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BIOCHEMICAL INDICATORS OF GREEN PHOTOSYNTHETIC BACTERIA *Chlorobium limicola* RESPONSE TO Cu^{2+} ACTION

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Photolithotrophic sulfur bacteria are involved in biota functioning and have a biotechnological potential for bioremediation of contaminated environment, but the mechanisms of xenobiotics, in particular of heavy metal ions damaging action and the pathways of photolithotrophic bacteria adaptation under these conditions have not been established. In this work, the biochemical indicators of green photosynthetic bacteria Chlorobium limicola response to Cu ions were studied. C. limicola cells were incubated during one hour in buffer containing copper (II) sulfate in 0.05–0.5 mM concentrations and grown for 8 days in GSB medium. The content of Cu^{2+} in cells was estimated by atomic absorption spectroscopy. The activity of enzymes of antioxidant defense, photosynthetic pigments and glutathione content, indexes of lipids unsaturation and membrane viscosity as markers of membrane fluidity were estimated. It was shown that the response of green photosynthetic bacteria C. limicola to Cu^{2+} action varied depending on cations concentration. Under the influence of metal salt at 0.05 mM concentration, the activity of antioxidant enzymes, GSH/GSSG ratio, the content of photosynthetic pigments and membrane fluidity indexes were higher as compared with control. Under the increase of copper (II) sulfate concentration to 0.25 mM, the activity of antioxidant enzymes was lower compared to the response of the cells under the influence of 0.05 mM copper (II) and the GSSG content was increased. Under the influence of 0.5 mM copper (II) the indexes of membrane fluidity did not differ from the control, but superoxide dismutase and peroxidase activity inhibition and the further decrease of GSH/GSSG ratio were observed followed by the highest Cu^{2+} cations accumulation in cells and significant decrease of bacteria biomass growth.

K e y w o r d s: green bacteria, Cu cations, antioxidant protection, membrane fluidity, adaptation.

Photolithotrophic sulfur bacteria play a significant role in the functioning of biota, have an influence on geochemical cycles of carbon, sulfur, nitrogen, and are widespread in different environments [1, 2]. Green photosynthetic bacteria *Chlorobium limicola*, isolated from Yavoriv Lake (Lviv region, Ukraine) [3], utilize as the donor of electrons hydrogen sulfide, produced during the sulfur reduction by sulfate- and sulfur-reducing bacteria, in the process of anoxygenic photosynthesis.

The anoxygenic phototrophic bacteria have the potential for bioremediation of recalcitrant dyes, pesticides, and heavy metals under anaerobic conditions. Finally, these organisms may be useful for overexpression of membrane proteins, photobiological production of H_2 and other valuable compounds [2].

Cu cations are one of the most prevalent pollutants of the environment [4]. Toxicity of Cu^{2+} is predetermined by cation participation in the production of reactive oxygen species (ROS), lipid peroxidation and oxidative modification of proteins which have a destructive influence on the functioning of bacteria [5].

In anaerobic microorganisms, the systems of antioxidative defense function to counteract the influence of ROS. In *Chlorobaculum tepidum* TLS (earlier *Chlorobium tepidum*) genome, the genes, the products of which are involved into defense against ROS and oxidative stress were found. Cytochrome *bd* quinol oxidase, rubredoxin:oxygen oxidoreductase, thiol peroxidases, alkyl hydroperoxide reductase, superoxide reductase and methionine sulfoxide reductase belong to such antioxidants [6, 7]. One of the main targets of heavy metals action are cell membranes and the adaptation to its is realized particularly by maintenance the appropriate level of membrane fluidity. Changes in fatty acids composition of membrane lipids are the most important reactions of bacterial cell to oxidative stress [8]. Activity of the enzymes of the antioxidant defense in cells of green photosynthetic bacteria under the influence of heavy metal ions has not been investigated.

The aim of the work was to estimate the activity of enzymes of antioxidant system superoxide dismutase and peroxidase, the content of glutathione and indexes of membrane fluidity as possible indicators of *C. limicola* adaptation to the influence of Cu cations.

Materials and Methods

Cultivation of bacteria. Chlorobium limicola bacteria were grown in GSB medium under microaerobic conditions, permanent illumination (wavelength 700−800 nm, luminosity 40 lux) and at temperature 28 °C [3]. To study the effects of copper (II) sulfate on the prooxidative-antioxidative indexes, *C. limicola* cells were incubated during one hour in 50 mM tris-HCl buffer (pH 7.0), which contained 0.05–0.5 mM copper (II) sulfate, inoculated into GSB medium and grown for 8 days. Metal salt was not added to control samples.

