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FOOD-BORNE PATHOGENS

Comparison of an immunochromatographic method and the TaqMan® *E. coli* O157:H7 assay for detection of *Escherichia coli* O157:H7 in alfalfa sprout spent irrigation water and in sprouts after blanching[1]

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An immunochromatographic-based assay (Quix™ *E. coli* O157 Sprout Assay) and a polymerase chain reaction (PCR)-based assay (TaqMan® *E. coli* O157:H7 Kit) were used to detect *Escherichia coli* O157:H7 strain 380-94 in spent irrigation water from alfalfa sprouts grown from artificially contaminated seeds. Ten, 25, 60, or 100 seeds contaminated by immersion for 15 min in a suspension of *E. coli* O157:H7 at concentrations of 10^6 or 10^8 cfu/ml were mixed with 20 g of non-inoculated seeds in plastic trays for sprouting. The seeds were sprayed with tap water for 15 s every hour and spent irrigation water was collected at intervals and tested. *E. coli* O157:H7 was detected in non-enriched water by both the TaqMan® PCR (30 of 30 samples) and the immunoassay (9 of 24 samples) in water collected 30 h from the start of the sprouting process. However, enrichment of the spent irrigation water in brain heart infusion (BHI) broth at 37°C for 20 h permitted detection of *E. coli* O157:H7 in water collected 8 h from the start of sprouting using both methods, even in trays containing as few as 10 inoculated seeds. The TaqMan® PCR assay was more sensitive (more positive samples were observed earlier in the sprouting process) than the immunoassay; however, the immunoassay was easier to perform and was more rapid. At 72 h after the start of the sprouting process, the sprouts were heated at 100°C for 30 s to determine the effectiveness of blanching for inactivation of *E. coli* O157:H7. All of the 32 samples tested with the TaqMan® assay and 16 of 32 samples tested with the Quix™ assay gave positive results for *E. coli* O157:H7 after enrichment of the blanched sprouts at 37°C for 24 h. In addition, the organism was detected on Rainbow® Agar O157 in 9 of 32 samples after 24 h of enrichment of the blanched sprouts. In conclusion, *E. coli* O157:H7 was detected in spent irrigation water collected from sprouts grown from artificially contaminated seeds by both the TaqMan® and Quix™ assays. The data also revealed that blanching may not be effective to completely inactivate all the *E. coli* O157:H7 that may be present in sprouts.
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Keywords: TaqMan®; PCR; immunoassay; *E. coli* O157:H7; Rainbow® Agar; alfalfa sprouts

Introduction

Raw seed sprouts have become important vehicles of food-borne disease in recent years [3,10,15]. *Escherichia coli* O157:H7 and *Salmonella* spp. are the pathogens most frequently associated with outbreaks linked to sprouts [15]. Contamination of sprouts can occur through use of seeds contaminated with pathogenic microorganisms or may occur during the sprouting process. The FDA has recently proposed testing of spent irrigation water (i.e., irrigation water that has flowed over and through the sprouts) during sprout production for the presence of pathogens as a means for determining the safety of individual batches of sprouts [5]. Consequently, rapid, sensitive, and reliable methods, which can detect pathogenic organisms at relatively low levels during the sprouting process, are needed to identify contaminated sprouts, which should not enter the food supply.

Immunologic assays employing antibodies specific for target organisms or genetic techniques, such as the polymerase chain reaction (PCR), are relatively rapid and sensitive methods that can be used for detection of specific pathogens in sprouts and other types of foods. However, these types of assays are generally not able to detect pathogens present in foods or other materials when the organisms are present at levels less than 1000 colony-forming units (cfu)/g or ml; therefore, a sample enrichment step to increase the concentration of the target organism is required. An enrichment step lengthens the amount of time required to perform the assay; however, the assay sensitivity is increased. A number of selective and differential agar media that can be used for isolation and enumeration of *E. coli* O157:H7 in foods are commercially available. One type of selective/differential agar medium, Rainbow® Agar O157,

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[1]Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture above others of a similar nature not mentioned.

enhances the primary isolation and the ability to enumerate *E. coli* O157:H7 in food enrichments [6].

