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Maciej WALCZAK[1] and Aleksandra BURKOWSKA[1]

UVB RADIATION IMPACT ON ACTIVITY OF DNA AND CELLULAR PROTEIN SYNTHESIS OF WATER ENVIRONMENT BACTERIA

WPŁYW UVB NA AKTYWNOŚĆ SYNTEZY DNA I BIAŁEK KOMÓRKOWYCH PRZEZ BAKTERIE ŚRODOWISKA WODNEGO

Abstract: The studies were carried out in 2007 based on water samples collected from SM and SW of pelagic zone of Brzezno lake. For the purpose of further research, representative collection of bacterial strains was isolated from collected samples. The progress of the research included estimation of the DNA and cellular protein synthesis activity of the bacteria subjected to UVB radiation. The survey was conducted in two experimental layouts: with and without *humic substances* (HS) playing role of compounds potentially protective from UV radiation. Conducted research demonstrated that under influence of UVB radiation the SM and SW bacteria activity of DNA and cellular protein synthesis were strongly inhibited. On the other hand, presence of HS have another impact on DNA and cellular protein synthesis.

Keywords: UV, DNA synthesis, protein synthesis, humic substances, surface microlayer

In the vertical plane, the external layer of water body is so-called *surface microlayer* (SM). It comprises as little as several hundred micrometers of the water body surface. Hence, the amount of solar radiation reaching SM is virtually the same as the mainland surface. The most biologically significant solar radiation component reaching SM, on account of harmful impact, is the mean UV radiation, so-called UVB λ = 290–320 nm and UVA λ = 320–400 nm [1]. It causes DNA defects (lethal effect) or hinders organisms' growth by inhibiting enzyme synthesis, reducing active transport and inducing mutations, which result in sublethal effect [1]. Therefore, insolation is one of the major factors determining number and activity of bacteria inhabiting that peculiar environment. Strong solar radiation as is known limits numbers of all microorganisms

[1] Department of Environmental Microbiology and Biotechnology, Institute of Ecology and Environment Protection, Nicolaus Copernicus University, ul. J. Gagarina 9, 87–100 Toruń, Poland, phone: +48 56 611 44 33, email: walczak@umk.pl

in the water body, however the amount of harmful UV radiation reaching deeper than SM water layers is definitely smaller because of absorption and diffuse. SM compared to *subsurface water* (SSW), for extreme temperature values or solar energy doses, is unstable environment, extreme in a sense.

There are studies reporting harmful impact of solar radiation, UVB in particular, on production of bacterial biomass and exoenzymes activity [2, 3]. On the other hand however, it is common knowledge that in SM water up to 50 % of *dissolved organic matter* (DOM) make up *humic substances* (HS) [4]. As is known, HS effectively absorb UV radiation protecting microorganisms from that radiation [5, 6]. During the exposure to radiation they undergo photooxidation and decomposition into simpler compounds, which become additional amount of easily assimilable organic matter that is likely to stimulate heterotrophic bacteria growth [7, 8]. Exact measurements of synthesis activity of particular cellular macromolecules in in situ conditions are extremely difficult to perform. It is due to the impossibility of control of particular physicochemical factors affecting organisms' activity in natural conditions. Therefore, this study examined UVB impact on activity of DNA synthesis and cellular proteins in manageable laboratory conditions. Furthermore, present work sought answer to the question whether or not HS can play role of protective agent from UVB radiation for bacteria.

Material and methods

The surveys were based on heterotrophic bacteria strains, isolated from the surface microlayer water and subsurface water.

Water samples collection. Water meant for analyses was collected in the summer, in pelagic zone of the Brzezno lake (latitude 53°57.5′; longitude 17°48.6′), which lies within the Tuchola Forest area. The surface area of the lake equals 71.6 ha, with a maximal depth of 9.7 m, length of 2405 m, and width of 560 m. It is situated at 139.8 m above sea level and is rated among eutrophic water bodies.

SM water samples were collected by a Garrett [9] technique using a Plexiglas plate, which collects a 150 μm water layer. Subsurface water was sampled from a depth of 25 cm using an automatic pump. Taken water samples were poured into sterile glass containers.

Isolation of bacterial strains. In order to isolate bacterial strains from accordingly dissolved water samples there was a surface screening carried out in three simultaneous experiments with 0.1 cm^3 on the *Tryptone Soy Agar* (TSA) (Difco) medium surface. After 6 days of incubation at 20 °C a representative strains collection was detached and transferred onto TSA medium bevels.

