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Sensitivity of pathogenic and attenuated *E. coli* O157:H7 strains to ultraviolet-C light as assessed by conventional plating methods and ethidium monoazide-PCR

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Abstract

The UV-C sensitivity of six pathogenic *Escherichia coli* O157:H7 strains associated with recent outbreaks of foodborne illnesses and five attenuated/nonpathogenic *E. coli* O157:H7 strains was investigated using conventional plating method, and real time PCR with and without ethidium monoazide (EMA) pretreatment. Results showed that the nonpathogenic/attenuated *E. coli* ATCC 25922, 6980-2, and 6982-2 had more or similar resistance to UV-C compared to the pathogenic strains. The UV-C inhibition on the PCR amplification of DNA correlated well with UV-C dose, as indicated by the cycle threshold (Ct), regardless of EMA pretreatment. The Ct values increased linearly with increasing UV-C doses. Cell membrane was damaged only at high doses when UV-C inactivated more than 6 log CFU of bacteria. Overall, our results suggest that *E. coli* strains have different sensitivities to UV-C and that EMA-PCR is a useful tool to assess cell damage caused by UV-C.

Practical applications

Ultraviolet (UV) light, as one of the postharvest interventions, is simple to implement and inexpensive for commercial applications. To conduct trials on a large scale or at a commercial setting, nonpathogenic/attenuated surrogates of pathogenic bacteria should be used, due to the difficulty for containment and post-treatment decontamination of equipment and the environment. We demonstrated the variations in UV-C sensitivity among pathogenic and nonpathogenic *E. coli* strains. Three attenuated/nonpathogenic strains of *E. coli* had similar sensitivities as the pathogenic strains, and therefore, could serve as surrogates. Furthermore, cell membrane was not affected by moderate doses of UV-C treatments, even though the bacteria were not cultivable. The information will be useful for researchers to conduct scale-up trials, and to optimize UV-C technology for microbial safety enhancement.

1 | INTRODUCTION

Ultraviolet (UV) light is a nonthermal/nonchemical intervention technology that employs physical light energy of a specific wavelength, or broad spectrum, to inactivate microorganisms. UV-C irradiation has been used for disinfection of water and other liquid foods (Hijnen, Beerendonk, & Medema, 2006; Koutchma, 2009; Yin, Zhu, Koutchma, & Gong, 2015). In addition, UV-C has been studied to inactivate various pathogenic bacteria on different foods (Ha & Kang, 2015; Yaun, Sumner, Eifert, & Marcy, 2004; Yun, Yan, Fan, Gurtler, & Phillips, 2013). For example, Bialka and Dimirci (2008) found that pulsed UV-C reduced *E. coli* O157:H7 and *Salmonella* populations inoculated on raspberries by 3.9 and 3.4 log at 72 and 59.2 kJ/m^2, respectively. On the surfaces of strawberries, maximum reductions achieved were 2.1 and 2.8 log CFU/g at 25.7 and 34.2 kJ/m^2, respectively. Similar reductions of pathogens were observed on blueberries (Bialka & Dimirci 2007). Escalona, Aguayo, Martínez-Hernández, and Artés (2010) found that UV treatment (24–240 mJ/cm^2) reduced *Listeria monocytogenes* populations on cut spinach by 2 logs. Despite the initial reductions of

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L. monocytogenes, populations of the pathogen on UV-C-treated leaves increased to levels similar to the controls during 14 days of storage at 4°C, indicating that the pathogen grew faster on UV-treated leaves than on the control. Yun et al. (2013) applied UV-C to inactivate *E. coli* O157:H7 and *Salmonella* spp. on the surface of apricots, and found that the bacterial populations decreased rapidly (1–2 logs) with increasing UV-C doses of 0 to 74 mJ/cm^2. Further increases in UV-C doses achieved only limited additional reductions in bacterial populations. It has been shown that microbial inactivation is directly related to the absorbed doses. To achieve significant microbial inactivation, the UV radiant exposure must be at least 40 mJ/cm^2 on all surfaces of the product (Sommer, Lhotsky, Haider, & Cabaj, 2000).

Various reductions of pathogens have been reported as a result of UV-C treatment. One of the reasons for the variation may be due to the use of bacteria with different UV-C sensitivities. Basaran, Quintero-Ramos, Moake, Churey, and Worobo (2004) compared the UV-C sensitivity of three strains of *E. coli* O157:H7 (EDL933, ATCC 43889, and ATCC 43895) and found that *E. coli* O157:H7 ATCC 43889 showed the most sensitivity to the disinfection process with an average 6.63-log reduction compared to an average log reduction of 5.93 for both strains EDL933 and ATCC 43895. The sensitivities of many pathogenic *E. coli* O157:H7 have not been evaluated.

