Isabel Poblotzki*, Fatmagül Öksüz* and Martin Heßling

Resistance Development in *Escherichia coli* Against Far-UVC Irradiation

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Abstract: Nosocomial pathogens spread in hospitals and are a permanent issue to patient safety [1]. Disinfecting medicinal equipment is therefore most important. Usually, chemical disinfection is applied but time consuming. Another known disinfection technique is irradiation with wavelength below 280 nm (UVC). UVC efficiently inactivates pathogens but usually cannot be applied in the presence of humans. The recently emerging Far-UVC with wavelengths between 200 and 230 nm is assumed to pose no danger to humans and is considered to be applied more frequently in the future. Therefore, this study investigates the potential radiation resistance development of *Escherichia coli* K12 caused by repeated exposure to 222 nm Far-UVC irradiation and the persistence of these adaptations. A cyclic irradiation experiment was conducted over ten cycles, followed by the assessment of resistance persistence over four subsequent cycles. The sensitivity of bacteria to irradiation was analyzed for exposure durations of 5, 10 and 15 seconds at an irradiation intensity of 1 mW/cm^2. The number of the bacteria after the incubation between the cycles after a fixed incubation time and morphological changes in bacteria colonies on Luria-Bertani (LB) plates were observed, indicating potential adaptation mechanisms. The results indicate that cyclic irradiation with this amount of irradiation intensity and the duration of several seconds does not induce significant and persistent Far-UVC radiation resistance but suggests transient adaptive responses which are not permanent. Adaptive reactions must be investigated more closely to avoid creating additional radiation resistance to existing drug resistances

Keywords: Far-UVC, bacterial resistance, cyclic irradiation, disinfection, *Escherichia coli (E. coli)*

***Corresponding author: Isabel Poblotzki:** Institute of Medical Engineering and Mechatronics, Ulm University of Applied Sciences, Ulm, Germany, poblis02@thu.de;
Fatmagül Öksüz: Institute of Medical Engineering and Mechatronics, Ulm University of Applied Sciences, Ulm, Germany, oeksfa01@thu.de;
Martin Hessling: Institute of Medical Engineering and Mechatronics, Ulm University of Applied Sciences, Ulm,Germany, martin.hessling@thu.de

1 Introduction

Due to the COVID-19 pandemic and the ever-increasing number of antibiotic-resistant bacteria, it is important to use technologies to disinfect surfaces and thus reduce resistant pathogens [2].

A proven technology for disinfection is the application of UVC radiation at 254 nm peak wavelength, which has an antimicrobial effect due to its DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) absorption and destruction properties [3].

A new option is to use the so called Far-UVC radiation for disinfection. Far-UVC radiation is ultraviolet radiation with a peak wavelength between 200 – 230 nm [4]. The advantage of applying Far-UVC radiation as a disinfection method is that it is unlikely to harm human skin [5,6] because of its strong absorption by the upper dead skin cells of the stratum corneum. This allows for the increased application of this type of radiation in public areas such as hospitals, public transport and schools - despite the presence of people or animals.

In view of this, research into the development of potential radiation resistance in bacteria and viruses is beneficial, in order to be prepared for a possible new pandemic and to ensure that this disinfection technology can continue to be applied effectively and sustainably to combat infectious diseases. In addition, the use of Far-UVC light is intended to reduce bacteria with possible resistance to antibiotics. To prevent bacteria from developing resistance to Far-UVC as well, possibilities of another type of resistance should be considered.

Therefore, this investigation deals with the possible development of radiation resistance and the change of sensitivity towards irradiation during cyclic irradiation of bacteria with Far-UVC radiation. The bacteria strain of *Escherichia coli* (*E. coli*) was selected as test microorganism.

E. coli were used because Alcantara Diaz in [3] has studied and proven an adaption effect of *E. coli* on cyclic irradiation with 254nm.

2 Methods

Escherichia coli K12 (DSM 498) were propagated in a shaking incubator overnight in liquid LB medium at 37 °C and 170 rpm (rotations per minute). For an irradiation cycle, a bacterial suspension in phosphate buttered saline (PBS) with a transmission of 30% at a wavelength of 222 nm was prepared. This was carried out in a quartz cuvette using a spectrophotometer type Specord 250 plus from Analytik Jena GmbH (Jena, Germany) . This transmission was selected because of the number of the cells in the suspension and the transmission during the irradiation cycle through the bacteria suspension. In this way it was be ensured that the bacteria at the bottom of the irradiated petri dish were also irradiated.