Preparation of test bacterial strains suspension. Isolated bacteria strains were generated for 3 days at 20 °C in 50 cm^3 of liquid *Tryptone Soy Broth* (TSB) medium. Afterward, from each culture taken was 30 cm^3 and spun for 5 minutes at 10K rpm, temperature of 10 °C. After supernants have been spun bacterial deposit was removed and suspended in 30 cm^3 of sterile Ringer's solution. Optic density of each strain bacterial

suspension was driven to equal value of 0.5 applying sterile physiological sodium salt solution as a diluting agent.

Exposure to UVB radiation. Prepared bacterial suspension of given strain was divided into 3 parts, 10 cm^3 each, and transferred into three parallel sterile Petri dishes. First one was a controlling agent and was not subjected to UVB radiation. Two other dishes containing bacterial suspension were exposed to UVB radiation (lamp Philips; 15 min, 50 $\mu W/cm^2$), while before exposure to radiation there was 0.1 cm^3 of HS (final concentration 100 mg $\cdot$ cm^{-3}) added to one of them.

DNA synthesis estimation. After exposure to radiation from all dishes (including the control one) 9 cm^3 of bacterial suspension was transferred to test-tube and 1 cm^3 of radioactive thymidine [^{3}H] solution was added. Thymidine concentration in a sample was 14 nM. Assimilation of radioactive thymidine by bacteria was estimated by Fuhrman and Azam method [10]. The amounts of taken thymidine formed a basis for estimation of DNA synthesis activity given that there is $2.5 \cdot 10^{-15}$g of DNA to one bacterial cell on average.

Estimation of cellular proteins synthesis. This procedure was carried out in comparable manner as DNA synthesis activity estimation, using labelled [^{3}H] leucine. Applied in the analysis leucine concentration was 15 nM. Assimilation of radioactive leucine by bacteria was estimated by Kirchman method [11]. The amount of leucine incorporated by bacterial cells formed a basis for measurement of produced protein. With this in view it was assumed that 1 mole of leucine constitutes 131.2 g and on this basis number of incorporated leucine moles was converted into leucine grams. Subsequently, obtained number of incorporated leucine grams was converted into grams of synthesized bacterial cells protein. The calculations were based on the assumption that leucine makes up 0.073 of bacterial protein mass on average [12].

Results and discussion

Results presented in this study regarding the UVB impact on DNA synthesis (Table 1) demonstrate that UVB radiation had hampering effect on DNA synthesis concerning all analysed strains of SM and SSW (Table 1). Experiments with HS application as potentially protective agent proved that DNA synthesis was still hampered, but inhibition amount was smaller than in case with no HS presence.

Among SM strains considerable decreased of inhibition at HS presence was observed in cases of 7 strains (2–7 and 9). For strains no. 8 and 10 noticed inhibition decrease was very slight. Strain no. 1 revealed continued further decrease of DNA synthesis activity at HS presence.

SSW strains response was very similar. Substantial inhibition decrease at HS presence was observed for 6 strains (11, 12, and 15–18). Strain no. 19 also demonstrated decreased inhibition at HS presence, but the difference was small. For strain no. 13 no difference was made, whereas for strains no. 14 and 20 the differences were insignificant.

Table 1

Influence of UVB radiation on activity of DNA synthesis

Strain No.	Control	UVB	UVB + HS
SM			
1	0.72*	0.08	0.06
2	**0.54**	**0.04**	**0.40**
3	**1.49**	**0.27**	**0.76**
4	**0.66**	**0.08**	**0.60**
5	**0.69**	**0.09**	**0.36**
6	**0.19**	**0.08**	**0.18**
7	**2.28**	**0.28**	**2.15**
8	2.29	1.04	1.14
9	**3.42**	**0.28**	**2.21**
10	3.05	1.32	1.86
SSW			
11	**0.52**	**0.18**	**0.32**
12	**1.62**	**0.02**	**0.31**
13	0.48	0.16	0.16
14	0.77	0.36	0.30
15	**1.30**	**0.19**	**0.96**
16	**4.27**	**0.08**	**0.90**
17	**1.07**	**0.20**	**1.00**
18	**6.30**	**0.07**	**2.21**
19	0.81	0.22	0.32
20	1.58	0.78	0.75

* – μg DNA.