Escherichia coli O157:H7 is one of the common pathogens linked to recent outbreaks of foodborne illness. Intervention technologies are needed to reduce the risk of the *E. coli* O157:H7 contamination. UV-C, as one of the postharvest interventions, is simple to implement and inexpensive for commercial applications. To conduct trials on a large scale or at a commercial setting, nonpathogenic/attenuated surrogates of pathogenic *E. coli* O157:H7 should be used due to the difficulty for containment and post-treatment decontamination of equipment and the environment. The nonpathogenic *E. coli* ATCC 25922 was identified as a potential surrogate organism for *E. coli* O157:H7 in apple cider, based on this microorganism's similar UV-C sensitivity (Basaran et al., 2004; Quintero-Ramos, Churey, Hartman, Barnard, & Worobo, 2004). However, it is unclear whether other attenuated strains of *E. coli* ATCC behave similarly as pathogens.

The inactivation of bacteria by UV-C and other intervention technologies is traditionally assayed in liquid (most probable number, MPN) or solid (plate count) media. In recent years, a novel approach has been developed to enumerate viable bacteria cells. In this method, PCR amplification of DNA is inhibited by treating bacteria with intercalating dyes such as ethidium monoazide (EMA) or propidium monoazide, prior to DNA extraction (Elizaquível, Aznar, & Sánchez, 2014; Lee & Levin, 2006; Nocker, Sossa, & Camper, 2007; Rudi, Moen, Drømtorp, & Holck, 2005; Wang & Mustapha, 2010). The dyes are able to penetrate membrane-compromised cells and covalently bind to nucleic acids after photoactivation. Cells with intact membranes or cell walls are generally capable of excluding DNA-binding dyes. DNA with the covalently-bound dyes cannot be amplified by PCR. Therefore, only viable cells can be detected by this method. The EMA-PCR method is also used to detect viable-but-nonculturable cells (VBNC). However, there has been limited studies on using this approach to evaluate UV-C sensitivity of pathogenic *E. coli*. Earlier studies demonstrated that the EMA-PCR method offered a useful, rapid and efficient tool for screening viable *E. coli* O157:H7 in raw and ready-to-eat meat products without being compromised by the presence of dead cells (Gordillo, Rodríguez, Werning, Bermúdez, & Rodríguez, 2014; Liu & Mustapha, 2014).

The damage caused by UV light on DNA is believed to be the primary reason for the bacterial inactivation in UV disinfection. Absorption of UV energy by nucleic acids (DNA and RNA) leads to formation of lesions such as cyclobutane pyrimidine dimers (Beauchamp & Lacroix, 2012). The formation of lesions can be repaired by bacteria using various mechanisms such as dark repair and photoinactivation (Oguma et al., 2001; Sommer et al., 2000). However, when the DNA damage is too severe and unrepaired, subsequent cell death occurs. In addition to the destruction of nucleic acids, proteins, membranes, and other cellular materials may be damaged (Elmnasser et al., 2007; Lopez-Malo & Palou, 2005).

The objectives of the present study were to evaluate the UV-C sensitivity of various pathogenic *E. coli* O157:H7 strains, to study the suitability of attenuated strains of *E. coli* O157:H7 as surrogates, and to investigate the mechanism of UV-C disinfection using PCR with and without EMA pretreatment.

2 | MATERIALS AND METHODS

2.1 | Bacterial strains

Six pathogenic *E. coli* O157:H7 strains (RM6535, RM7386, 06F00475, Sakai, RM1484, and EDL933) and four attenuated *E. coli* O157:H7 (6980-2, 6982-2, CV267, 700728) strains along with a nonpathogenic generic *E. coli* (ATCC 25922) were used in the present study. The pathogenic bacteria were chosen due to their association with recent outbreaks of foodborne illnesses. The attenuated strains were selected as they are similar to pathogenic counterparts without the Shiga toxin genes. *Escherichia coli* O157:H7 RM6535 (2006 U.S. Iceberg lettuce outbreak), RM7386 (Romaine lettuce outbreak), 06F00475 (spinach outbreak), RM1484 (apple juice outbreak), and *E. coli* O157:H7 Sakai (sprout outbreak) strains were provided by Dr. Robert E. Mandrell (USDA, ARS, WRRC). *E. coli* EDL933, isolated from hamburger, was from the ERRC collection.

