Research Note

Response of *Salmonella* and *Escherichia coli* O157:H7 to UV Energy

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**ABSTRACT**

To determine the efficacy of a UV light treatment at 253.7 nm (UVC light) on microbial growth, plates containing tryptic soy agar plus 50 ppm of nalidixic acid (TSAN) were inoculated with known concentrations of *Salmonella* and *Escherichia coli* O157:H7 and subjected to different UVC treatments. The concentration of the cocktail inoculum was determined with TSAN prior to inoculation. Serial dilutions were carried out, and inoculation levels of 10⁰ to 10⁶ CFU/ml were tested for each pathogen. Multiple replications of doses of UV light ranging from 1.5 to 30 mW/cm² were applied to different cocktail concentrations, and doses of >8.4 mW/cm² resulted in a 5-log reduction of *Escherichia coli* O157:H7, while a 5-log reduction of *Salmonella* was observed with doses of >14.5 mW/cm². Results for both organisms yielded sigmoidal inactivation curves. UVC light is effective in reducing microbial populations of pathogens on agar surfaces.

UV light at 200 to 280 nm is classified as UVC light. This range of the UV spectrum has a germicidal effect on bacteria and viruses (6, 10). UVC light is effective in air, in liquid media, and on treatment surfaces (14). The success of UVC light is dependent on a system that is capable of delivering the necessary dose to the surfaces of foods or food contact surfaces. UVC light has commonly been used on processing equipment and packaging material, but its application to foods has been limited, perhaps owing to its low level of penetration of products and to the fact that shadows and crevices on the sample itself may shield bacteria from the harmful wavelengths (4). However, UVC light is more effective in the disinfection of smooth surfaces because of the direct path of the beam and the absence of light scattering.

Bachmann (1) reported wide variation in the UVC susceptibility levels of different bacteria. Previous studies by other researchers have produced mixed results with respect to doses required for the inactivation of pathogens. Kuo et al. (8) reported a ~6-log decrease of *Salmonella Typhimurium* on the surface of brilliant green agar with UV light doses of >37 mW/cm². Yousef and Marth (15) reported a 3-log reduction of *Listeria monocytogenes* on the surface of tryptose agar with a dose of approximately 9 mW/cm² and a 7-log reduction with doses of >36 mW/cm². Sumner et al. (13) indicated that doses of 3.1 mW/cm² resulted in up to a 7-log reduction of a nalidixic acid–resistant strain of *Salmonella Typhimurium* on brain heart infusion plates. Wong et al. (14) reported a >5-log reduction of *Escherichia coli* on the surface of tryptic soy agar with doses of >12 mW/cm².

Research by Sommer et al. (12) indicated significant differences between three strains of *E. coli* O157:H7 that were inactivated by UV light in a water system. The most UV-susceptible strain (CCUG 29199) was reduced by 6 log units when exposed to 12 J/m², whereas the most resistant strain (CCUG 29193) required a dose of >50 J/m² for a 4-log reduction. Data from the study of Sommer et al. (12) demonstrate that the use of a single strain of a particular species is not adequate for the determination of a specific dose for a given log reduction. In September 1997, an EPA Scientific Advisory Panel specifically identified *Salmonella*, *L. monocytogenes*, and *E. coli* O157:H7 as pathogens of public health concern. The panel also recommended the testing of five outbreak-related strains in a cocktail for each pathogen (5). In light of these previous experiments and recommendations, the overall objective of this study was to define the UVC dose required to effectively reduce the numbers of multistrain cocktails of *Salmonella* and *E. coli* O157:H7 on agar surfaces.

