UV Inactivation, Liquid-Holding Recovery, and Photoreactivation of *Escherichia coli* O157 and Other Pathogenic *Escherichia coli* Strains in Water

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**ABSTRACT**

Drinking water, water used in food production and for irrigation, water for fish farming, waste water, surface water, and recreational water have been recently recognized as a vector for the transmission of pathogenic *Escherichia coli*, especially serotype O157:H7. We investigated the UV (253.7 nm) inactivation behavior and the capability of dark repair (liquid-holding recovery) and photoreactivation of seven pathogenic (including three enterohemorrhagic *E. coli*) strains and one nonpathogenic strain of *E. coli* (ATCC 11229) with respect to the use of UV light for water disinfection purposes. Because most bacteria and yeast are known to be able to repair UV damage in their nucleic acids, repair mechanisms have to be considered to ensure safe water disinfection. We found a wide divergence in the UV susceptibility within the strains tested. A 6-log reduction of bacteria that fulfills the requirement for safe water disinfection was reached for the very most susceptible strain O157:H7 (CCUG 29199) at a UV fluence of 12 J/m², whereas for the most resistant strain, O25:K98:NM, a UV fluence of about 125 J/m² was needed. Except for one strain (O50:H7) liquid-holding recovery did not play an important role in recovery after UV irradiation. By contrast, all strains, particularly strains O25:K98:NM, O78:K80:H12, and O157:H7 (CCUG 29193), demonstrated photorepairability. For a 6-log reduction of these strains, a UV fluence (253.7 nm) up to 300 J/m² is required. The results reveal that the minimum fluence of 400 J/m² demanded in the Austrian standard for water disinfection is sufficient to inactivate pathogenic *E. coli*. A fluence of 160 J/m² (recommendation in Norway) or 250 J/m² (recommendation in Switzerland) cannot be regarded as safe in that respect.

The impact of pathogenic *Escherichia coli* strains has become of special interest since enterohemorrhagic *E. coli*, especially serotype O157:H7, was recognized as a severe risk affecting human health. Other types of pathogenic *E. coli* strains are known as enteroinvasive *E. coli*, enterotoxigenic *E. coli*, and enteropathogenic *E. coli* (7) that are differentiated on the basis of three major surface antigens, O (somatic), H (flagella), and K (capsule). Possible routes of their transmission are fecally contaminated food and water. In the latter respect, drinking water, water used in food production, water for irrigation, water for fish farming, waste water, surface water, and recreational water have been recently recognized as vectors for these pathogenic microorganisms (1, 4, 6, 7, 13, 16, 24, 26). A recent study shows that *E. coli* O157:H7 is a hardy pathogen that can survive for long periods of time, up to 91 days in water, especially at low temperatures (26). Depending on its origin and its hygienic quality, water has to be treated and disinfected before its use in human consumption and food production. For disinfection purposes, UV irradiation has become a credible alternative to the chemical disinfectants like chlorine and ozone for both water for human consumption and sewage water. In order to ensure safe water disinfection, accurate and complete information about the inactivation behavior of pathogenic microorganisms causing waterborne diseases is necessary. Chlorine inactivation of *E. coli* O157: H7 has been described by two working groups (6, 11). Both did not find any difference in resistance to chlorine between pathogenic and nonpathogenic *E. coli* strains and concluded that the normally used conditions during water disinfection by chlorine are sufficient to inactivate pathogenic *E. coli*. Regarding UV disinfection, only one working group has published data on two pathogenic *E. coli* strains thus far (25). The UV inactivation of microorganisms is based on damage to nucleic acids by UV rays. It is known that several bacteria and yeast possess enzymes able to repair these types of nucleic acid damage (9). Such enzymes are involved in dark repair pathways, such as liquid-holding recovery (induced especially by excision enzymes), and in photoreactivation due to light (by the enzyme photolyase). The effect of liquid-holding recovery—first reported by Roberts and Aldous in 1949—means an enhanced survival of UV-irradiated cells after keeping them for several hours in nutrient-free buffer in the dark before cultivation on media. This effect was observed by the authors in *E. coli* B that has become notorious for its great responsiveness for the liquid-holding recovery effect (9).

Photoreactivation is a very potent repair mechanism that is caused by the enzyme photolyase. This enzyme has to be activated by light energy in the near-UV or violet-
blue spectral range. In drinking water disinfection, this photorepair pathway usually does not play an important role, because drinking water is usually stored and transported under dark conditions in containers and distribution systems. However, sewage water and water used for irrigation and fish farming is exposed to visible light directly after UV disinfection. Therefore the ability of health-relevant microorganisms to undergo photorepair has to be considered to ensure conditions for hygienically safe water disinfection.