Four strains of Shiga toxin-negative *E. coli* O157:H7 used in this study include 6980-2, 6982-2, CV267, and ATCC 700728 which do not have *stx1* or *stx2* genes (Feng, Dey, & Abe, 2001; Harris et al., 2012). Strain 700728 (aka, strain BDMS T4169) was purchased from American Type Culture Collection (ATCC, Manassas, VA). Ampicillin-resistant *E. coli* O157:H7 6980-2 (isolated from beef), 6982-2 (isolated from beef), and CV267 (isolated from cattle) were graciously provided by Drs. Cathy Webb and Michael Doyle (University of Georgia, Center for Food Safety, Griffin, GA). *Escherichia coli* ATCC 25922 was obtained from ATCC. Since many strains we obtained were ampicillin-resistant, to counter the possible effects of ampicillin-resistance, we made all bacteria ampicillin resistant by successive transfers of the bacteria in Tryptic Soy Broth (TSB) containing increasing concentrations of the

TABLE 1 Primer sequences used for real-time PCR analysis

Gene	Forward primer sequence	Reverse primer sequence	Amplicon size (bp)
mdh	AGTACGCCCGTTTCTTCTCT	CAGGGCGATATCTTTCTTGA	140
arcA	GTTGGGAACTGGACATCAAC	CGGTCATTTTCTTCAGCAGT	150
gapA	ACGAAGTTGGTGTTGACGTT	GTTAGCGCCTTTAACGAACA	149

antibiotic to a final level of 100 μg/ml during a approximately 10-day period.

2.2 | UV-C irradiation

All bacteria were grown separately in 40 ml TSB at 37°C overnight to reach final stationary populations of approximately 10^9 CFU/ml. When bacteria stored in a −80°C freezer were used, bacteria were grown three times overnight in 10 ml TSB to obtain active cells. After centrifugation (5,000 rpm, 10 min), pellets were washed with phosphate buffer saline (PBS, pH 7.4) twice. The pellets were then suspended in PBS and vortexed sufficiently to disrupt aggregations. Aliquots of bacterial suspensions (10-ml) were placed on the entire surface of a Petri-dish (100 × 7 mm) plastic surface with a sterilized stir bar (2 cm long, 3 mm diameter). The Petri dish was then placed on a Thermix Stirrer (model 120mR) stir plate (Fisher Scientific, Nepean, Ontario, Canada) in a UV-C treatment chamber for UV-C treatment. The bacteria were stirred using the stir plate at a speed setting of 1 during the entire irradiation period. The custom-built UV treatment chamber contained a 0.61-m 55 Watt UV-C emitting bulb (SaniLIGHTTM, Atlantic Ultraviolet, White Plains, NY). UV treatment was performed at ambient temperature (~23°C). The UV-C (at 254-nm wavelength) intensity at the same distance as the Petri dish was measured using a UVX-25 radiometer (UVP Inc., Upland, CA, USA), and the UV dose was calculated with the following equation: UV dose (mJ/cm^2) = irradiance (mW/cm^2) × exposure time (s). The UV-C radiation doses were 0, 51, 203, and 913 mJ/cm^2 at a UV intensity of 10.14 mw/cm^2 after exposure times of 0, 5, 20, and 90 s, respectively, at the fixed distance.

After irradiation, the bacterial suspension was transferred to a sterile tube. After appropriate dilutions (when needed), bacteria (100 μl) of each sample were plated onto TSA and SMAC (Becton, Dickinson and Company, Sparks, MD). The plates were incubated at 37°C overnight before colonies on the plates were counted.

2.3 | EMA treatment, DNA extraction, and EMA-PCR assay

EMA treatment of different bacteria and a follow-up quantitative PCR (qPCR) were carried out (Rudi et al., 2005) for RM6535 and EDL933. Based on the data from the UV-C sensitivity study, RM6535 was more sensitive than EDL933, especially at a high dose. Briefly, within 1 hr after UV-C treatments, 1 ml portions of UV-C irradiated or nonirradiated bacteria cell suspensions were treated with 20 mg/ml of EMA in the dark for 5 min and subsequently exposed to a 650-W halogen light for 1 min. Cells were then immediately washed with PBS (pH, 7.2) and subjected to DNA extraction using a DNeasy tissue kit (Qiagen, Valencia, CA), according to the manufacturer's instructions. DNA was also extracted from UV-C treated cells without the treatment of EMA. All DNA preparations were eluted twice with 100 μl of elution buffer supplied with the kit and then combined. Three house-keeping genes (*mdh*, *gapA*, and *arcA*) were selected as targets for detection in qPCR. Primers selected based on the gene sequences of *E. coli* O157:H7 EDL933 strain (GenBank accession# NC002655) were designed using Primer3 software (version 0.4.0, Whitehead Institute for Biomedical Research, Center, Cambridge, MA). The primer sequences are listed in Table 1. Malate dehydrogenase (Mdh) catalyzes the reversible oxidation of malate to generate oxaloacetate, using NAD+ as an electron acceptor. Mdh carries out the oxidation reaction as part of the TCA cycle, the glyoxylate cycle, and gluconeogenesis and reduces oxaloacetate to generate malate as part of anaerobic respiration and mixed-acid fermentation. The *gapA* gene codes for the glyceraldehyde-3-phosphate dehydrogenase A, a key enzyme of the glycolytic and gluconeogenesis pathways. In *E. coli*, the two-component regulatory ArcAB system functions as a major control system for the regulation of genes encoding enzymes involved in both aerobic and anaerobic catabolic pathways.