**MATERIALS AND METHODS**

**Preparation of inoculum.** Five strains each of *Salmonella* and *E. coli* O157:H7 were used in this study. Three of five strains of *E. coli* O157:H7 and all five strains of *Salmonella* used were isolated from outbreaks associated with raw vegetables or unpasteurized fruit juices. *E. coli* O157:H7 strain H1730 was isolated from a lettuce-associated outbreak, *E. coli* O157:H7 strain F4546 from an alfalfa sprout–associated outbreak, *E. coli* O157:H7 strain E0019 from a beef outbreak, and *E. coli* O157:H7 strain 994 from a salami outbreak. *Salmonella Montevideo* was isolated from a tomato-associated outbreak, *Salmonella Agona* from an alfalfa sprout–related outbreak, *Salmonella Baildon* from a lettuce-and-tomato–associated outbreak, *Salmonella Michigan* from a canta-
loupe-associated outbreak, and *Salmonella* Gaminara from an orange juice-associated outbreak. All serotypes were obtained from Dr. Larry Beuchat at the Center for Food Safety and Quality Enhancement at the University of Georgia (Griffin, Ga.). All strains were resistant to 50 ppm of nalidixic acid. Cultures were maintained at 28°C in tryptic soy broth (TSB; Becton Dickinson, Sparks, Md.) supplemented with 50 ppm of nalidixic acid (ICN Biomedicals, Aurora, Ohio) (TSBN). Cultures were grown in TSB at 35°C and transferred three times at consecutive 24-h intervals prior to their use as inocula. Incubation for 24 h allowed the respective bacteria to approach the stationary phase of growth at a concentration of ca. 10^8 CFU/ml. Equal aliquots of each individual strain were vortexed and then aseptically combined in a sterile dilution blank to produce a cocktail of five strains. Serial dilution of the inoculum, enumerated after 24 h of incubation at 35°C, was carried out to determine the inoculum concentration.

**Plate inoculation.** Inocula were serially diluted in 9.0 ml of sterile 0.1% peptone (Becton Dickinson). Duplicate spread plates with TSA plus 50 ppm of nalidixic acid (TSAN) were used for each dilution (dilutions ranged from 10^-1 to 10^-6). Multiple dilutions were tested in order to achieve a quantifiable logarithmic reduction. Higher inoculation levels were required to determine whether a 5-log reduction of the target organism was feasible. Spread plates were used in order to obtain the highest possible concentration of cells on the surface of the agar. After inoculation, plates were allowed to dry for at least 30 min in a laminar flow hood prior to treatment.

**Ultraviolet chamber.** The chamber used for the UVC irradiation of plates was fabricated at the Virginia Tech Department of Food Science and Technology. The chamber is approximately 1 m long and contains a single G36T6 Model 4136 germicidal light unit emitting 253.7 nm of UV light (Fuller Ultra Violet, Frankfort, Ill.). This particular light supplies an intensity of ca. 100 mW/s/cm^2 at a distance of 1 m. The light source is suspended on a chain and may be moved to either increase or decrease the intensity of the light. Light intensity is inversely proportional to the distance between the source and the target; therefore, as the source is moved closer to the target, intensity increases. The interior is lined with a highly reflective material (Solar Bright, Fuller Ultra Violet) designed to increase UVC intensity and to minimize any shadowing effect on irregular-shaped samples. Access is gained through a hinged bifold door. The UVC dose was measured with a dosimeter calibrated to read specifically at 253.7 nm (Spectronics, Westbury, N.Y.). The meter was calibrated and standardized by the manufacturer prior to the study.

**UV treatment.** Inoculated plates were randomized and individually subjected to different doses of UVC light. UVC intensity was determined prior to treatment by measuring the output of the light (mW/s/cm^2), and the applied dose received by the inoculated plate was calculated by the formula \(D = LT\), where \(D\) is the dosage received, \(L\) is the applied intensity (mW/s/cm^2), and \(T\) is the irradiation time (s). The intensity was kept constant, and various exposure times were employed to allow different doses (1.5 to 30 mW/cm^2) to be applied to the surface of the agar plate. Exposure times ranged from 5 to 75 s and were measured with a standard laboratory timer. Light intensity was evaluated several times during the experiments to ensure consistent output.

