparison of the RAPTOR biosensor assay with samples of buffer (PBS) and spent sprout irrigation water (spent irrigation water) spiked with* Salmonella *Typhimurium. Samples were automatically injected into the RAPTOR coupon containing streptavidin and biotinylated KPL CSA-1 coated waveguides. The detection antibody (at 10 μg/ml) consisted of Cy-5-lableled BiosPacific* Salmonella *Typhimurium monoclonal antibody. The ΔpA previous signal minus the detection limit was calculated as described in "Materials and Methods." (A) A representative assay for the detection of* Salmonella *Typhimurium. Four waveguides were used for testing buffer or spent irrigation water samples. (B) The normalized values for the mean of the ΔpA previous signal minus the detection limit when four waveguides were interrogated in the biosensor assays. Error bars represent ±1 SD for the mean of the four waveguides.*

TABLE 1. *RAPTOR biosensor assays results for spent sprout irrigation water collected 19, 43, and 67 h after germination of* Salmonella *Typhimurium–inoculated sprout seeds*

Concn (CFU/g) of *Salmonella* Typhimurium inoculated into seeds	Collection time (h) after seed germination	Concn (CFU/ml) of *Salmonella* Typhimurium in spent irrigation water	Normalized Mean ΔpA previous signal minus detection limit ± SD
0	19	$<10^a$	−2.6 ± 1.8
1.02×10^2	19	$<10^a$	−3.1 ± 3.1
1.02×10^3	19	$<10^a$	0.4 ± 2.0
1.02×10^4	19	1.1×10^6	11.8 ± 1.5
0	43	$<10^a$	−1.1 ± 0.3
1.02×10^3	43	4.0×10^4	5.6 ± 0.3
1.02×10^4	43	3.6×10^4	3.7 ± 0.3
1.02×10^5	43	3.0×10^6	12.1 ± 1.0
0	67	$<10^a$	−2.5 ± 1.3
0.5	67	5.0×10^2	−1.4 ± 0.6
5	67	2.0×10^4	0.1 ± 0.6
5.0×10^1	67	5.7×10^5	2.7 ± 0.6
5.0×10^2	67	6.7×10^5	36 ± 2.5
5.0×10^3	67	2.0×10^6	58 ± 5.7

[a] 0.1 ml plated on XLD agar.

ter on day 1 (19 h) and day 2 (43 h) of sprouting only when the contamination level of *Salmonella* Typhimurium in the seeds was high (10^4 and 10^3 CFU per g of seed, respectively). After 3 days of sprouting (67 h), however, *Salmonella* Typhimurium could be positively detected in spent sprout irrigation water even when the initial *Salmonella* Typhimurium contamination level for the seeds was as low as 5 CFU/g. The positive signal of 0.1 ± 0.6 for the normalized ΔpA previous signal minus the detection limit for spent irrigation water collected from seeds inoculated with 5 CFU of *Salmonella* Typhimurium per g, however, was very close to 0 and should be considered a suspect positive value. In addition, after 67 h of sprouting, spent irrigation water collected from sprouts produced from seeds inoculated with higher *Salmonella* Typhimurium levels (50 and 500 CFU per g of seeds) resulted in increased normalized values of 2.7 and 36 for the ΔpA previous signal minus the detection limit, respectively. These spent irrigation water samples also contained non–*Salmonella* Typhimurium bacteria at levels ranging from 10^5 to 10^6 CFU/ml (data not shown).

ELISA. Both the KPL and the BiosPacific antibodies were evaluated for specificity by ELISA. According to KPL, the *Salmonella* CSA-1 antibody is cross-reactive with other members of *Enterobacteriaceae.* BiosPacific has determined that the monoclonal antibody that was used in these studies is not cross-reactive with *Salmonella* Paratyphi A, *Salmonella* Choleraesuis, *Salmonella* Newport, *Salmonella* Enteritidis, *Salmonella* Anatum, or *Salmonella* Selandia. The specificity of the KPL and BiosPacific antibodies to *Salmonella* Typhimurium, *C. freundii, K. pneumoniae, S. flexneri, Y. enterocolitica, E. coli* ATCC 29417, *E. coli* ATCC 23724, and *E. coli* O157:H7 was determined by dividing the fluorescence measurement for each strain tested by the fluorescence measurement for the negative control (background). The KPL antibody reacted with *Salmonella* Typhimurium, *C. freundii, S. flexneri, E. coli* O157:H7, and *E. coli* ATCC 23724, giving fluorescence values of 5.6, 3.2, 2.1, 2.1, and 2.6 RFU, respectively, over the background. The BiosPacific antibody reacted only with *Salmonella* Typhimurium, giving a fluorescence value of 3.2 RFU over background.

Recovery of *Salmonella* Typhimurium from waveguides after completion of RAPTOR assay. Kramer et al. *(11)* have shown that viable *E. coli* O157:H7 cells can be isolated from the waveguide after a fiber-optic waveguide biosensor assay has been completed. The cells are recovered from the waveguide through incubation of the previously tested waveguide in an enrichment broth for 4 h followed by the streaking of 10 μl for isolation or by the plating of 100 μl of the enrichment broth onto *E. coli* O157:H7–selective differential agar. The next day, after incubation overnight, isolated colonies of *E. coli* O157:H7 had grown on the agar. With the use of a modified protocol, isolated colonies of *Salmonella* Typhimurium were recovered from the waveguides. A single sample of spent sprout irrigation water collected from sprouts grown from *Salmonella* Typhimurium–contaminated seeds was tested on each waveguide. Each waveguide was then added to mLB broth and incubated for 2.5 to 4 h. The mLB broth was then plated onto XLD agar and incubated overnight. Table 2 lists the different concentrations of *Salmonella* Typhimurium inoculated into the seeds and shows the recovery of *Salmonella* Typhimurium from the waveguides used to detect *Salmonella* Typhimurium in the spent irrigation water. It was possible to recover *Salmonella* Typhimurium colonies from waveguides when the spent sprout irrigation water that was tested had been collected from sprouts grown from seeds contaminated with at least 5 CFU of *Salmonella* Typhimurium per g of seeds.