2.4 | Real-time PCR amplification and analysis

Real-time PCR assay was performed on a 7500 real-time PCR system (ABI, Carlsbad, CA). Each 20-μl PCR mixture contained 0.25 × EvaGreen dye (Biotium, Hayward, CA), 0.25 μM of each primer, 2 μl (50 ng) of DNA template, 5 units of Platinum Taq DNA polymerase, and buffer (Invitrogen, Inc., Carlsbad, CA). The amplification program was set at 50°C for 2 min and 95°C for 10 min, followed by 35 cycles at 95°C for 15 s and 60°C for 1 min. Reactions without template were used as negative controls. Fluorescence data were collected at the 55°C annealing step. Results were analyzed using the SDS Software provided with the thermocycler. All samples were analyzed in triplicate. Data analysis was performed using the $2^{-\Delta\Delta Ct}$ method, as described (Pfaffl, 2001), and Ct is the threshold cycle value for the amplified gene.

2.5 | Statistical analyses

Data were subjected to General Linear Model analysis using SAS Version 9.4 (SAS Institute, Cary, NC, USA). Treatment differences were compared using Duncan's Multiple Range test method at $p = .05$. The nonlinear regression and curve fitting were conducted using Sigmaplot, Window Version 11.0 (Systat Software Inc. Chicago, IL, USA).

TABLE 2 UV-C sensitivity of pathogenic and attenuated/nonpathogenic *E. coli* O157:H7

	TSA				SMAC			
	0[a]	51	203	913	0	51	203	913
Pathogenic								
RM6535	0.00[b] ± 0.06	5.31 ± 0.12c[c]	6.57 ± 0.36b	8.54 ± 0.25d	0.00 ± 0.08	4.81 ± 0.17cd	5.30 ± 0.17ab	8.08 ± 0.00b
RM7386	0.00 ± 0.15	5.09 ± 0.07c	5.30 ± 0.10ab	8.80 ± 0.74d	0.00 ± 0.04	4.71 ± 0.16cd	4.70 ± 0.06a	7.72 ± 0.46b
06F00475	0.00 ± 0.11	4.54 ± 0.07bc	4.80 ± 0.26a	7.08 ± 0.69abc	0.00 ± 0.04	4.21 ± 0.06abcd	4.72 ± 0.27a	6.74 ± 0.73a
Sakai	0.00 ± 0.18	5.19 ± 0.41c	5.93 ± 0.52ab	6.60 ± 0.45a	0.00 ± 0.04	5.50 ± 0.53d	6.22 ± 0.22bc	6.29 ± 0.33a
EDL933	0.00 ± 0.19	4.95 ± 0.29c	5.87 ± 0.41ab	7.17 ± 0.32abc	0.00 ± 0.02	4.49 ± 0.13abcd	5.82 ± 0.44abc	6.88 ± 0.22a
RM1484	0.00 ± 0.13	4.82 ± 0.06bc	5.98 ± 0.96ab	6.97 ± 0.33ab	0.00 ± 0.11	4.08 ± 0.03abcd	5.20 ± 0.19ab	6.61 ± 0.09a
Attenuated								
6980-2	0.00 ± 0.16	3.29 ± 0.50ab	5.54 ± 0.13ab	6.51 ± 0.82a	0.00 ± 0.19	3.10 ± 0.38ab	5.60 ± 0.22abc	6.42 ± 0.74a
6982-2	0.00 ± 0.14	2.81 ± 0.10a	5.52 ± 0.72ab	6.49 ± 0.24a	0.00 ± 0.27	2.99 ± 0.16a	5.44 ± 0.57ab	6.21 ± 0.13a
CV267	0.00 ± 0.61	4.31 ± 0.74abc	5.67 ± 0.51ab	7.60 ± 0.52bc	0.00 ± 0.44	4.39 ± 0.32abcd	6.11 ± 0.55abc	7.98 ± 0.29b
700728	0.00 ± 0.02	4.56 ± 1.27bc	6.24 ± 0.73ab	7.99 ± 0.29cd	0.00 ± 0.02	4.52 ± 0.69bcd	6.93 ± 0.79c	7.90 ± 0.29b
25922	0.00 ± 0.11	4.07 ± 1.01abc	5.63 ± 0.85ab	6.58 ± 0.80a	0.00 ± 0.52	3.64 ± 0.92abc	5.22 ± 0.74ab	6.34 ± 0.47a

Bacteria cultures were exposed to UV-C for 0, 5, 20, and 90 s, corresponding to doses of 0, 51, 203, and 913 mJ/cm^2.
[a]Values represent UV-C dosage in mJ/cm^2.
[b]Values represent log reductions. When calculating the log reductions, half the limit of detection (0.5 log CFU/fruit) was used when a sample did not yield any colonies by the direct plating.
[c]Means followed by the same letters within the same column are not significantly ($p > .05$) different.

