The survival of *Escherichia coli* upon exposure to irradiation with non-coherent polychromatic polarized light

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ABSTRACT: The purpose of this work was to examine the influence of non-coherent polarized light upon the growth of *Escherichia coli*. Experiments were designed to test the main hypothesis that this kind of polychromatic light can produce decimal reductions in numbers of *E. coli* bacteria. Two strains of *E. coli* - isolated from ground pork and commercial culture *E. coli* ATCC 25922, were both exposed to light for 20, 30, 40 and 60 minutes. The source of non-coherent polarized light was a Bioptron-1 lamp (Zepter, Swiss) with the following technical characteristics: wavelength 400–2000 nm, polarization > 95%, and constant radiation dose 2.4 J/cm^2 per minute. The result for both strains showed a slight increase in bacterial count in response to an irradiation time of 20 min and decreases in bacterial counts for irradiation times of 30, 40 and 60 min without characteristics of a decimal reduction. Bacterial counts after treatments displayed a linear relationship with the total count of bacteria before treatments as well as the percentage surviving bacteria and irradiation time. Data analysis (ANOVA two factors with replication) showed that the survival of bacteria was influenced significantly by duration time ($P < 0.01$), bacterial culture ($p<0.05$), and interaction between duration time and bacterial culture ($P < 0.01$). Neither *E. coli* ATCC 25922 nor *E. coli* isolated from ground pork showed a decimal reduction after irradiation with non-coherent polychromatic polarized light.

Keywords: bacteria; growth; light; plate count

List of abbreviations

ANOVA = analysis of variance; BGLB = brilliant green lactose bile broth; CFU = colony forming units; I = energy impute; IMViC = indole, methyl red, Voges-Proskauer, and citrate; N_0 = plate count before treatments; N = plate count after treatments; R^2 = coefficient of determination; S = survival; SD = standard deviation; VRBA = violet red bile agar; VTEC = verotoxin-producing Escherichia coli

Many *Escherichia coli* strains are harmless and are commonly found in the intestinal tract of warm-blooded organisms. Other strains such as verotoxin-producing *E. coli* (VTEC) serotype, especially serotype O157:H7, cause serious poisoning in humans (Caprioli, 2007; Alexa et al., 2011). Transmission of *E. coli* occurs via the faecal-oral route and illness is most commonly associated with meat and meat products (Gansheroff and O'Brien, 2000; Conedera et al., 2007; Miko et al., 2009). Meat becomes contaminated during slaughter, when infected animal intestines or faeces come into contact with the carcass. Ground or mechanically tenderized meats are considered riskier than intact cuts of meat because *E. coli* bacteria can be mixed throughout the meat in the grinding process or during tenderization. However, a routine foodstuff control for *E. coli* is obligatory in the Republic of Serbia and according to regulations regarding microbiological criteria for foodstuffs (Pravilnik o mikrobioloskoj

ispravnosti namirnica u prometu, Sluzbeni list SRJ, 26/93, 53/95, 46/02) the presence of these bacteria is not allowed in 0.001g of grounded fresh meat.

Despite the significant advances that have been made towards a better understanding of *E. coli* transmission and pathogenicity, the levels of food/water-borne infection still remain high. Research on factors affecting the survival of *E. coli* is of great interest due to the importance of this microorganism as an indicator of faecal pollution in food and water, especially because its reported high level of resistance against antibiotics (Idrees et al., 2011). Concern over this situation has led to a search for alternative non-thermal technologies such as ultraviolet (UV) irradiation and pulsed electric filed (PEF) for pasteurization and sterilisation purposes (Donahue et al., 2004; Noci et al., 2008; Gabriel and Nakano, 2009).

The natural habitat of *E. coli* is the intestinal tract of humans and animals and it normally lives in darkness. Exposure to light, results in different effects depending on wavelength, intensity and the type of test light (Djurdjevic-Milosevic et al., 2003).

