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RELATIONSHIPS BETWEEN PROOXIDANT AND ANTIOXIDANT PARAMETERS OF BACTERIA OF SULFUR CYCLE UNDER THE INFLUENCE OF HEAVY METAL COMPOUNDS

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INTRODUCTION

Man-made environments are characterized by a complex of unfavorable factors for living organisms. Such areas often contain high concentrations of heavy metal compounds, chlorides, nitrates, toxic organic compounds, hydrogen sulfide, sulfates etc. Considering the extreme conditions of existence, we can assume that in the cells of microorganisms that inhabit these environments, there are effective mechanisms for adaptation to adverse conditions. Bacteria isolated from man-made environments are resistant to environmental factors and are often characterized by valuable biotechnological properties.

Isolated from Yavorivske lake (Lviv region, Ukraine), that was created in result of flooding of the sulfur quarry, green photosynthetic bacteria *Chlorobium limicola* IMV K-8 use hydrogen sulfide formed in the process of sulfur reduction by sulfate- and sulfur-reducing bacteria as an electron donor in the process of anoxygenic photosynthesis, thus providing purification of environment from this toxic compound. These bacteria produce glycogen and form electric current during growth in different wastewaters, so they can be considered as promising objects of ecobiotechnology[1]. Bacteria *Desulfuromonas acetoxidans* IMV B-7384, that were also isolated from this lake[2], are able to oxidize organic compounds in the tricarboxylic acid cycle with simultaneous reduction of

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[1] Segin T., Hnatush S., Maslovska O., Komplikevych S. Synthesis of glycogen by *Chlorobium limicola* IMV K-8 during growth in wastewater. Visnyk of Lviv University. Biological series. 2020. Vol. 83. P. 67–73.

[2] Гудзь С. П., Гнатуш С. О., Мороз О. М., Перетятко Т. Б., Василів О. М. Свідоцтво про депонування штаму бактерій *Desulfuromonas acetoxidans* Ya-2006 у Депозитарії Інституту мікробіології і вірусології ім. Д. К. Заболотного НАН України з наданням реєстраційного номеру ІМВ В-7384 від 10 квітня 2013 року.

extracellular electron acceptors, in particular Fe (III) ions[3]. The isolated strain is resistant to heavy metal compounds (in particular, iron, copper, cobalt, nickel) in concentrations exceeding the maximum permeasible concentration and able to oxidize organic compounds (acetate, propionate, long chain fatty acids, etc.) with simultaneous reduction of sulfur[4]. These microorganisms are characterized by exoelectrogenic properties during growth on infiltrates of the solid waste landfill and wastewater of an alcohol or yeast plant and are able to remediation of these substrates from organic pollution[5]. Sulfur-reducing bacteria *D. acetoxidans* together with green photosynthetic bacteria *C. limicola* play an important role in the sulfur cycle, as they are involved in the reduction and oxidation link of this cycle[6,7]. The combination of different types of metabolism, in particular phototrophic and chemotrophic, using photosynthetic bacteria *C. limicola* and heterotrophic exoelectrogens *D. acetoxidans* is a new approach of optimization of the process of exoelectrogenesis[8,9].

Pollution of the environment with heavy metal compounds has reached a significant scale. Copper and iron compounds are one of the most

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[3] Мороз О., Яворська Г., Муравель Н., Клим І. Відновлення феруму (III) сульфатвідновлювальними та сірковідновлювальними бактеріями. Біологічні Студії / Studia Biologica. 2012. Т. 6. № 2. С. 161–172.

[4] Чайка О. М., Перетятко Т. Б., Гудзь С. П. Сірковідновлювальні бактерії водойм Язівського сіркового родовища. Науковий вісник Ужгородського університету Серія Біологія. 2010. Вип. 28. С. 52–55.

