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The Effect of UV-A and Different Wavelengths of Visible Lights on Survival of *Salmonella typhimurium* in Seawater Microcosms

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In this article, the effect of UV-A and different wavelengths of visible light combined with or without photosensitizer Methylene Blue (MB) on the establishment of viable but non-culturable state (VBNC) and survival of *Salmonella typhimurium* were investigated in sea water. The inhibition rate of various light sources in the presence or absence of MB on *S. typhimurium* in seawater was ranked UV-A>red light> white light> blue light> green light (from the greatest to the least effective respectively). *S. typhimurium* survived for 24.1, 59, 61.6 and 66.4 hour under the effect of red, white, blue, green light exposure, for 15.2 h UV-A exposure in the absence of MB according to t_{99}. Red light was the most efficient light source among the studied visible light wavelengths on the survival of *S. typhimurium* with or without MB. Some cells were still capable of respiration and entered the VBNC state. It was seen that red light had a stronger effect than white, blue and green light in entry VBNC state of *S. typhimurium* in the seawater. This study has shown for the first time that red light alone is fairly effective on survival of *S. typhimurium*.

Key words: Photooxidation, Viable but non-culturable state, *Salmonella typhimurium,* Visible light, Red light, Seawater.

The disinfection of water and wastwater which is the largest reservoir of human enteric bacteria is very important for public health. *Salmonella typhimurium* which is one of the most important pathogens among these enteric bacteria is transmitted via different ways to water sources. *S. typhimurium* is a worldwide facultative intracellular pathogen that causes salmonellosis in human and animals[1]. *Salmonella* infections are initiated when a pathogenic strain is ingested into a suitable host via contaminated food or water[1]. In the aquatic environment, there are many factors that effect the survival rate of enteric bacteria such as including *S. typhimurium.* These factors can be listed as temperature, osmolarity, pH, starvation, protozoa, and natural sunlight *etc.*, [2-13]. One of these factors is photooxidative stress which is generated by sunlight and photosensitizer substances and this stress has a very important effect on the

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survival of enteric bacteria in marine environments[14-17].

Oxidative stress causes cell damage directly by the formation of either hydrogen peroxide and hydroxyl radicals from water, or singlet oxygen and superoxide radicals from molecular oxygen[18]. Besides this, photooxidative stress on microorganism occurs via the absorbance of light by a sensitizer[19]. There are many photosensitizer which are endogeneous or exogeneous in aquatic environment. These photosensitizers are chlorophyll, porphyrin, humic acid, methylene blue, toluidine blue *etc*. [20]. Methylene blue (MB) is known to be one of the important exophotosensitizers that affect the survival of bacteria[21, 22]. The reactive oxygen species cause very serious damage in lipits, DNA and proteins of bacteria [23-27]. Dunlop *et al.* (2002) demonstrated that the cell membrane acts as the primary site of oxidative attack[28]. The cellular damage which is caused by sunlight results from the reactions involving these photosensitizers that become excited on the absorption of different light spectrums.

Sunlight has long been known to improve water quality. In recent years, disinfection technology developed by using sunlight (SODIS) is used in the disinfection of water[29, 30]. Besides this, photodynamic therapy (PDT) is based on using photosensitizer interaction with visible light, and this interaction causes short-lived cytotoxic species *in situ*. [31-33]. This photosensitizer is converted from singlet to triplet state in two ways. In the first mechanism (type 1), it reacts with the surrounding molecules and yields radical species and hydrogen peroxide[34]. In the second mechanism (type 2), it transfers its energy to molecular oxygen and produces singlet oxygen[34]. The second type is a more effective type which generates a toxic singlet oxygen. Therefore, some organic dyes have been suggested for PDT such as MB. Because the absorption of MB is effective in PDT to kill microorganisms[35].