Obtaining of cell-free extracts. Cells were centrifuged and disintegrated at ultrasonic disintegrator UZDN-2T (22 kHz, 5 min, 0 °C) [9]. Cell debris was separated by centrifuging (8000 g, 30 min, 4 °C). The concentration of protein in cell-free extracts was measured by Lowry method [10].

Accumulation of Cu^{2+} ions. Suspension of *C. limicola* cells at the exponential phase of growth, washed by Tris-HCl buffer (50 mM, pH 7.0), was incubated with 0.05–0.5 mM copper (II) sulfate solution during one hour. Metal salt was not added to control samples. After incubation suspension of bacteria was divided into two equal parts. The first part was washed once with Tris-HCl buffer (50 mM, pH 7.0) and centrifuged during 60 min (3000 g), the second was washed twice and centrifuged during 60 min (3000 g). Determination of Cu^{2+} cations in both samples was carried out using atomic absorption spectrometry (Zeeman Atomic Absorption Spectrometer AA240Z Varian with GTA 120 Graphite Tube Atomizer). Argon flow was 0.3 l/min, the temperature of ashing stage – +800 °C, the temperature of atomization stage – +2300 °C. Detection of Cu^{2+} cations was carried out at 327 nm. The value, obtained after atomic absorption analysis of twice-washed cells, was considered as Cu^{2+} content inside cells. Cu^{2+} content at cells surface was counted as the difference between copper cations content in cells, washed once and twice.

Obtaining of pigment samples from bacterial cells. C. limicola cells, previously incubated in medium with copper (II) sulfate and grown on GSB medium, were centrifuged (3000 g, 60 min), resuspended in acetone (Sigma-Aldrich, France) and disintegrated at ultrasonic disintegrator UZDN-2T (22 kHz, 5 min, 0 °C). Extraction of pigments was performed by method [11]. Chromatographic separation of pigments was performed using high-performance liquid chromatography system [11].

Determination of glutathione content. 0.05 ml of 7.0 M perchloric acid was added to 1 ml of cell-free extract and vigorously mixed during 1 min. Dry potassium carbonate (general mass – 80 mg) was slowly added to the obtained solution. Formed $KClO_4$ sediment was centrifuged (2500 g, 5 min). 1 ml of supernatant was taken and derivatization of glutathione was performed using 1-fluoro-2,4-dinitrobenzene [12]. Glutathione content was measured using high-performance liquid chromatographer Agilent 7890A (Agilent Technologies, USA) with MZ Aqua Perfect C18 column (250×4.6–5 mm, Agilent Technologies, USA). Chromatograms were recorded at 350 nm. Na-K-phosphate buffer (0.05 M, pH 2) and methanol were used as a mobile phase.

Glutathione content determination was performed at 40 °C. Results were processed using fresh standard solutions of reduced and oxidized glutathione (Sigma-Aldrich, France).

Determination of carbonyl groups in proteins. Carbonyl groups in proteins were determined in reaction with 2,4-dinitrophenylhydrazine, as a result of which 2,4-dinitrophenylhydrazones are formed [13]. To 0.5 ml of the cell-free extract, 1 ml of trichloroacetic acid was added to a final concentration of 10%

and centrifuged (2500 g, 10 min). To the precipitate, obtained after centrifugation of the cell-free extract, 1 ml of 10 mM solution of 2,4-dinitrophenylhydrazine in 2 M HCl was added. To the control solution, 1 ml of 2 M solution of HCl was added instead. The mixture was stirred and incubated for 1 h at room temperature, then centrifuged (2500 g, 10 min). The precipitate was washed three times with 1 ml of a mixture of ethanol and ethyl acetate (1:1) and centrifuged (2500 g, 10 min). The washed precipitate was dissolved for 30 min in 6 M solution of guanidine hydrochloride. The undissolved material was separated by centrifugation (2500 g, 10 min). In supernatants, extinction of carbonyl groups of proteins was determined at 370 nm.

Determination of SOD and peroxidase activities. The SOD activity was estimated by the extent of quercetin oxidation inhibition by superoxide anion at 406 nm [14]. To determine the SOD activity, reagent 1 was prepared by mixing of 100 ml of 0.08 mM EDTA solution and 100 ml of 0.1 M potassium phosphate buffer (pH 7.8). To the obtained mixture, a concentrated solution of N,N,N,N-tetramethylethylenediamine was added to bring the pH to 10 (C reagent). Quercetin in a concentration of 1.4 μM was dissolved with dimethyl sulfoxide and immersed in hot water. Immediately before the determination quercetin solution was diluted by 10 times with distilled water. To the control test tube, 1 ml of C reagent, 2.4 ml of H_2O, 0.1 ml of quercetin were added.

