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Effect of Environmental Conditions on *Escherichia coli* Survival in Seawater

Slaven Jozić and Mladen Šolić

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Abstract

We investigated separate and simultaneous effect of temperature, salinity and solar radiation, as well as bacterial strain and origin on *Escherichia coli* (*E. coli*) survival in seawater in experimental conditions. The experiments were carried out by placing the bottles filled with seawater of different salinity (15.0, 30.0 and 36.5 psu) and contaminated by bacterial cultures in three light-protected air incubators set to different temperatures (6, 12, 18 and 24°C), or by placing the bottles in plastic containers filled with water of controlled temperature and exposing them to direct solar light. In experiments in the dark, two typed and two wild *E. coli* strains were tested. The mean T_{90} values were 33.55 h for *E. coli* ATCC 8739, 42.50 h for *E. coli* ATCC 35218, 72.8 h for *E. coli* originating from seagull feces and 278.6 h for *E. coli* originating from sewage, indicating differences between survival abilities among strains. The effect of temperature on T_{90} was significant only in seagull *E. coli* at 36.5 psu and sewage *E. coli* at 30.0 psu and was positive. The effect of salinity was significant only in seagull strain and also was positive. No interactive effect of temperature and salinity was recorded. Experiments in the presence of solar radiation, carried out with two ATCC *E. coli* strains, demonstrated its dominate harmful effect on bacterial cells, reducing T_{90} of both strains to 0.30–0.82 h for *E. coli* ATCC 35218 and 0.31–5.93 h for *E. coli* ATCC 8739. Within the ultraviolet A (UVA) and photosynthetically active radiation (PAR) spectrum of solar radiation, the wavelengths of 320–360 nm were found as most bactericidal. By comparing survival of cultivated *E. coli* cells to those in natural seawater samples, significantly higher survival *E. coli* cells in natural seawater samples was found.

Keywords: *Escherichia coli*, bacterial strain, survival, temperature, salinity, solar radiation

1. Introduction

Fecal pollution of seawater can present a serious problem due to potentially introducing of intestinal pathogens—bacterial, viral, and parasitic. Because of a wide variety of pathogenic

microorganisms that can enter the sea, time-consuming and complex procedures of their determination, the assessment of microbiological quality of seawater has traditionally been based on the determination of indicator microorganisms, bacteria that suggest the presence of pathogens [1]. In the last decade, European Union member states have accepted *Escherichia coli* as the indicator for assessment of microbiological quality of bathing and shellfish waters [2, 3]. Apart from its human and warm-blooded animal feces origin, selection of *E. coli* as indicator organisms is based on scientific understanding and reliable research of one of the most important criteria which good indicator have to meet: resistance to environmental conditions.

When released into the sea, *E. coli* cells are exposed to the impact of a very hostile environment. That impact is reflected in the negative effect of the complex array of biotic and abiotic factors of the marine environment. Among abiotic factors, the effect of temperature, salinity, and particularly solar radiation is most pronounced [4–7]. The distribution and abundance of indicator bacteria in seawater depend mostly on their input, but also on the intensity of aforementioned environmental factors [8] and bacterial cell adaptation capacity. Due to their minor adaptation capacity to marine conditions, *E. coli* cells suffer a sublethal injury and enter a dormant, viable but nonculturable state, in which they can still maintain some metabolic activity [9–11], infective capacity and potential for pathogenicity [12, 13]. The "viable but non-culturable" state concept was introduced to describe cells that remain metabolically active but are unable to divide in or on nutritional media that normally support their growth. The capacity to form colonies on a solid medium is the first ability that enteric bacteria lose in seawater [14]. That means that they cannot be detected during standard routine monitoring by culturing method unless resuscitation methods on liquid media were used. With the pro-longed exposure, particularly in the presence of solar radiation, *E. coli* cells are irreversibly inactivated and they die [15]. For how long they will maintain culturability on standard media depends on many factors, such as the intensity of environmental factors and the characteristics of the bacterial cell, where besides the origin and previous growth history, the bacterial strain plays an important role [7, 16, 17]. Although there are many studies in which the effects of the aforementioned factors have been addressed, their simultaneous effects have been poorly investigated. In this chapter, the separate and combined effect of temperature, salinity and solar radiation, as well as the growth history, strain and origin of *E. coli* cells on their survival in seawater were presented. Since microbial pollution of bathing waters is routinely moni-tored by culturing methods on standard solid media, and viable but nonculturable cells usu-ally cannot be detected, for the purposes of this chapter, the term "survive" means to maintain culturability.

2. Materials and methods

2.1. Experiments with pure bacterial cultures

Bacterial cultures used in experiments were always freshly prepared by incubation of pure culture on mineral-modified glutamate broth (MMGB). Bacterial suspensions were taken in the exponential phase of growth and serially diluted in phosphate buffer solution. After

determination of the concentration of *E. coli* cells by epifluorescence microscopy method, appropriate dilution, cca 10^3 CFU/mL, was kept at 4°C until the beginning of the experiments (cca 20 h).

Experiments in the dark were performed in two sets. In the first set, experiments were carried out at six different experimental conditions corresponding to Adriatic sea natural range of temperatures (12°C—mean winter temperature, 18°C—mean spring and autumn temperature and 24°C—mean summer temperature) and salinities (30.0 psu—lower salinity corresponding to areas near the mouth of rivers or sewage outfalls and 36.5 psu—typical salinity in coastal seawater). Two different *E. coli* strains were used: *E. coli* ATCC 35218 originating from canine feces and *E. coli* ATCC 8739, originating from human feces. Bacterial suspension was added to 500 mL borosilicate glass bottles containing autoclaved seawater of appropriate salinity (30.0 and 36.5 psu). Bacterial concentration in bottles was targeted to 10^3 CFU *E. coli*/100 mL. The bottles were then stirred and placed in a temperature controlled air incubators at three different temperatures (12, 18 and 24°C).

In the second set, additional experiments were performed at lower temperature (6°C) and lower salinity (15 psu). Two new bacterial strains were also introduced—wild *E. coli* strain isolated from sewage and wild *E. coli* strain isolated from seagull feces.