Bacteria has mechanisms of protection against oxidative stress. These mechanisms can be divided into enzymatic (Catalase, SOD, glutathione peroxidase, glutathione S-transferases, *etc*.) and non enzymatic (glutathione, alpha tocopherol, beta carotene, *etc*.). That bacteria without differentiation properties, such as endospor, is very diffucult to overcome so many of these stresses have been shown to lead to the establishment of the VBNC state in this bacteria[36, 37]. This state leads to apparent loss of viability in bacteria and they don't grow and don't produce colonies on agar media. However, the fact that the cells may still retain the ability to carry out respiration was observed by direct microscopy and therefore they can often be resuscitated given the appropriate optimal conditions. Recent studies showed that exposure of *E. coli* and *S. typhimurium* to photo-oxidation caused the cells to become viable but non-culturable (VBNC) and to lose the ability to form colonies[37-40]. However, the relationship between photooxidation under different wavelengths of visible light and entry to the VBNC state has not also been clarified. *S. typhimurium* cells in the VBNC state, particularly in food or water, pose a health risk because they still show metabolical activities but cannot be detected by standard laboratory methods. Similarly, in the marine environment, the induction of the VBNC state can lead to potential health problems due to the under-estimation of potentially harmful bacteria in the environment[37]. Besides, viable but non-culturable cells may cause serious human health problems, especially if the cells have a pathogenic potential.

Few studies have been carried out on VBNC cells under irradiation using different wavelengths of visible light in sea water. The purpose of this study was to investigate the effect of different wavelengths of light (Blue, Green, Red and White) and UV-A in the presence and absence of MB on VBNC state of *S. typhimurium* in seawater. The relationship between different wavelengths of light and the survival of *S. typhimurium* in seawater was also investigated.

MATERIAL AND METHODS

Bacterial strains

Salmonella typhimurium LT2 was grown in nutrient broth (Oxoid) overnight at 37ºC. The culture (10 ml) was harvested by centrifugation at 3440xg for 10 min, the cells washed twice with distilled water, the pellet finally resuspended in distilled water (10 ml).

Light sources

Bacterial samples were exposed to UV-A

(4-lamp Osram Eversun K 40w/79k) and four different wavelengths of visible light (white light, 400-700nm; blue light 400-500nm; green light 500-600nm; and red light 600-700nm, Osram L 18w/66 4 flourescens lamps). The distance between the light source and the exposed sample surface was adjusted by controlling power densities for each case. The light intensity was measured in beakers with a radiometer (PMA 2100, Lutron, Siber Tip, Turkey).

Microcosm work

Re-suspended washed culture were transferred to a beaker containing filter-autoclaved seawater (100 ml) to give a final concentration of approx. 5.10^6 cfu/ml. The tops of the beakers were wrapped with cling film to prevent contamination of the microcosm. Methylene Blue dye (Merck) (final concentration of 1,5 µM) was added as a photosensitizer to all the beakers, except the control. The microcosm were incubated at 25ºC. A series of samples were also incubated in the dark with and without MB. Samples were taken at different time intervals in order to investigate the effects of different wavelengths and sources of light and their interactions with photo-sensitizers on survival and VBNC. The respiring cell count, viable count and direct total count were carried out at each time interval.

Plate count

The culturability of *S. typhimurium* was assessed by a standard surface-spread plate technique to enumerate viable cells in the sample. All microcosm were sampled immidately after inoculation and at regular intervals thereafter samples were serially diluted in Ringer's solution and aliquots (100 µl) spread on triplicate nutrient agar plates. The plates were incubated at 37ºC for 24 h and counted manually. The results are expressed as mean colony forming units per ml (cfu/ml).