Since decontamination methods for seeds and sprouts may not always be effective, and pathogens including *E. coli* O157:H7 can grow to levels as high as 10^7 cfu/g during the sprouting process [14], it has been suggested that heating or cooking of sprouts prior to consumption may ensure that pathogenic bacteria such as *E. coli* O157:H7 and *Salmonella* are inactivated [12]. The objectives of this study were to compare the use of a commercial immunochromatographic assay (the Quix™ *E. coli* O157 Sprout Assay) and the PCR-based TaqMan® *E. coli* O157:H7 assay for detection of *E. coli* O157:H7 in alfalfa sprout spent irrigation water and to employ the Quix™ and TaqMan® assays in combination with plating on Rainbow® Agar O157 to determine if a short period of blanching of the contaminated sprouts eliminates viable *E. coli* O157:H7.

Materials and methods

Inoculation of seeds

E. coli O157:H7 strain 380-94 (salami isolate, obtained from the USDA Food Safety and Inspection Service, Athens, GA) was cultured at 37°C for 18 h in brain heart infusion broth (BHI; Difco Laboratories, Detroit, MI). The culture was diluted in 250 ml of BHI to obtain concentrations of 10^6 and 10^8 cfu/ml. Alfalfa seeds (*ca.* 15 g; Caudill Seed, Louisville, KY) were soaked in each of the bacterial suspensions for 15 min at ambient temperature. The suspension was decanted, and the seeds were spread evenly on wire racks covered with cheesecloth, then dried in a laminar flow hood for 24 h at room temperature.

Preparation of seeds for sprouting

Uninoculated alfalfa seeds (20 g) were mixed in 250-ml beakers with 10, 25, 60, or 100 seeds inoculated as described above, whereas the control samples contained no inoculated seeds. Tap water was added to cover the seeds, and after gentle stirring by hand for 3 min at ambient temperature, the water was decanted. The seeds were then washed with standard household bleach diluted 1:50 with tap water (final NaOCl concentration of approximately 0.1%, equivalent to 1000 ppm) for 3 min with gentle stirring, then three times with tap water, leaving the seeds in the third rinse water for 2 h prior to draining. A second set of samples was not treated with the NaOCl solution, but was simply washed three times with water, leaving the seeds in the third rinse water for 2 h prior to draining. These samples are referred to as "nb" (no bleach).

Sprouting of seeds

The seeds were spread evenly in plastic trays (sterilized plastic lids of pipet tip boxes, 87×124 mm) in which three small holes were made along the length of one side. The trays were positioned at a 15° angle to allow water to drain through the holes into sterile solution basins (55 ml; Labcore Products, Frederick, MD). The seeds were sprayed using a spray bottle with *ca.* 15–20 ml of tap water for 15 s every hour during the day (for 9 h), and the spent irrigation water was collected from the basins at 8, 30, 48, and 72 h after the start of sprouting for testing as described below. Trays were covered with paper towels and kept at ambient temperature during sprouting.

Enrichment of spent irrigation water and detection of E. coli O157:H7

Spent irrigation water was tested immediately after collection, incubated at room temperature for 20 h prior to testing, and subjected to enrichment by adding 9 ml of the water to 1 ml of 10× strength BHI containing novobiocin (0.02 mg/ml; Sigma, St. Louis, MO) and incubated at 37°C at 150 rpm for 20 h. A few drops of the enriched and non-enriched water samples were tested for the presence of *E. coli* O157:H7 with the Quix™ *E. coli* O157 Sprout Assay (Guardian Scientific, Columbia, MD) according to the manufacturer's instructions. The rest of the 37°C enrichment (approximately 9 ml) were centrifuged to collect the bacteria, and DNA extraction was performed using the PrepMan™ Sample Preparation Reagent (PE-Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Ten milliliters of non-enriched spent irrigation water was also subjected to DNA extraction using the PrepMan™ reagent. All samples incubated for 20 h at room temperature were tested using only the Quix™ assay. All samples subjected to DNA extraction using the PrepMan™ reagent were tested using the TaqMan® *E. coli* O157:H7 Kit according the manufacturer's instructions. The 96-well reaction plates were read using an ABI PRISM 7200 Sequence Detector (PE-Applied Biosystems). All experiments were performed at least twice.