To the test tube, 1 ml of C reagent, 2.3 ml of H_2O, 0.1 ml of a cell-free extract, 0.1 ml of quercetin (Sigma Aldrich, USA) were added. Measurements were performed at 406 nm at zero point (immediately after the addition of quercetin) and at 20 min. SOD activity was determined in standard units of activity per 1 mg of cellular protein. The concentration of protein which provides 50% inhibition of quercetin oxidation was taken as one unit of activity.

Peroxidase activity was determined by reaction of H_2O_2 with ammonium molybdate [14]. The reaction mixture contained 2.8 ml of 0.1 M solution of H_2O_2 and 0.1 ml of a cell-free extract diluted by n times. The reaction was carried for 5 min and stopped by addition of 1.0 ml of a 0.3 M $(NH_4)_2MoO_4$ solution. The control contained H_2O instead of cell-free extract.

Determination of membrane fluidity indexes. Extraction of lipids from bacterial cells was performed by E. Bligh method [15], after which the mixture of chloroform with methanol was dried on water bath and methylation of obtained fatty acids using 2 M sodium methylate was performed. Obtained fatty acids methyl esters were analyzed using gas chromatography [16]. Results were processed using the standard kit of fatty acids methyl esters (Supelco, USA). Index of lipids unsaturation and index of membrane viscosity were determined by [8].

Statistic processing of results. Experiments were repeated three times with three parallel formulations for each variant of experimental and control conditions. The obtained data were processed by generally accepted methods of variation statistics. The reliability of the difference was evaluated using ANOVA. Differences between the samples were considered reliable at $P < 0.05$.

Results and Discussion

Decrease of Cu^{2+} content in the incubation medium (Fig. 1, *A*) for 12–16 %, compared to samples without cells, was found in all samples after 1 h of *C. limicola* cells incubation in buffer with copper (II) sulfate at concentrations 0.05–0.5 mM. Metal ions were accumulated both at the surface of *C. limicola* cells and inside the cells (Fig. 1, *B*). Cu^{2+} cations content at the surface of cells and inside cells increased with the increase of metal salt concentration in the incubation medium.

Accumulation of Cu^{2+} cations in *C. limicola* cells had negative influence on the functional state of cells, as a consequence accumulation of *C. limicola* biomass decreased. In the previous works [9] we have established that in case of addition of copper (II) sulfate at concentrations 0.05–0.5 mM to incubation medium *C. limicola* biomass decreased by 10–70%, compared to control. We suppose that the damage of bacteria cells under the influence of copper (II) sulfate was connected with the free radical damage of cellular molecules, because lipid peroxidation products were accumulated in cells [9]. It is known that protein molecules also are the targets of ROS attack. Copper cations are involved in the processes of oxidative modification of proteins [17]. The content of carbonyl groups in side chains of amino acids is the biomarker of proteins free radical damage [17]. These groups can also be formed in proteins as a consequence of metal-catalyzed oxidative attack of proline, arginine, lysine and threonine [17].

The content of carbonyl groups in proteins of *C. limicola*, incubated without copper (II) sulfate, decreased with the increase of duration of bacterial growth. The content of these groups in *C. limicola* proteins after incubation with 0.05–0.5 mM cop-