Experiments in the presence of solar radiation were performed with two ATCC *E. coli* strains, also combining the same temperatures (12, 18 and 24°C) and salinities (30.0 and 36.5 psu). Bacterial suspension was added to 500 mL ultraviolet B (UVB)—blocking borosilicate glass bottles containing autoclaved seawater of appropriate salinity. Bacterial concentration in bottles was also targeted to 10^3 CFU *E. coli*/100 mL. The bottles were then stirred and left in dark for 15 min to homogenize. Bacterial cells in bottles were then exposed to different intensities of the natural range and spectrum of solar radiation. The exposure to solar light was carried out in two ways: by placing the bottles in 50 L (60 × 30 × 30 cm) transparent plastic containers, filled with water of appropriate and controlled temperature, and placed on the open space in front of the laboratory;—by hanging the bottles from research vessel, vertically on rope to different depths (0.2, 5, 15 and 30 m) of the water column, to expose them to different intensities of solar light (for *E. coli* ATCC 8739 only). In both cases, the irradiance, Ed (μWcm^{-2}nm^{-1}), of ultraviolet A (UVA) and photosynthetically active radiation (PAR) was measured by optical radiometer PRR800 (Biospherical Inc.).

All experiments were performed in triplicates. The initial concentrations of culturable *E. coli* cells in all experiments were determined by taking 10 mL subsamples 15 min after adding bacterial suspension. After exposing the bottles to desired conditions, the concentrations of *E. coli* were monitored by taking 10 mL subsamples every 24 h in experiments performed in the dark and every 10 or 20 min in experiments performed in solar light.

2.2. Experiments with natural samples

Natural samples of moderately polluted seawater were collected near sewage outfalls and were stored at 4°C. Sampling was performed in the morning (9 o'clock AM) and at noon. The number of culturable *E. coli* was determined after sampling and every 24 h until their

reduction to zero. After isolation and counting on selective agar plates, one colony of *E. coli* cells that survived T_{90} (the time required to reduce culturability by 90%) or longest was randomly selected from each plate of samples collected in the morning. Cultures were incubated on MMGB and processed in the same way as pure cultures of ATCC strains. A bacterial suspension was added to 500 mL borosilicate bottles, containing autoclaved, 36.5 psu seawater, and stored in dark at 4°C. The number of culturable *E. coli* was determined every 24 h until their reduction to zero.

2.3. *E. coli* determination

The concentration of *E. coli* in suspensions was determined by the direct method of epifluorescence microscopy [18]. The number of culturable *E. coli* cells in suspension and seawater was determined by the modified ISO/TS 16649-1 method [19] using membrane filtration technique and expressed as CFU *E. coli*/100 mL.

2.4. Statistical analysis

Data were processed using statistical package Statistica 8.0 (StatSoft Inc., 2007). High and significant ($R^2 > 0.9$, $p < 0.01$) fittings of raw die-off data of *E. coli* to exponential function, which can be expressed by Eq. (1), were found in the dark as well as in the presence of solar radiation. Consequently, T_{90} was derived from Eq. (1) and was calculated by Eq. (2),

$$N_t = N_0 e^{kt} \tag{1}$$

$$T_{90} = \frac{-\ln(0.1)}{k} \tag{2}$$

where N_0 and N_t are the number of culturable *E. coli* at the beginning of the experiment and at the time of subsampling (t).

The inactivation energy S_{90} (the insolation required to reduce culturability by 90%) was calculated by Eq. (3),

$$S_{90}(\text{Whm}^{-2}) = \sum_{n=0}^{T_{90}} E_n T_n \tag{3}$$

where E_n (Wm^{-2}) = the intensity of solar radiation.

3. Results and discussion

3.1. Experiments in the absence of solar radiation

In the natural range of temperature and salinity, the T_{90} values in this study were 31.9–51.7 h (mean 42.5 h) for *E. coli* ATCC 35218 and 29.4–37.9 h (mean 33.55 h) for *E. coli* ATCC 8739 (**Figure 1**). The results were mostly consistent with the results of previous studies. The T_{90} values of *E. coli* reported in earlier studies were from 26 h, which was found in estuarine water at 20°C [20], to 115 h, which was found for fecal coliforms in seawater at 8–10°C [5].

The results of two-way ANOVA revealed that there were no statistically significant ($p > 0.05$) separate and/or interactive effects of the variations in temperature and salinity in their natural range, on changes in T_{90} values of ATCC *E. coli* strains. Unlike the T_{90} values, these results were not consistent with those of previous studies. In general, most studies showed enhanced stability of indicator bacteria at lower temperatures [4, 5], although Anderson et al. [21] found a lower stability and negative effect. The increased inactivation of indicator microorganisms at higher temperatures was mostly attributed to increased metabolic activities in terms of reduced nutrient concentration [22], increasing predation, grazing and the deleterious effect of solar radiation [23]. One of the possible explanations for the absence of clear effect of temperature in our experiments might be the absence of grazing and predation, since seawater was sterilized by autoclaving. Anderson et al. [21] attributed a positive effect of temperature in their study mostly to sublethal stress that was induced by laboratory manipulation and related to the pre-exposure history of the used isolates. In our additional experiments with *E. coli* ATCC 8739 at 6°C, significantly higher T_{90} values were recorded (**Figure 1**), indicating more significant negative effect of temperature in its natural range than at lower values. A positive effect of temperature in whole measured range was observed in seagull *E. coli* at 36.5 psu and sewage *E. coli* at 30.0 psu (**Figure 2**).
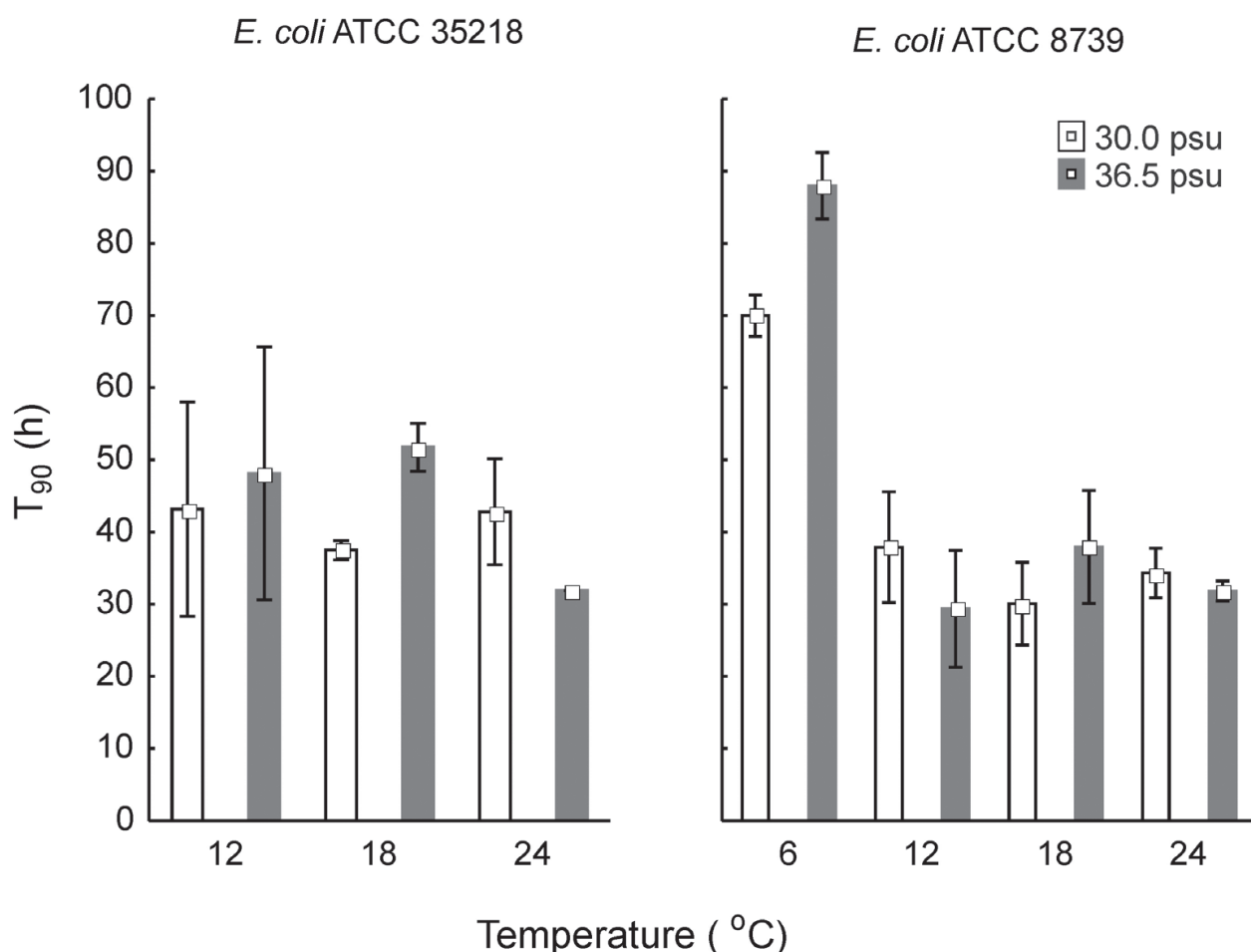

