

## CHANGES OF FATTY ACID COMPOSITION OF *CHLOROBIVM LIMICOLA* IMV K-8 CELLS UNDER THE INFLUENCE OF COPPER (II) SULFATE

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**Aim.** To investigate the changes of fatty acid composition of green sulphur bacteria *Chlorobium limicola* IMV K-8 cells at the influence of copper (II) sulfate. **Methods.** Microbiological, biochemical, biometrical. **Results.** The increase of content of long chain saturated fatty acids of *C. limicola* IMV K-8 cells, in particular pentadecanoic, hexadecanoic, heptadecanoic and octadecanoic acids was observed under the influence of copper (II) sulfate in concentrations which caused decrease of biomass accumulation up to 70 %. Among the first reactions of adaptation of *C. limicola* IMV K-8 cells under these conditions are cis/trans isomerisation of monounsaturated fatty acids and synthesis of cyclopropane fatty acids. Maintenance of appropriate level of membrane fluidity is provided by branched chain fatty acids. **Conclusions.** Under the influence of copper (II) sulfate on *C. limicola* IMV K-8 cells fatty acids composition of membranes is changed, which causes the increase of membranes fluidity, and, probably, is contributed to more efficient efflux of  $\text{Cu}^{2+}$  ions.

*Keywords:* green photosynthetic bacteria, *Chlorobium limicola*,  $\text{Cu}^{2+}$  ions, fatty acids.

An increase of anthropogenic influence caused the formation of technogenically transformed territories that occupy large areas. They contain increased amount of organic matters, sulfur compounds and heavy metal ions that affect the vital functions of organisms [1, 2, 3]. Sulfur bacteria, isolated from these territories, could be successfully applied for development of biotechnologies for contaminated environments remediation from organic matter with the simultaneous toxicity neutralization of heavy metal ions and sulfur compounds [4]. Green photosynthetic bacteria *Chlorobium limicola* take an essential part in functioning of biocenoses of man-made water storages, since it influences carbon and sulfur cycles because of using hydrogen sulfide as the electron donor. It results in  $\text{H}_2\text{S}$  elimination from natural water environments [5].

The toxicity of heavy metal ions, in particular ferrum and copper, is caused by their participation in Fenton and Haber-Weiss reactions. It results in reactive oxygen species (ROS) formation. One of the main targets of ROS harmful influence are membranes. It's due to intensification of lipid peroxidation processes and oxidative modification of proteins while bacterial cell damage with free radicals [6, 7, 8]. An important part in bacteria adaptation to the influence of stress factors is caused by the modification of membrane fatty acid composition [9]. Many studies are devoted to investigations the changes of fatty acid composition of bacterial membranes of genera *Pseudomonas* and

*Vibrio* at the influence of toxic organic substances, in particular toluene, xylol, nitrotoluene, 4-chlorophenol etc [10, 11, 12, 13]. It has been determined that these microorganisms adapt to the influence of toxic organic compounds because of *cis/trans* isomerization of monounsaturated fatty acids [14]. The change of the ratio of *anteiso*-fatty acids to *iso*-fatty acids was found in *Arthrobacter chlorophenolicus* bacteria at the influence of high temperatures and various phenol concentrations [15, 16]. The reactions of synthesis of cyclopropane fatty acids are important for stabilization the membrane of bacteria under stressful environmental conditions [17, 18]. Overexpression of *cfa* genes of *Clostridium acetobutylicum* cells causes increase of cyclopropane fatty acids content and resistance to butanol [19]. It is known that changes of degree of saturation of cell lipids due to the influence of toxic compounds are one of the main mechanisms of gram-negative bacteria adaptation at these conditions. An increase of the degree of lipids saturation of *Geobacter sulfurreducens* bacteria, grown with 2,4-dichlorophenol, benzene, toluene, xylene and ethylbenzene has been determined [20]. There is almost no information according to the influence of heavy metals compounds on fatty acid composition of bacterial lipids with the exception of several studies [20, 21, 22]. The investigation [21] describes an increase of the degree of lipids saturation in *Klebsiella pneumoniae* and *Enterobacter intermedius* bacteria at the influence of cadmium, nickel, zinc and copper ions.