Based on our previous studies (5, 19–23), detailed prescriptions have been in place for the application of UV irradiation of drinking water since 1993 in Austria (2, 3) and recently in Germany (8). In these standards the main demands are that the dose (wavelength 253.7 nm) measured by biodosimetry has to be at least 400 J/m² and that only biodosimetrically proven and certified UV plants shall be installed in public water works. Thus, the aims of our study were first to determine the UV (wavelength 253.7 nm) inactivation behavior of selected pathogenic E. coli strains and second to investigate whether these strains possess dark repair and photoenzymatic repair. Third, we wanted to determine if the required 400-J/m² UV dose for drinking water disinfection is adequate to inactivate these bacteria sufficiently, taking into consideration their abilities of photoreactivation and liquid-holding recovery.

MATERIALS AND METHODS

Bacteria. The following seven pathogenic strains were investigated in our study: E. coli O157:H7 (CCUG 29197; isolated from a hamburger during an outbreak in the United Kingdom), O157:H7 (CCUG 29199; isolated from a hemolytic-uremic syndrome patient in North Carolina), O50:H7 (CCUG 29198; isolated from a patient with hemorrhagic colitis), O157:H7 (CCUG 29193; isolated from human feces), O78:H11 (ATCC 35401; isolated from human feces), O25:K98:NM (ATCC 43886; isolated from a human during an outbreak on a ship), and O25:K80:H12 (ATCC 43896; isolated from a child with diarrhea). For comparison a nonpathogenic E. coli strain ATCC 11229 that is often used in research was included. The strains were obtained from the Culture Collection University of Göteborg, Sweden (CCUG).

Cultivation and enumeration of the test organisms. The E. coli strains were cultured onto Columbia agar (CM 331; Oxoid Division, Unipath Co., Ogdenburg, N.Y.) for 24 h at 37°C. Cells were carefully removed from the agar layer, suspended in sterile 0.9% saline, and homogenized using a vortex mixer. The suspension was diluted with saline to a concentration of 10⁶ to 10⁷ CFU/ml using a calibration curve of the optical density at 578 nm versus the bacterial concentration (CFU/ml). In pretests we did not find a significant difference in the UV inactivation behavior of these E. coli strains, if the cells were grown in that way or in liquid medium (trypticase soy agar; Oxoid) followed by centrifugation and washing (data not shown). Therefore we decided to use the less time-consuming cultivation method. By investigation with a microscope it was ensured that no aggregates of bacteria occurred in the test suspensions. The enumeration of the bacteria as CFU before and after UV irradiation and photoreactivation was performed by pour plating with plate count agar (CM 325; Oxoid) and incubation at 37°C for 24 h.

UV irradiation in the laboratory device. A bank of 10 low-pressure mercury UV lamps (EK 36; length 500 mm, wavelength 253.7 nm, Katadyn, Wallisellen, Switzerland) was horizontally suspended over the irradiation vessel. A diaphragm (100 mm by 15 mm) was fixed directly below the UV lamps that could be additionally equipped with optical wiremeshes to enable low fluence rates (2.0 W/m², 0.2 W/m², and 0.02 W/m²). Additionally, the intensity of the UV lamps could be regulated by a transformer. The test suspensions (volume 25 ml) were irradiated in sterile petri dishes (diameter 90 mm) during permanent stirring. The depth of the layer was 4 mm. The UV fluence (253.7 nm) was measured online with a research radiometer IL 1700 SED 240 (International Light, Newburyport, Mass.), taking into consideration the transmission of the test suspension and the reflection on the surface as previously described (21). This device was evaluated for its reliability in an international laboratory trial for laboratory UV devices (21). Experiments were done at room temperature.

Photoreactivation in the laboratory device. Aliquots of 5 ml of the UV-irradiated suspension of test organisms were placed in sterile petri dishes (diameter 60 mm) under a bank of five horizontally fixed daylight fluorescent lamps (L 18W/20, Osram, Munich, Germany) emitting light in the 360- to 700-nm wavelength range. The distance between the surface of the test suspension and the lamps was 60 mm. The illuminance (lx) was measured online with a research radiometer IL 1700 SED 033 (International Light). The mean intensity of the illumination was 23,500 lx, which is equivalent to the illumination on an overcast summer day in Middle Europe (14).

To optimize the time period for the photoreactivation experiments we used test suspensions that have been irradiated with a UV fluence of 80 J/m² and illuminated the samples for 30, 60, 90, and 120 min (23,500 lx).

In each experiment, a sample of non-UV-irradiated test organisms was illuminated as a control. Experiments were done at room temperature.

