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Toxic effect of ciprofloxacin on the photosynthesis reactions in microalga *Scenedesmus quadricauda* (Turp.) Bréb.

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Ciprofloxacin (CIP) is widely used broad-spectrum antimicrobial drug of fluoroquinolone family. The widespread use of ciprofloxacin increases its release into the environment. Ciprofloxacin is detected in aquatic ecosystems potentially harming aquatic organisms. The CIP effect on photosynthetic organisms is not fully studied. In this study we examined the CIP effect on green freshwater microalgae *Scenedesmus quadricauda* (Turp.) Bréb. using chlorophyll fluorescence methods (JIP test parameters and rapid light curves). A significant decrease in the cell number was observed at ≥10 mg/L of ciprofloxacin in comparison with control. Analysis of chlorophyll fluorescence parameters obtained from OJIP transients revealed the changes in photosynthetic reactions in ciprofloxacin treatment. Ciprofloxacin was found to inhibit electron transport rate in photosystem II (PSII). The decrease in the quantum yield of electron transport in photosystem II (φ_{Eo}) was accompanied by the decrease in performance index (PI_{ABS}) and an increase in energy dissipation (DI_0/RC). Ciprofloxacin enhanced the photosensitivity of microalgae but did not inhibit the recovery of photosynthetic activity after the photooxidative stress. In this regard the effect of CIP differs from that of the well-known antibiotic chloramphenicol that inhibits the resynthesis of plastid proteins and, accordingly, the recovery of photosynthetic activity associated with the resynthesis of PSII protein D1. Among the fluorescence parameters, PI_{ABS} was found to be the most stress-specific; therefore it can be proposed to detect an early toxic CIP effect in microalgae.

Keywords: ciprofloxacin, *Scenedesmus quadricauda*, chlorophyll fluorescence, photosynthesis, bioassay.

УДК 577.355

Токсическое действие ципрофлоксацина на реакции фотосинтеза микроводоросли *Scenedesmus quadricauda* (Turp.) Bréb.

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Ципрофлоксацин (ЦИП) – часто используемый в мире антимикробный препарат широкого спектра действия, относящийся к семейству фторхинолонов. Широкое использование ЦИП привело к увеличению его выброса в окружающую среду. Ципрофлоксацин обнаруживается в водных экосистемах, что потенциально может нанести вред водным организмам. Влияние ЦИП на фотосинтезирующие организмы до конца не изучено. В этой работе мы исследовали влияние ЦИП на зелёные пресноводные микроводоросли *Scenedesmus quadricauda* (Turp.) Bréb. с использованием методов флуоресценции хлорофилла (параметры JIP теста и быстрые световые кривые). Значительное снижение числа клеток наблюдали при ≥10 мг/л ЦИП. Анализ параметров флуоресценции хлорофилла, полученных из OJIP кривых, выявил изменения в фотосинтетических реакциях в присутствии ЦИП. Было обнаружено, что ЦИП ингибирует скорость транспорта электронов в фотосистеме II. Снижение квантового

выхода электронного транспорта в фотосистеме II (φ_{E_0}) сопровождалось снижением индекса производительности (PI_{ABS}) и увеличением диссипации энергии в тепло (DI_0/RC). Ципрофлоксацин повышал фоточувствительность микроводорослей, но не тормозил восстановление фотосинтетической активности после фотоокислительного стресса. В этом отношении действие ЦИП отличается от действия известного антибиотика хлорамфеникола, ингибирующего ре-синтез пластидных белков и, соответственно, восстановление фотосинтетической активности, связанное с ресинтезом белка D1 фотосистемы II. Среди параметров флуоресценции PI_{ABS} оказался наиболее стресс-специфичным, поэтому его можно предложить для выявления раннего токсического действия ЦИП на микроводоросли.

Ключевые слова: ципрофлоксацин, *Scenedesmus quadricauda*, флуоресценция хлорофилла, фотосинтез, биотестирование.