Figure 1. Effect of temperature and salinity on the survival of ATCC 8739 and ATCC 35218 *E. coli* strains in the absence of solar radiation (mean values ± SD).
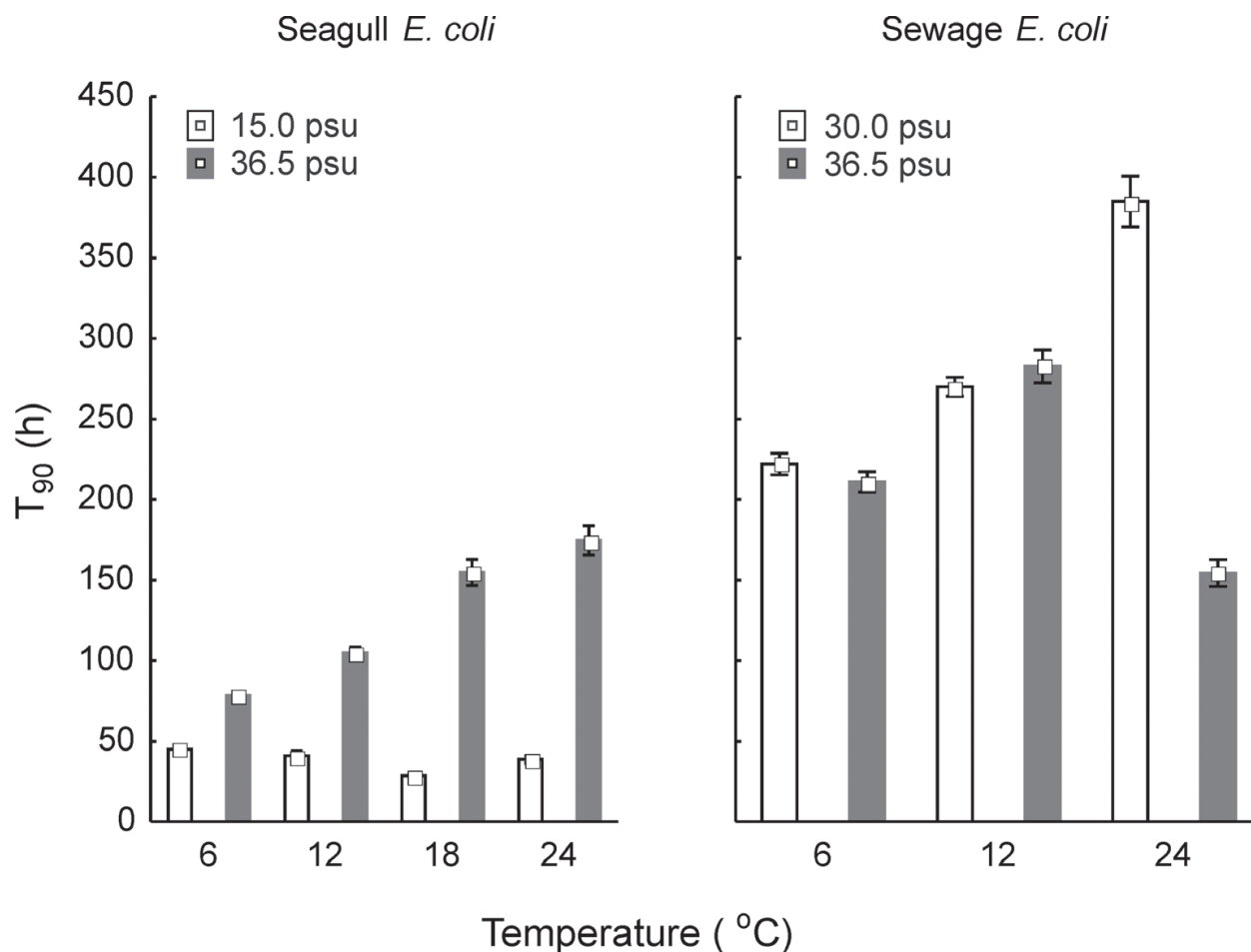
Figure 2. Effect of temperature and salinity on the survival of *E. coli* strains originating from seagull feces and sewage in the absence of solar radiation (mean values ± SD).