Green photosynthetic bacteria are often found in the consortia with sulfur-reducing bacteria *Desulfuromonas acetoxidans* [23]. It was established that at the influence of Fe (III) ions on the membranes of bacteria *D. acetoxidans* IMV B-7384 the unsaturation of cellular lipids decreases and the content of cyclopropane fatty acids and branched chain fatty acids increases. It allows the cell to diminish the probability of cellular lipids damage and maintain an appropriate level of membrane fluidity [22]. The influence of heavy metal ions on the fatty acid composition of photosynthetic bacteria cells, in particular *C. limicola*, has not been investigated. The determination of adaptation reactions of bacteria isolated from technogenically transformed territories is necessary for understanding the ways of metabolism regulation of microorganisms of aquatic environments contaminated by heavy metal compounds. Therefore, the aim of work was to investigate the changes of fatty acid composition of *C. limicola* IMV K-8 at the influence of copper (II) sulfate.

**Materials and methods.** Bacteria *Chlorobium limicola* IMV K-8, isolated from Yavorivske lake (Lviv region, Ukraine) have been investigated. *C. limicola* IMB K-8 belongs to the collection of microorganisms of D.K. Zabolotny Institute of Microbiology and Virology of NAS of Ukraine [24]. The bacteria were grown in the GSB cultivation medium which contains g per liter:  $\text{KH}_2\text{PO}_4$  – 0.03;  $\text{NH}_4\text{Cl}$  – 0.34;  $\text{KCl}$  – 0.34;  $\text{CaCl}_2 \times 2\text{H}_2\text{O}$  – 0.15;  $\text{MgSO}_4 \times 7\text{H}_2\text{O}$  – 0.5. After sterilization were added 10%  $\text{NaHCO}_3$  – 25 ml;  $\text{Na}_2\text{S} \times 9\text{H}_2\text{O}$  – 5ml; 10% sodium pyruvate – 10 ml; 10% sodium acetate – 10 ml; solution of vitamin  $\text{B}_{12}$  (2 mg per 100 ml of  $\text{H}_2\text{O}$ ) – 1 ml; solution of microelements – 1 ml. Microelements solution contain per liter: 25%  $\text{HCl}$  – 10 ml;  $\text{FeSO}_4 \times 7\text{H}_2\text{O}$  – 2.0 g;  $\text{CoCl}_2 \times 6\text{H}_2\text{O}$  – 190 mg;  $\text{MnCl}_2 \times 4\text{H}_2\text{O}$  – 100 mg;  $\text{ZnCl}_2$  – 70 mg;  $\text{Na}_2\text{MoO}_4 \times 4\text{H}_2\text{O}$  – 36 mg;  $\text{NiCl}_2 \times 6\text{H}_2\text{O}$  – 24 mg;  $\text{H}_3\text{BO}_3$  – 6 mg;

$\text{CuCl}_2 \cdot x \text{2H}_2\text{O}$  – 2 mg. pH of medium were 6.7–6.8. Bacteria were cultivated under microaerophilic conditions and constant illumination (wavelength 700–800 nm, intensity – 40 lux), at a temperature 28 °C until the exponential phase of growth [5, 6]. Microaerophilic conditions have been created by filling the test tubes with medium in the way that exclude the presence of air under the rubber stopper. The oxygen content in the medium was reduced by its rapid cooling after sterilization [25].

For investigation the influence of copper (II) sulfate on fatty acids content of *C. limicola* IMV K-8, cells were precipitated by centrifugation (3000 g, 60 min) in sterile centrifuge tubes and washed with 50 mM Tris-HCl buffer (pH 7.0). The precipitated cells were incubated for one hour in 50 mM Tris-HCl buffer (pH 7.0) containing copper (II) sulfate in concentration 0.05-0.50 mM. Investigated metal salt was not added into control sample. After incubation bacterial cells were precipitated by centrifugation (3000 g, 60 min) in sterile centrifuge tubes, washed with 50 mM Tris-HCl buffer (pH 7.0) and added into GSB medium (inoculum density was 0.2 g /l). Selected concentrations of copper (II) sulfate cause reduction of biomass accumulation from 10 % to 70 % that allows to estimate the changes of bacterial cells fatty acid composition with the increase of inhibitory effect [6]. On the seventh day of cultivation biomass was precipitated by centrifugation (3000 g, 60 min), washed with 50 mM Tris-HCl buffer (pH 7.0), resuspended in a cooled 50 mM Tris-HCl buffer (pH 7.5) and used for obtaining the cell-free extracts. It was achieved by cells destruction with application of the ultrasound homogenizer UZDN-2T (22 kHz, 5 min, 0 °C). Extraction of lipids was carried out according to the method described by Bligh and Dyer [26]. After extraction of lipids, the chloroform and methanol mixture was dried on a water bath. 2 ml of hexane was added into the residue and stirred. 0.5 ml of 2 M sodium methylate was added to the mixture, stirred and heated on a water bath (50 °C, 5 min). The top layer was taken and filtered through a paper filter. Hexane was dried on a water bath at the temperature 100 °C. Fatty acid methyl esters were dissolved in methanol and analyzed by using a gas chromatograph (Agilent Technologies, USA) with a flame-ionization detector and the capillary column HP-1 (size 50 m × 0.320 mm, Agilent Technologies, USA). The thickness of phase was 1 μm, the temperature range was + 130 ... + 250 °C, the temperature gradient was + 4 °C / min. Helium was used as a gas-carrier. The results were interpreted by using a standard set of methyl esters of bacterial fatty acid (Supelco, USA). The content of separate fatty acids was determined as a percentage of the total peak area. The degree of unsaturation of fatty acids was determined as described in [27].