Direct viable count

A method modified from Kogure *et al*. (1979) was used to determine the direct viable count in the samples[41]. Nucleopore Track-Etch membrane (25 diameter, pore size 0,1 µm) filters were used for epifluorescent microscopic counts. Filters were immersed in a solution of Sudan Black B for 48 h. Stained filters were washed with double distilled water before passage of the sample through the stained filter supported on a filter discs (Swinnex, 25 mm diameter, Millipore, Whatford, Herts, UK). After filtration, acridine orange (Sigma-0,5 ml, 0,01% /w/v in 6,6 mmol/ l phosphate buffer, pH 6,7) was dropped onto the filter for few minutes. The filter was air dried and cut into pieces. One piece was covered with immersion oil with a cover slip and examined using a UV microscope (Nikon Eclipse E 600). The number of bacteria was determined in each square of eyepiece graticule. Bacteria were counted from 20 random fields from the central point to edge. Three time longer than normal bacteria was counted. The sum up of viable (longer) and inactive bacteria was taken to be the total bacterial count.

Respiring cell count

5-Cyano-2,3 ditolyl tetrazolium chloride (CTC)(Fluka) was used to obtain a respiring cell count in the samples using the method of Rodriguez *et al*. (1992)[42]. 1 ml sample was transferred to the test tube, then CTC was added to tubes and final concentration adjusted to 2 mM. The test tubes were wrapped with aluminium foil and shaken at 160 rpm at 37°C for 3 hours. After that, 0.5% of formaldehyde was added to tubes in order to stop bacterial activity. After filtration (Nuclepore track-etched membrane), 0.5 ml DAPI (Sigma-final concentration 15 µg/ml) was dropped onto the filters for 10 minutes. The filters were dried in the air and counted under the epifluorescence microscopy with an appropriate optical standard filter.

Data analysis and statistics

All count data were expressed as mean values of log counts from three replicated experiments. The gradients of the log linear survival curves are expressed as the time taken for a two-log drop in number expressed as t_{99} values. All primary data are presented in mean of standard deviations. Differences were tested for statistical significance by general linear model. Tukey test was performed for multiple comparisons. Probabilities less than 0.05 were considered significant at $p<0.05$.

RESULTS

The effect of different wavelength of visible light on survival of *S. typhimurium* in seawater

The series of experiments were carried out at 25°C in sea water to investigate the relationship

Table 1. t_{99} values for *S. typhimurium* incubated with or without methylene blue (MB) in various light regimes or in the dark in sea water

Light Irradiation	Presence of methylene blue (hour)	Absence of methylene blue (hour)
UVA	07.4	15.2
Red	12.7	24.1
White	19.5	59.0
Blue	25.7	61.6
Green	28.0	66.4
Dark	>>72	>>72

Table 2. Comparision of plate count (PC), respiring cell count (RCC), direct viable count (DVC) and total count (TC) of *S. typhimurium* under the effect of white, blue and green light irradiation with the presence of MB in filtered-autoclaved seawater. The values are the averages of three independent experiments ± standard deviations

Viable count	Incubation time (h)	White light	Blue Light	Green light	MB+Dark control	Dark control
PC	0	6.77±0.03	6.48±0.05	6.48±0.05	6.52±0.04	6.42±0.09
	16	4.63±0.07	4.91±0.08	5.18±0.04	6.39±0.10	6.38±0.06
	32	4.06±0.04	4.01±0.03	4.19±0.06	6.34±0.07	6.33±0.04
RCC	0	6.39±0.06	6.30±0.03	6.32±0.04	6.42±0.03	6.39±0.05
	16	5.08±0.06	5.23±0.03	5.65±0.07	6.38±0.07	6.36±0.08
	32	4.72±0.12	5.05±0.04	5.18±0.06	6.32±0.09	6.28±0.06
DVC	0	6.21±0.07	6.32±0.03	6.44±0.04	6.38±0.02	6.27±0.10
	16	4.64±0.07	5.00±0.05	5.22±0.04	6.33±0.05	6.16±0.04
	32	4.14±0.06	4.10±0.08	4.22±0.06	6.29±0.07	6.15±0.03
TC	0	6.59±0.04	6.43±0.05	6.41±0.05	6.45±0.04	6.59±0.08
	16	6.41±0.02	6.39±0.04	6.41±0.05	6.41±0.04	6.50±0.08
	32	6.20±0.05	6.16±0.09	6.26±0.07	6.37±0.06	6.50±0.05