**Enumeration.** UVC-treated plates were incubated at 35°C for 24 h. Random colonies were selected and confirmed after each trial. Confirmation for *Salmonella* was carried out with xylene lysine deoxycholate agar (Becton Dickinson) and with API 20E test strips (Biomerieux, Hazelwood, Mo.). *E. coli* O157:H7 was confirmed with sorbitol MacConkey agar (Becton Dickinson) and with the use of a Visual Immunoprecipitate Assay (Biocontrol, Bellevue, Wash.).

**Statistical analysis.** Experiments were replicated more than 10 times with multiple dilutions for each ultraviolet dose tested. Data presented are the average levels of pathogen recovery from treated plates, along with the standard errors of the means. Means and standard errors were calculated from a commercial spreadsheet (Microsoft Excel, Redmond, Wash.). Survival data were treated according to Chick’s law as \(\log(N_s/N_0)\), where \(N_s\) is the density of survivors and \(N_0\) is the initial concentration of bacteria, which was calculated as the inoculum concentration.

**RESULTS AND DISCUSSION**

UVC light was effective in reducing microbial populations of *Salmonella* serovars and *E. coli* O157:H7 on the surface of TSAN. *Salmonella* cocktail cultures averaged 2.6 × 10^9 CFU/ml of inoculum. Concentrations of *E. coli* O157:H7 cocktail cultures averaged 2.0 × 10^9 CFU/ml of inoculum. Figure 1 shows the average log reductions of both *Salmonella* and *E. coli* O157:H7 on the surface of TSAN. Data points represent the average log reduction as calculated from all dilutions showing quantifiable reduc-
tions for each dose. The overall UVC dose required to reduce a microbial population of Salmonella was higher than that required to achieve the same reduction in an equivalent population of E. coli O157:H7. By plotting the equation of the best-fit line, it is possible to predict the point at which a 5-log reduction is achieved. For E. coli O157:H7, a 5-log reduction is achieved at a dose of >8.4 mW/cm². In contrast, Salmonella required a dose of >14.5 mW/cm².

The results of this study agree with those of other studies in which similar log reductions on different agar surfaces were observed (3, 8, 14). The results of this study also agree with those of a recent study by Kim et al. (7) in which Salmonella Typhimurium proved to be more resistant to UVC light than E. coli O157:H7 did. Chang et al. (3) found that doses required for a 3-log reduction of vegetative bacteria were similar among pathogens (3). The dose required for a 5-log reduction of the five-strain E. coli O157: H7 cocktail was approximately half the dose required for a 5-log reduction of the five-strain Salmonella cocktail.

Other researchers have described the inactivating effect of UVC light on microorganisms as sigmoidal (1, 11, 15). The initial exposure of bacteria to UVC is believed to injure cells. As increasing doses of UV light are received, mutations arise in the DNA code as neighboring pyrimidine bases begin to form cross-linkages that impede cellular replication. Cellular death occurs after the threshold of crosslinked DNA is exceeded (6, 11). Similar results were observed in this study, with the use of UVC light to reduce microbial populations leading to the formation of a sigmoidal curve. The tail of the inactivation curve has been explained by multiple-hit phenomena (15), the lack of a homogenous population (2), and the presence of suspended solids (11). It is possible that the use of multiple strains that may vary in their susceptibility to UVC light produced the tailing effect, as demonstrated by Sommer et al. (12). Other possible explanations for a sigmoidal curve include varying abilities of cells to repair DNA mutations through either light or dark pathways (9) and the shadowing effect that may have been produced by the edge of the petri dish.

In summary, UVC light was found to be an effective means of reducing microbial populations on agar surfaces. Logarithmic reductions of >6 log units are possible with an appropriate dose of radiation. In this study, the use of a multiple-strain inoculum demonstrated that E. coli O157: H7 was more susceptible to UVC light than Salmonella was.

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