Most studies showed a negative correlation between survival time of indicator bacteria, including *E. coli*, and salinity [3, 7, 24, 25]. Troussellier et al. [26] found negative effect of salinity only in the presence of solar light. An unclear correlation between T_{90} and salinity in our study was partly attributed to a narrow salinity range. In order to extend the range of salinity and to clarify its effect on T_{90}, we performed additional experiments with *E. coli* ATCC 8739 at 15 psu. At higher temperatures (18 and 24°C), negative effect of lower salinity was less pronounced than the effect of salinity in its natural range, while at 6°C, lower salinity significantly lowered T_{90}, making the effect of salinity additionally unclear (**Figure 3**). A negative effect of salinity is attributed to specific characteristics of sea water, such as osmotic pressure [27] and the toxicity of inorganic salts [6]. Survival largely depends on the osmoregulatory ability of each strain or group of bacteria [28]. In order to equalize osmotic pressure and avoid drastic loss of water from the cytoplasm, bacterial cells can accumulate or synthesize specific osmoprotectant molecules [29] which increase cell resistance to seawater. This depends, among other things, on preadaptation media. Since *E. coli* in experiments was incubated on MMGB media, accumulation of glutamate, which is recognized as osmoprotectant [30], probably caused an unclear effect of salinity on *E. coli* survival.
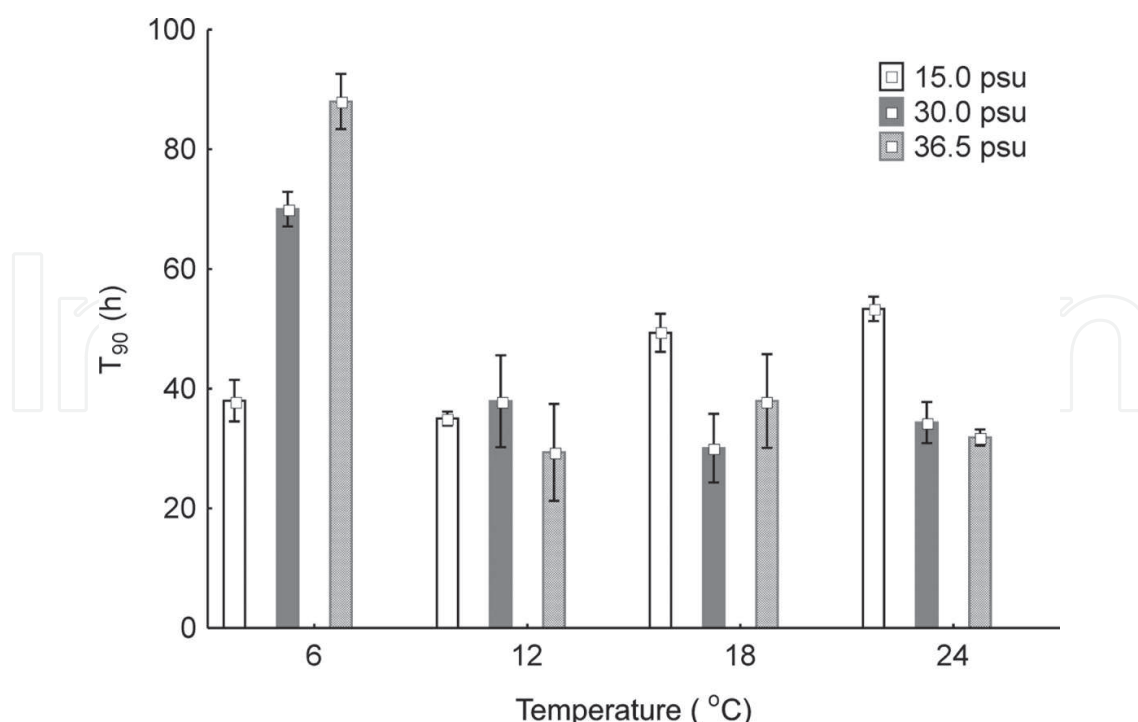
Figure 3. Effect of temperature and salinity in its wider range (15.0, 30.0 and 36.5 psu) on the survival of ATCC 8739 *E. coli* strain in the absence of solar radiation (mean values ± SD).

Although not statistically significant, *E. coli* ATCC 35218 survived longer than *E. coli* ATCC 8739 in almost all tested conditions. A significantly higher T_{90} was found in pure culture of *E. coli* isolated from seagull feces, 28.5–173 h (mean 72.8 h) and particularly in *E. coli* isolated from sewage outfall, 150–390 h (mean 278.6) (**Figure 3**). This suggested the importance of cell strain and origin to their survival in sea water and partially contributed to better understanding of the variations in results of previous studies. Since all tested *E. coli* strains were exposed to the same environmental conditions, the obtained results probably illustrated the extensive genetic and phenotypic diversity exhibited within *E. coli* strains, which could explain the different survival abilities in this study and aquatic environments generally [31]. On the basis of genomic information, *E. coli* species have been divided into eight phylogenetic groups, A, B1, B2, C, D, E, F, and clade I [32]. Strains belonging to phylogroups A and B1 are highly adapted to humans and vertebrate animals, the A phylogroup strains being predominant in humans and the B1 strains in animals [33, 34]. Significant differences in survival in water environment were found among strains belonging to different phylogroups. Recent studies showed that *E. coli* B1 strains can persist longer in water than strains of the other phylogroups [31] and supported the hypothesis that persistent genotypes have an adaptive advantage in the secondary habitat outside the host [35]. Consequently, once released into water, *E. coli* strains could be selected on the basis of their survival ability, and the resulting population differed from the original one in terms of phenotypic traits. *E. coli* strain isolated from sewage outfall and used in this study was in the seawater for an unknown period and probably passed selection that explains its superiority in survival over other tested strains.

Anderson et al. [7] found significant differences in the survival of indicator bacteria, *E. coli* and enterococci, depending on their origin. Among three investigated sources of bacteria; soil, sewage and dog feces, bacteria that originated from soil showed the highest resistance to environmental factors. These results are very important, particularly because apart human feces, there are many potential sources of enteric bacteria that enter the sea, such as untreated piggery effluents [36], and the feces of wild birds [37] and other warm-blooded animals.