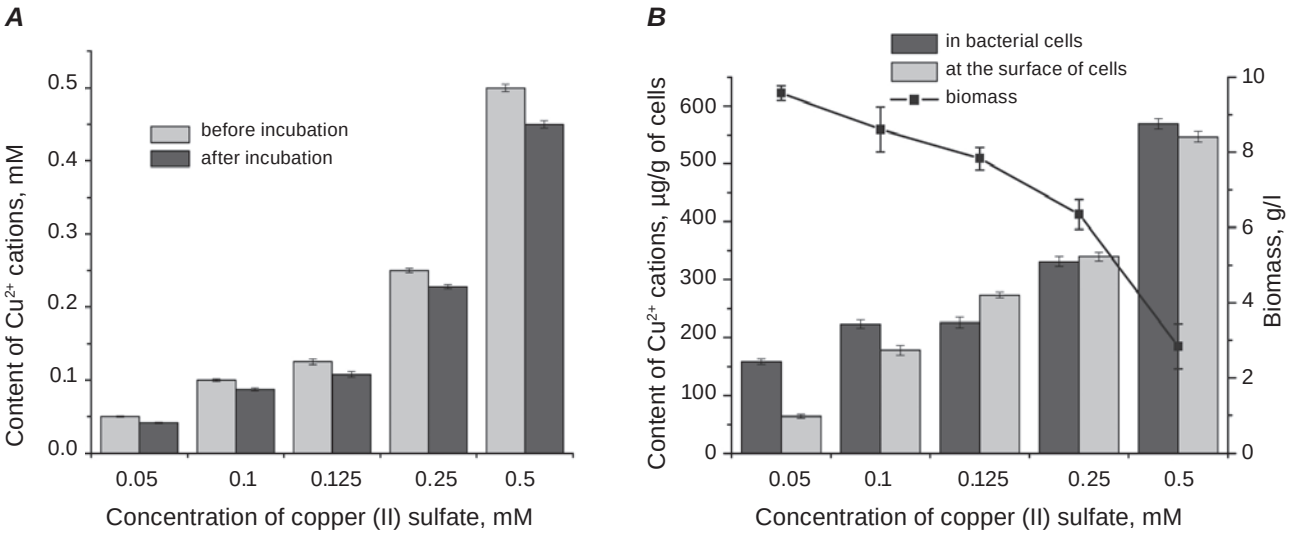

*Fig. 1. Dose-dependent dynamics of Cu^{2+} cations content in the incubation medium (**A**) and in bacteria C. limicola (**B**) (n = 9)*

per (II) sulfate was lower, compared to control, and changed depending on metal salt concentration in the medium and duration of cultivation (Fig. 2).

The decrease of carbonyl groups content in *C. limicola* proteins under the influence of copper (II) sulfate can be caused by degradation of damaged proteins in proteasomes. Amino acid sequences of α- and β-subunits of 20S proteasome of *Chlorobium limicola* DSM 245 (GenBank: ACD90186.1) [18] and ATP-dependent subunit of HslV protease (GenBank: KUL29135.1) [18] are found in GenBank database. We suppose that proteolysis of damaged proteins may be intensified under the influence of Cu^{2+} cations. The other possible cause of the carbonyl groups content decrease in *C. limicola* proteins under the influence of Cu^{2+} cations is the formation of protein aggregates as the consequence of the increased content of oxidatively damaged proteins.

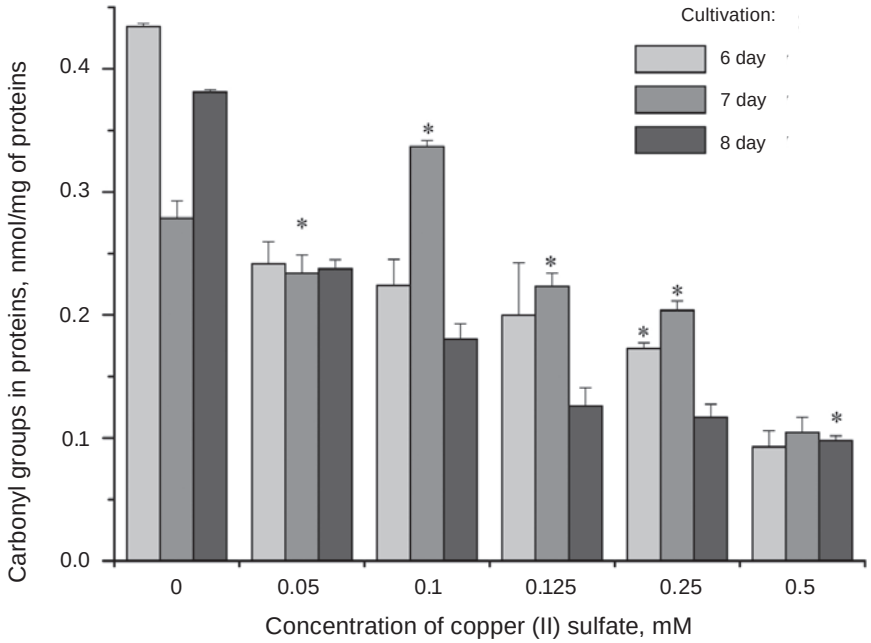

*Fig. 2. Dose- and time-dependent effect of copper (II) sulfate on carbonyl groups content in proteins of C. limicola bacteria (*P ≥ 0.95, n = 9 – credible changes, compared to control)*

Probably, not all carbonyl groups in the aggregates are exposed to the medium and some of them are inside the aggregate, complicating the detection of these groups by 2,4-dinitrophenylhydrazine.

Photosynthesis is an important physiological and biochemical process in *C. limicola*. Bacteriochlorophylls *a*, *c*, *d* and chlorobactene, which are the main pigments of green photosynthetic bacteria [19] were found in cell-free extracts of *C. limicola* (Fig. 3).