Enumeration of E. coli O157:H7 and blanching of sprouts

Seventy-two hours after the start of the sprouting process, 10 g of sprouts were pummeled in 90 ml of 0.1% peptone water using a Stomacher (Model 400; Tekmar, Cincinnati, OH), and dilutions were plated onto Rainbow® Agar O157 (Biolog, Hayward, CA) using a Spiral Plater (Model D; Spiral Biotech, Bethesda, MD) to determine the level of *E. coli* O157:H7 in the sprouts. The remaining sprouts were blanched for 30 s (timing was started a few seconds after immersion of sprouts when water resumed boiling), drained using a sterile colander, placed on ice for 1 min, then added to 500 ml of tryptic soy broth (Difco) and enriched for 20 h at 37°C at 150 rpm. The enrichment broth was tested using the Quix™ strips, and a portion (10 ml) was subjected to the PrepMan™ procedure followed by testing with the TaqMan® assay. Dilutions of the enrichment were also plated on Rainbow® Agar O157 for enumeration and to confirm the presence of *E. coli* O157:H7.

Results and discussion

The Quix™ *E. coli* O157 Sprout Assay is an immunochromatographic assay employing anti-*E. coli* O157 polyclonal antibody conjugated with colloidal gold particles immobilized on the test strip. Positive results using the Quix™ strips were generally observed earlier in trays containing 60 and 100 contaminated seeds compared to trays containing 10 or 25 contaminated seeds. This is more evident in samples of spent irrigation water tested using Quix™ strips after enrichment at 37°C (Table 3). After broth enrichment of the water collected 8 h after the start of the sprouting process, all of the water samples from trays that contained 60 or 100 inoculated seeds were positive, whereas five of eight of the water samples collected from trays that contained 10 inoculated seeds or 25 inoculated seeds were positive.

Generally, incubation of spent irrigation water at room temperature for 20 h prior to testing did not enhance detection

Table 1 Testing of non-enriched spent irrigation water from sprouts grown from seeds artificially contaminated with *E. coli* O157:H7

Number of inoculated seeds	Inoculum[a] (cfu/ml)	8 h[b]		30 h		48 h		72 h
		TaqMan PCR	Quix assay	TaqMan PCR	Quix assay	TaqMan PCR	Quix assay	Quix assay
10	10^6	–[c]/+[c]	NT/NT[c]	+/+	–/NT	NT/NT	–/–	–/–
	10^6 nb[c]	–/–	NT/NT	+/+	–/NT	NT/NT	–/–	–/–
	10^8	–/+	NT/NT	+/+	–/NT	NT/NT	–/–	–/–
	10^8 nb	–/+	NT/NT	+/+	–/NT	NT/NT	–/–	–/–
25	10^6	–/–	NT/NT	+/+	–/–	NT/NT	–/–	–/–
	10^6 nb	+/+	NT/NT	+/+	–/–	NT/NT	–/–	–/–
	10^8	+/+	NT/NT	+/+	+/–	NT/NT	NT/+	NT/+
	10^8 nb	+/+	NT/NT	+/+	+/+	NT/NT	NT/+	NT/NT
60	10^6	–/+	NT/NT	+/+	NT/–	NT/NT	–/–	–/–
	10^6 nb	+/–	NT/NT	+/+	NT/–	NT/NT	–/–	–/–
	10^8	+/+	NT/NT	+/+	NT/+	NT/NT	–/NT	–/NT
	10^8 nb	+/+	NT/NT	+/+	NT/+	NT/NT	–/NT	–/NT
100	10^6	+/+	NT/NT	+/+	–/–	NT/NT	–/–	–/–
	10^6 nb	+/+	NT/NT	+/+	–/–	NT/NT	+/–	+/–
	10^8	+/+	NT/NT	+/+	+/+	NT/NT	+/NT	NT/NT
	10^8 nb	+/+	NT/NT	+/+	+/+	NT/NT	+/NT	NT/NT