Statistical analysis of the results was carried out. The main statistical indices of the obtained data (arithmetic mean – M, standard deviation of the arithmetic mean – m) were calculated. To assess the reliability of the difference between the statistical characteristics of the three alternative sets of data, Student coefficient was counted. The difference was considered reliable when the reliability index P was > 0.95 [28]. MS Excel 2003 and Origin 8 were applied for data processing.

**Results.** The main fatty acids of *C. limicola* cells IMV K-8 incubated in the medium without copper (II) sulfate, were tetradecanoic (14:0), hexadecanoic (16:0) and *cis*-9-hexadecanoic (16:1  $\omega$ 9c) acids. These fatty acids form more than 64 % of all fatty acid content of *C. limicola* IMV K-8 cells. Branched chain fatty acids were found in the cells of *C. limicola* IMV K-8. There are 12- and 13-methyltetradecanoic acids (*iso*-15:0 and *anteiso*-15:0 respectively), 14-methylpentadecanoic (*iso*-16:0) and 15-methylhexadecanoic acid (*iso*-17:0) (Table 1). The presence of fatty acids with straight and branched chains in *C. limicola* IMV K-8 indicates on its probable involvement into the biosynthesis of two synthases (synthetase of branched chain fatty acids and synthetase of straight chain fatty acids), which function independently and use different primers for biosynthesis [29]. Probably, the synthesis of unsaturated fatty acids (16:1  $\omega$ 9c, *cis*18:1  $\omega$ 9c and *trans* 18:1  $\omega$ 9c) occurs in the anaerobic desaturation path [30].

Table 1 shows the fatty acid composition of two other strains, in particular *C. limicola* DSM 245 and *C. limicola* BF8010, according to the results described in [31]. Fatty acids composition of *C. limicola* IMV K-8 differs from it of *C. limicola* DSM 245 and *C. limicola* BF8010. There were identified dodecanoic (12:0), 12-methyltetradecanoic (*iso*-15:0), 14-methylpentadecanoic

**Table 1**

**Fatty acid composition of *C. limicola* IMV K-8 cells**

Fatty acids	<i>C. limicola</i> IMV K-8*	<i>C. limicola</i> DSM 245**	<i>C. limicola</i> BF8010**	The average content for green photosynthetic bacteria**
12:0	0.14	-	-	nd
14:0	29.49	16.61	13.00	trace quantities – 27.10
16:0	11.18	19.93	24.25	3.52–29
18:0	1.79	1.60	0.87	trace quantities – 3.30
15:0	0.94	14.14	9.38	2.74–14.14
17:0	0.70	0.28	0.22	nd
<i>iso</i> -15:0	0.72	-	-	nd
<i>anteiso</i> -15:0	1.02	-	0.48	nd
<i>iso</i> -16:0	1.07	-	-	nd
<i>iso</i> -17:0	0.31	-	-	nd
<i>anteiso</i> -17:0	-	-	0.25	nd
<i>cy</i> -17:0	0.46	-	-	0.70–21
14:1	-	0.30	0.50	nd
16:1 $\omega$ 9c	23.59	38.26	41.81	37.3–64.0
<i>cis</i> 18:1 $\omega$ 9c	0.66	2.13	2.82	trace quantities – 15.59
<i>trans</i> 18:1 $\omega$ 9c	5.29	-	-	nd
14:2-OH	0.55	-	-	nd

**Note:** The content of fatty acids is expressed as the percentage from the total content of fatty acids; \* - the error in the three alternative sets did not exceed 5%; \*\* – results obtained by analyzing fatty acid methyl esters by gas chromatography and highlighted in the review [31]; “nd” – no data; «-» – fatty acid was not detected. References strains were cultivated in medium which contain Na<sub>2</sub>S x 9H<sub>2</sub>O as electron donor and sodium bicarbonate as carbon donor.