Photosynthetic pigments content significantly changed under the influence of Cu^{2+} cations. It decreased in cell-free extracts of *C. limicola*, compared to control, under the influence of all investigated concentrations of copper (II) sulfate with the exception of 0.05 mM. Under the influence of copper (II) sulfate in this concentration bacteriochlorophyll *a*, *c* and chlorobactene content increased by 2–2.5 times, compared to control. Content of lycopene, which is the precursor in chlorobactene biosynthesis in green photosynthetic bacteria [20], significantly increased under the influence of 0.05 mM copper (II) sulfate.

We suppose that changes in the content of photosynthetic pigments in cell-free extracts of *C. limicola* under the influence of Cu^{2+} cations lead to the change of photosynthetic units composition and, as a consequence, could disturb the process of photosynthesis.

Reduced (GSH) and oxidized (GSSG) glutathione, together with other compounds, for example, $NADPH_2$, provide the maintenance of redox status of cell [21]. Glutathione is also involved in metabolism of some metal cations, particularly, copper, and formation of resistance to the heavy metal cations with variable valence, particularly, to Cr^{6+}, Zn^{2+}, Cd^{2+} and Cu^{2+} in *Escherichia coli* [22].

General content of glutathione in *C. limicola* cells increased under the influence of Cu^{2+} with the increase its concentration in the incubation medium (Fig. 4). Probably, *C. limicola* synthesize glutathione to maintain appropriate redox potential of cytoplasm. With the further increase of metal salt concentration, the ratio between GSH and GSSG is abruptly decreased compared to control (Fig. 4). It is known that under normal conditions, the content of GSH significantly exceeds the content of GSSG [21]. Maintenance of GSH/GSSG ratio is strictly controlled at the transcriptional, translational and post-translational level [21, 22]. In the previous work, we established that glutathione system is an important part of the antioxidative defence of *C. limicola* under the influence of copper (II) sulfate [23]. We suppose that the increase in GSSG level in cell-free extracts of *C. limicola* under the influence of copper (II) sulfate can be the consequence of the glutathione peroxidase or glutathione-S-transferase activity in-

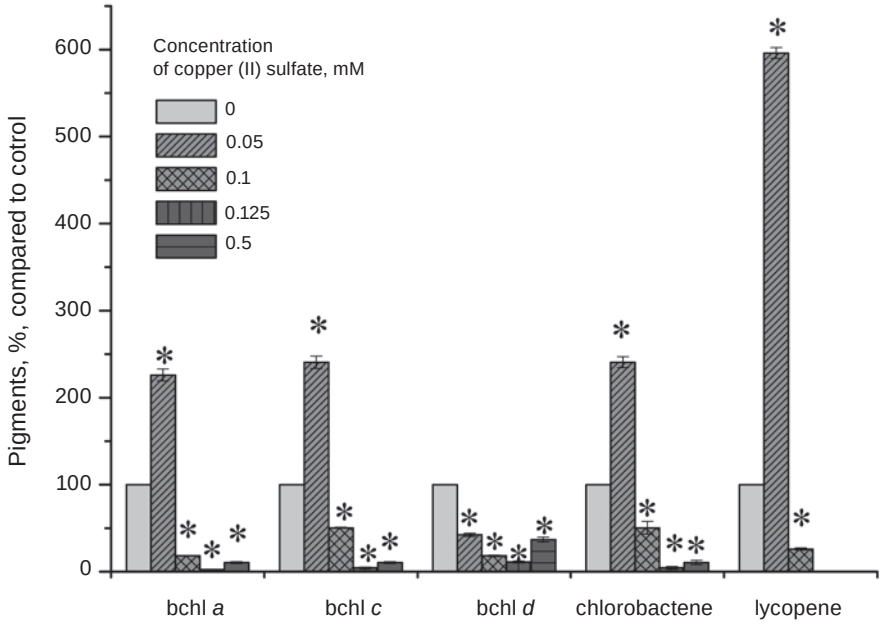

*Fig. 3. Dose-dependent effect of Cu^{2+} cations on the content of pigments of photosynthesis in cell-free extracts of C. limicola bacteria (bchl a – bacteriochlorophyll a, bchl c – bacteriochlorophyll c, bchl d – bacteriochlorophyll d; *P ≥ 0.95, n = 9 – credible changes, compared to control)*