[a]Seeds were soaked in BHI broth containing *E. coli* O157:H7 at 10^6 and 10^8 cfu/ml.
[b]Hours after the start of the sprouting process, spent irrigation water was collected for testing. Results of two separate experiments are shown.
[c]nb=no bleach (seeds not rinsed in NaOCl solution); NT=not tested; –=negative; +=positive.

of *E. coli* O157:H7 by the Quix™ strips compared to testing of the water immediately after sampling. An exception occurred with one set of samples containing 60 inoculated seeds (10^8, 10^8 nb) collected at 48 h, which was positive after incubation at room temperature (Table 2), but negative when not enriched (Table 1). Compared to no enrichment or incubation at room temperature, enrichment of the spent irrigation water at 37°C enhanced the ability to detect *E. coli* O157:H7. Of all of the enriched water samples (from two separate experiments) collected 8 h after the start of the sprouting process, 26 of 32 were positive (Table 3), whereas 21 of 32 non-enriched water samples were still negative by the Quix™ assay at 72 h after the start of the sprouting process (Table 1).

The TaqMan® *E. coli* O157:H7 fluorogenic PCR-based assay utilizes the 5′ to 3′ nuclease activity of *Taq* DNA polymerase to digest a probe, which binds to a region of DNA internal to the two primer binding sites. The probe is labeled with a fluorescent reporter dye at the 5′ end and a fluorescent quencher dye at the 3′ end. As the polymerase extends from the PCR primer, it cleaves the probe separating the quencher dye from the reporter dye resulting in an increase in fluorescence intensity. Using a TaqMan®-based PCR assay, Witham *et al* [17] could detect as few as 0.5 cfu of

Table 2 Testing of spent irrigation water incubated at room temperature for 20 h from sprouts grown from seeds artificially contaminated with *E. coli* O157:H7

Number of inoculated seeds	Inoculum[a] (cfu/ml)	Quix assay			
		8 h[b]	30 h	48 h	72 h
10	10^6	–[c]/–	–/–	–/–	–/–
	10^6 nb[c]	–/–	–/–	–/–	–/–
	10^8	–/–	–/–	–/–	–/–
	10^8 nb	–/–	–/–	–/–	–/–
25	10^6	–/–	–/–	–/–	–/–
	10^6 nb	–/–	–/–	–/–	–/–
	10^8	+[c]/–	+/–	NT[c]/+	NT/NT
	10^8 nb	+/–	+/+	NT/+	NT/NT
60	10^6	–/–	–/–	–/–	–/–
	10^6 nb	–/–	–/–	–/–	–/–
	10^8	–/–	+/+	+/NT	NT/NT
	10^8 nb	–/+	–/+	+/NT	NT/NT
100	10^6	–/–	–/–	–/–	–/–
	10^6 nb	–/–	–/–	+/–	+/–
	10^8	+/–	+/+	+/NT	NT/NT
	10^8 nb	+/–	+/+	+/NT	NT/NT

[a]Seeds were soaked in BHI broth containing *E. coli* O157:H7 at 10^6 and 10^8 cfu/ml.
[b]Hours after the start of the sprouting process, spent irrigation water was collected for testing. Results of two separate experiments are shown.
[c]nb=no bleach (seeds not rinsed in NaOCl solution); NT=not tested; –=negative; +=positive.