(*iso*-16:0), 15-methylhexadecanoic (*iso*-17:0) and *trans*-9-octadecanoic (*trans* 18:1  $\omega$ 9c) acids in the cells of *C. limicola* IMV K-8 that were not detected in *C. limicola* DSM 245 and *C. limicola* BF8010. Content of the main fatty acids of *C. limicola* IMV K-8 cells also differs from the content of these fatty acids in the cells of *C. limicola* DSM 245 and *C. limicola* BF8010, as well as from the average content of fatty acids in the cells of green photosynthesis bacteria. It is known that the composition of fatty acids is not only species-specific, but also a strain-specific particularity.

The content of fatty acids depends on the bacterial growth phase and the composition of growth medium [31]. We assume that the composition and the content of fatty acids of *C. limicola* IMV K-8 cells were formed in the process of evolution due to the adaptation of these bacteria to the influence of environmental factors (in particular, heavy metal ions) at the territory of sulfur deposit that was transformed into Yavorivske lake, where this strain was isolated.

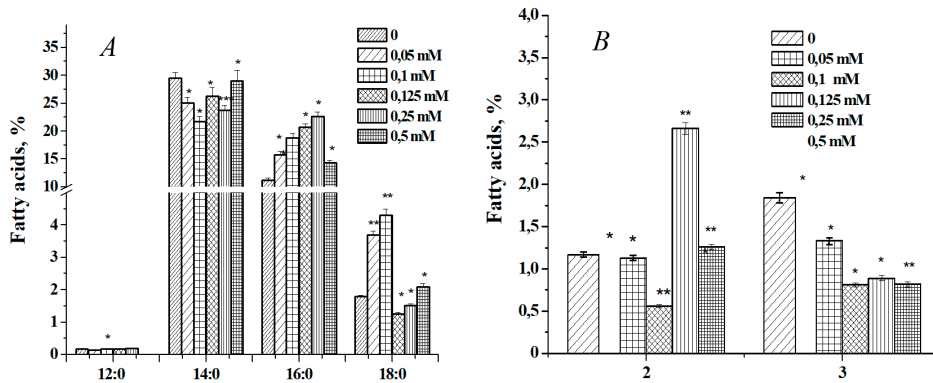
For interpretation the effect of copper (II) sulfate on *C. limicola* IMV K-8 bacteria, identified fatty acids were divided into even-numbered saturated fatty acids with, odd-numbered saturated fatty acids, branched chain fatty acids, cyclopropane fatty acids, hydroxy acids and unsaturated fatty acids.

Addition of copper (II) sulfate as a stress factor into the incubation medium of *C. limicola* IMV K-8 caused changes of fatty acid composition in comparison with control. Among even-numbered saturated fatty acids, the content of hexadecanoic and octadecanoic acids changed under the influence of copper (II) sulfate. The content of hexadecanoic acid enhanced while increase of metal salt concentration from 0.05 to 0.25 mM.

Hexadecanoic acid content of the cells, which were incubated with 0.5 mM copper (II) sulfate, also exceeded its value in a control, but decreased by 30-40% in comparison with its content at the influence of 0.05-0.25 mM copper (II) sulfate. The increase of octadecanoic acid content by 2 and 2.4 times was observed for *C. limicola* IMV K-8 cells, incubated in a medium containing 0.05 mM and 0.1 mM copper (II) sulfate respectively. Increase of metal salt concentration in the incubation medium caused that the content of octadecanoic acid either slightly decreased or did not change in comparison with control. The content of dodecanoic acid in bacterial cells, which were incubated in a medium with copper (II) sulfate, did not differ significantly comparing to control. A slight decrease of the tetradecanoic acid content was observed at the influence of all investigated concentrations of copper (II) sulfate (Fig. 1A).