The antimicrobial effect of UV light is primarily due to its effects on the pyrimidine bases of microbial nucleic acids. When *E. coli* were exposed to fluorescent light after a 99.9% inactivation by UV irradiation, UV-induced pyrimidine dimers in DNA were continuously repaired and colony-forming ability recovered gradually (Oguma et al., 2001). Most micro-organisms can repair UV-damaged DNA with enzymes in light or dark conditions. Repair processes of UV-damaged biomolecules other than DNA, however, have yet to be elucidated.

The ability of wavelengths in the solar spectrum above 300 nm to kill small bacteria (< 10 microns) has been known for a long time (Ward, 1893). However, the biological molecules or chromophores affected have yet to be identified. Since proteins and nucleic acids show little or no absorption above 340 nm there must be other chromophores with sufficient absorbency to result in the death of small micro-organisms.

Visible light is not as photochemically reactive as UV radiation, but under appropriate conditions can be quite detrimental (McGinty and Fowler, 1982; Liou et al., 2011; Nair et al., 2011). Several authors have deduced that visible light has a negative effect on *E. coli* cells in freshwater (Fujijoka, 1981; Bracina et al., 1989). This was revealed by a decrease in the numbers of metabolically active *E. coli* cells. Light above 400 nm had marked differential effects on active transport processes in *E. coli* (Barran et al., 1974) and had selective effects on *E. coli* ML-308: several processes or enzymes were strongly inhibited, whereas others were relatively unaffected (D'Aoust et al., 1974). Bailey et al. (1983) observed that visible light provokes a decrease in the active transport of radioactively labelled amino acids in natural bacterial populations from a marine medium.

The aim of this study was to examine the influence of non-coherent polarized light with wavelengths in the visible spectrum and part of IR spectrum (λ = 400–2000 nm) on the survival of two strains *E. coli*. Taking into account all that is known regarding the natural habitat of *E. coli* and its sensitivity to light, the application of this kind of light could decrease bacterial counts. If such treatment would have a significant effect, then the application of this light could become a useful tool against *E. coli*.

MATERIAL AND METHODS

Culture

Two strains of *E. coli* – an isolate from ground pork and a commercial culture from the ATCC 25922 (Torlak, Belgrade, Serbia) were used in this project. *E. coli* ATCC 25922 is utilized as a surrogate microorganism to check the effects of UV light on the pathogenic *E. coli* O157: H7 (Quinero-Ramos et al., 2004). This strain is in particular used widely used as a non-pathogenic microorganism in assessing the efficiency of disinfection of fruit products with high pH values, since the D_{80} is nearly identical to *E. coli* O157: H7 (Pao, 2001).

Isolation of *Escherichia coli*

A total of 33 samples of ground pork were analyzed for the presence of *E. coli* and one was found to be positive. For the isolation of *E. coli*, National Provisions on microbiological methods for the analysis of food were used (Pravilnik o mikrobioloskoj ispravnosti namirnica u prometu, Sluzbeni list SRJ, 26/93, 53/95, 46/02) and media were prepared according to the instructions of the manufacturer (Institute Torlak, Belgrade, Serbia).

Twenty g of each sample were transferred to a 250 ml Erlemeyer flask and were homogenized with 180 ml saline (0.85% w/v of NaCl) for 15 min.

Successive dilutions were then made. One ml of a 10^{-3} dilution was transferred to Brilliant green lactose bile broth (BGLB) with a Durham tube and incubated for 24–48 h at 44 °C. A loopful was then streaked by gassing a BGLB tube onto a Violet red bile agar (VRBA) plate before incubation at 44 °C for 24–48 h. The plates were observed for the growth of *E. coli* and isolated colonies were picked for the preparation of smears and stained with Gram's stain for the examination of staining and morphological characters of the isolate using a bright field microscope. The culture characteristics of the isolates were confirmed by inoculating the pure colonies on a slant with Nutrient agar (24 h at 37 °C) and then the performance of an IMViC test to confirm *E. coli.*

Pure cultures of isolates were preserved at 4 °C on Nutrient agar slants.