Comparison of mean values (Fig. 1) received for SM and SSW strains confirms that regardless of the water layer of surveyed strains origination UVB radiation similarly hampered DNA synthesis. Data presented in the figure demonstrate also substantial differences of DNA synthesis inhibition degree depending on HS presence. Similar results concerning UV impact on DNA synthesis activity and/or secondary production of bacteria were published by Davidson [13], Herndl et al [3, 8] and Kaiser and Herndl [2].

The above-mentioned works comprise evidences of DNA synthesis activity decrease based on exposure of bacteria to UV radiation up to more than 50 % compared with a control sample [3], which remains in a full accordance with results of present study. The experiments using various fractions of organic matter as protective agents proved thymidine incorporation activity reduction based on UV effect and HS presence at as small degree as 20 % [8]. Also, investigations carried out in *in situ* conditions demonstrated significant limitations of bacterioneuston DNA synthesis activity [14].

Scully et al [7] reported that bacteria exposure to UVB radiation causes substantial decrease of survival. The same author proved that the survival was less hindered when fulvic acids solution was added to bacterial suspension. A study conducted by Vosjan

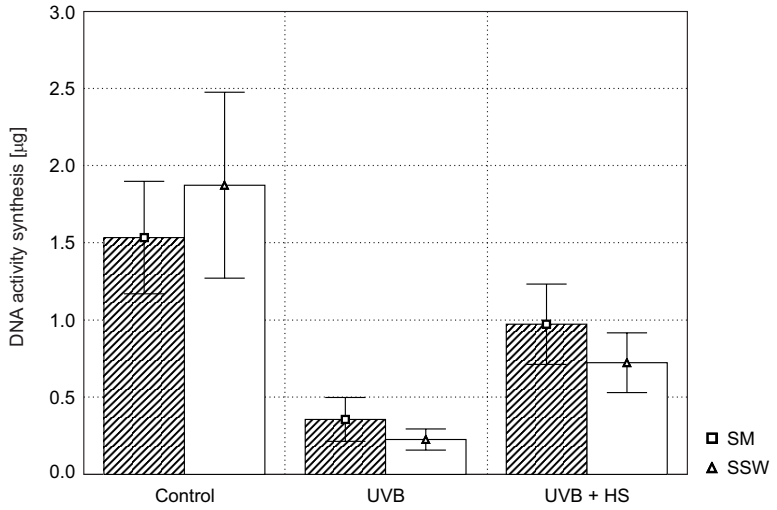

Fig. 1. Influence of UVB on DNA activity synthesis (verticalal bars represent standard error; SM – surface microlayer; SSW – subsurface water)

and Zdanowski [15] demonstrated that UV significantly hampered bacteria metabolic activity expressed in amounts of synthesized ATP.

Results of this study proved that UVB radiation has hampering impact on cellular proteins synthesis (Table 2).

Table 2

Influence of UVB radiation on activity of cellular protein synthesis

Strain No.	Control	UVB	UVB + SH
SM			
1	14.45*	7.49	0.57
2	19.00	12.17	5.05
3	2.62	1.43	1.07
4	59.23	30.95	6.51
5	156.34	41.74	3.78
6	14.25	5.90	5.31
7	44.84	34.92	27.20
SSW			
11	4.82	2.24	**2.64**
12	50.20	50.16	4.97
13	17.88	3.35	1.67
14	4.37	3.66	1.17
15	9.88	5.54	2.21
16	295.72	31.51	7.78
17	8.00	8.07	2.81

* – µg of protein.

For SM strains limitation of proteins synthesis under influence of UVB was noted in cases of nearly all strains, except for strain no. 7. Nevertheless, comparison of UVB operation impact on DNA and proteins synthesis activities clearly shows that proteins synthesis process is hindered to much lower degree than DNA. Similar results pertain to SSW strains. With respect to them two strains did not demonstrate any respond to UVB radiation (strains no. 12 and no. 17), whereas strain no. 14 reaction was very slight. Moreover, as previously stated, activity of cellular proteins synthesis by SSW strains exposed to UVB radiation was significantly less hindered that DNA synthesis process. Also, Denward's et al study [16] proves that leucine incorporation process was not significantly disturbed by UV radiation. There is also a study, which suggests that bacteria proteins synthesis process would undergo more intensively when affected by UV radiation [17]. That author, however exposed to radiation water sampled from a lake, not excluding bacteria. In such an experiment UVB operation resulted among other things in organic matter photooxidation. Low-molecular matter, produced in this way could have caused increase in general cells activity, including protein synthesis.