In seagull *E. coli*, a positive effect of salinity was recorded in whole range of temperature, and at 36.5 psu higher temperature enhanced bacteria survival (**Figure 2**). Some *E. coli* cells may adapt to a range of concentrations of sodium chloride, possibly by means of osmoregulatory mechanism induced by salts or some structural modification in the outer membrane of bacteria [27]. Seagulls are marine birds and sea environment is their natural habitat. They feed mainly marine animals that contain high content of sodium chloride and drink both, fresh and seawater. Before being processed and excreted through salt gland in order to maintain osmotic balance, sodium chloride is present in intestinal tract content in a relative high concentration [38]. Consequently, seagull intestinal microflora, including *E. coli*, is highly adapted to saline environment. Such cells probably survive longer in seawater than other cells. Seagulls present a big problem to shellfish farms because they usually gather on buoys and other farm constructions, and huge quantities of feces enter the sea and contaminate it. Since up to 10^7 CFU *E. coli* g^{-1} was found in seagull feces in this study, and the results suggested better survival of *E. coli* originating from seagull feces, there is higher probability of their accumulation in filter feeding bivalves than *E. coli* that survive shorter.

3.2. Experiments in the presence of solar radiation

The intensity of solar radiation in this study was in the range 258–693 Wm^{-2}, with a mean contribution of the UVA spectrum of 9.3–11.1%. In the experiments carried out in the laboratory, a strong ($R^2 > 0.82$) and statistically significant ($p < 0.01$) negative correlation between the T_{90} of *E. coli* and the intensity of the UVA spectrum of solar radiation was found (**Figure 4**). The T_{90} of *E. coli* ATCC 35218 was in the range 0.30–0.82 h, whereas the T_{90} of *E. coli* ATCC 8739 ranged from 0.31 to 5.93 h. A significant, 15- to 70-fold reduction in T_{90}, compared with that recorded in the absence of solar radiation indicated the dominant effect of solar radiation on the survival of *E. coli* in seawater. Multiple linear regressions were used to investigate the combined effect of temperature, salinity, and the intensity of solar radiation on changes in the T_{90} of *E. coli* (**Table 1**). High and statistically significant ($p < 0.01$) coefficients of multiple determination (R^2) were found, which revealed that most of the variance in T_{90} can be explained by independent variables. However, only variations in solar radiation had a significant effect on T_{90}.

Because of inadequate weather conditions (a relative cloudy sky), in situ experiments were carried out at lower intensities of solar radiation. We also found a strong vertical decline in the intensity of solar radiation (UVA and PAR) and a significant decrease in the contribution of the UVA spectrum in the water column (**Figures 5** and **6**). Therefore, only a few data from surface layer could be used in our calculations. Unlike the experiments carried out in the laboratory, T_{90} of *E. coli* fitted exponential function (**Figure 7**) which can be expressed by Eq. (4).

$$T_{90}(h) = 31.305\, e^{-0.17E_{UVA}} \tag{4}$$

According to the function, when $E_{UVA} = 0\ \text{Wm}^{-2}$, T_{90} value of *E. coli* ATCC 8739 amounted to 31.305, which corresponds to its value in the previous experiments in the absence of sunlight (**Figure 1**), which confirms the validity of function.
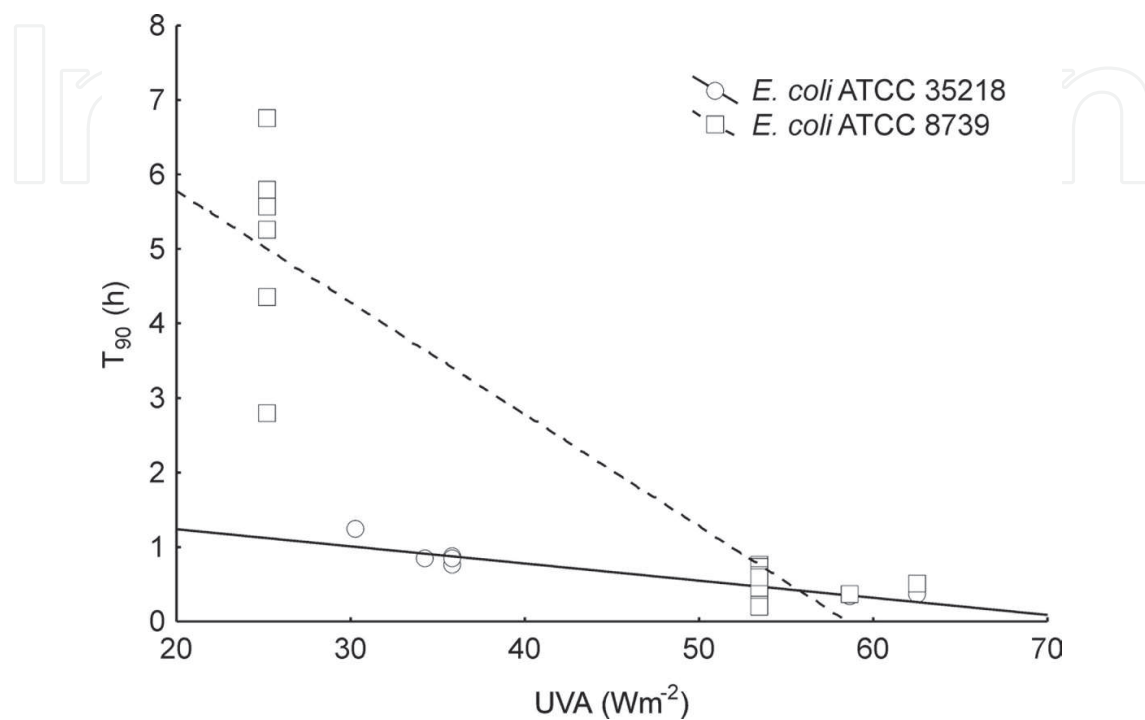

Figure 4. Effect of the UVA part of the solar radiation spectrum on the survival of ATCC 8739 and ATCC 35218 *E. coli* strains.

Variable	r_p	Beta	a	b	R^2
E. coli ATCC 35218					
T	−0.116	−0.208	1.183	−0.011	0.8797*
S	0.380	0.143		0.011	
UVA	−0.830*	−0.955		−0.024	
PAR	0.211	0.323		0.001	
E. coli **ATCC 8739**					
T	−0.463	−1.043	7.882	−0.488	0.911*
S	0.319	0.107		0.711	
UVA	−0.8465*	−0.816		−0.131	
PAR	0.438	0.924		0.017	

*p < 0.01.