[5] Hnatush S. O., Maslovska O. D., Segin T.B., Vasyliv O. M., Kovalchuk M. M., Malovanyy M. S. Waste water treatment by exoelectrogenic bacteria, which were isolated from technogenically transformed territories. Ecological Questions. 2020. Vol. 31, № 1. P. 35–44. http://doi. org/10.12775/EQ.2020.005

[6] Madigan M. T. The *Chlorobiaceae*, *Chloroflexaceae*, and *Heliobacteriaceae*. *Modern Topics in the Phototrophic Prokaryotes* / M. T. Madigan, N.A.V. Schaaf, W. M. Sattley; (eds) Hallenbeck P. Cham.: Springer, 2017. P. 139–161. https://doi.org/10.1007/978-3-319-46261-5_4

[7] Мороз О., Гуль Н., Галушка А., Звір Г., Борсукевич Б. Використання різних акцепторів електронів бактеріями *Desulfuromonas sp.*, виділеними з озера Яворівське. Вісн. Львів. ун-ту. Сер.біол. 2014. Вип. 65. С. 322–334.

[8] Badalamenti J. P., Torres C. I., Krajmalnik-Brown R. Coupling dark metabolism to electricity generation using photosynthetic cocultures. Biotechnology and Bioengineering. 2013. Vol. 9999. P. 1–9. http://doi. org/10.1002/bit.25011

[9] Hnatush S. O., Maslovska O. D., Segin T. B., Vasyliv O. M., Kovalchuk M. M., Malovanyy M. S. Waste water treatment by exoelectrogenic bacteria, which were isolated from technogenically transformed territories. Ecological Questions. 2020. Vol. 31, № 1. P. 35–44. http://doi. org/10.12775/EQ.2020.005

common pollutants[10,11]. Numerous studies have been conducted on the effects of heavy metal compounds on the cells of microorganisms and protection strategies under these conditions. It was found that the main strategies for counteracting the cells of microorganisms under the influence of heavy metal compounds are extracellular sequestration of metal ions, biotransformation into insoluble or less toxic metal compounds, intense efflux, synthesis of metallothioneins, exopolysaccharides etc.[12]. One of the main causes of cell damage and death due to ROS is lipid peroxidation and oxidative modification of proteins[13]. Antioxidant defense systems function to counteract the effects of ROS in anaerobic microorganisms[14]. Cell membranes are one of the main targets of heavy metal ions, so the adaptive mechanisms to the influence of their compounds are to maintain the appropriate level of membrane fluidity. Changes in the fatty acid composition of membrane lipids are the most important reactions of the cell under oxidative stress[15].

The mechanisms of damage to cellular structures and the adaptation response of the typical member of the family *Chlorobiaceae – C. limicola* and sulfur-reducing exoelectrogens *D. acetoxidans* under the influence of these heavy metals ions have not been established. The authors have been working on this issue for many years. Multiple complementary models of the influence of chemical pollutants on the physiological and biochemical properties of microorganisms and prediction of their adaptive potential to stressors will be valuable not only to cope with pollution through new ecobiotechnologies, but also to scientifically substantiate conservation and

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[10] Briffa J., Sinagra E., Blundell R. Heavy metal pollution in the environment and their toxicological effects on humans. Heliyon. 2020. Vol. 6. Iss. 9. P. e04691. https://doi.org/10.1016/j.heliyon.2020.e04691

[11] Gadd G. M. Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology. 2010. Vol. 156. P. 609–643. https://doi.org/10.1099/mic.0.037143-0

[12] Mathivanan K., Chandirika J.U., Vinothkanna A., Yin H., Liu X., Meng D. Bacterial adaptive strategies to cope with metal toxicity in the contaminated environment. A review. Ecotoxicol. Environ. Saf. 2021. Vol. 226. P. 112863. https://doi.org/10.1016/j.ecoenv.2021.112863

[13] Ezraty B., Gennaris A., Barras F., Collet J. Oxidative stress, protein damage and repair in bacteria. Nat. Rev. Microbiol. 2017. Vol. 15, № 7. P. 385–396. https://doi.org/10.1038/nrmicro.2017.26

[14] Скороход І., Курдиш І. Низькомолекулярні антиоксиданти мікроорганізмів. Мікробіологічний журнал. 2014. Вип. 76. С. 48–59. http://nbuv.gov.ua/UJRN/MicroBiol_2014_76_3_10

[15] Baysse C. Role of membrane structure during stress signaling and adaptation in *Pseudomonas*. *Pseudomonas*: *book* / C. Baysse, F. O'Gara. Springer, 2007. P. 193–224. https://doi.org/10.1007/978-1-4020-6097-7_7

sustainable use of natural resources. Factor analysis allows to establish the relationship between the processes of free radical damage of lipids and proteins, the accumulation of biomass of bacteria of the sulfur cycle and the concentration of heavy metal compounds in the medium. The analysis of the main factors allows to determine the structure of the data and to establish the relationship between prooxidant-antioxidant parameters of the studied bacteria. Its main objectives are to reduce the number of variables (data reduction) and to determine the structural relationships between them (classification of variables)[16].