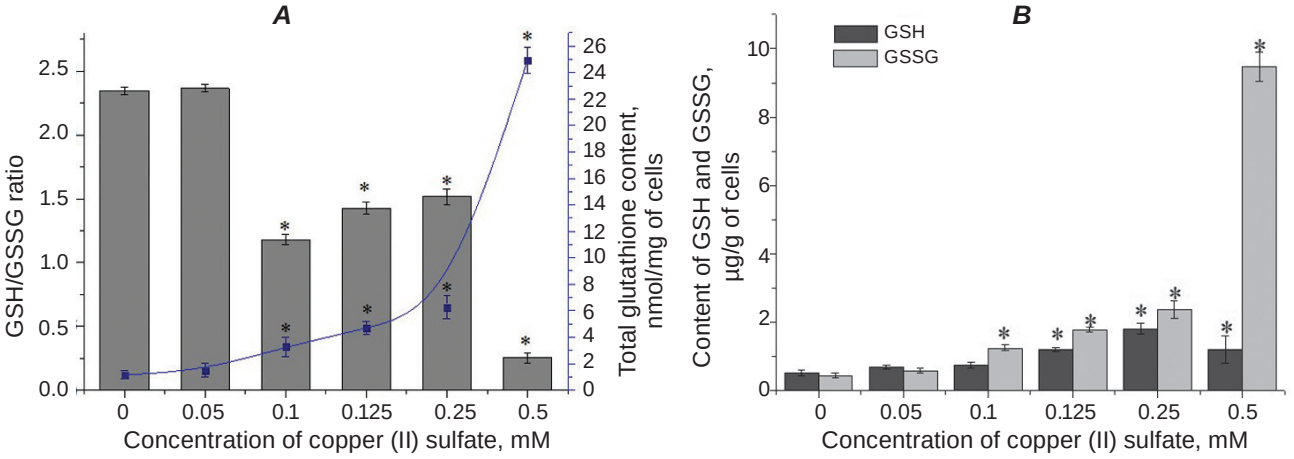

*Fig. 4. Dose-dependent effect of Cu^{2+} cations on GSH, GSSG content and GSH/GSSG ratio in cell-free extracts of C. limicola bacteria (**A** – GSH/GSSG ratio and total glutathione content (curve); **B** – reduced (GSH) and oxidized (GSSG) glutathione content; *P ≥ 0.95, n = 9 – credible changes, compared to control)*

crease or the direct oxidation of GSH by the free-radical compounds.

Disruption of the GSH/GSSG ratio as a consequence of Cu^{2+} cations accumulation may indicate the development of oxidative stress in *C. limicola*.

Superoxide dismutase (SOD) is an important part of the antioxidative defense of anaerobic bacteria [7]. *SodA* gene, which encodes this enzyme, is revealed in green photosynthetic bacteria genomes, particularly in representatives of *Chlorobiaceae* family [18]. Amino acid sequences of SOD from green photosynthetic bacteria *C. limicola* (WP_059138934.1), *Chlorobaculum parvum* (WP_012502390.1), *Chlorobaculum limnaeum* (WP_069809705.1), *C. tepidum* (WP_010932882.1), *Pelodictyon phaeoclathratiforme* (WP_012508276.1), *Pelodictyon luteolum* (WP_011357958.1), *Chlorobium phaeobacteroides* (WP_011744857.1), *Chlorobium phaeovibrioides* (WP_011890217.1), *Prosthecochloris aestuarii* (WP_012505759.1), *Chlorobium chlorochromatii* (WP_011361998.1), *Chlorobium ferrooxidans* (WP_006365846.1) are deposited in GenBank database [18]. Amino acid sequence of *C. limicola* SOD is the most identical to C. parvum (83%) and *C. limnaeum* (81%) SOD. Interestingly, amino acid sequence of SOD from the representatives of *Chlorobium* genus, particularly, *C. phaeovibrioides*, *C. ferrooxidans*, *C. chlorochromatii* is identical by 76–78%.

Enzymatic activity changed depending of the duration of cultivation and concentrations of copper (II) sulfate in the incubation medium. Superoxide dismutase activity was the highest for the eighth day of cultivation under the influence of all investigated concentrations of copper (II) sulfate. It was established that under the influence of metal salt at concentrations 0.05–0.125 mM SOD activity increases, compared to control. In the case of addition of copper (II) sulfate at concentrations 0.25–0.5 mM to the incubation medium SOD activity was lower during the sixth and the seventh day of cultivation or didn't significantly differ from the control.

Peroxidases have a significant role in the antioxidative defense of bacteria. GenBank database lacks the information about the presence of genes, encoding catalase, in genomes of green photosynthetic bacteria, particularly, in *C. tepidum*, which genome of is fully sequenced and annotated [7]. Enzymes of thioredoxin system are the widespread antioxidants, which control the content of peroxides in bacterial cells [24]. Genes and amino acid sequences of thioredoxin dependent thiol peroxidase (NC_002932.3) and 2-Cys-peroxiredoxin (NC_002932.3) of *C. tepidum* TLS are deposited in the GenBank database [18]. Thiol peroxidases and 2-Cys-peroxiredoxins provide the degradation of long-chain fatty acids peroxides and hydrogen peroxide [25]. Cytochrome *c* peroxidase, which can also be involved in the antioxidative defense, is revealed in *C. chlorochromatii* [18].