DISCUSSION

Microorganisms such as pathogenic strains of *E. coli* and *S. enterica* can grow to exponential numbers in sprouts when present even at low levels in the sprout seeds, and these high levels are also found in the sprout irrigation water *(8, 9, 13).* The FDA requires sprout producers to test spent irrigation water from each production lot for the presence of pathogens at least 48 h after seeds have germinated *(2).* Conventional analysis for the detection of *Salmonella* is labor-intensive and takes days to complete *(1).* A rapid (20-min) automated fiber optic–based biosensor assay for the detection of *Salmonella* in sprout rinse water was developed in this study. This assay could directly detect a minimum of 5×10^5 CFU of *Salmonella* Typhimurium per ml in spent alfalfa sprout irrigation water (Fig. 3). The spent irrigation water collected from sprouts grown from alfalfa seeds contaminated with various concentrations of *Salmonella* Typhimurium was assayed with the RAPTOR, an automated fiber optic–based biosensor assay system (Table 1). *Salmonella* Typhimurium could be detected in spent irri-

TABLE 2. *Representative example of recovery of* Salmonella *Typhimurium from waveguides used to detect* Salmonella *Typhimurium in samples of spent sprout irrigation water*

Salmonella Typhimurium concn (CFU/g) in sprout seeds	ΔpA previous signal above detection limit of RAPTOR assay	Enrichment incubation time (h)	Volume plated (μl)	No. of black colonies isolated on XLD agar[a]
0	−17	4	100	0
0.5	−11	4	100	0
5	−21	4	100	53
50	6.4	4	10	100
500	59	2.5	100	50
5,000	1,303	2.5	10	TNTC

[a] At least three colonies per XLD agar plate were tested for agglutination reaction with the use of BiosPacific *Salmonella* Typhimurium monoclonal antibody. In every instance, all colonies demonstrated positive agglutination. TNTC, too numerous to count.

gation water that had been collected 19 and 43 h after seed germination, but to obtain positive detection, the concentration of *Salmonella* Typhimurium in the seeds had to be high (10^4 and 10^3 CFU/g of seed for 19- and 43-h postgermination spent irrigation water, respectively). *Salmonella* Typhimurium could be positively identified in spent irrigation water that had been collected 67 h after seed germination when the *Salmonella* Typhimurium concentration in the seeds was only 50 CFU/g of seed. It has been shown that the conditions generated during sprout production are favorable for the exponential growth of bacteria *(8, 9).* When the spent sprout irrigation water is collected 3 days (67 h) after seed germination, the additional days of sprouting allow the bacterial counts for the sprouts and irrigation water to reach saturation levels *(8, 9).* The alfalfa sprouting process takes 5 to 7 days, so even after 3 days, the sprouts have not finished sprouting. Collection and biosensor testing of spent sprout irrigation water from 3-day-old sprouts would therefore be a very workable and commercially feasible alternative solution for the sprouting industry. Ideally, an in-line biosensor assay should be capable of detecting *Salmonella* in spent irrigation water produced from seeds contaminated with *Salmonella* levels even lower than 50 CFU/g of seed. Studies to improve the sensitivity of the biosensor assay are ongoing in our research laboratories.

It was possible to recover *Salmonella* Typhimurium colonies from waveguides after the completion of the biosensor assay when the spent sprout irrigation water that was tested had been collected from sprouts that were grown from seeds contaminated with at least 5 CFU of *Salmonella* Typhimurium per g of seed. Therefore, *Salmonella* could be recovered from waveguides even when the waveguide used in the biosensor assay was barely able to positively detect *Salmonella* Typhimurium. The recovery of *Salmonella* cells from the waveguide proves that *Salmonella* cells have been captured by the waveguide.

There are >2,300 serovars of *S. enterica,* and *Salmonella* Typhimurium represents only one of these serovars *(15).* Therefore, there is still a need to develop a rapid assay that will detect most pathogenic strains of *Salmonella*. The development of such an assay will depend on the construction or discovery of a single molecule or of only a few molecules that have the ability to bind specifically to the majority of *Salmonella* serovars. The specificity of the biosensor assay described here lies in the specificity of the BiosPacific antibody. The assay does not detect the background bacterial flora indigenous to spent sprout irrigation water; no positive detection occurred when the biosensor assay was used to test spent irrigation water from uninoculated seeds even though the spent irrigation water contained high levels of background bacteria. The assay therefore shows how a biosensor assay that detects the majority of *Salmonella* strains that cause foodborne illness could be used.

This biosensor assay system has the potential to be directly connected to water lines within the sprout-processing facility and to operate automatically, requiring manual labor only for preventative maintenance. Therefore, the presence of *Salmonella* Typhimurium in spent irrigation water could be continuously and rapidly determined 3 to 5 days before the completion of the sprouting process. The RAPTOR coupon could be reused up to 40 times within a 24-h testing period. Positive detection by the RAPTOR could be linked to a warning system so that the production crew would be immediately notified of pathogen contamination. Upon completion of an assay demonstrating positive detection, the coupon containing the pathogen could be removed and the presence of the pathogen could be confirmed through isolation of the target pathogen from the waveguides.

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