Table 1. Simultaneous effect of temperature (T), salinity (S), and solar radiation (UVA and PAR) on the T_{90} of *E. coli* (r_p—coefficient of partial correlation; beta-regression coefficients; a—intercept; b—coefficient of multiple linear regression; R^2—coefficient of multiple determination).
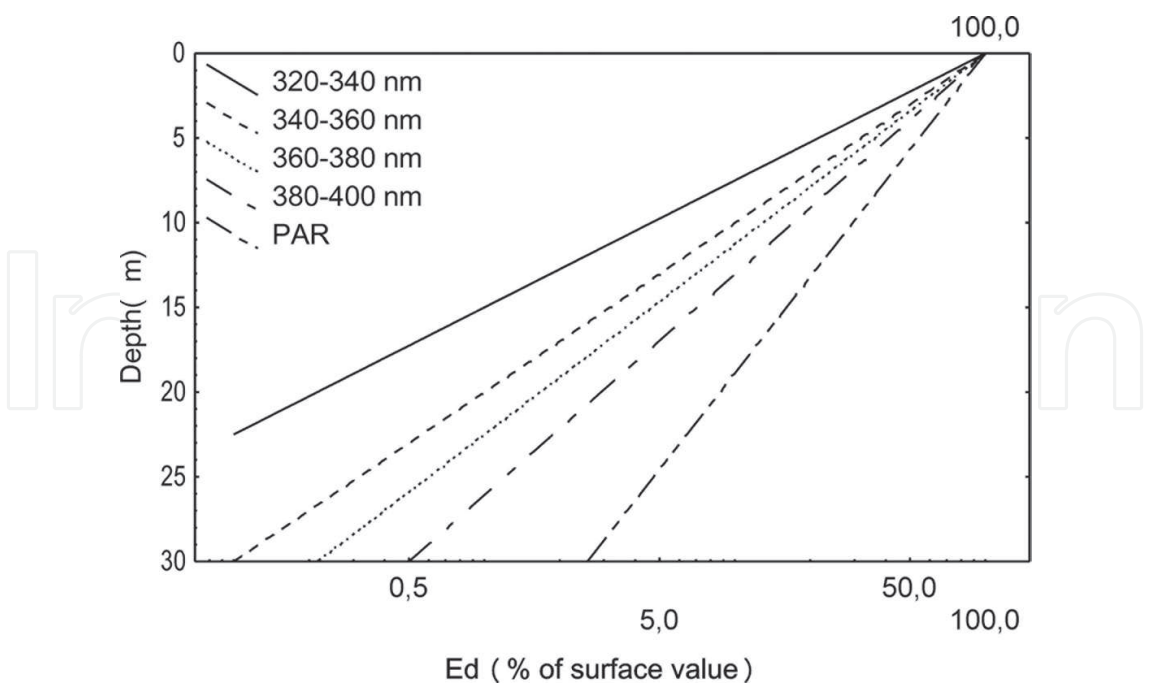
Figure 5. Attenuation of irradiance (% of surface value) as a function of sea depth.
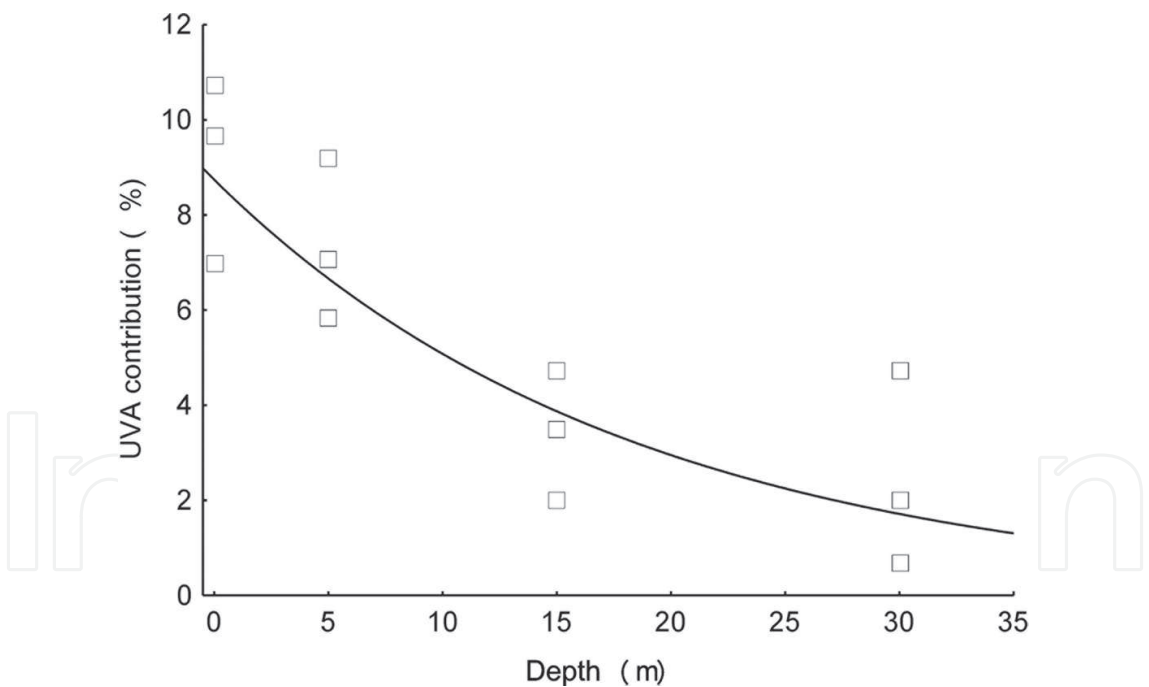

Figure 6. The reduction in the contribution of the UVA component of solar radiation as a function of sea depth.