Ciprofloxacin (CIP) is a second-generation fluoroquinolone broad-spectrum antibiotic against aerobic Gram-negative and Gram-positive bacteria. Ciprofloxacin is widely used in human and veterinary medicine as well as in aquaculture [1]. This antibiotic was universally used during the COVID-19 pandemic to suppress the bacterial co-infections [2]. Ciprofloxacin mechanism of action is based on interference with deoxyribonucleic acid (DNA) synthesis by binding to DNA gyrase; this prevents the replication [1]. The widespread use of fluoroquinolones has led to their appearance in the environment, especially in aquatic ecosystems. The consequences of the occurrence of fluoroquinolones in the environment are not fully understood, but these chemicals are known to be toxic to plants and aquatic organisms [3–9].

It is definitely known that bacteria are target organism of antibiotics instead of eukaryotic algae. However, antibiotics may induce adverse effects in algae interfering with chloroplast metabolism such as photosynthesis process and interrelated protein synthesis. These effects are due to chloroplast similarity to bacteria in structures and even evolutionary origin, so they are often targeted by antibiotics. Antibiotics can interrupt chloroplast gene expression and inhibit chlorophyll synthesis [10]. For instance, the antibiotic chloramphenicol interferes with chlorophyll biosynthesis by inhibiting protein synthesis [11].

Photosynthetic microalgae are the base of the food webs in aquatic ecosystems and any alteration in it can lead to alterations in other levels of food web. Therefore, microalgae are suitable indicator for anthropogenic pollution and water quality. Freshwater green microalgae genus *Scenedesmus* and *Chlorella* are widely employed in bioassay in the current practice adopted in the Russian Federation [12, 13]. The effect of tested substances on unicellular algae is assessed in terms of the changes in cell number [14] as well as chlorophyll fluorescence. The above is recommended and used among other certified methods for bioassay of natural and waste waters [13, 15].

Chlorophyll fluorescence methods such as PAM-fluorometry and analysis of OJIP transients with JIP-test actively applied in recent papers to study photosynthetic electron transport within photosystem II (PSII) and between photosystems (PSII and PSI) as well as to assess total photosynthetic activity [15–21] by toxic substances [21].

The aim of the present work is to study the toxic effects and CIP-induced mechanisms on photosynthetic reactions in *Scenedesmus quadricauda* through the measurement of chlorophyll fluorescence parameters.

Objects and methods of research

The unicellular freshwater green microalga *Scenedesmus quadricauda* (Turp.) Bréb. is a test organism. Culture was grown in Uspensky's medium supplied with microelements [22] at 24 °C. It was continuous illumination by luminescent lamps at photosynthetic photon flux density (PPFD) of 40 μmol photons m^{-2} s^{-1} up to 10^6 cells/mL. Exponentially growing cells were inoculated into 100 mL Erlenmeyer flasks with 50 mL of the Uspensky's medium, so that the concentration was $5 \cdot 10^4$ cells/mL for each flask. The ciprofloxacin (CIP) antibiotic (98%; Kelun-Kazpharm, Kazakhstan) was a toxicant. It was added into flasks to achieve 10, 25, and 50 mg/L final concentrations. Samples with CIP and control (non-exposed culture) were incubated for 72 h in the described above conditions.

The microalgae growth rate was determined by cell counting using the Goryaev chamber (haemacytometer).

The light-induced chlorophyll *a* fluorescence (OJIP transients) was recorded with an Aquapen–C 100 fluorometer (Photon System Instruments, Czech Republic). PPFD and duration of the blue actinic flash (λ = 455 nm) were 3000 μmol photons m^{-2} s^{-1} and 2 s, respectively. The samples were adapted to the darkness for 10 min before measurements. OJIP transients were analyzed with JIP-test [17] to obtain the following parameters: F_V/F_M – maximum

quantum yield of primary photochemistry in photosystem II (PSII); V_J – relative variable fluorescence at the J-step (reflects the number of closed reaction centers (RCs) in relation to the total number of PSII RCs); φ_{Eo} – quantum yield of electron transport (at t = 0); ABS/RC – absorption flux per RC; DI_0/RC – dissipation energy per active reaction center; PI_{ABS} – performance index on absorption basis (indicator of functional activity of PSII) [17].