3 | RESULTS AND DISCUSSION

3.1 | UV-C sensitivity of pathogenic and attenuated/nonpathogenic *E. coli* strains

Survival populations of various strains of *E. coli* after UV-C treatments are shown in Table 2. As UV-C doses increased, populations of all *E. coli* strains decreased as assayed on both TSA and SMAC. Similar to previous studies (Bialka & Dimirci, 2007; Yun et al., 2013), on bacteria inoculated on different foods, there was a rapid decrease in *E. coli* O157:H7 populations enumerated on TSA and SMAC as UV dose increased from 0 to 51 mJ/cm^2. UV-C treatment at 51 mJ/cm^2 resulted in 4.54–5.31 and 2.81–4.56 log CFU/ml reduction of pathogenic and attenuated/nonpathogenic *E. coli* strains, respectively, as assessed on TSA. It seems that most pathogenic *E. coli* strains were significantly ($p < .05$) more sensitive than the attenuated 6980-2 and 6982-2 strains at the low UV-C dose. Additional reductions in *E. coli* populations with further increases in UV-C doses were not as profound as those in the low UV-C dose range (0–51 mJ/cm^2).

There were some variations in UV-C sensitivity among the *E. coli* strains. Even though there were no significant or consistent differences in UV-C- induced log reductions among the six strains of *E. coli* O157:H7 at the low dose range (0–203 mJ/cm^2), UV-C at 913 mJ/cm^2 achieved significantly ($p < .05$) higher reductions on RM6535 and RM7386 than other pathogenic strains, when plating on both TSA and SMAC. Therefore, among these six pathogenic *E. coli* O157:H7 strains, RM6535 and RM 7386 were the most sensitive to UV-C irradiation. More than 8 log CFU/ml reductions on TSA were achieved after 913 mJ/cm^2 UV-C treatment. Among the nonpathogenic/attenuated strains, ATCC 25922, 6980-2, and 6982-2 were more UV-C resistant than ATCC 700728 and CV267. Unluturk, Atılgan, Baysal, and Unluturk (2010) found that ATCC 700728 was less resistant to UV-C irradiation than that of the nonpathogenic serotype strain (*E. coli* K-12) in liquid egg white. The ATCC 700728 strain does not have *stx1* and *stx2* genes but has the single base mismatch at +93 in the *uidA* gene, characteristic of *E. coli* O157:H7 (Moyne et al., 2011). Strains RM6535 and RM7386 were more sensitive to UV than other pathogenic *E. coli*. It is unclear what is responsible for the UV sensitivity of the strains. Curli are adhesive fimbriae of *Enterobacteriaceae* and are involved in surface attachment, cell aggregation, and biofilm formation (Carter et al., 2011). Whether curli production is related to UV-C sensitivity of *E. coli* O157:H7 needs further study.

Statistical analysis of individual strains of attenuated *E. coli* O157:H7 in comparison with pathogenic strains as a whole revealed that ATCC 25922, 6980-2, and 6982-2 had similar or more UV-C resistance than the pathogenic strains at all of the doses tested. Therefore, the three attenuated *E. coli* O157:H7 can serve as surrogates in place of pathogenic *E. coli* O157:H7. Of course, for applications on solid food, the suitability of surrogate bacteria needs to be verified as the ability for attachment, biofilm formation, and other characteristics may differ between O157:H7 and nonpathogenic/attenuated *E. coli* strains. Orlowska, Koutchma, Kostrzynska, and Tang (2015) found that *E. coli* ATCC 8739 met the criteria for a UV-C surrogate organism for O157:H7 and non-O157 STEC strains. Oteiza, Peltzer, Gannuzzi, and Zaritzky