Table 3. Comparision of plate count (PC), respiring cell count (RCC), direct viable count (DVC) and total count (TC) of *S. typhimurium* under the effect of red light and UV-A light irradiation with the presence of MB in filtered-autoclaved seawater. The values are the averages of three independent experiments ± standard deviations

Viable count	Incubation time (h)	Red light	+MB Dark control	Dark control	Incubation time (h)	UV-A
PC	0	6.60 ± 0.09	6.58±0.06	6.39±0.10	0	6.45±0.08
	8	5.60 ± 0.05	6.52±0.04	6.35±0.04	5	5.60±0.09
	16	4.06 ± 0.05	6.47±0.07	6.29±0.02	10	3.92±0.07
RCC	0	6.30±0.06	6.37±0.09	6.36±0.03	0	6.27±0.05
	8	5.37±0.10	6.33±0.09	6.32±0.04	5	5.92±0.05
	16	4.81±0.04	6.29±0.04	6.27±0.03	10	4.60±0.06
DVC	0	6.32±0.06	6.38±0.06	6.41±0.05	0	6.15±0.05
	8	5.51±0.03	6.34±0.0.8	6.36±0.07	5	5.68±0.08
	16	4.23±0.07	6.28±0.03	6.32±0.05	10	4.06±0.10
TC	0	6.32±0.06	6.34±0.04	6.34±0.09	0	6.44±0.07
	8	6.15±0.04	6.29±0.07	6.31±0.03	5	6.36±0.06
	16	6.00±0.02	6.24±0.03	6.24±0.09	10	6.05±0.04

between the survival of *S. typhimurium* and UV-A and different wavelength of visible light, plate counts (PC), direct viable counts (DVC), respiring cell counts (RCC) and total counts (TC). In present experiments, all count data were expressed as a mean of log count values from three replicated experiments. The gradients of the log linear survival curves are expressed as the time taken for a two-log drop in number. It is also expressed as t_{99} values. The experiments were carried out in the presence and absence of MB dye.

Fig. 1 and 2 show the survival of *S. typhimurium* at different wavelength of visible light in sea water with or without MB. The number of *S. typhimurium* cells did not decline in the seawater samples at dark control with or without MB (Fig. 1 and 2). It was seen that the number of bacteria were decreased in all of the light sources with or

Table 4. Comparision of plate count (PC). respiring cell count (RCC). direct viable count (DVC) and total count (TC) of *S. typhimurium* under the effect of white. blue and green light irradiation with the absence of MB in filtered-autoclaved seawater. The values are the averages of three independent experiments ± standard deviations

Viable count	Incubation time (h)	White light	Blue light	Green light	Dark control
PC	0	6.73±0.07	6.47±0.09	6.40±0.03	6.46±0.11
	24	6.27±0.08	6.04±0.04	6.08±0.05	6.43±0.04
	48	5.74±0.06	5.25±0.05	5.52±0.09	6.38±0.05
	72	4.07±0.05	4.15±0.07	4.26±0.06	6.32±0.11
RCC	0	6.31±0.05	6.32±0.06	6.40±0.05	6.43±0.06
	24	6.08±0.04	5.92±0.07	6.10±0.04	6.42±0.04
	48	5.84±0.09	5.52±0.07	5.83±0.08	6.35±0.05
	72	5.46±0.07	4.71±0.09	5.19±0.04	6.28±0.02
DVC	0	6.21±0.07	6.32±0.04	6.40±0.06	6.32±0.09
	24	5.97±0.05	5.86±0.04	5.94±0.03	6.28±0.07
	48	5.73±0.11	5.40±0.05	5.55±0.09	6.21±0.02
	72	4.42±0.06	4.28±0.09	4.35±0.12	6.10±0.05
TC	0	6.42±0.04	6.43±0.04	6.45±0.07	6.38±0.09
	24	6.41±0.06	6.35±0.07	6.46±0.05	6.34±0.04
	48	6.39±0.08	6.27±0.05	6.48±0.06	6.30±0.05
	72	6.26±0.04	6.16±0.06	6.17±0.07	6.28±0.07