Source of light

The source of visible non-coherent polarized light was a Bioptron-1 lamp (Zepter, Swiss) with the following technical characteristics: wavelength λ = 400–2000 nm, linear polarization > 95%, power 40 mW/cm^2 and constant radiation dose of 2.4 J/cm^2 per minute. Bioptron polarized light is produced on a special multilayered mirror which produces: (1) polarization – all emitted waves are oscillating, (2) incoherence – every light wave is oscillating at its own wavelength and amplitude, and (3) polychormy – covering the visible light bond and a slight part of the infrared bond (Colic et al., 2004).

Determination of bacterial survival and irradiation procedure

The survival of bacterial cells following irradiation was monitored by plate count before and after exposure of the suspended bacteria to light. This was performed by counting the number of colony forming units (CFU) on Nutrient agar plates and calculating their number per ml.

Before the irradiation treatment a loopful of culture was transferred from a slant with Nutrient agar to Brilliant green lactose bile broth (BGLB) and incubated for two hours at 37 °C. One ml from BGLB was transferred to a tube with 9 ml of saline (0.85% w/v of NaCl) and serial dilutions were prepared. One ml was transferred from tubes with dilutions of 10^{-6}, 10^{-7} and 10^{-8}, inoculated with Nutrient agar in duplicate and incubated (48 h/37 °C) for the counting of *E. coli* before the treatment (No). One ml from BGLB was transferred to an empty Petri plate and the suspension of bacteria was irradiated under sterile conditions at a distance of 5 cm from the source of light. After exposure (20, 30, 40 or 60 min) in Petri plates 9 ml of saline was added to the irradiated bacterial suspension (0.85% w/v of NaCl) and prepared serial dilutions. Dilutions of 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} and 10^{-10} were used for inoculation in duplicate and incubation (48 h/37 °C) for the counting of *E. coli* after the treatment (N). Plates with 30–300 colonies were chosen for counting.

This procedure was performed five times (n = 5) for each irradiation time (20, 30, 40 and 60 min).

Calculation

The percentage of surviving bacteria (S) was calculated according to the following equation:

$$S\ (\%) = (N - N_0)/N_0 \times 100 \quad (1)$$

where:
N_0 = plate count of *E. coli* before treatments
N = plate count of *E. coli* after treatments

Constant radiation dose 2.4 J/cm^2 per minute and duration of treatments (t) were used in an equation to calculate the energy impute (I) of linear non-coherent polarized light:

$$I\ (\text{J/cm}^2) = 2.4\ (\text{J/min/cm}^2) \times t\ (\text{min}) \quad (2)$$

Data analysis and statistics

Differences in plate counts of bacteria before and after irradiation were tested using a linear model. For each irradiation time (20, 30, 40 and 60 min) experiments were performed five times. All primary data were calculated as the percentage of surviving bacteria and presented as the mean of the standard deviation. Two ways analysis of variance (ANOVA) with replication was performed to test two null hypotheses:

H_{01} = applied times of irradiation have equal effects on bacterial counts

H_{02} = Both strains of *E. coli* have equal counts after irradiation

The post hoc Duncan test was used to determine the significance of differences between group means. Probabilities of less than 0.05 were considered significant at $P < 0.05$.

The data were analyzed using Microsoft Office Excel 2007 and SigmaPlot11 (Sysstat Software, Inc. USA).

RESULTS AND DISCUSSION

Two strains of *E. coli* were exposed to a source of non-coherent polarized light which covered visible spectra and part of the IR spectra (λ = 400 to 2000 nm). Our results show that the numbers of the two *E. coli* strains after the performed treatments were in a linear relationship with the number of bacteria before treatments (Figures 1 and 2).

Thus, N is a linear function of N_0 with various slopes for different treatment durations and bacterial strains (Table 1). The slope for 20 min irradiation of *E. coli* ATCC 25992 as well as for 20 min irradiation of the *E. coli* isolate indicated a slight increase in numbers of bacteria. Decreases in numbers of bacteria were observed after irradiation of 30, 40 and 60 min for both strains and were 0.919, 0793, 0.749 and 0.853, 0.680, 0.466, for *E. coli* ATCC 25992 and the *E. coli* isolate, respectively. The effect of irradiation differed from one strain to the other, leading to the conclusion that the *E. coli* isolated from ground pork was more sensitive to non-coherent polarized light.