Obtained results of experiments with application of HS as potentially protective substance from UVB are completely different from those received in DNA synthesis investigations. Use of HS did not cause increase in protein synthesis activity and even further collapse of that activity was observed (Fig. 2). These statements pertain virtually to all surveyed bacterial strains, except for strain no. 11. Presented results indicate that HS can play a role of protective agent, but only for some selected kinds of metabolic activity. For others they not only do not constitute protective substances, but have even further hampering effect (Fig. 2).

Similar results making up an evidence for stronger UV impact on DNA than protein synthesis were published in connection with survey conducted *in situ* [14, 18]. Chrost and Faust [18] noticed substantial limitation of bacteria secondary production in the

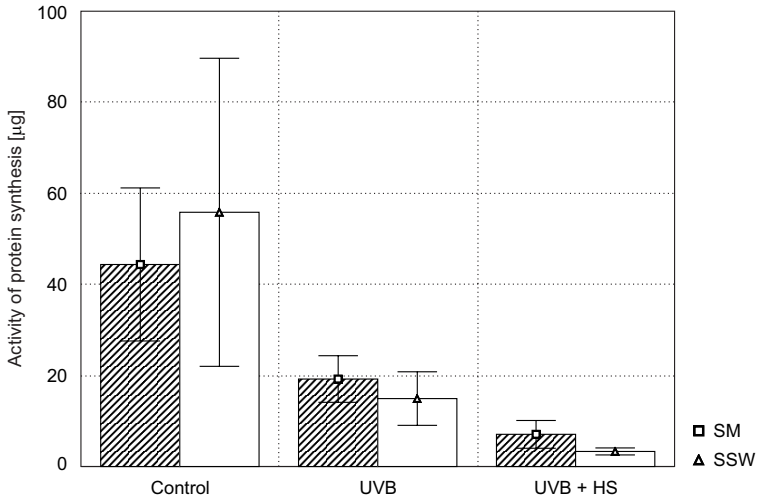

Fig. 2. Influence of UVB on cellular protein activity synthesis (verticalal bars represent standard error; SM – surface microlayer; SSW – subsurface water)

afternoon that is after strong solar radiation periods. Simultaneously the protein production level was stable. Analogous results signifying strong UV impact on DNA synthesis and weak impact on proteins synthesis of SM bacteria were registered during experiments conducted in twenty-four hour cycle [14].

To conclude it should be emphasised that UVB radiation has unfavourable impact on activity of DNA and cellular proteins synthesis. However, DNA synthesis process is much more sensitive to this radiation exposure. In addition, presented results prove that HS not always operate as protective substances. HS regarded as a filter of a kind, which delivers protection from UV [5, 6] demonstrate such impact only for some sorts of bacterial cells activity. For others they can act like additional inhibitor.

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WPŁYW UVB NA AKTYWNOŚĆ SYNTEZY DNA I BIAŁEK KOMÓRKOWYCH PRZEZ BAKTERIE ŚRODOWISKA WODNEGO

Zakład Mikrobiologii Środowiskowej i Biotechnologii
Uniwersytet Mikołaja Kopernika w Toruniu

Abstrakt: Badania prowadzono w 2007 r., pobierając próbki wody z MP i WPP w strefie pelagialu jeziora Brzeźno. Z pobranych próbkach wody izolowano reprezentatywną kolekcję szczepów, na których wykonywano dalsze badania. W toku badań oznaczano aktywność syntezy DNA i białek komórkowych bakterii poddanych promieniowaniu UVB. Badania prowadzono w dwóch układach doświadczalnych: bez substancji humusowych (SH) i w obecności tych substancji jako związków o działaniu potencjalnie ochronnych przed UV. Przeprowadzone badania wykazały, że pod wpływem promieniowania UVB aktywność syntezy DNA i białek komórkowych ulegały silnej inhibicji. Natomiast obecność SH inaczej wpływa na syntezę DNA i białek.

Słowa kluczowe: UV, synteza DNA, synteza białek, substancje humusowe, mikrowarstwa powierzchniowa