The content of pentadecanoic and heptadecanoic acids in *C. limicola* IMV K-8 cells increased at the influence of all investigated concentrations of copper (II) sulfate (Fig. 1, B). The highest content of pentadecanoic acid was observed while bacterial cells incubation in the medium with 0.25 mM copper (II) sulfate. The content of pentadecanoic acid increased by 78% at these conditions. The increase of heptadecanoic acid content depended on the concentration of metal salt in the incubation medium. It increased by 2.6 and 1.9 times at the influence of 0.05 and 0.1 mM copper (II) sulfate respectively. The addition of 0.125-0.5 mM copper (II) sulfate caused a slight increase of heptadecanoic acid content in comparison with control.





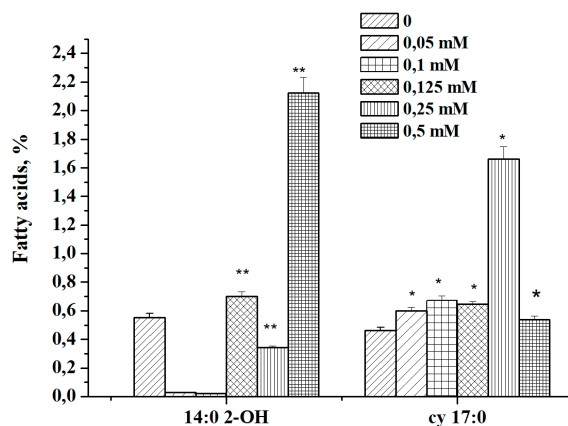
**Fig. 1. The content of even-numbered saturated fatty acids (A) and odd-numbered fatty acids (B) of *C. limicola* IMV K-8 cells at the influence of copper (II) sulfate (\* -  $p \geq 0.95$ ,  $n = 3$ ; \*\* -  $p \geq 0.99$ ;  $n = 3$  – herein and further are reliable changes in comparison with the control)**

Consequently, the addition of copper (II) sulfate into the incubation medium causes increase of the content of fatty acids with long carbon chain, in particular pentadecanoic, hexadecanoic and octadecanoic acids, and almost does not change the content of dodecanoic and tetradecanoic acids. It is known that fatty acids with a long carbon chain reduce fluidity of the cytoplasmic membrane [9]. We assume that such changes of fatty acid composition are caused by adaptive response of *C. limicola* IMV K-8 cells to the influence of copper (II) sulfate.

2-hydroxytetradecanoic acid, which belongs to hydroxy acids, was revealed in the cells of *C. limicola* IMV K-8 (Fig. 2). While bacteria cultivation without addition of copper (II) sulfate the content of 2-hydroxytetradecanoic acid equaled 0.57 % of all cellular fatty acids. At the addition of metal salt into the incubation medium in concentration 0.05–0.1 mM the trace amounts of 2-hydroxytetradecanoic acid were detected. Although at the influence of 0.125–0.5 mM copper (II) sulfate the content of investigated fatty acid increased by 1.3–3.8 times. Hydroxy fatty acids are components of the lipopolysaccharide in the cells of gram-negative bacteria [21]. Probably, due to the effect of copper (II) sulfate the changes of lipopolysaccharide fatty acid composition occurs, and one of such modifications is the change of 2-hydroxytetradecanoic acid content.

Cyclopropane fatty acids are needed to stabilize the bacteria membrane at stress conditions. Synthesis of these fatty acids occurs by binding the methyl group to a double bond in the chain of unsaturated fatty acid. The biophysical properties of cyclopropane fatty acids are similar to those of *cis*-unsaturated fatty acids, but cyclopropane acids are more resistant to the influence of reactive oxygen species than *cis*-unsaturated fatty acids [29].

*Cis*-9,10-methylene hexadecanoic acid, which belongs to the group of cyclopropane fatty acids, was detected in *C. limicola* IMV K-8 cells. Changes of the content of *cis*-9,10-methylene hexadecanoic acid of *C. limicola* IMV K-8 cells depended on the concentration of metal salt in the incubation medium. At the addition of 0.05–0.125 mM copper (II) sulfate the increase by 29–45%



**Fig. 2.** The content of hydroxy acids (14:0 2OH) and cyclopropane fatty acids (cy 17: 0) in *C. limicola* IMV K-8 cells at the influence of copper (II) sulfate (\* -  $p \geq 0.95$ ,  $n = 3$ ; \*\* -  $p \geq 0.99$ ,  $n = 3$ )

of *cis*-9,10-methylene hexadecanoic acid content was observed in comparison with control. The addition of 0.25 mM copper (II) sulfate into the incubation medium resulted in a 3.6-fold increase of the content of *cis*-9,10-methylene hexadecanoic acid compared to control.