Table 3 Testing of spent irrigation water enriched at 37°C for 18 h from sprouts grown from seeds artificially contaminated with *E. coli* O157:H7

Number of inoculated seeds	Inoculum[a] (cfu/ml)	8 h[b]		30 h		48 h		72 h
		TaqMan PCR	Quix assay	TaqMan PCR	Quix assay	TaqMan PCR	Quix assay	Quix assay
10	10^6	+[c]/ –[c]	+/ –	NT[c]/ +	NT/ +	NT/NT	NT/NT	NT/NT
	10^6 nb[c]	+/+	– /+	+/+	+/NT	NT/NT	NT/NT	NT/NT
	10^8	+/+	– /+	+/+	+/+	NT/NT	NT/NT	NT/NT
	10^8 nb	+/+	+/+	NT/NT	NT/NT	NT/NT	NT/NT	NT/NT
25	10^6	– / –	– / –	+/+	+/+	NT/NT	NT/NT	NT/NT
	10^6 nb	+/ –	– /+	+/NT	+/NT	NT/NT	NT/NT	NT/NT
	10^8	+/+	+/+	NT/NT	NT/NT	NT/NT	NT/NT	NT/NT
	10^8 nb	+/+	+/+	NT/NT	NT/NT	NT/NT	NT/NT	NT/NT
60	10^6	+/+	+/+	NT/NT	NT/NT	NT/NT	NT/NT	NT/NT
	10^6 nb	+/+	+/+	NT/NT	NT/NT	NT/NT	NT/NT	NT/NT
	10^8	+/+	+/+	NT/NT	NT/NT	NT/NT	NT/NT	NT/NT
	10^8 nb	+/+	+/+	NT/NT	NT/NT	NT/NT	NT/NT	NT/NT
100	10^6	+/+	+/+	NT/NT	NT/NT	NT/NT	NT/NT	NT/NT
	10^6 nb	+/+	+/+	NT/NT	NT/NT	NT/NT	NT/NT	NT/NT
	10^8	+/+	+/+	NT/NT	NT/NT	NT/NT	NT/NT	NT/NT
	10^8 nb	+/+	+/+	NT/NT	NT/NT	NT/NT	NT/NT	NT/NT

[a]Seeds were soaked in BHI broth containing *E. coli* O157:H7 at 10^6 and 10^8 cfu/ml.
[b]Hours after the start of the sprouting process, spent irrigation water was collected for testing. Results of two separate experiments are shown.
[c]nb=no bleach (seeds not rinsed in NaOCl solution); NT=not tested; – =negative; + =positive.

Shiga toxin-producing *E. coli* per gram of ground beef after 12 h of enrichment. It was possible to detect as few as 50 cfu of *Listeria monocytogenes* employing a TaqMan® system based on the *L. monocytogenes hlyA* gene as target [1]. In the present study, the TaqMan® PCR assay was more sensitive than the Quix™ *E. coli* O157 Sprout Assay. *E. coli* O157:H7 was detected in non-enriched spent irrigation water collected 8 h after the start of the sprouting process from all trays containing 100 inoculated seeds and from three of four trays from each experiment containing 10, 25, and 60 inoculated seeds, with the exception of one experiment in which 10 inoculated seeds were used and no water samples were positive (Table 1). Generally, the negative results were obtained from trays containing seeds that were inoculated in *E. coli* suspensions at 10^6 cfu/ml. All of the non-enriched water samples collected at 30 h were positive by the TaqMan® assay. Positive results using the Quix™ assay were obtained for all of the spent irrigation water samples collected at 30 h only when the water was subjected to enrichment at 37°C (Table 3).