The aim of our work was to establish the relationship between the processes of free radical damage to lipids and proteins, indicators of antioxidant defense, fatty acid content, accumulation of biomass of bacteria *Chlorobium limicola* IMV K-8 and *Desulfuromonas acetoxidans* IMV B-7384 and concentration of copper (II) sulfate and ferric citrate in the medium.

1. Physiological and biochemical properties of *Desulfuromonas acetoxidans* IMV B-7384 under the influence of ferric citrate

Iron is necessary for the functioning of many heme-containing proteins, including cytochromes, which are involved in photosynthesis and electron transfer in the respiratory chain. However, Fe^{2+} ions are potentially toxic agents that cause free radical damage to cell macromolecules[17]. In our previous work, it was found that the addition of ferric citrate into the culture medium intensifies the processes of lipid peroxidation and oxidative modification of proteins (OMP) in *D. acetoxidans* IMV B-7384 cells, which indicates free radical damage to cellular macromolecules (table 1)[18,19]. Probably, due to the intensification of lipid and protein damage processes, the fluidity of the cytoplasmic

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[16] 1.Marley W. W. Determining parallel analysis criteria. Journal of modern applied statistical methods. 2006. Vol. 5, № 2. P. 344–346. https://doi.org/10.22237/jmasm/1162354020

[17] Bradley J. M., Svistunenko D. A., Wilson M. T., Hemmings A. M., Moore G. R., Le Brun N. E. Bacterial iron detoxification at the molecular level. J. Biol. Chem. 2020. Vol. 295. Iss. 51. P. 17602–17623. https://doi.org/10.1074/jbc.REV120.007746

[18] Масловська О., Гнатуш С. Інтенсивність процесів перекисного окиснення ліпідів і показники системи антиоксидантного захисту клітин *Desulfuromonas acetoxidans* IMB B-7384 за впливу ферум (III) цитрату. Вісник Львівського університету. Серія біологічна. 2014. Вип. 64. С. 270–278.

[19] Maslovska O., Hnatush S. Oxidative modification of proteins and specific superoxide dismutase activity of *Desulfuromonas acetoxidans* IMV B-7384 under the influence of ferric (III) citrate. Мікробіологія і біотехнологія. 2015. Т. 30, № 2. С. 34–40. https://doi.org/10.18524/2307-4663.2015.2(30).48072

membrane changes, resulting in changes in ATP-hydrolases of *D. acetoxidans* IMV B-7384 cells[20,21].

Table 1

Biochemical indicators of *Desulfuromonas acetoxidans* IMV B-7384 under the influence of ferric citrate

Indicators	**Concentration of ferric citrate, mM**					
	0	**10**	**12**	**14**	**16**	**20**
1	**2**	**3**	**4**	**5**	**6**	**7**
Biomass, g/l	1.5±0.02	1.3±0.02	1.2±0.02	1.1±0.05	1.0±0.05	0.5±0.02
Carbonyl groups, nmol/mg protein	7.5±0.2	13±0.5	8.5±0.5	9.0±0.5	9.0±0.5	9.0±0.5
Diene conjugates, μmol/mg protein	1.9±0.2	5.1±0.3	4.7±0.3	3.6±0.4	4.0±0.4	3.3±0.4
Lipid hydroperoxides, cond. units/g protein	20.0±1.5	96.0±3.0	115.0±3.0	98±3	63±3	130±5
TBARS, nmol/mg protein	26±2	143±3	125±3	110±5	75±4	80±4
ATP-hydrolase activity, units of activity/mg protein	2.4±0.1	3.5±0.1	3.3±0.1	1.3±0.1	1.5±0.1	2.4±0.2
Mg^{2+}-hydrolase activity, units of activity/mg protein	1.7±0.1	2.1±0.1	1.7±0.1	1.4±0.1	1.3±0.1	1.3±0.1
Azide sensitive ATP-hydrolase activity, units of activity/mg protein	1.4±0.1	2.0±0.1	0.6±0.1	0.7±0.03	0.7±0.03	0.7±0.03
SOD, units of activity/min·mg protein	120.0±5.0	630.0±5.0	550.0±5.0	810.0±10.0	820.0±15.0	800.0±15.0
Catalase, units of activity/mg protein	1.0±0.05	2.4±0.20	2.5±0.20	2.7±0.30	3.0±0.30	4.3±0.40