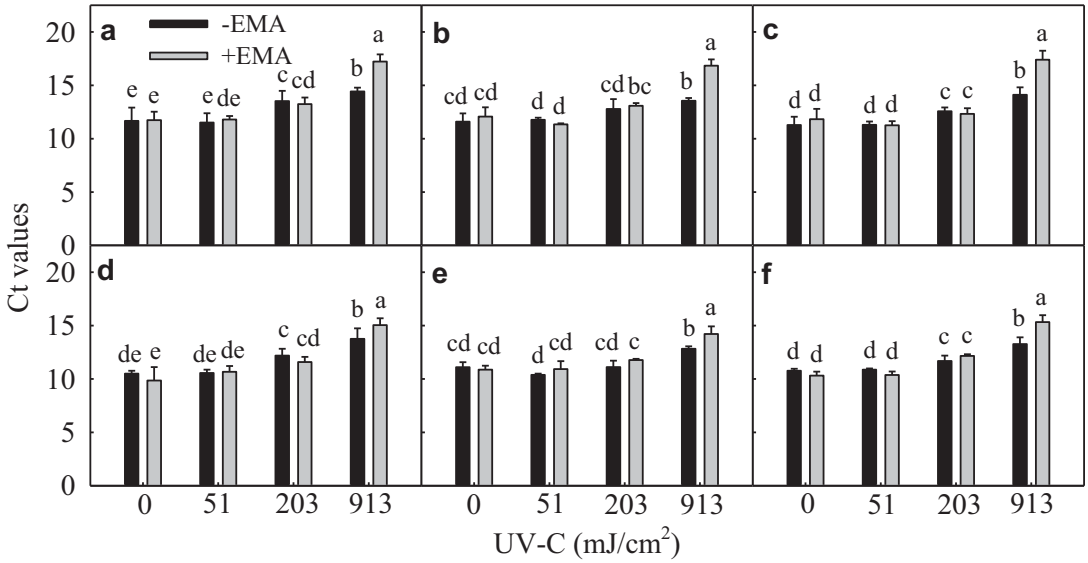

FIGURE 1 Influence of UV-C exposure and ethidium monoazide (EMA) treatment on the cycle threshold (Ct) values for *mdh* (a,d), *gapA* (b, e), and *arcA* (c,f) as determined by PCR. *Escherichia coli* O157:H7 RM6535 (a–c) and EDL933 (d–f) were treated with different doses of UV-C and then treated either with or without EMA. *mdh*, *gapA*, and *arcA* genes were then amplified with PCR. UV-C treatment times 0, 5, 20, and 90 s. Vertical lines above the bars represent standard deviations, and bars with the same letter within the same individual graph are not significantly different ($p > .05$)

(2005) found that EDL933 had a similar sensitivity to UV-C in juices as did ATCC 25922. Previous studies have shown that ATCC 25922 is a suitable surrogate for *E. coli* O157:H7 in apple cider (Quintero-Ramos et al., 2004; Sauer & Moraru, 2009). Our results confirmed the suitability of ATCC 25922 as a surrogate. Furthermore, our results suggest that 6980-2 and 6982-2 can also serve as surrogates in addition to ATCC 25922.

3.2 | EMA real-time PCR analysis

In real-time PCR, Ct is defined as the cycle number at which a fluorescent signal exceeds a fixed threshold and corresponds to the number of cycles of target amplification necessary for detection. Samples with higher numbers of a target gene require fewer cycles of amplification to reach the threshold. Therefore, a larger Ct number without EMA pretreatment indicates a lower concentration (or lower copy number) of template DNA, presumably due to DNA damage or degradation. Figure 1 shows the Ct values for *mdh*, *gapA*, and *arcA* of *E. coli* O157:H7 RM6535 and EDL933, as a function of UV-C and EMA treatments. Without EMA pretreatment, Ct values for all three genes increased with increasing UV-C doses. Significant increases in Ct values for each individual gene were observed at higher UV-C doses, particularly at 913 mJ/cm^2. The Ct values averaged from the three genes correlated well with the increase in UV-C doses and with the UV sensitivity of the two bacteria (RM6535 and EDL933). In the low dose range (0–203 mJ/cm^2 range), with each 10 mJ/cm^2 increase in UV-C dose, Ct values (averaged from the three genes) increased by 0.54 and 0.87% for EDL933 and RM6535, respectively. The R^2 for the linear relationship between Ct values and UV-C doses in the 0–203 mJ/cm^2 ranges were 0.9975 and 0.9749 for EDL933 and RM6535, respectively. The difference in Ct values between the two strains can be explained as UV-C causing more DNA damage to the UV sensitive strain (RM6535) than on the UV-C resistant strain (EDL933). The increased Ct values were also correlated with an increased fading of PCR amplified products visualized on an agarose gel (data not shown).

With EMA pretreatment, the Ct values increased with increasing UV-C doses, but the relationship was not as strong as for that without EMA pretreatment, especially for EDL933 at the low UV-C dose range. On average, Ct values increased by 0.15 and 0.47% for EDL933 and RM6535, respectively, in the UV-C dose range of 0–203 mJ/cm^2. The results on Ct values from both EMA treated or nontreated cells suggested that more profound increases in Ct values as a function of increasing UV-C were observed for the UV-C sensitive strain (RM6535) of *E. coli* compared with the less sensitive EDL933 strain. EMA-PCR was developed to measure membrane damage of the cell. There were no significant differences in Ct values (ΔCt) between EMA-treated and non-treated cells in the UV-C range of 0–203 mJ/cm^2, suggesting that UV-C did not cause significant membrane damage or affect membrane permeability in relatively low UV-C doses. However, at the highest dose tested (913 mJ/cm^2), EMA-PCR with pre-EMA treatment yielded significantly ($p < .05$) higher Ct values than those without EMA. The difference between EMA treated and nontreated cells suggest that UV-C resulted in membrane damage at the high dose. The cell membrane damage caused by the high doses of UV-C may be an indirect result of DNA damage, because membrane damage can be the ultimate result of extensive damage of the UV-treated cell, even though UV-C treatment does not directly target the cell membrane.