Rapid light curves (RLCs) were recorded with a Water-PAM fluorometer (Walz, Effeltrich, Germany) using a pre-installed software routine based on 8 actinic increasing light levels (50, 100, 200, 400, 600, 800, 100 and 1200 μmol photons m^{-2} s^{-1}); duration of light steps was 20 s. At each light level the effective PSII quantum efficiency (Yield = $(F_M' - F_t)/F_M'$) (with F_t being the current fluorescence yield in the light measured just before the saturation pulse) was measured by the saturation pulse technique. Whereby a saturating light pulse of 3000 μmol photons m^{-2} s^{-1} was applied for 0.8 s to measure the maximum fluorescence yield, F_M'. The relative electron transport rate (rETR) was calculated as the product of light utilization efficiency (Yield) and PPFD (rETR = Yield · PPFD · 0.5). The several characteristic parameters were calculated from rETR RLCs: the RLC initial slope before the saturation onset (α), the relative maximum electron transport rate ($rETR_{max}$) and saturating irradiance ($E_k = rETR_{max}/\alpha$) [23]. The non-photochemical quenching (NPQ), that reflects light energy dissipation by heat, was calculated as NPQ = $(F_M - F_M')/F_M'$. Designation and description of photosynthetic parameters are presented according to [16].

The delayed fluorescence (DF) induction curves in the millisecond decay interval (2–3 ms) were measured by the phosphoroscopy setup described in [15].

The data was analyzed and processed using the OriginPro software package. Means and confidence intervals ($M \pm CI$) (P = 0.95) were calculated for each parameter.

Results and discussion

The table shows the decrease in growth rate of *S. quadricauda* after 72 h incubation with CIP. Starting from the 1st incubation **day the antibi**otic concentration equal to 50 mg/L or higher reduces the number of *S. quadricauda* microalgal cells. The growth inhibition of other microalgal species by CIP was described earlier [3–9]. It is noted that eukaryotic microalgae are generally less sensitive than prokaryotes [3].

It was found that low CIP concentrations (10 mg/L) increased the cultured cells number at early stages of cultivation (1st day) compared to control. This hormesis effect manifested in the facilitated microalgae development at low concentrations of the toxicant was previously noted in [3, 4, 6].

The microalgae fluorescence F_0 excited by low-intensity light correlates with the chlorophyll content [24]. The experimental results showes the correlated increase in the algal cell number (N) determined by direct counting in cultures with ciprofloxacin as well as in the fluorescence index F_0, with the correlation coefficient of at least 0.90 (Table). This fact confirms the suitability of fluorescence index F_0 for bioassay using microalgae *S. quadricauda* as a test object. The intensity of millisecond delayed chlorophyll fluorescence also correlated with the cell number and F_0 (Table).

Fluorescence induction parameters were measured to evaluate in detail the changes in photosynthetic activity in *S. quadricauda*. Fluorescence parameters derived from OJIP transients are summarized in the Table. The maximum quantum yield of the primary photochemical reactions of PSII (F_V/F_M) in control cells was as high as 0.72 (Table). By contrast, F_V/F_M decreases affected by various concentrations of CIP, indicating the disturbance of photosynthetic processes. Changes in F_V/F_M were mainly due to the decrease intensity of maximum fluorescence F_M. In CIP-treated cells, the quantum yield of electron transport in PSII (φ_{Eo}) was also reduced. This indicates one of the primary targets of antibiotic action localized on the PSII acceptor side.

The absorption flux per RC (ABS/RC) in CIP-treated cells was higher than in control samples due to the decreased proportion of active PSII RC in the treated cells.

The performance index on absorption basis (PI_{ABS}) is an indicator of PSII functional activity. PI_{ABS} was higher in control cells than in CIP-treated cells. Low PI_{ABS} values in CIP-treated algae indicate the low PSII functional activity due to the decrease in the proportion of active RCs and the increase in excitation quenching in the antenna. A decrease in the efficiency of excitation energy transfer from the light-harvesting complex to the RCs should be accompanied by an increase in the light energy dissipation. The efficiency of energy dissipation (DI_0/RC) in CIP-treated cells was indeed at a high level.

The rapid light curves (RLCs) were recorded in order to study photosynthetic characteristics in response to sequential increase of irradiance in CIP-treated samples ((data is not shown).