Sodium or calcium hypochlorite at various concentrations has been used to disinfect seeds prior to sprouting [11,15]. In the

Table 4 Testing for *E. coli* O157:H7 in sprouts before and after blanching

Number of inoculated seeds	Inoculum[a] (cfu/ml)	Sprouts[b]	Blanched sprouts[c]		Blanched sprouts[c]
		$\log_{10}$ cfu/g	TaqMan PCR	Quix assay	$\log_{10}$ cfu/ml
10	10^6	4.10/<3.32[d]	+[e]/+	–[e]/ –	<3.32/<3.32
	10^6 nb[e]	3.80/<3.32	+/+	– / –	<3.32/<3.32
	10^8	<3.32/3.92	+/+	– /+	<3.32/<3.32
	10^8 nb	4.10/3.32	+/+	– / –	<3.32/<3.32
25	10^6	<3.32/<3.32	+/+	– / –	6.80/<3.32
	10^6 nb	3.32/<3.32	+/+	+/ –	8.54/<3.32
	10^8	3.43/4.49	+/+	+/+	8.58/<3.32
	10^8 nb	4.94/5.47	+/+	+/+	8.42/<3.32
60	10^6	<3.32/3.50	+/+	– / –	<3.32/<3.32
	10^6 nb	2.84/3.60	+/+	+/ –	7.02/<3.32
	10^8	4.73/4.72	+/+	+/+	<3.32/<3.32
	10^8 nb	5.01/5.39	+/+	+/+	<3.32/<3.32
100	10^6	3.92/4.75	+/+	+/+	3.13/<3.32
	10^6 nb	5.32/<3.32	+/+	– / –	<3.32/<3.32
	10^8	5.05/4.90	+/+	+/+	2.60/<3.32
	10^8 nb	5.16/5.54	+/+	+/+	7.68/8.88

[a]Seeds were soaked in BHI broth containing *E. coli* O157:H7 at 10^6 and 10^8 cfu/ml.
[b]After 72 hours, 10 g of sprouts was pummeled in 90 ml of 0.1% peptone water and plated on Rainbow® Agar O157.
[c]Blanched sprouts were enriched in TSB at 37°C (150 rpm) for 20 h, plated on Rainbow® Agar O157, and enrichments were tested by the TaqMan® PCR and the Quix™ assay.
[d]Overgrowth of mucoid colonies raised the limit of detection on Rainbow® Agar O157.
[e]nb=no bleach (seeds not rinsed in NaOCl solution); NT=not tested; – =negative; + =positive.

present study, the seeds were washed with a relatively mild NaOCl solution (1000 ppm NaOCl wash for 3 min) prior to sprouting to determine whether the NaOCl treatment would influence the ability to detect *E. coli* O157:H7 in the spent irrigation water. There was no appreciable difference in the ability to detect *E. coli* O157:H7 using the Quix™ or TaqMan® assays in spent irrigation water collected from samples in which the seeds had been treated with the NaOCl solution compared to samples in which the seeds were not treated with NaOCl prior to sprouting (Tables 1–3). Further, there was no appreciable difference in the $\log_{10}$ cfu/g of *E. coli* O157:H7 obtained by plating on Rainbow® Agar O157 sprout samples grown from seeds treated and not treated with the NaOCl solution (Table 4). Future studies will examine the effect of using seed wash solutions containing higher concentrations of NaOCl or $Ca(OCl)_2$ on the ability to detect *E. coli* O157:H7 in spent irrigation water and on the level of the organism in raw sprouts.

For enumeration of *E. coli* O157:H7 in sprouts and in enrichments of blanched sprouts, samples were plated onto Rainbow® Agar O157, a selective chromogenic agar on which *E. coli* O157:H7 appears as black colonies [2]. The level of *E. coli* O157:H7 in the sprouts grown for 72 h ranged from 2.84 to 5.54 $\log_{10}$ cfu/g (Table 4). Growth of *E. coli* O157:H7 and *Salmonella* in sprouts can reach levels of 10^7 cfu/g in $\leq$72 h [8,14]. It is likely that lower levels were obtained in the present study because all of the seeds were not contaminated; only 10, 25, 60, or 100 inoculated seeds were mixed with 20 g of uninoculated seeds prior to sprouting. In many cases, enumeration on Rainbow® Agar O157 was difficult due to overgrowth of bacteria with mucoid phenotypes on plates containing lower dilutions of samples. This led to an increase in the limit of detection on Rainbow® Agar O157 from 1.32 to 3.32 $\log_{10}$ cfu/g. Many *Pseudomonas* and *Klebsiella* spp. form mucoid colonies on agar medium and are found on plant seeds and in high numbers in sprouts [4,13]. We found that a portion of the mucoid colonies was fluorescent pseudomonads by plating several of the sprout samples on *Pseudomonas* Agar F (Difco) and observing for fluorescent colonies using an ultraviolet lamp (data not shown).