The Ct values of EMA-treated cells were significantly higher ($p < .05$) than that of the untreated cells, especially at the highest dose (913 mJ/cm^2) tested (Figure 1). Furthermore, the difference in Ct values between EMA-treated and nontreated cells was significantly

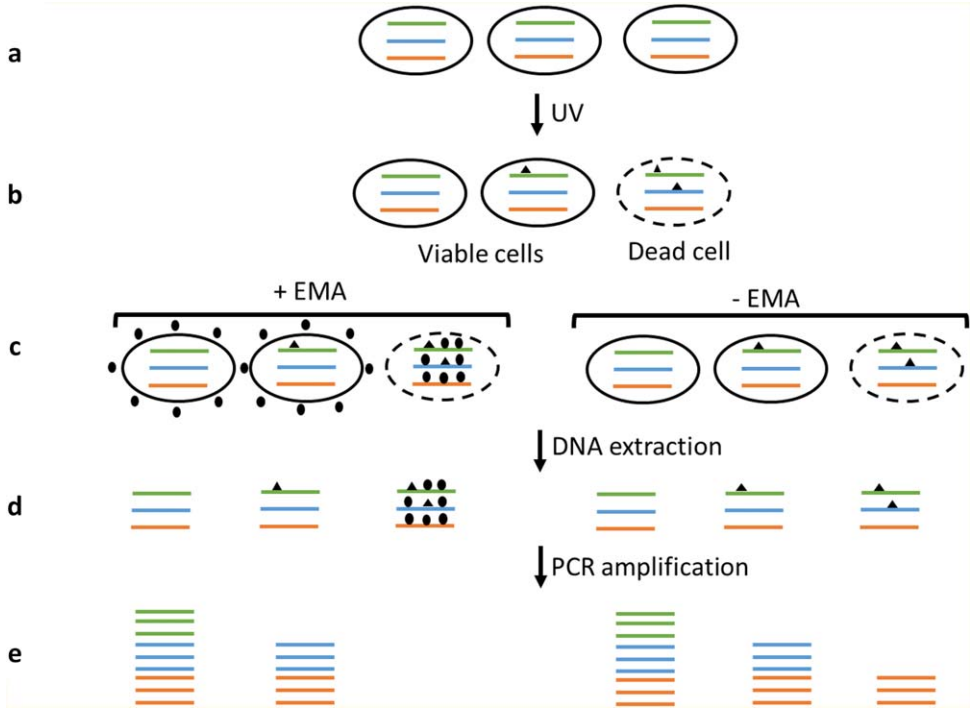

FIGURE 2 Proposed schematic representations of UV-C and ethidium monoazide (EMA) treatments on the fate of *E. coli* cells and DNA amplification with PCR. Viable *E. coli* cells (a) are treated with UV-C, resulting in the formation of mutagenic and cytotoxic lesions (such as cyclobutane-pyrimidine dimers) on DNA and death of some cells (b). When EMA was added to the mixture of viable and dead cells, EMA penetrated the dead cells and covalently bound to the DNA (c. left side). After DNA extraction (d), only DNA without EMA binding or lesions were amplified (e, left side). When the cells after UV-C treated were not treated with EMA, only DNA without lesions were amplified (e, right side). The solid triangle symbols indicate DNA lesions while the solid cycles represent EMA molecules

($p < .05$) larger for RM6535 than for EDL933, in concordance with the results from direct plating results that RM6535 was more UV-C sensitive than EDL933, particularly at 913 mJ/cm^2. At the dose of 913 mJ/cm^2, EMA-treated cells had 5.1 and 20.9% increases in Ct values for EDL933 and RM6535, respectively, compared to those without EMA treatment, suggesting that 5.1 and 20.9% of cells are membrane damaged (dead cells) for EDL933 and RM6535, respectively. Therefore, the differences in Ct values correlated well with the sensitivity of *E. coli* to UV-C.

Formation of pyrimidine dimers in genomic DNA is primarily responsible for UV inactivation of *E. coli* (Eischeid & Linden, 2007). However, it is known that cells with intact membranes can show metabolic activity and repair, and are able to multiply, unless their DNA is damaged beyond repair or they cannot generate a positive energy balance (Nebe von Caron et al., 2000). Schenk, Raffellini, Guerrero, Blanco, and Alzamora (2011), using flow cytometry and other techniques, showed that mechanisms of UV-C induced cellular damage differed according to time of exposure. When *E. coli* were exposed to fluorescent light after a 3-log UV inactivation, UV-induced pyrimidine dimers in the DNA were continuously repaired and the colony-forming ability recovered gradually (Oguma et al., 2001). When kept in darkness after the UV inactivation; however, *E. coli* showed neither repair of pyrimidine dimers nor recovery of colony-forming ability.