Table

Cell number and chlorophyll fluorescence parameters of microalga *S. quadriqauda* under the effect of CIP at 10, 25, and 50 mg/L after 72 hours of incubation

Parameter	Control	10 mg/L	25 mg/L	50 mg/L
Cell number, 10^3 per mL	180±4.3 (100±2.4)	147±5 (82±2.8)	126±3.9 (70±2.2)	95±2.8 (53±1.6)
F_O, rel. units	2787±94 (100±3.4)	2277±111 (82±4)	1997±98 (72±3.5)	1309±102 (46±3.7)
DF intensity (stationary level) rel. units	200±8 (100±4)	192±3 (86±3)	160±3 (80±3)	84±2 (42±2)
Parameters derived from OJIP transients via JIP-test				
$F_V/F_{M,}$ rel. units	0.75±0.01 (100±1.3)	0.62±0.01 (83±1.3)	0.56±0.01 (75±1.3)	0.54±0.01 (72±1.3)
V_J	0.5±0.01 (100±2)	0.54±0.01 (108±2)	0.55±0.02 (110±4)	0.56±0.02 (112±4)
φ_{Eo}	0.37±0.1 (100±2.7)	0.29±0.1 (78±2.7)	0.26±0.1 (70±2.7)	0.25±0.2 (68±5.4)
ABS/RC	3.04±0.02 (100±0.6)	3.04±0.01 (100±0.3)	3.05±0.02 (103±0.6)	3.19±0.01 (105±0.3)
DI_0/RC	0.92±0.01 (100±1)	0.97±0.02 (105±2)	0.93±0.01 (101±1)	0.92±0.01 (100±1)
PI_{ABS}	1.03±0.05 (100±4.5)	0.34±0.04 (33±3.6)	0.22±0.01 (21±0.9)	0.21±0.01 (20±0.9)
Parameters derived from RLCs				
$rETR_{max}$, rel. units	186±11 (100±5.9)	111±12 (60±6.5)	101±9 (54±4.8)	97±13 (52±7)
α, rel. units	0.25±0.01 (100±4)	0.18±0.01 (72±4)	0.18±0.01 (72±4)	0.17±0.01 (68±4)
E_k, μmol photons $m^{-2}s^{-1}$	733±9 (100±1.2)	614±10 (84±1.4)	569±12 (78±1.6)	560±8 (76±1.1)
NPQ at 600 μmol photons $m^{-2}s^{-1}$	0.32±0.01 (100±3)	0.43±0.01 (134±3)	0.39±0.01 (121±3)	0.4±0.01 (125±3)

Note: Non-treated culture designated as control. Means values and confidence intervals (P = 0.95) are presented. Values in parentheses are percentages (%) of control values.

Photosynthetic parameters of relative electron transport (rETR) were obtained from RLCs of rETR (Table). The relative maximum transport rate ($rETR_{max}$) and α were decreased in CIP-treated samples which correlated with the changes in fluorescence parameters derived from OJIP transients.

Presented in the Table non-photochemical quenching (NPQ) values were taken from the RLCs of NPQ (data not shown). NPQ is a protective mechanism against over reduction of the photosynthetic electron transport chain by dissipation of excess absorbed light energy in the PSII antenna system as heat [16]. The CIP exposure to *S. quadricauda* microalgae was accompanied by the NPQ increase, which is consistent with DI_0/RC increase (Table).

It is known that elevated irradiance may cause the photoinhibition of photosynthesis and induce the protective nonradiative dissipation of excess light energy into heat. As shown in [25], photoinhibition of photosynthesis is mainly associated with the protein D1 degradation. This protein is encoded by the chloroplast genome and synthesized on plastid ribosomes. Protein D1 is one of the crucial proteins of PSII RC. Post-photoinhibition recovery of PSII activity is associated with the resynthesis of protein D1 in the chloroplast. The intracellular concentration of active PSII RCs depends on the proportion of the photooxidative destruction and recovery rates that can be monitored by F_V/F_M [26, 27]. Figure shows the change of F_V/F_M when exposed to increased irradiance (3000 μmol photons m^{-2} s^{-1}) for 45 min and the subsequent adaptation of cells to darkness.