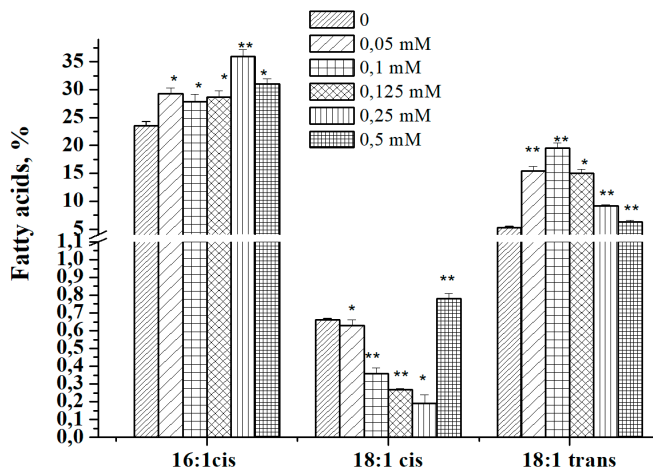
The predecessor of the synthesis of *cis*-9,10-methylenehexadecanoic acid is *cis*-9-hexadecanoic acid [19]. Thus, as the response of *C. limicola* IMV K-8 bacteria to the influence of copper (II) sulfate the conversion of monounsaturated fatty acids to cyclopropane acids occurs.

*Cis*-9-hexadecanoic, *cis*-9-octadecanoic and *trans*-9-octadecanoic acids, which belongs to the group of unsaturated fatty acids, were identified in *C. limicola* IMV K-8 cells.

The content of *cis*-9-hexadecanoic acid in *C. limicola* IMV K-8 cells increased by 17–45% at the influence of investigated concentrations of copper (II) sulfate. *Cis*-9-hexadecanoic acid is one of the main unsaturated fatty acids of gram-negative bacteria, including bacteria of *Chlorobium* genus. Its content of various representatives of green photosynthesis bacteria is 37.3–64.0% [31]. We assume that at these conditions there occurs the renovation of membrane lipid composition, which results in the synthesis of *cis*-9-hexadecanoic acid.

The isomerization of *cis*-isomers into *trans*-isomers of unsaturated fatty acids is considered as an alternative way of controlling the fluidity of the cytoplasmic membrane at the influence of toxic compounds [32]. The increase of the *cis/trans* index at the influence of various stress factors on the cell, such as osmotic stress, heavy metal ions, heat shock and antibiotics, which destroy the membranes, was determined [33, 34, 35]. The double bond in *cis*-configuration forms the bend of the chain at an angle 30°, which makes impossible the dense packaging of solid phospholipids in the membrane and low phase transition temperature of membrane. Membranes that contain unsaturated fatty acids in *cis*-configuration are characterized by relatively high fluidity. In contrast, fatty acids in the *trans*-configuration lose bend in the chain and integrate into the membrane as saturated fatty acids. The isomerization of *cis*-isomers of unsaturated fatty acids into *trans*-isomers reduces the fluidity of the cytoplasmic membrane [36].

While addition of 0,05–0,25 mM copper (II) sulfate into the incubation medium the content of *cis*-9-oktadekanoic acid of bacterial cells decreased from 5 % to 71 %, with increasing the metal salt concentration in comparison with control (Fig. 3).



**Fig. 3** The content of unsaturated fatty acids in *C. limicola* IMV K-8 cells at the influence of copper (II) sulfate (\* -  $p \geq 0.95$ ,  $n = 3$ ; \*\* -  $p \geq 0.99$ ,  $n = 3$ )

At these conditions the content of *trans*-9-oktadekanoic acid increases, which probably indicates on *cis/trans* isomerisation of *cis*-9-oktadekanoic acid. However, at the influence of 0.5 mM metal salt there was determined a slight increase (18 %) of *cis*-9-oktadekanoic and *trans*-9-oktadekanoic acids content. Ferrum-containing enzyme *cis/trans*-isomerase catalyzes the double-bond isomerization reaction [14]. It is known that enzymes that contain ferrous ions in the active center are susceptible to oxidative damage [37]. In our previous studies, it was found that a significant amount of lipid peroxidation products, which are capable to damage proteins, lipids and nucleic acids, are formed in the cells of *C. limicola* IMV K-8 at the influence of copper (II) sulfate [6]. We assume that activity of *cis/trans* isomerase decreased at the influence of 0.5 mM  $\text{CuSO}_4$  because of its interaction with lipid peroxidation products or as a result of damage of genes responsible for *cis/trans* isomerase expression by the free radical compounds. Also *C. limicola* IMV K-8 cells may accumulate copper ions after incubation that initiates new free radical reactions.