E. coli O157:H7 was detected in 32 of 32 enrichments of blanched sprouts using the TaqMan® assay and in 16 of 32 enrichments using the Quix™ strips (Table 4). Several of the enrichments, which were positive using the Quix™ assay, had levels of *E. coli* O157:H7 as high as 7–8 $\log_{10}$ cfu/ml. However, many of the enrichments, which were positive with the TaqMan® assay, had levels of *E. coli* O157:H7 less than 3.32 $\log_{10}$ cfu/ml, as did several enrichments which were positive with the Quix™ assay. Enumeration on Rainbow® Agar O157 was again problematic due to overgrowth of mucoid colonies of the background microflora. Using microscopy techniques, Itoh *et al* [7] observed viable *E. coli* O157:H7 on the outer surfaces and also in the inner tissues and stomata of cotyledons of sprouts grown from artificially contaminated seeds. Therefore, it is possible that blanching for 30 s is insufficient to inactivate *E. coli* O157:H7 found in the inner sprout tissues. Although the color of the sprouts did not appear very different after blanching for 30 s, they were less crisp. Therefore, blanching times longer than 30 s were not examined. The sensitivity of TaqMan®-based assays may be as low as 50 cfu [1]; therefore, it is possible that there was viable *E. coli* O157:H7 in the enrichments, but at levels lower than what was detectable on Rainbow® Agar O157. The same could be true for samples tested using the Quix™ assay, although the analytical sensitivity of the Quix™ *E. coli* O157 Sprout Assay reported by the manufacturer is 3.4×10^4 cfu/ml. On the other hand, it is possible that the presence of DNA from inactivated *E. coli* O157:H7 could, in some cases, result in a false-positive result with the TaqMan® *E. coli* O157:H7 assay due to the technique's high sensitivity. The antibody employed in the Quix™ assay reacts with lipopolysaccharide (LPS). Since LPS is heat-stable, release of the LPS from cells into the enrichment broth and the presence of heat-inactivated *E. coli* O157:H7 released from sprouts into the enrichment broth may potentially cause a false-positive result with the Quix™ assay if *E. coli* O157:H7 is initially present at sufficient levels. Others have reported that heating bacterial preparations enhanced reactivity of antibodies with target antigens in immunoassays [9].

Conclusion

Testing of spent irrigation water is a simple and effective way to monitor the presence of *E. coli* O157:H7 in growing sprouts; however, the method employed for testing is important, since the sensitivity of different assays may vary considerably. In general, PCR-based systems are highly sensitive, and commercially-available assays such as TaqMan® are easy to perform. In the present study, enrichment of spent irrigation water enhanced detection of *E. coli* O157:H7 by both the TaqMan® *E. coli* O157:H7 assay and the Quix™ *E. coli* O157 Sprout Assay. These data indicate that it may be important to include an enrichment step in the protocols for testing spent irrigation water to detect low levels of pathogens. Since the infectious dose of *E. coli* O157:H7 is relatively low [16], methods employed for detection must have the ability to detect very low levels of the pathogen to ensure the microbial safety of sprouts. To our knowledge, this is the first study evaluating methods for detection of *E. coli* O157:H7 in spent irrigation water from sprouts and evaluating blanching for inactivation of the organism in sprouts. Future studies include comparison of Rainbow® Agar O157 to other commercially available selective media for isolation of *E. coli* O157:H7 from sprouts and spent irrigation water and also the development/evaluation of assays for detection of *Salmonella* spp. in spent irrigation water.

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