The effect of increased irradiance decreases F_V/F_M in control and CIP-treated samples. Significant F_V/F_M decrease (about 0.1) was observed in CIP-treated sample indicating major photoinhibition effects in comparison with con-

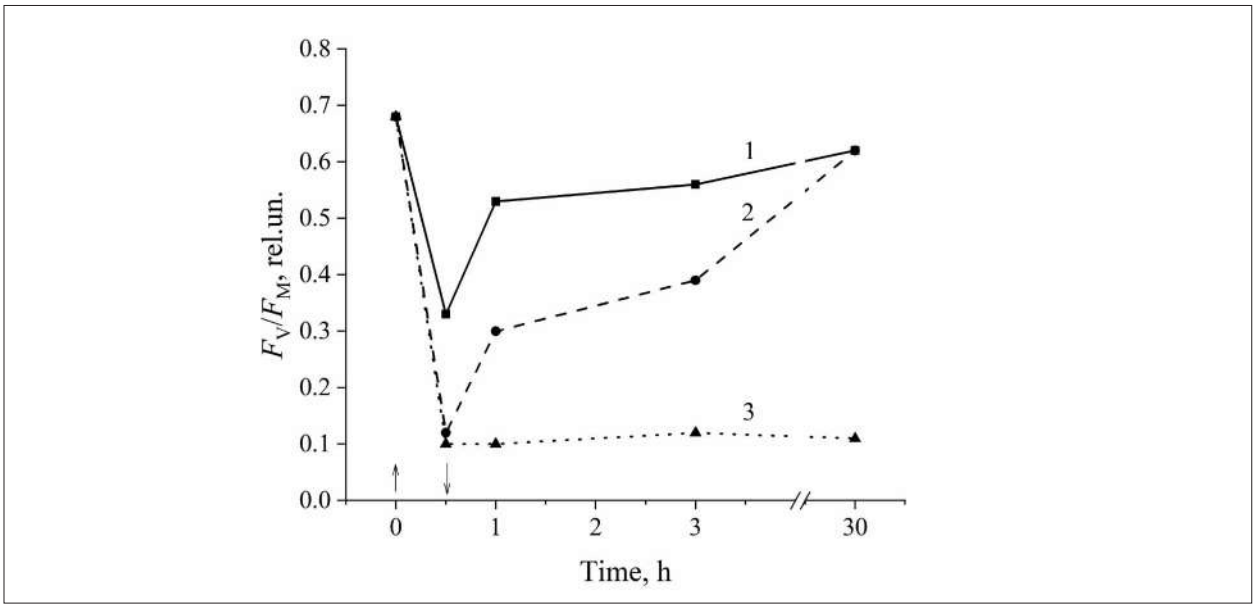

Fig. F_V/F_M changes in 45 min photoinhibition with 3000 μmol photons $m^{-2}s^{-1}$ light and in the darkness. 1 – control culture of green algae *S. quadricauda*, 2 and 3 – samples incubated for 24 hours with CIP and chloramphenicol at 100 and 300 mg/L, respectively. The up and down arrows mark the moments when the light is turned on and off

trol. However, F_V/F_M in CIP-treated sample was completely recovered to initial F_V/F_M (before light stress) after continuous incubation in shaded conditions. This result indicates that the CIP-treated microalgae become photosensitive but retain the ability to recover the photosynthetic activity after photoinhibition. Chloramphenicol inhibited the repair of photodamaged PSII RCs in algal cells; this corresponds to well-known mechanism of its action on protein synthesis in plastids [25–27].

Conclusion

Ciprofloxacin, a derivative of fluoroquinolones is currently one of the most widely used broad-spectrum antibiotics. In our study CIP suppresses the growth rate of green microalgae *S. quadricauda* at 10 mg/L. Analysis of chlorophyll fluorescence demonstrates the changes in photosynthetic characteristics of CIP-treated *S. quadricauda* cells. The fluorescence parameters comprehensive analysis obtained from OJIP transients and RLCs allowed revealing changes in photosynthetic characteristics in CIP-treatment. Thus, CIP reduces the maximum quantum yield of PSII (F_V/F_M), the maximum relative electron transport rate ($rETR_{max}$), the maximum utilization of light energy coefficient (α), the electron transport efficiency (φ_{E_0}), and productivity index (PI_{ABS}), while enhancing the energy dissipation (DI_0/RC). Among the above set of parameters, PI_{ABS} is considered as the most sensitive fluorescence parameter and proposed as indicator of algae physiological state in CIP treatment. We showed that CIP enhances the microalgae photosensitivity. Ciprofloxacin did not eliminate the recovery photosynthetic processes, indicating that this antibiotic does not affect the processes of protein D1 synthesis on plastid ribosomes in contrast to chloramphenicol.

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