Branched chain fatty acids (*iso*-12 and *anteiso*-13-methyltetradecanoic acids (*iso*-15:0 and *anteiso*-15:0, respectively)) regulate cytoplasmic membrane fluidity in bacterial cells. At the addition of metal salt into the incubation medium, the content of *iso*-15:0 and *anteiso*-15:0 acids varied depending on the concentration of metal salt in the medium (Fig. 4). For example, at the influence of 0.05 mM and 0.1 mM copper (II) sulfate, their content was higher in comparison with control. The addition of 0.125 mM and 0.25 mM copper salt caused the decrease of content of *iso*-15:0 and *anteiso*-15:0 acids with the increase of metal salt concentration in the medium. At the influence of these concentrations of copper (II) sulfate, the *iso*-15:0 acid content decreased by 17 % and 71 % respectively. The increase of *iso*-15:0 and *anteiso* -15:0 acids



content by 41 % and 32 % was observed at the addition of 0.5 mM copper (II) sulfate in comparison with control.

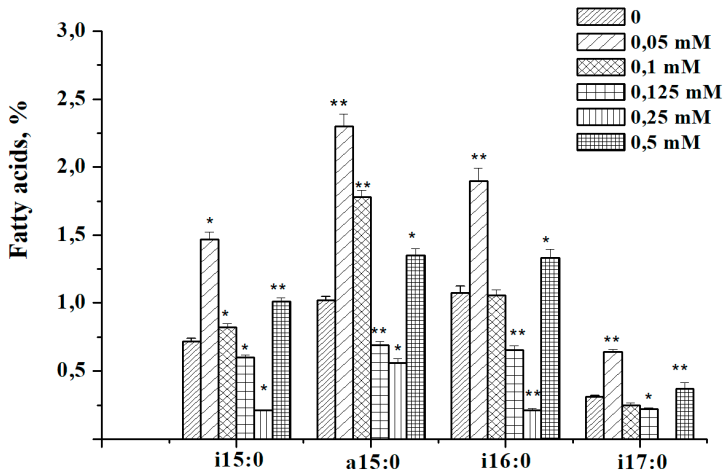


Fig. 4. The content of branched fatty acids of *C. limicola* IMV K-8 cells under the influence of copper (II) sulfate (\* -  $p \geq 0.95$ ,  $n = 3$  \*\* -  $p \geq 0.99$ ,  $n = 3$ )

Significant changes of the content of 14-methylpentadecanoic acid (*iso*-16:0) in bacterial cells at the influence of various concentrations of copper (II) sulfate have been determined in comparison with control. While the addition of 0.05 mM metal salt, the content of this acid increased by 77 % in comparison with control. It is worth to note that under the influence of 0.1; 0.125 and 0.25 mM copper (II) sulfate, the content of *iso*-16:0 decreased in comparison with control. Significant growth (by 24 %) of 14-methylpentadecanoic acid content was observed while 0.5 mM copper (II) sulfate addition into the growth medium.

In addition to *iso*-15:0, *anteiso*-15:0 and *iso*-16:0 fatty acids of *C. limicola* IMV K-8 cells, the *iso*-15-methylhexadecanoic acid (*iso*-17:0) has been investigated. Changes of *iso*-17:0 acid content of *C. limicola* IMV K-8 cells depended on the concentration of metal salt in the incubation medium. At the influence of 0.05 mM and 0.5 mM copper (II) sulfate the content of *iso*-17:0 acid increases by 108 % and 21 %. While addition of 0.1 mM and 0.125 mM of metal salt the content of investigated fatty acid decreased in comparison with control. *Iso*-17:0 acid was not detected under the influence of 0.25 mM copper (II) sulfate.