The populations of viable cells after UV-C treatment as quantified by EMA-PCR were significantly higher as determined by EMA-PCR than those on TSA (data not shown). Even though TSA plating indicated 51 mJ/cm^2 UV-C reduced the populations of RM6535 and EDL933 by 5.31 and 4.95 log CFU/mL, respectively (Table 1), EMA-PCR did not detect significant changes in viable cell populations between the nontreated and UV-C (51 mJ/cm^2) treated cells. Only when UV-C doses increased to 203 mJ/cm^2, was EMA-PCR able to detect significant changes in the populations of viable cells. Therefore, EMA-PCR is not a sensitive method to detect changes in populations of the bacteria due to low doses of UV-C.

Several researchers have studied the maintenance of some metabolic functions after treatment with germicidal UV radiation (Blatchley, Dumoutier, Halaby, Levi, & Laîné, 2001; Fiksdal & Tryland, 1999; Said, Masahiro, & Hassen, 2010). The VBNC state has been implicated as a result of UV-C treatment (Said et al., 2010). Villarino, Bouvet, Regnault, Delautre, and Grimont (2000) and Rager, Grimont, and Bouvet (2003) demonstrated that cells inactivated by UV lost their ability to grow in culture. However, these cells are not lysed, preserving both their membrane integrity and membrane potential. The loss of bacterial cultivability may not be synonymous with death of bacteria. Zhang, Ye, Lin, Lv, and Yu (2015) concluded that UV-C induced a VBNC state on *E. coli* and *Pseudomonas aeruginosa*, though their results have been criticized (Linden, Hull, & Rodriguez, 2015). A more recent study (Garvey, Stocca, & Rowan, 2016) suggested that pulsed UV light induced a VBNC state in treated *Bacillus* cells. Our results also indicate that the cell membrane integrity of *E. coli* was not affected by low dose UV-C treatments, even

though the cell populations as determined by the direct TSA and SMAC plating methods showed significant decreases. Furthermore, the populations of viable cells after UV-C treatment, as estimated by quantitative EMA-PCR, were significantly higher than those determined by direct TSA plating method (data not shown). Whether the lack of membrane damage and the higher viable cell populations are indications of VBNC state needs further study.

It is known that the main cellular targets of short wave UV-C light are nucleic acids (Douki & Cadet, 2001). The formation of lesions (dimers) as a result of UV-C exposure inhibited the replication and amplification of the DNA both in vivo and in vitro (Trombert, Irazoqui, Martín, & Zalazar, 2007) due to the blockage of DNA-polymerase at pyrimidine dimer sites. Consequently, the Ct values increased in PCR analysis as Taq DNA polymerase cannot pass the lesion site, and a lower size of the amplicons was generated. Humphrey et al. (2015) showed that UV-C treatment of primers before PCR increased the cycle threshold in a dose-dependent manner, due to damage sustained by oligonucleotide primers.

The interaction of UV-C and PCR amplification of DNA with and without EMA pretreatment may be illustrated using a diagram (Figure 2). Exposure of viable *E. coli* to UV-C induces two types of damage on *E. coli* cells, depending on dosage: DNA lesions (cyclobutane-pyrimidine dimer formation) and membrane damage (indirect or direct). Therefore, there would be three types of cells in the bacterial suspension after UV-C treatment: undamaged or repaired cells (viable), cells with DNA lesions, and cells with membrane damage (dead cells). When EMA is added to the mixture of three types of cells, EMA only penetrates those cells with compromised membranes, and covalently binds DNA. During PCR amplification of purified DNA from the cell mixture, both EMA-bound DNA and DNA with lesions hamper the amplification of DNA by PCR, resulting in higher Ct values. When the cells after UV-C treatment are not treated with EMA, the amplification of the DNA was hindered when pyrimidine dimers are present (Trombert et al., 2007; Humphrey et al., 2015). Only DNA without lesions is amplified.

4 | CONCLUSIONS

Our results demonstrated that the sensitivity of *E. coli* strains to UV-C varied among the pathogenic and attenuated strains. Among the pathogenic strains, RM6535, a strain associated with the 2006 lettuce outbreak, was the most sensitive strain while CV267 and ATCC 700728 were more sensitive than other strains among the attenuated strains, especially at high UV-C doses. Three attenuated/nonpathogenic strains (ATCC 25922, 6980-2, and 6982-2) of *E. coli* O157:H7 had similar sensitivities or were more resistant to UV-C than the pathogenic strains; therefore, could serve as surrogates. EMA-PCR analyses suggest that UV-C- induced membrane damage occurred only at higher doses. Further research is needed to evaluate whether UV-C induces VBNC state in *E. coli* O157:H7.

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