The negative effect of sunlight exposure was also confirmed in the study with wild cultures of *E. coli* kept at 4°C. A significantly lower survival ($T_{90} = 98 \pm 22$ h, n = 18) was found in *E. coli* sampled at noon than in those sampled in the morning ($T_{90} = 153 \pm 35$ h, n = 18). This might be attributed to prolonged exposure to solar radiation and its higher intensity.
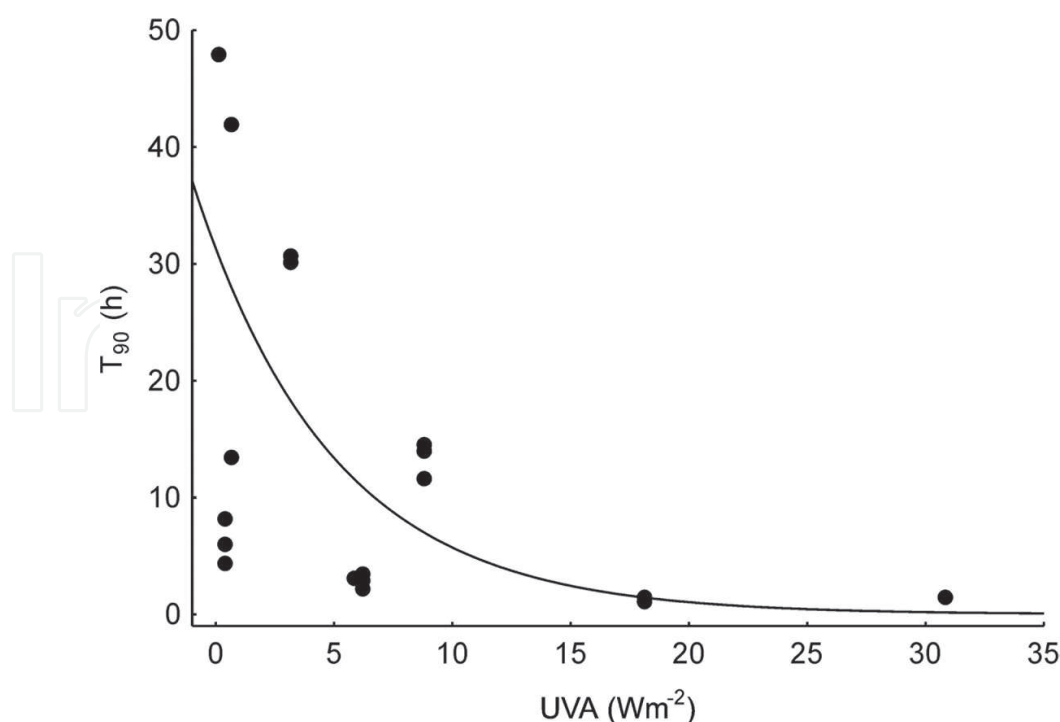
Figure 7. Effect of the lower intensity of UVA part of the solar radiation spectrum on the survival of ATCC 8739 *E. coli* strain.

Solar light is considered to be the most important factor to bacterial reduction in the sea, although its effects are restricted to shallow depths [5], also observed in this study. The negative effects of solar radiation to bacterial cells operate via two different mechanisms: firstly, direct photobiological mechanisms break DNA bonds in bacterial cells [39, 40], while secondly, indirect photochemical mechanisms damage bacterial cells by photosensitized reactions initiated by some of endogenous and/or exogenous sensitizers [41]. Photochemical mechanisms become more important at higher wavelengths, and it is more injurious in the presence of oxygen [5]. Both effects cause a rapid decrease in colony forming ability [25, 26]. Dominant toxic effect of sunlight was also observed in previous studies. Šolić and Krstulović [4] found that within the investigated range of intensity of solar radiation (510–830 Wm^{-2}), the T$_{90}$ of fecal coliforms exponentially decreased by about 40% when the intensity of solar radiation increased by 100 Wm^{-2}. Large differences (30- to 40-fold) in survival with or without exposure to sunlight were observed by Fujioka et al. [25], who noted a T$_{90}$ of fecal coliforms exposed to solar radiation in the range of 0.5–1.5 h. The reduction in survival time in the presence of solar radiation was also recorded by Chandran and Hatha [20], where the T$_{90}$ of *E. coli* was reduced from 26 h in the dark to 4 h in the presence of solar radiation.

According to Davies-Colley et al. [42], T$_{90}$ is a better indicator for expressing inactivation in the absence of solar radiation and also inactivation caused by solar radiation of an equable and higher irradiance, whereas the S$_{90}$ better expresses the inactivation caused by solar radiation of variable intensity, as exists in nature. The energy of solar radiation absorbed by bacterial cells and which is responsible for cell injury is a product of the intensity of solar radiation and exposure time.

The mean values of S_{90} recorded in this study were 250 Whm^{-2} for *E. coli* ATCC 35218, and 610 Whm^{-2} for *E. coli* ATCC 8739. We found a medium-strong ($R^2 = 0.457$) and strong ($R^2 = 0.8282$) statistically significant ($p < 0.01$) negative linear correlation between the intensity of the UVA spectrum of solar radiation and S_{90} values for both *E. coli* strains (**Figure 8**).

A high and significant correlation between the intensity of the UVA spectrum of solar radiation with T_{90} and S_{90} in this study suggests that within the studied spectrum of solar radiation, this part of the measured spectrum was most responsible for the inactivation of microorganisms. The linear regression-slope coefficients suggested the wavelengths 320–360 nm to be the most bactericidal within the UVA spectrum (**Table 2**).

Acra et al. [43] found that up to 70% of the solar inactivation of bacteria can be attributed to the effect of the UVA part of the solar radiation spectrum, whereas Sinton et al. [5] found that 50% of the inactivation of indicator microorganisms could be attributed to solar radiation wavelengths up to 360 nm, with a wavelength of about 330 nm being most bactericidal. Calkins and Barcelo [44] find the UVB (280–330 nm) portion of the solar spectrum the most bactericidal, causing direct photobiological DNA damage. Although bactericidal effect of solar radiation is mainly associated with shorten wavelengths, the synergistic effect of UVB, UVA, and PAR explained most variations found in culturability of *E. coli* [45].