Generally, previously reported results indicate opposing effects of non-coherent polarized light on eukaryotic and prokaryotic cells. The favourable effects of polarized light rays were discovered during studies on laser light rays, and in 1981 a group of physicists from Hungary proved that polarized light has a stimulating effect on the natural defence and repair functions of organisms and cells (Kertesz et al., 1982; Fenyo, 1984; Kubasova et al., 1988). These cells include endothelial cells (Moore et al., 2005), keratinocytes (Yu et al., 2003), macrophages (Fujimaki et al., 2003), and several classes of leukocytes such as neutrophils (Young et al., 1989). Earlier research on bacteria showed a significant difference ($P < 0.05$) in the number of *Bacillus subtilis* after treatment with non-coherent polarized light, darkness or visible light (Solaja et al., 2003). Apart from changes in bacterial counts of *E. coli* after irradiation with non-coherent polarized light in different media (Djurdjevic-Milosevic et al., 2003), changes in quantities of metabolic products after irradiation of *Lactobacillus delbureckii* subsp. *bulgaricus* and *Steptococcus thermophilus* have been reported; namely, a statistical difference in the titratable acidity of yogurts ($P < 0.05$; Stijepic et al., 2008) and a decrease in the lactose content of milk (Stijepic et al., 2009).

Most experiments, which have concentrated on wavelengths in the visible light part of the spectrum and its effect on *E. coli*, have considered the effect of monochromatic laser light with precisely defined wavelengths. A key difference between laser and polarized light is that laser light is coherent and monochromatic. Its wavelengths can add up their energies so that in the area of application they have very high energy. Some published works have raised the possibility of mutagenic effects of visible light (Voskanyan, 1990) or lethal effects on bacteria

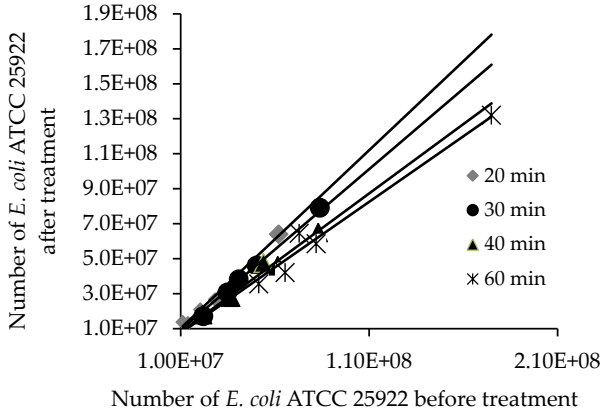

Figure 1. Change in plate counts of *E. coli* ATCC 25922 in response to treatments with non-coherent polychromatic polarized light λ = 400–2000 nm for different durations

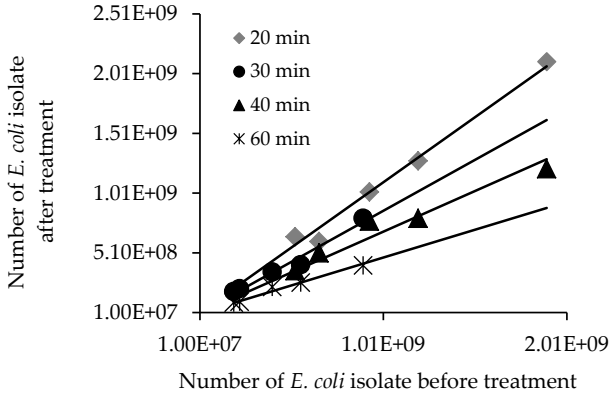

Figure 2. Change in plate counts of the *E. coli* isolate in response to treatments with non-coherent polychromatic polarized light λ = 400–2000 nm for different durations