The irradiation setup is given in Figure 1. One millilitre of the suspension was transferred to a quartz Petri dish with a diameter of 6 cm and irradiated with an irradiation intensity of 1 mW/cm^2 at 222 nm for 5 seconds. This intensity was measured and adjusted before each irradiation using the X15 SN 51417 optometer of Gigahertz Optics (Tuerkenfeld, Germany). A fixed point on the table for the Optometer and later the bacteria suspension in the petri dish was used and the lamp was moved horizontally to meet the required intensity. The vertical distance was kept constant. The irradiation was carried out as described in Figure 1 step 1.

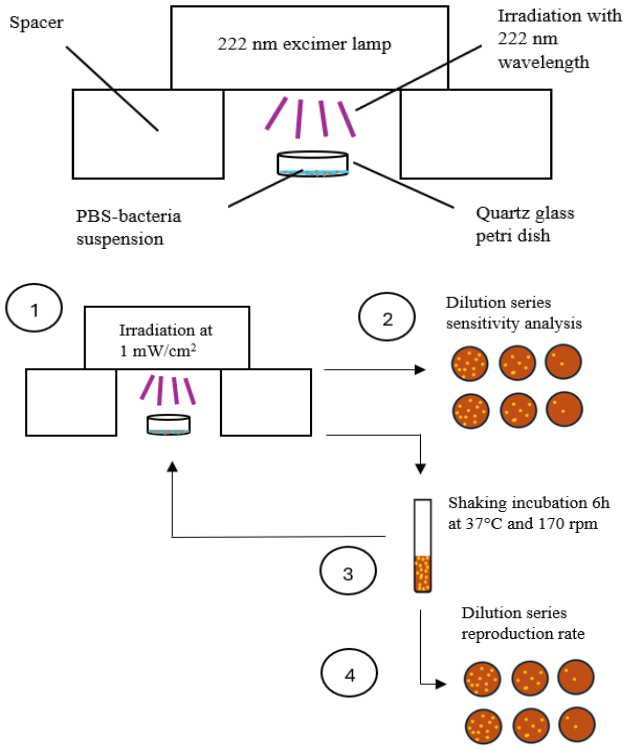

Fig. 1: Top: schematic irradiation setup; below: procedure steps (1-4) for the irradiation and analysis.

The suspension was then transferred to a culture vessel containing 5 ml of liquid Luria-Bertani medium. The shaking incubation time was 6 h at 37 °C and 170 rpm. This is illustrated in Figure 1 step 3. After incubation 500 µL of the bacterial suspension was removed and plated on LB agar plates. This is depicted in step 4 of Figure 1.

The remaining suspension was centrifuged, the supernatant was pipetted off and suspended in 5 ml of PBS. Then centrifuge a second time pipetted off the supernatant and add 5 ml PBS again. The bacterial suspension was adjusted to 30% transmission at 222 nm. For the next cycle, the bacteria suspension is irradiated for 5 seconds at 1 mW/cm^2 as in step 1 and the shaking incubation started.

Separate experiments were carried out to measure the radiation sensitivity of the bacteria. The bacterial suspension with 30% transmission was used and irradiated in three different samples as above. The irradiation time for the sensitivity tests was 5, 10 and 15 seconds. The irradiated bacteria samples from step 1 were plated on LB agar plates and compared to an unirradiated control of the bacterial suspension. This is shown in Figure 1 step 2. A total of 10 irradiation cycles were performed.

To test the adaptation reactions and the stability, the cyclic procedure was carried out as above, except that the irradiation was omitted after setting the 30% transmission suspension. The sensitivity irradiations after the incubations were carried out as for the first 10 cycles. The sequence was therefore: preparation of the 30% suspension, step 3, following step 1 for the sensitivity analysis. For the next cycle only the 30% suspension was prepared after step 3 and a new suspension is incubated.

To estimate the change in bacterial sensitivity to irradiation, a reference value was determined. This was carried out using bacteria taken in culture. The irradiation procedure from Figure 1 step 1 was carried out and then the bacteria were plated on LB plates and colonies counted after one day in a 37 °C incubator. The mean value of three dilution series was calculated for the reference value.