**Discussion.** Analysis of the results shows that as the response to the influence of copper (II) sulfate on the cells of *C. limicola* IMV K-8 the content of saturated long chain fatty acids, in particular pentadecanoic, hexadecanoic, heptadecanoic and octadecanoic acids increases. Cell adaptation reactions of investigated bacteria to the influence of  $\text{Cu}^{2+}$  ions are *cis/trans* isomerization of monounsaturated fatty acids and the conversion of monounsaturated

fatty acids into cyclopropane fatty acids. Since *cis/trans* isomerization and formation of cyclopropane fatty acids occurs post-synthetically, we assume that these reactions are among the first protection mechanisms of *C. limicola* IMV K-8 cells at the influence of  $\text{Cu}^{2+}$  ions. However, such changes in fatty acid composition of lipids can lead to the decrease of membrane fluidity. Therefore, investigated bacteria cells synthesize branched chain fatty acids that increase membrane fluidity and possess higher resistance to the influence of reactive oxygen species. Fatty acid composition of *C. limicola* IMV K-8 lipopolysaccharide under the influence of copper (II) sulfate also undergoes changes, which are observed by the increase of 2-hydroxytridecanoic acid content.

**Conclusions.** As the response of *C. limicola* IMV K-8 cells to the influence of copper (II) sulfate fatty acid composition of membranes is changed, which causes the increase of membranes fluidity, and, probably, is contributed to more efficient efflux of  $\text{Cu}^{2+}$  ions.

## ЗМІНИ ЖИРНОКИСЛОТНОГО СКЛАДУ КЛІТИН *CHLOROBIVM LIMICOLA* IMB K-8 ЗА ВПЛИВУ КУПРУМ (II) СУЛЬФАТУ

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### Резюме

**Мета.** Встановити зміни жирнокислотного складу клітин зелених сіркобактерій *Chlorobium limicola* IMB K-8 за впливу купрум (II) сульфату. **Методи.** Мікробіологічні, біохімічні, біометричні. **Результати.** Встановлено зростання вмісту довголанцюгових насичених жирних кислот, зокрема пентадеканової, гексадеканової, гептадеканової і октадеканової, у клітинах *C. limicola* IMB K-8 за впливу купрум (II) сульфату у концентраціях, які зумовлювали зниження накопичення біомаси до 70 %. Одними з перших реакцій адаптації клітин *C. limicola* IMB K-8 за цих умов є *cis/trans* ізомеризація мононенасичених жирних кислот і синтез жирних кислот з циклопропановим кільцем. Підтримання необхідного рівня текучості мембрани за цих умов забезпечують жирні кислоти з розгалуженим карбоновим ланцюгом. **Висновки.** Під впливом купрум (II) сульфату на клітини *C. limicola* IMB K-8 змінюється жирнокислотний склад мембран, що забезпечує зростання їх текучості, та, ймовірно, сприяє більш ефективному викачуванню іонів  $\text{Cu}^{2+}$  з клітини.

**Ключові слова:** зелені фотосинтезувальні бактерії, *Chlorobium limicola*, іони  $\text{Cu}^{2+}$ , жирні кислоти.

# ИЗМЕНЕНИЯ ЖИРНОКИСЛОТНОГО СОСТАВА КЛЕТОК *CHLOROBIVM LIMICOLA* ИМВ К-8 ПОД ВЛИЯНИЕМ КУПРУМ (II)СУЛЬФАТА

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## Резюме

**Цель.** Установить изменения жирнокислотного состава клеток зеленых серобактерий *Chlorobium limicola* ИМВ К-8 под влиянием купрум (II) сульфата. **Методы.** Микробиологические, биохимические, биометрические. **Результаты.** Установлено увеличение содержания насыщенных жирных кислот с длинной цепью, в частности пентадекановой, гексадекановой, гептадекановой и октадекановой, в клетках *C. limicola* ИМВ К-8 под влиянием купрум (II) сульфата в концентрациях, которые повлекли снижение накопления биомассы до 70%. Одними из первых реакций адаптации клеток *C. limicola* ИМВ К-8 в этих условиях является *цис/транс* изомеризация мононенасыщенных жирных кислот и синтез жирных кислот с циклопропановым кольцом. Поддержание необходимого уровня текучести мембраны при этих условиях обеспечивают жирные кислоты с разветвленной карбоновой цепью. **Выводы.** Под влиянием купрум (II) сульфата на клетки *C. limicola* ИМВ К-8 меняется жирнокислотный состав мембран, что обеспечивает возрастание их текучести, и, вероятно, способствует более эффективному выкачиванию ионов  $\text{Cu}^{2+}$  из клетки.

**Ключевые слова:** зеленые фотосинтезирующие бактерии, *Chlorobium limicola*, ионы  $\text{Cu}^{2+}$ , жирные кислоты.

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