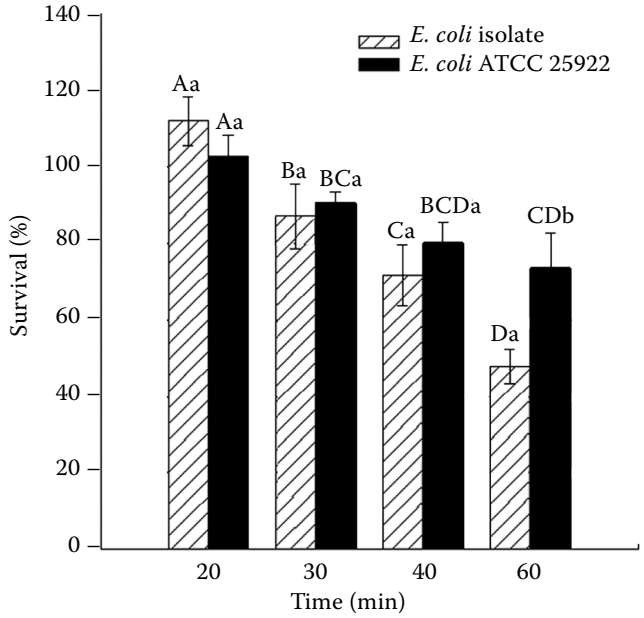

Figure 3. Mean ± SD of survival (%) of *E. coli* ATCC 25922 and *E. coli* isolated from ground pork after application of non-coherent polychromatic polarized light λ = 400–2000 nm for durations

[ABCD]different capital letters denote the results, in different groups, which are significantly different ($P < 0.05$, Duncan test)
[ab]different small letters denote the results, within a particular groups, which are significantly different ($P < 0.05$, Duncan test)

(Sikora et al., 2003). D'Aoust et al. (1974) reported that light above 400 nm had selective effects on *E. coli* ML-308: several processes or enzymes were strongly inhibited, whereas others were relatively unaffected. On the basis of previous findings on the lethal effects of 633 nm laser irradiation on *E. coli* K-12 (Arutyunyan, 1988), Voskanyan (1999) tested the induction of Lac^- mutations in *E. coli* K-12 (Hfr) under 633 nm laser irradiation and noted lethal and mutagenic effects on bacteria.

Under natural conditions, *E. coli* lost its ability to form colonies in marine environments when exposed to artificial continuous visible light (Gourmelon et al., 1997). Experiments on the application of sunlight for water disinfection showed that the destruction of different types of bacteria requires different times of exposure to light, and that *E. coli* possesses a relative susceptibility to solar radiation compared to other bacteria (Acra et al., 1990).

Our bacterial plate counts following exposure to non-coherent polarized light were expressed in percentage surviving bacteria according to equation (1). The survival of *E. coli* ATCC 25992 was in the range: 93% to 123%, 89% to 94%, 75% to 88% and 64% to 88% for irradiation time of 20, 30, 40 and 60 min, respectively. The survival of *E. coli* isolated from ground pork was in the range: 92% to 128%, 73% to 95%, 64% to 83% and 44% to 56% for irradiation times of 20, 30, 40 and 60 min, respectively. Neither *E. coli* ATCC 25922 nor *E. coli* isolated from ground pork showed decimal reductions after irradiation with non-coherent polychromatic polarized light, and this kind of light, therefore, is not an efficient tool against *E. coli*.

Data analysis (ANOVA two factors with replication) showed that the survival of bacteria was influenced significantly by duration of exposure ($P < 0.01$), bacterial culture ($P < 0.05$), and interaction between duration and bacterial culture ($P < 0.01$). This indicates the existence of a further factor which was not observed in experiments, but had an influence on the plate count of *E. coli.*

The post hoc Duncan test showed that the strain of *E. coli* was a source of variation for plate count only after irradiation of 60 min. The recorded increase in plate counts after irradiation of 20 min did not show any statistical difference between the *E. coli* strains. The time of irradiation was a strong source of variation for the plate count of the *E. coli* isolate, but in case of *E. coli* ATCC 25922 no statistical difference was observed between irradiation times of 30 and 40 min, and 40 and 60 min (Figure 3).