The values of S_{90} observed in this study were significantly lower than those in similar studies. S_{90} of fecal coliforms found by Gameson [22] was 1.290 Whm^{-2}, whereas Sinton et al. [5] found a significantly higher value, 1.660 Whm^{-2} (6.0 MJm^{-2}). Significant differences could be
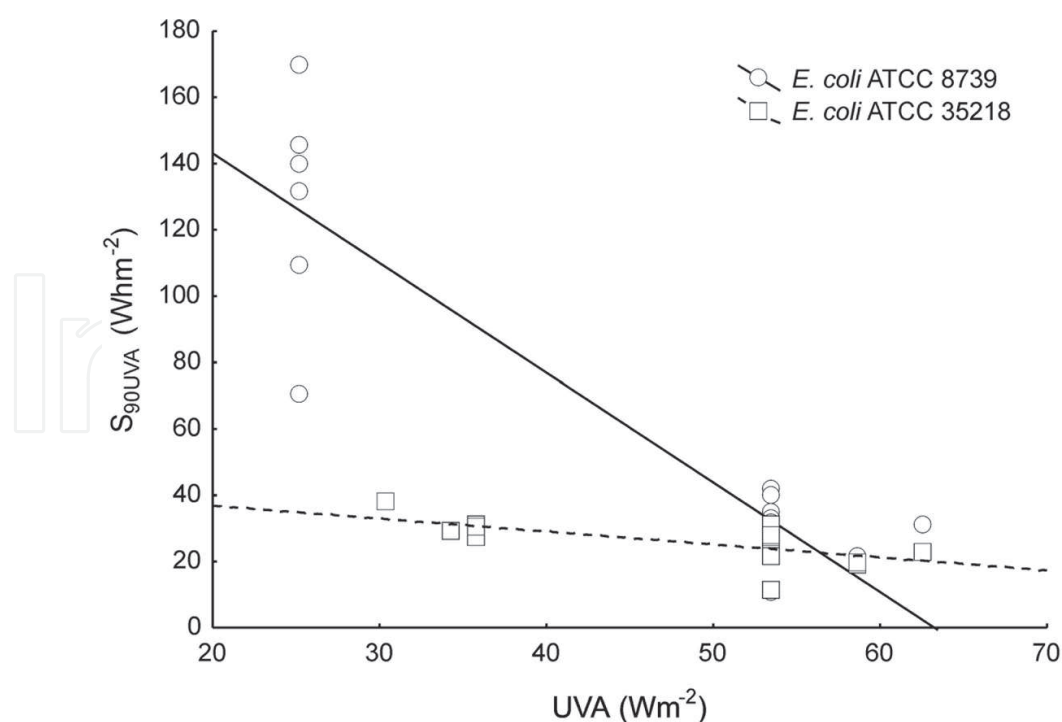

Figure 8. The S_{90} of ATCC 8739 and ATCC 35218 *E. coli* strains as a function of the intensity of the UVA part of the solar radiation spectrum.

	Wavelength (nm)				
	320–340	340–360	360–380	380–400	400–700 (PAR)
E. coli **ATCC 35218**					
b	−0.147	−0.124	−0.072	−0.052	−0.001
R^2	0.442	0.663	0.848	0.848	0.192
p	<0.01	<0.01	<0.01	<0.01	>0.05
E. coli **ATCC 8739**					
b	−1.095	−0.812	−0.488	−0.364	−0.014
R^2	0.741	0.834	0.851	0.864	0.635
p	<0.01	<0.01	<0.01	<0.01	<0.01

Table 2. Contribution of different wavelengths of measured solar radiation to T_{90} of *E. coli* expressed through slopes of linear regression (b—coefficient of multiple linear regression; R^2—coefficient of multiple determination; p—level of significance).

attributed to the different intensity of solar radiation and to variations in intensity, but also to different groups of microorganisms tested.

Lower T_{90} and S_{90} values in this study might partly be explained by the previous growth history of cell cultures until exposure to the environment, different origin and strain of bacterial cells, as well as by the different intensity of solar radiation and other environmental factors. There are many ways in which bacterial cells can enter the sea. In some cases, they are discharged directly from boats or bathers, and in others, they remain for different periods in wastewater reservoirs and/or are carried out in the sea through natural rivers or artificial conduits [14]. The change from a nutrient-rich environment to nutrient-poor one, places *E. coli* cells under starvation stress, which is less pronounced in bacteria from the stationary-phase because they have already experienced a starvation adaptation period. As a response to starvation stress, bacterial cells can induce protective mechanisms against UVA stress [46]. Furthermore, starved cultures taken in the stationary-phase of growth also showed higher osmotic [47] and temperature [48] resistance than those taken in a logarithmic-phase. This is due to specific protein synthesis during stationary-phase starvation [43]. In this study, *E. coli* cells were taken during the logarithmic-phase and were directly transferred to phosphate buffer without washing of nutrients. Since these cells did not experience starvation during the growth phase or during culture transfer, this probably made them less resistant to the negative effects of seawater and oxidative processes of solar radiation than cells that had experienced starvation [47, 49, 50]. Consequently, wild bacterial cells that have not experienced growth in a rich medium and are taken from the stationary phase of growth should show a higher resistance than cultivated cells, as was confirmed in this study. We found a statistically significant, at least threefold lower T_{90} (50.6 ± 6 h, n = 18) in cultivated wild *E. coli* cells than in their mother cells that were isolated from seawater (153.3 ± 35 h, n = 18), although the latter were pre-exposed to solar light and hostile environment for unknown period before seawater samples collected.

As mentioned previously, *E. coli* ATCC 35218 survived longer than *E. coli* ATCC 8739 in almost all experimental conditions in the dark, but if exposed to solar radiation, *E. coli* ATCC 35218 survived significantly shorter than *E. coli* ATCC 8739. This suggests that these microorganisms probably do not have equally developed mechanisms of protection against various abiotic environmental factors and that the protection rate also depends on which mechanism is more effective under the same conditions.

4. Conclusions

This study showed that in the absence of solar radiation, there were no statistically significant effects of temperature and salinity in their natural range (12, 18 and 24°C; 30.0 and 36.5 psu) on the survival of two ATCC strains. The survival of *E. coli* ATCC 8739 was enhanced only at 6°C, suggesting a negative effect of temperature at temperatures lower than natural range. At 15.0 psu, significantly lower T_{90} values of *E. coli* ATCC 8739 were observed at lower temperatures (6 and 12°C) than at higher ones (18 and 24°C). In the natural range of temperature and salinity, mean T_{90} values varied significantly, but only as a function of bacterial strain and origin. The importance of bacterial strain and origin for the survival of *E. coli* was demonstrated by significantly longer survival of *E. coli* cells isolated from seagull feces and sewage outfall. In the same conditions, their survival surpassed the survival of ATCC strains up to 10-fold. A dominate effect of solar light on *E. coli* survival was confirmed by recording a more rapid *E. coli* died-off in cells exposed to a natural range of solar radiation. The T_{90} was 15- to 70-fold shorter than in the absence of solar radiation. Within the investigated range of solar radiation (320–700 nm), only the effect of UVA spectrum was found to be statistically significant. The wavelengths 320–360 were the most bactericidal. The bacterial strain that had a shorter T_{90} in the absence of solar radiation showed a significantly higher T_{90} and S_{90} when exposed to solar light, suggesting different mechanisms of adaptation to studied abiotic environmental factors and a different efficiency of mechanisms under the same conditions. Cultivated bacteria showed threefold shorter T_{90} than their mother cells isolated from seawater. This suggested the importance of previous growth history of bacterial cells prior to exposure to the environment, for their adaptation capacity. The observed superiority of wild bacterial cultures over cultivated ones was probably a result of starvation experience during the stationary-phase, and/or due to a lack of nutrients.

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