Peroxidase activity deacreased with increasing of duration of bacterial growth to eight day (Fig. 5, *B*). During the sixth day peroxidase activity was the highest under the influence of all metal salt concentrations in the incubation medium. On the eighth day of cultivation a dose-dependent decrease of peroxi-

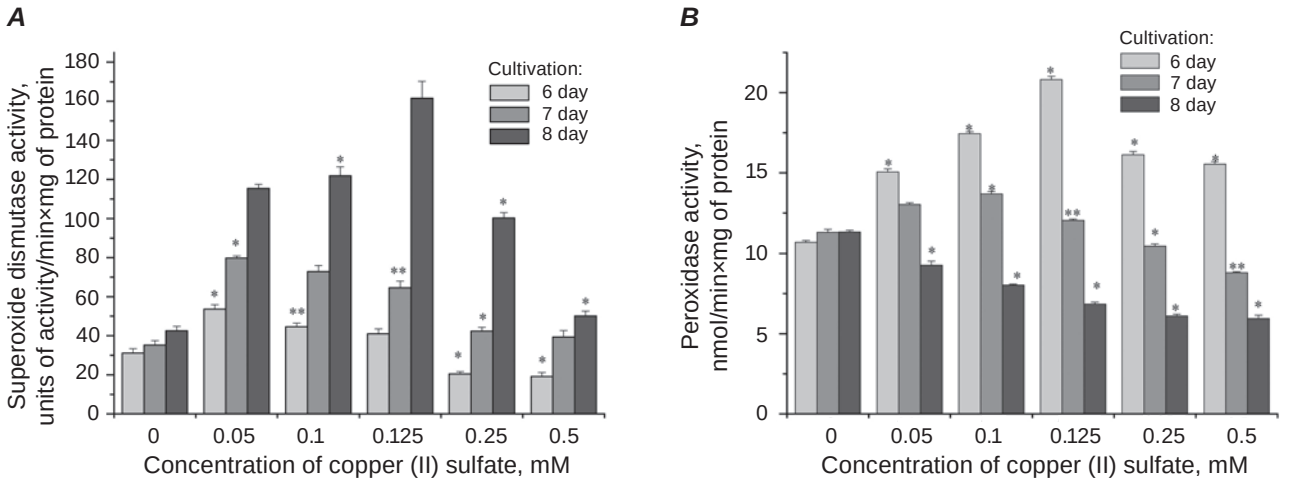

*Fig. 5. Dose- and time-dependent effects of copper (II) sulfate on SOD (**A**) and peroxidase activity (**B**) of cell-free extracts of C. limicola bacteria (*P ≥ 0.95, n = 5; ** P ≥ 0.99, n = 9 – credible changes, compared to control)*

dase activity under the influence of copper (II) sulfate in studied concentrations was detected as compared to control.

The maintenance of the appropriate level of cytoplasm membrane fluidity is an important mechanism of cells adaptation to environmental factors [8]. Changes in the fatty acids composition of membrane lipids, or "homeophase adaptation", are important for the maintenance of the optimal bacterial membrane fluidity under the influence of toxic compounds [8]. As it was established in our previous work [25] cis/trans isomerization of monounsaturated fatty acids and synthesis of cyclopropane fatty acids are the first reactions of *C. limicola* cells adaptation under the influence of Cu^{2+} cations. For the estimation of cytoplasm membrane fluidity, we used the index of cellular lipids unsaturation and the index of membrane viscosity [8]. Under the influence of Cu^{2+} cations at 0.05–0.25 mM concentrations the index of lipids unsaturation increased, and index of membrane viscosity decreased, which indicated the increase of *C. limicol*a cells membrane fluidity compared to control (Table). No significant difference of studied indexes from the control under the influence of Cu^{2+} cations at 0.5 mM concentration was detected, but the accumulation of biomass was shown to decrease by 70%.

In the majority of gram-negative bacteria, the efflux of copper cations from cytoplasm into periplasmic space is provided by Cu^{+}-ATP-hydrolases. In the periplasmic space, copper cations are detoxified by oxidases, metalchaperones, and by RND systems [26]. There are no data on the influence of membrane fluidity on the copper cations transport systems. We suggest that the increase of membrane fluidity induced by 0.05–0.25 mM copper (II) sulfate facilitated the efflux of accumulated Cu^{2+} cations from the cells. Probably, the mechanisms, involved in the regulation of *C. limicola* cells membrane fluidity became damaged when Cu^{2+} concentration was increased to 0.5 mM and in consequence Cu^{2+} cations were accumulated in cells and the growth of biomass was inhibited.