Liquid-holding recovery. Aliquots of 0.5 ml of UV-irradiated test suspensions were added to 4.5 ml of buffer solution and incubated for 48 h at 22°C in the dark. The composition of the buffer was according to Harm (9): 7 g Na₂HPO₄·12H₂O, 3 g KH₂PO₄, 4 g NaCl dissolved in 1,000 ml deionized water; 4 ml of sterile 0.5 M MgSO₄ solution was added after autoclaving. In each experiment a sample of non-UV-irradiated test organisms was treated in the same way as a control. In pretests two other buffers were tested (0.9% saline and phosphate-buffered saline, Dulbecco’s). Both of them gave less recovery compared with the buffer according to Harm, therefore all tests of the study were done using Harm buffer (9).

Statistical analysis. All experiments were replicated three times and the data are reported as averages with standard deviations (SD). The concentrations of CFU/ml of the nonirradiated (N₀) and irradiated (N) samples were converted to log₁₀ values, and the reductions log(N₀/N) were calculated. In the figures, error bars indicate the standard deviations. Values below the quantitative detection limit (DL: 30 CFU/ml equivalent to log 1.48) are expressed by the term <DL.

To characterize the inactivation curves of the bacteria the curve parameters k (slope; m²/l) and d (shoulder broadness, intercept of the linear part of the survival function with the ordinate in semilogarithmic representation, decadic logarithm, unit 1) were calculated by using a curve-fitting program for the function, 

\[
\frac{N}{N_0} = 1 - (1 - 10^{-kH_0^d})
\]

where H₀ means fluence (J/m²).
The larger the parameter \( k \) is and the smaller the parameter \( d \) is, the higher is the sensitivity of the microorganism. The parameter \( d \) is also a measure for the ability of the bacterium to repair damage (9).

### RESULTS

**UV inactivation.** The UV inactivation behavior of the eight *E. coli* strains differed considerably (Fig. 1). A 6-log reduction was reached for the very most susceptible strain O157:H7 (CCUG 29199) at a UV fluence of 12 J/m\(^2\), whereas for the most resistant strain, O25:K98:NM, a UV fluence of about 125 J/m\(^2\) was needed. The three strains of the same serotype O157:H7 did not show similar UV inactivation at all. The curve parameters are shown in Table 1.

**Photoreactivation.** Figure 2 represents the relationship between the recovery of *E. coli* and the time period of illumination at constant illuminance (23,500 lx) for *E. coli* ATCC 11229 as an example. Direct plating of the UV-irradiated suspension showed a 6-log reduction. Thirty and 60 min of illumination led to a distinct increase in the recovery for about 1 and 3 logs, respectively, whereas further prolongation of the illumination time gave only a weak improvement in recovery. Based on these results, an illumination time of 120 min was chosen as an optimum for the photoreactivation experiments.

Photoreactivation was much more effective in allowing repair of UV damage than liquid-holding recovery with the only exception being strain O50:H7, in which both repair mechanisms revealed the same enhanced recovery (Figs. 3 and 4). The effect of photorepair was most pronounced in the strains O25:K98:NM, O78:K80:H12, and O157:H7 (CCUG 29193). An about two- to three-fold higher UV fluence (up to 300 J/m\(^2\)) was necessary to reduce these strains for 6 log after photoreactivation for 2 h with 23,500 lx. The curve parameters are shown in Table 1.

The controls (non-UV-irradiated but illuminated test suspensions) showed neither an increase nor a decrease in the concentrations of test organisms.

**Liquid-holding recovery.** Except for O50:H7, the *E. coli* strains yielded no or only a minor higher recovery after holding the UV-irradiated cells in buffer for 48 h under dark conditions (Figs. 3 and 4). The curve parameters are shown in Table 1. For strain O50:H7 an about 1.7-fold higher UV fluence was needed at a reduction level of 6 log. We even observed a slightly negative liquid-holding effect in the strain O157:H7 (CCUG 29199), where the survival decreased during the time of holding in buffer.

### TABLE 1. Curve parameters \( k \) (slope; \( m^2/J \)) and \( d \) (shoulder broadness) for the UV inactivation (253.7 nm) of seven pathogenic and one nonpathogenic *E. coli* strains with and without consideration of photoreactivation and liquid holding (decadic logarithm)