Table 5. Comparision of plate count (PC), respiring cell count (RCC), direct viable count (DVC) and total count (TC) of *S. typhimurium* under the effect of red and UV-A light irradiation with the absence of MB in filtered-autoclaved seawater. The values are the averages of three independent experiments ± standard deviations

Viable Count	Incubation time (h)	Red light	Dark control	Incubation time (h)	UV-A
PC	0	6.32±0.06	6.34 ± 0.09	0	6.46±0.06
	12	5.19±0.06	6.19 ± 0.10	8	5.60±0.08
	24	4.55±0.06	6.14 ± 0.06	15	4.76±0.07
RCC	0	6.31±0.06	6.27 ± 0.06	0	6.26±0.05
	12	5.54±0.10	6.18 ± 0.04	8	5.82±0.08
	24	5.19±0.06	6.11 ± 0.05	15	5.09±0.06
DVC	0	6.29±0.07	6.29 ± 0.10	0	6.18±0.05
	12	5.24±0.07	6.14 ± 0.08	8	5.62±0.06
	24	4.69±0.08	6.06 ± 0.08	15	4.75±0.06
TC	0	6.22±0.05	6.36 ± 0.04	0	6.43±0.05
	12	6.12±0.06	6.28 ± 0.07	8	6.15±0.07
	24	6.09±0.07	6.26 ± 0.09	15	6.07±0.04

Table 6. VBNC state bacterial logaritmic count: The difference between PC and RCC for in the presence (16 h incubation) or absence (24 h incubation) of the photosensitizer (methylene blue) in various light regimes or in the dark of *S. typhimurium*

Light Irradiation	Presence of methylene blue (16 h)	Absence of methylene blue (24 h)
Red	0.75	0.64
White	0.44	0.19
Blue	0.32	0.12
Green	0.47	0.02
Dark	0	0

without MB (Fig. 1 and 2). Survival experiment showed that the viable count of *S. typhimurium* cells in the samples declined almost a two-log drop in 7.4, 12.7, 19.5, 25.7 and 28.0 h owing to the effect of inactivation by UV-A, red, white, blue and green light irradiation in the presence of the photosensitizer MB, respectively ($p<0.05$; Table 1). The highest decrease in viability of *S. typhimurium* after exposure to UV-A was 2.53 log in 10 h incubation period with MB. Red light is the most efficient light sources among other light sources (white, blue and green) (Table 1). *S. typhimurium* survived 24.1 h, 59.0 h, 61.6 h and 66.4 h under of red, white, blue and green light exposure without MB according to t_{99} value respectively. In the case of UV-A irradiation, *S. typhimurium* survival was determined as 15.2 h. It was interesting to determine that red light alone was more effective than white, blue and green light on the survival of *S. typhimurium* (Table 1) ($p<0.05$).

The relationship between various light sources and the entry VBNC in *S. typhimurium*

The data obtained from the studies to investigate the relationship between VBNC state and different wavelengths of light sources are given in Table 2, 3, 4 and 5. It was showed by RCC data that there is a relationship between different wavelength of visible light and VBNC state of *S. typhimurium* with MB. The difference between the PC and RCC gave us the rate of VBNC cell numbers. The total count did not show a significant change in all samples ($p<0.05$)(Table 2-5). Regarding the results, the number of VBNC bacteria was detected as 0.75 log. in red light, 0.44 in white light, 0.32 log. in blue light and 0.47 log in green light for 16 h standart incubation with MB in sea water (Table 6). Significantly, the highest reduction in viability and also the highest VBNC rate of *S. typhimurium* cells was obtained with the red light exposure ($p<0.05$). In the same way, samples of sea water without MB, the alone effect of light, VBNC rate of