Obtained results indicate that free-radical damage of macromolecules occur in *C. limicola* cells as a result of Cu^{2+} cations accumulations. Under these conditions, the process of photosynthesis – one of the most important metabolic processes of green photosynthesis bacteria is undergoing change. In response to the influence of Cu^{2+} cations in the cells of

Indices of membrane fluidity of C. limicola bacteria under the influence of copper (II) sulfate (M ± m; n = 9)

Indices of membrane fluidity	Concentration of copper (II) sulfate, mM					
	0	0.05	0.1	0.125	0.25	0.5
Lipid unsaturation	0.38 ± 0.02	0.45 ± 0.05	0.48 ± 0.01	0.44 ± 0.03	0.45 ± 0.05	0.38 ± 0.03
Membrane viscosity	58 ± 2	49 ± 2	49 ± 3	54 ± 3	54 ± 1	56 ± 3

C. limicola, GSH is synthesized and enzymes of the antioxidant system function, in particular superoxide dismutase and peroxidase. Important mechanism of *C. limicola* adaptation to the influence of Cu^{2+} cations is the change of membrane fluidity, directed at more effective efflux of metal cations from cell [27].

Under the increase of copper (II) sulfate concentration to 0.25 mM, the activity of antioxidant enzymes was lower compared to the response of the cells under the influence of 0.05 mM copper (II) and the GSSG content was increased, indicating on disturbance of oxidation-reduction homeostasis. We assume that under the influence of 0.25 mM concentration of Cu^{2+} cations adaptation reaction was not sufficiently effective to prevent or to compensate cell damage induced by Cu ions. Under the influence of 0.5 mM copper (II) sulfate, the indexes of membrane fluidity did not differ from the control, but superoxide dismutase and peroxidase activity inhibition and the further decrease of GSH/GSSG ratio were observed followed by the highest Cu^{2+} cations accumulation in cells and a significant decrease of bacteria biomass growth.

We concluded that such indexes as activity of antioxidant enzymes, glutathione content, the content of photosynthetic pigments and membrane fluidity could be used as biochemical indicators of adaptive or toxic effects induced by metal salt in green bacteria cells.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

БІОХІМІЧНІ ІНДИКАТОРИ РЕАКЦІЇ ЗЕЛЕНОЇ ФОТОСИНТЕЗУВАЛЬНОЇ БАКТЕРІЇ *Chlorobium limicola* НА ДІЮ ІОНІВ Cu

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Фотолітотрофні сіркобактерії залучені до функціонування біоценозів та мають біотехнологічний потенціал для біоремедіації забруднених середовищ, однак механізми пошкоджувальної дії ксенобіотиків, зокрема іонів важких металів, та способи адаптації клітин фотолітотрофних бактерій за цих умов не встановлено. У роботі досліджено біохімічні індикатори реакції бактерії *Chlorobium limicola* на дію іонів Cu. Клітини *C. limicola* інкубували впродовж однієї години в буфері, який містив купрум (II) сульфат у концентраціях 0,05–0,5 мМ, та вирощували впродовж 8 діб. Вміст Cu^{2+} в клітинах оцінювали методом атомної абсорбційної спектроскопії. Вивчали активність ензимів антиоксидантного захисту, вміст глутатіону та фотосинтезувальних пігментів, індекси ненасиченості ліпідів та в'язкості як маркерів текучості мембран. Показано, що відповідь зелених фотосинтезувальних бактерій *Chlorobium limicola* на дію Cu^{2+} залежить від концентрації катіона. За дії 0,05 мМ солі металу активність антиокситантних ензи-

мів, співвідношення GSH/GSSG, вміст фотосинтезувальних пігментів та індекс мембранної текучості були вищими порівняно з контролем. За підвищення концентрації сульфату (II) міді до 0,25 мМ активність антиоксидантних ензимів виявилась нижчою, ніж за дії 0,05 мМ Cu^{2+}, а вміст GSSG підвищився. За дії 0,5 мМ Cu^{2+} індекси мембранної текучості не відрізнялись від контролю, проте супероксидазна та пероксидазна активність пригнічувалась, співвідношення GSH/GSSG падало, що супроводжувалось найвищим рівнем акумуляції Cu^{2+} в клітинах та значним пригніченням росту бактеріальної біомаси.

Ключові слова: зелені бактерії, катіони Cu, антиоксидантний захист, текучість мембрани, адаптація.

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