3 Results

Figure 2 illustrates the behavior of the examined *E. coli* after one irradiation cycle. These values were used as reference values to compare bacteria which have been irradiated several times. Figure 2 displays the percentage bacterial survival rate of the bacteria after irradiation compared to an initial value of the not irradiated bacteria suspension. The irradiation

durations of 5, 10, 15 and 20 seconds at 1 mW/cm^2 irradiation intensity are plotted.

The figure reveals a reduction in the number of bacteria with increasing irradiation energy. The red line indicates a reduction in bacteria by one log level. The irradiation references are approx. 80% survival rate at 5 s, approx. 20% survival rate at 10 s and approx. 3% survival rate at 15 s.

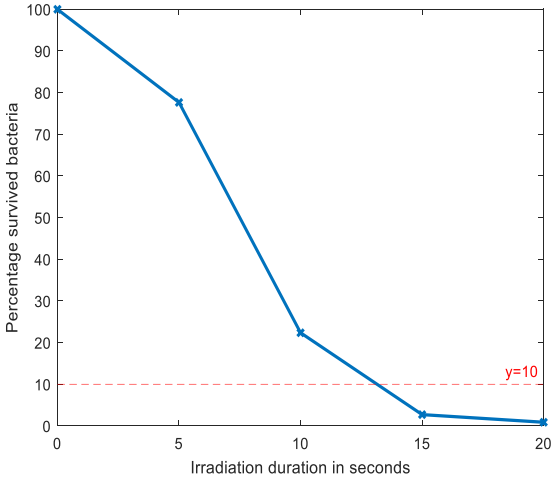

Fig. 2: Reference bacterial survival rates after exposure to 5, 10, 15and 20 seconds of irradiation

The irradiated bacteria of the different cycles in Figure 3 reveal different behaviors on the corresponding irradiation energy. The first value at the irradiation duration of 15 s is missing because the plated samples of the dilution series was too high for the first cycle. The three functions do not exhibit consistent behavior. The survival rates fluctuate, and do not indicate a continuous trend.

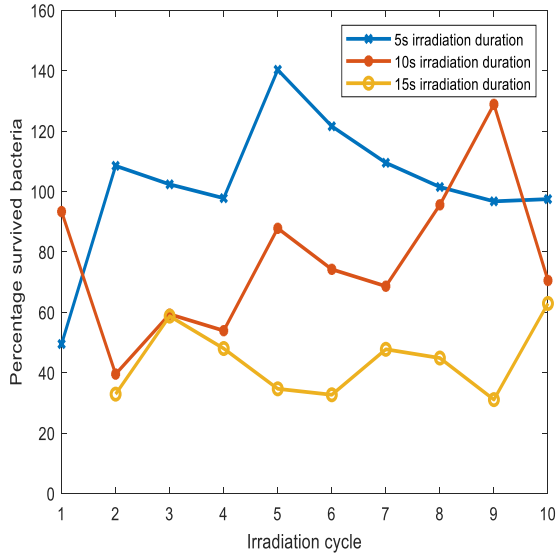

Fig. 3: Survival percentages over multiple incubation cycles on different irradiation durations

For the sample irradiated for 5 s for the sensitivity measurement, a decreasing trend occurs after cycle 5. Prior to this, the survival rate is greater than 100%, which means that more bacterial colonies have grown than in the non-irradiated control. The function of the survival rate of the 5 s irradiated bacteria shows a behavior that the function approaches the 100% survival rate after 10 cycles.

The 10 and 15 second samples give a higher survival rate than expected at the beginning. Both curves reveal a rather irregular course and have survival values at cycle 10 that are significantly higher than the initial and expected values. At 70% and 63%, the bacterial survival rates of the irradiated cycles are significantly higher than the initial value.