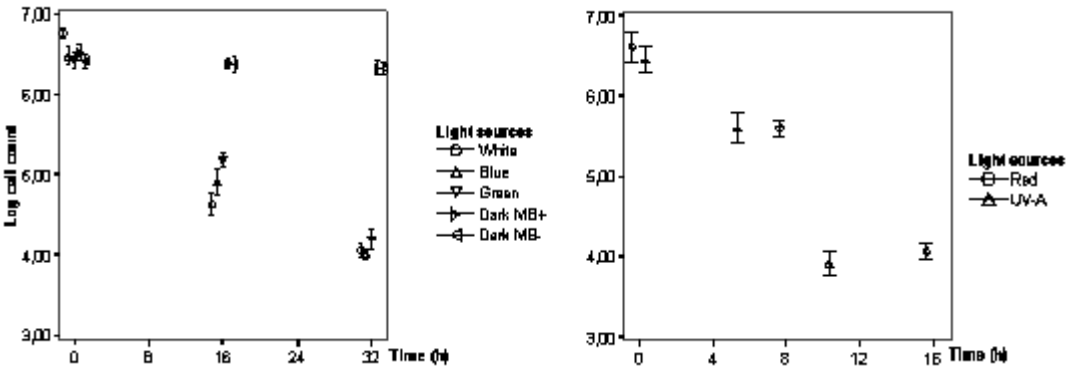

Fig. 1. Comparisons by plate count (PC) of irradiation of *S. typhimurium* samples with white light, red light, green light, blue light and UV-A in the presence of methylene blue in filtered-autoclaved seawater. The values are the averages of three independent experiments ± SD (cfu/ml)

S. typhimurium cells determined 0,64 log., 0,19 log., 0,12 log., and 0.02 log for red light, white light, blue light and green light, respectively (Table 6). In the absence or presence of the photosensitizer it is likely that exposure to the various light irradiations in filtered-autoclaved seawater led to entering a possible VBNC state of *S.typhimurium*. Especially, It was showed that the data which was obtained from the red light, is very important. In the case of the controls incubated in the dark, there was no significant reduction in numbers in the presence or absence of MB (Table 1 and 3)($p<0.05$).

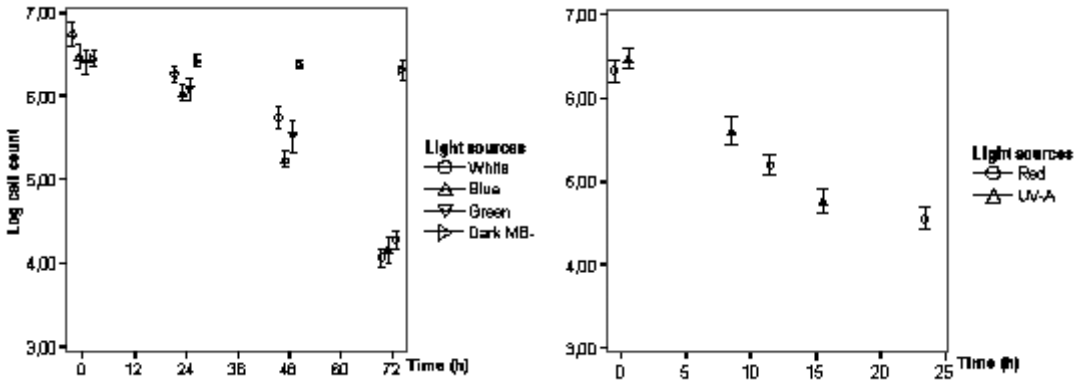

Fig. 2. Comparisons by plate count (PC) of irradiation of *S. typhimurium* samples with white light, red light, green, blue light in the absence of methylene blue in filtered-autoclaved seawater. The values are the averages of three independent experiments ± SD (cfu/ml)