A diagram of the experimental results presents a decrease in *E. coli* survival as a logarithmic function of the energy impute in the interval 48–144 J/cm^2. The logarithmic function has different coefficients for each one of strains. The equation for *E. coli* ATCC 25922 is $S = -27.3 \ln(I) + 207.6$ with $R^2 = 0.976$, and for the *E. coli* isolate it is $S = -57.9 \ln(I) + 336$ with $R^2 = 0.999$. In neither case are there characteristics of decimal reduction.

Extrapolation to zero irradiation doses shows polynomial functions in between percentages of surviving bacteria and the irradiation doses

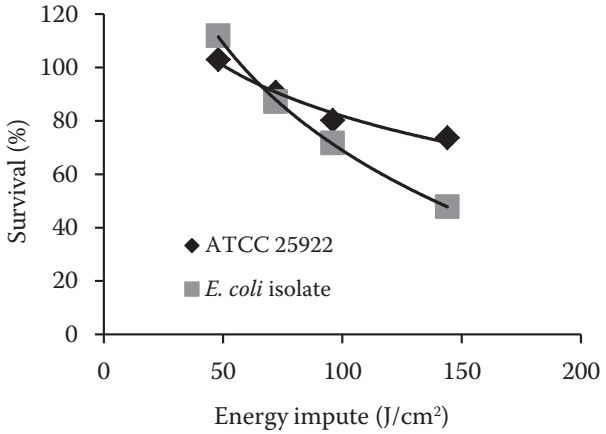

Figure 4. Influence of energy impute in the interval 48–144 J/cm^2 of non-coherent polychromatic polarized light on the survival of bacteria

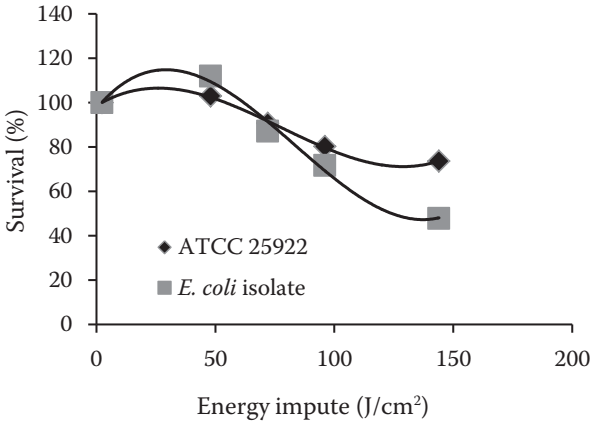

Figure 5. Extrapolation of energy impute of non-coherent polychromatic polarized light to the beginning of irradiation and influence on the survival of bacteria

themselves. The equation for *E. coli* ATCC 25922 is $S = 7E\text{-}05I^3 - 0.014I^2 + 0.588I + 100$ with $R^2 = 0.994$, and the equation for the *E. coli* isolate is $S = -0.025I^2 + 1.162I + 100$ with $R^2 = 0.985$.

These functions have one maximum in the interval 0–48 J/cm^2 and one minimum in the interval 96–144 J/cm^2. The maximum area and reduction in irradiation time could be very interesting avenues for further research, because as well as an investigation of dimensional coefficients those can support the equation of trend line survival-energy in the dimensional sense (Figures 4 and 5).

In conclusion, in this study, it was determined that different durations of treatment with non-coherent polarized light irradiation have different effects on the survival of *E. coli*. It was also established that 20 min of irradiation supported the growth of bacteria while irradiations of other durations (30, 40 and 60 min) provoked a decrease in bacterial plate counts. *E. coli* isolated from ground meat was more sensitive to non-coherent polarized light compared to *E. coli* ATCC 25922. Neither *E. coli* ATCC 25922 nor *E. coli* isolated from ground pork showed decimal reduction after irradiation with non-coherent polychromatic polarized light, and thus this kind of light does not represent an efficient tool against *E. coli*.

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