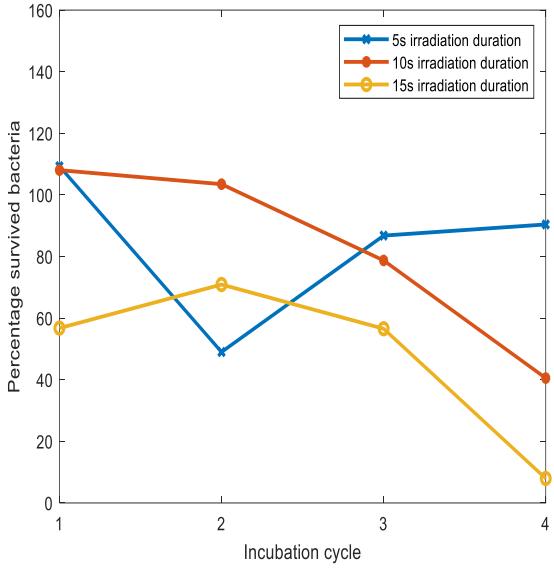

Fig. 4: Survival percentages over multiple incubation cycles confirming that adaptation effects are reversible

Figure 4 presents the *E. coli* radiation sensitivity for the cycles where no irradiation in between the cycles was carried out. The bacteria suspension was irradiated only before the sensitivity analysis.

The curves in Figure 4 do not give a uniform picture. The 5s sample first decreases in survival rate before the survival rate increases to approximately 90 %. For the samples that were irradiated for 10 and 15 seconds, it can be seen that the sensitivity to radiation at 222 nm increases significantly again. After 4 cycles, all three values are again close but a little above the initial values of the reference sample of the once irradiated bacterial suspension from Figure 2.

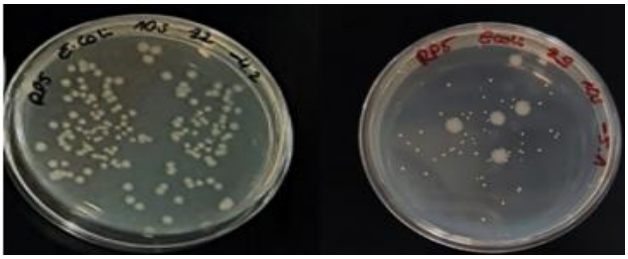

Fig. 5: Plated bacteria from cycle 2 on the left and cycle 9 on the right bacteria adapted to radiation

The change in morphology between the initial cycles in Figure 5 on the left and the later cycles on the right is clearly recognizable. Both images were taken by sensitivity analysis after 10 seconds of irradiation. The bacteria show a clear reduction in size after repeated irradiation. Nevertheless, some bacteria reveal a slightly larger shape than the initial size.

4 Discussion

The results indicate that *E. coli* does not develop stable resistance to cyclic Far-UVC irradiation but exhibits temporary adaptation during repeated radiation cycles. The fluctuations in bacterial survival suggest metabolic adjustments or stress-response mechanisms rather than heritable resistance.

Compared to studies on UV (254 nm), where genetic mutations contributed to increased UV resistance [3], no direct comparable trend was observed under Far-UVC conditions. The difference may stem from Far-UVC´s lower penetration depth. The irradiation at 222 nm is mainly absorbed by the proteins of the cell. This slows down and possibly also reduces mutations in the DNA compared to 254 nm [3].

The survival rate fluctuated across cycles, showing increased resistance in some instances but overall declining sensitivity at higher irradiation durations (15 s). The differences suggest a temporary stress response rather than a stable resistance mechanism. Also, higher stress in form of a higher irradiation energy needs more cycles to adapt. This means the cell must compensate for the amount of the irradiation and use more of its energy to keep the cell alive. Therefore, the adaptations on longer durations take more time.

To examine whether the observed bacterial adaptations persisted, four additional growth cycles were conducted without irradiation. The sensitivity of the bacteria was then reassessed.

This study demonstrates that cyclic Far-UVC irradiation does not induce stable resistance in *E. coli* K12 at least for the selected test organism. While transient adaptive responses were observed, the overall bacterial sensitivity remained unchanged in the long term.

These findings support the safe and effective application of Far-UVC for disinfection applications as long as there are also non-irradiated periods between the irradiation cycles.

This trend suggests that bacteria exposed to repeated irradiation experience a consistent reduction, but the overall decline stabilizes after multiple cycles, indicating that the remaining bacterial population is not gaining resistance but rather reaching a threshold of survival.

The reduction in the size of the bacteria may have been caused by a reduction in the surface area due to mutation. By reducing the surface area, the bacteria no longer have to absorb the radiation that leads to harmful changes. The delayed reaction on some bacteria colonies on the irradiation with the shrinkage in size is a missing mutation at that cycle. The cell can compensate for the irradiation without changing size.

Author Statement

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