DISCUSSION

Enteric pathogens such as *E. coli*, *S. typhimurium* are regarded as the most important indicator of water pollution. These bacteria are effected by different environmental stress. Reactive oxygen sources (OH*, $^1O_2$, O_2^-, H_2O_2) is one of the factors that effect survival of bacteria in aquatic environments. These radicals could oxidize many biological molecules such as proteins, nucleic acids and lipids. Survival of enteric bacteria in the seawater is greatly affected by sunlight. Sunlight is composed of UV and visible light wavelengths. The UV-B and UV-C portion of the solar spectrum are the most bactericidal part, due to direct photobiological DNA damage[43, 44]. Davies and Evison (1991) demonstrated[14] that when *E. coli* and *S. typhimurium* were exposed to light in seawater, bacterial count (DVC and CFU) decreased by 4-5 log for 10 h. In our study, when we examined the effect of light alone, the shortest survival of *S. typhimurium* was detected in the UV-A irridation. This effect of sunlight on culturability has been reported by numerous authors in both fresh and sea water[14, 45, 44].

Our study determined that red light (600-700) among the different wavelengths of visible light (400-700 nm) has been very effective without MB. While 2 log. reduction at white light was seen in 59 h, red light showed 2 log. reduction in 24.1 h. There was no significant difference in the survival of bacteria in the dark in the presence or the absence of MB (Table 2-3). Vermeulen *et al.* (2008) demonstrated that for treatment wavelengths from 250 to 488 nm, UV radition at 265 nm was the most effective in killing the *E. coli* cells and the lethal dose was also increased with increasing wavelength[46]. At the same time, Vermuelon *et al.* (2008) said that while the radiation dosages required for a one log reduction of *E. coli* density at 458 and 488 nm were 5.5 and 6.9 log mJ/cm^2, laser treatment at 515 and 532 nm did not produce a significant reduction in the viability of *E. coli* cells with radiation doses up to 7 log mJ/cm^2 [46]. In our study also, survival of *S. typhimurium* was more effected by white light (59 h at 400-700 nm) and

blue light (61.6 h at 400-500 nm) in comparison to green light (66.4 h at 500-600 nm)(Table 1). Idil *et al.* (2010) demonstrated that survival of *E. coli* was more affected by white light (56.6 h) and blue light (59.5 h) than green light (66.0 h) [47]. According to this data, *S. typhimurium* has lived longer than *E. coli* in the effect of white, blue and green light. But red light was more effective on *S. typhimurium* (24.1 h) than *E. coli* (24.4 h) in sea water[47] so *E. coli* should be not indicator bacteria for *S. typhimurium*. Winfield and Groisman (2003) said that the different rates of survival of *Salmonella* and *E. coli* in nonhost environments suggest that *E. coli* may not be an appropriate indicator of *Salmonella* contamination[48]. However, Chandran and Hatha (2005) demonstrated better survival capacity of *E. coli* cells under sunlight when compared to *S. typhimurium*[49]. But in our study, it is demonstrated for the first time that *E. coli* may not be an appropriate indicator for *S. typhimurium* under the effect of visible light in a natural environment. When *E. coli* is used as a indicator for *S. typhimurium*, this can create a risk for public health.

When *S.typhimurium* was exposed to white, blue and green light irradiation in filtered-autoclaved seawater in the presence of MB, a two log drop in numbers occured after 19.5, 25.7 and 28 h respectively according to t_{99} value. Under the red light and UV-A irridations, a 2 log reduction in cell numbers occured faster (12.7 and 7.4 h respectively). The inhibition rate of various light sources in the presence of MB on *S. typhimurium* in seawater was ranked UV-A>red light>white light>blue light>green light (from gratest to least activation). Soukos *et al.* (1998) reported that MB and toluidine blue absorbed red light and showed a bactericidal effect[50]. Methylene blue, which is a well known dye, has high light absorption at 665 nm and is effective in PDT. Recently, various alternative light sources have been researched for photooxidation. When considering the studies targeting both gram (+) and gram (-) bacteria with a lamp light sources and the same photosensitizer, it seems that there are a lot of studies. Various lamps as light sources are used with different photosensitizer [see review for details, [51]]. The hazardous effect of solar radition may be lightened by dissolved organic material[52]. Therefore, bacteria in water are primarily exposed to visible light (400-775 nm) and to a lesser extent, UV-A (329-400 nm) radiation[52].