<table>
<thead>
<tr>
<th><em>E. coli</em> strain</th>
<th>UV inactivation</th>
<th>With photoreactivation</th>
<th>With liquid holding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( k )</td>
<td>( d )</td>
<td>( k )</td>
</tr>
<tr>
<td>O157:H7 (CCUG 29193)</td>
<td>0.096</td>
<td>1.848</td>
<td>0.034</td>
</tr>
<tr>
<td>O157:H7 (CCUG 29197)</td>
<td>0.087</td>
<td>0.878</td>
<td>0.051</td>
</tr>
<tr>
<td>O157:H7 (CCUG 29199)</td>
<td>0.455</td>
<td>0.000</td>
<td>0.252</td>
</tr>
<tr>
<td>O50:H7</td>
<td>0.136</td>
<td>1.878</td>
<td>0.065</td>
</tr>
<tr>
<td>O78:K80:H12</td>
<td>0.102</td>
<td>3.627</td>
<td>0.029</td>
</tr>
<tr>
<td>O25:K98:NM</td>
<td>0.079</td>
<td>4.069</td>
<td>0.048</td>
</tr>
<tr>
<td>O78:H11</td>
<td>0.150</td>
<td>4.956</td>
<td>0.040</td>
</tr>
<tr>
<td>ATCC 11229</td>
<td>0.082</td>
<td>1.656</td>
<td>0.039</td>
</tr>
</tbody>
</table>
FIGURE 3. UV inactivation (253.7 nm) of *E. coli* strains O157:H7 (CCUG 29193), O157:H7 (CCUG 29197), O157:H7 (CCUG 29199), and O50:H7 under consideration of photoreactivation (PR) and liquid holding (LH). <DL = below detection limit.

**DISCUSSION**

The UV inactivation behavior of pathogenic *E. coli* strains showed greater variation than expected from the data on nonpathogenic strains (9, 10, 18, 23). One of the strains, O157:H7, was extremely susceptible, similar to a repair-deficient strain described by Harm (9). Because the UV susceptibility of the three O157:H7 strains tested diverged widely from each other, no relation between UV resistance and serotype can be assumed.

In our study, dark repair by liquid-holding recovery did not play an important role in reactivation of *E. coli* after UV irradiation compared to photoreactivation. With one exception, strain O50:H7, we did not observe enhanced recovery in the nonpathogenic strain ATCC 11229 and in the remaining six pathogenic strains. Therefore the strong dark repair effect that was observed in the particularly responsive *E. coli* strain B (9) seems not to be valid for *E. coli* species in general. This is also in accordance with the findings on *E. coli* of Kashimada et al. (12), who did not notice dark repair in strains K12 and B. However, other bacteria, like fish pathogenic species of *Aeromonas*, *Yersinia*, and *Vibrio* are able to repair UV damage by liquid holding (15).

Data on photoreactivation in the nonpathogenic *E. coli* strain ATCC 11229 after an illumination period of 2 h—
FIGURE 4. UV inactivation (253.7 nm) of E. coli strains O58:K80:H12, O25:K98:NM, O78:H11, and ATCC 11229 under consideration of photoreactivation (PR) and liquid holding (LH). <DL = below detection limit.

without further specification of the fluence rate—yield a maximum recovery of 3.4 log (10) and 3 log (18). In our study the maximum recovery of this E. coli strain due to illumination with 23,500 lx for 2 h was slightly higher (3.56 log). Nevertheless, Kashimada et al. reported on two non-pathogenic E. coli strains, B and K12, that do not possess the ability to photoreactivate (12). These results indicate that the photoreactivation characteristic varies distinctly among the various E. coli strains.

The only UV inactivation data on pathogenic E. coli thus far have been published by Tosa and Hirata (25). They found a 2-log reduction for one strain of O157:H7 at a UV fluence of 33 J/m² and a 4-log reduction for one strain of O26 at 130 J/m². In this study, photoreactivation was detected only for strain O26 but not for strain O157:H7. This is in contrast to our results, where all E. coli strains, including three O157:H7 strains, showed a more or less enhanced recovery after illumination. This divergence could be explained either by biological differences in photorepair activity of the strains or by the fact that the intensity of the illumination used in the study of Tosa and Hirata (25) seems to be rather weak compared to daylight.

Our results clearly point out that a UV dose (253.7 nm) of 400 J/m² measured by biodosimetry as demanded in the Austrian standard for UV water disinfection is sufficient to ensure an inactivation of 6 logs of pathogenic E. coli, including strains of enterohemorrhagic E. coli, even with considerations of dark repair and photoreactivation. UV flu-
ences as recommended in other countries like Switzerland (250 J/m²) or Norway (160 J/m²) cannot be regarded safe with respect to the hygienic requirements of water disinfection. Furthermore, it must be stressed that the microbical effective UV dose applied by flowthrough water disinfection plants cannot be calculated nor predicted by mathematical modeling but must be proven by standardized biodosimetric fullscale testing \((3, 5, 19)\).

**ACKNOWLEDGMENT**

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**REFERENCES**