The viable but noncultrable state is thought to be a survival strategy adopted by nondifferenting bacteria in response to unfavorable environmental conditions. A lot of workers investigated the effect of light, but VBNC state has not been studied at different wavelegth of visible light for *S. typhimurium* in sea water. The relationship between VBNC state and the different wavelengths of visible light on *S. typhimurium* has not been clarified yet. Therefore, this study was carried out at different wavelengths of light sources (UV-A, red light, white light, blue light and green light). Our results showed that red light is the most important wavelength (600-700 nm) for *S. typhimurium* in sea water with or without MB. It is interesting that red light without MB is more effective than any other wavelength of visible light. Idil *et al.* (2010) has shown that red light is most effective wavelegth on *E. coli* in sea water[47]. At the same time, *E. coli* entered more VBNC state in red light than other visible light sources in all of their samples without MB (white, blue, green). Troussellier *et al.* (1998) showed a similar effect with *E. coli* kept for 40 h in the presence of white light irradiation in artificial seawater, losing colony formation ability but still retaining the ability to respire active in a VBNC state[40]. Generally, our results agree with recent findings showing that *S. typhimurium* enters a dormant state and loses colony forming ability under the effect of visible light and sun light with the TC and DVC remaining unchanged[37, 53, 54]. There are also studies showing the effect of visible light on progressive dormancy of *Escherichia coli* cells during the survival process in natural fresh water and wastewater[55-58]. These results shows that red light is the major VBNC inducing factor for *S. typhimurium* among other visible light sources in sea water

The molecular mechanism of VBNC state has not been known. But, Darcan *et al.* (2003) showed that there was a relationship between VBNC state and EnvZ osmosensor and the sensor protein EnvZ may have an important role in triggering the entry into the VBNC state of

E. coli [59]. Also Darcan *et al.* (2009) showed that pH and osmolarity changes did not affect this relationship[60]. Muela *et al.* (2008) also reported changes in *E. coli* OMPs under environmental conditions effecting viable but non culturable state[61]. Moreover, Asakura *et al.* (2008) suggested that outer membrane protein W (OmpW) expression was induced in VBNC state in *E. coli* O157:H7 [62]. There is intense debate regarding the biological significance of this phenomenon. Gram (–) bacteria appear to be more resistant to photosensitized alteration than gram (+) bacteria[63]. This stuation is due to the permeability of the outer membrane. Therefore porins and EnvZ may play a role for relationship with VBNC in oxidative stress.

The use of photochemical reaction for water disinfection was previously studied[45, 64]. PDI is a procedure in the killing of resistant bacteria using a combination of photosensitizer and light. Our study shows that red light may be more effective for this procedure. The red light at high dosage may be prefered for disinfection of bacteria with SODIS or PDT. At the same time, the alone effect of red light on bacteria should be investigated by researcher.

We have shown that some *S. typhimurium* cells are not dead due to the effects of light irradiation in sea water and are still active as VBNC in microcosms as determined in respiring and active cell counts. The red light was found to be the most effective between different light sources in the presence or absence of MB on *S. typhimurium* survival in seawater. Besides this, *S. typhimurium* was determined to be more VBNC state in red light than other light wavelengths.

CONCLUSION

Ours is the first research that shows that the red light is the most effective visible light wavelengths in causing the inactivation of *S. typhimurium* in seawater. The addition of MB has an activator effect which increases the deleterious effects on bacteria by providing a source of the active free radicals required to cause internal damage to the bacteria. This is the first time that red light has been shown to be the most effective light sources among different wavelegths of visible light sources with or without MB on VBNC state and survival of *S. typhimurium* in sea water.

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