[20] Масловська О., Гнатуш С., Галушка А. Зміни жирнокислотного складу клітин *Desulfuromonas acetoxidans* ІМВ В-7384 за впливу ферум цитрату. Біологічні Студії / Studia Biologica. 2014. Т. 8, № 3–4. С. 87–98.

[21] Масловська О., Гнатуш С. Вплив ферум (ІІІ) цитрату на АТФ-гідролази *Desulfuromonas acetoxidans* ІМВ В-7384. Вісник Дніпропетровського університету. Біологія. Екологія. 2013. Т. 21, № 1. С. 3–8.

Continuation table 1

1	2	3	4	5	6	7
GSH, mmol/g cells	0.2±0.05	0.4±0.05	0.5±0.05	0.4±0.03	0.4±0.03	0.3±0.03
Glutathione reductase, units of activity/mg protein	4.6±0.2	7.5±0.3	7.5±0.3	9.1±0.5	10.0±1.2	8.0±1.1
Glutathione peroxidase, units of activity/mg protein	7.0±1	27.0±2,0	35.0±2.0	37.0±2.0	48.0±2.0	46.0±2.0
Glutathione-S-transpherase, units of activity/mg protein	4.0±0.5	25.0±2.0	22.0±2.0	17.0±1.5	22.0±1.5	54.0±3.0

Note: *TBARS – thiobarbiturate reactive species, SOD – superoxide dismutase, GSH – reduced glutathione*

It was determined that in response of *D. acetoxidans* IMV B-7384 cells to the influence of ferric citrate the enzymatic link of the antioxidant defense system is involved: increase superoxide dismutase (SOD) and catalase activity, increase glutathione peroxidase, glutathione S-transferase, glutathione reductase activity and the content of reduced glutathione (GSH).

It was found that in response to the influence of ferric citrate in the membranes of *D. acetoxidans* IMV B-7384 the unsaturation of cellular lipids is reduced, the content of branched chain fatty acids and fatty acids with cyclopropane ring increased, which provides the reduction of probability of damage of lipids and maintains the appropriate level of fluidity (table 2) [22]. The activity of all studied components of the antioxidant defense system of *D. acetoxidans* IMV B-7384 cells varies depending on the concentration of metal salt in the medium and the duration of cultivation[23].

[22] Масловська О., Гнатуш С., Галушка А. Зміни жирнокислотного складу клітин *Desulfuromonas acetoxidans* ІМВ В-7384 за впливу ферум цитрату. Біологічні Студії / Studia Biologica. 2014. Т. 8, № 3–4. С. 87–98.

[23] Maslovska O., Hnatush S., Katernyak S. The activity of enzymes of glutathione antioxidant system of *Desulfuromonas acetoxidans* IMV B-7384 under the influence of ferric (III) citrate. Вісник Львівського університету. Серія біологічна. 2015. Вип. 70. С. 213–220.

Table 2

The content of fatty acids of *Desulfuromonas acetoxidans* IMV B-7384 under the influence of ferric citrate

Fatty acids, % from the total area of the peaks	Concentration of ferric citrate, mM					
	0	**10**	**12**	**14**	**16**	**20**
12:0	0.05±0.001	0.25±0.05	0.3±0.01	0.3±0.05	0.5±0.05	0.45±0.05
14:0	2.45±0.2	4.7±0.3	4.4±0.3	4.5±0.5	4.6±0.5	5.4±0.5
16:0	30.7±0.4	33±3	31.5±3	32.5±2	30±2	31±2
18:0	0.6±0.01	1±0.1	1.3±0.1	1±0.1	1.5±0.1	1.1±0.1
20:0	0.3±0.01	0.1±0.05	0.05±0.001	0	0	0
13:0	0.01±0.005	0.1±0.05	0.2±0.05	0.23±0.05	0.25±0.05	0.07±0.05
15:0	2.5±0.5	1.6±0.2	2.2±0.2	1.7±0.1	2.4±0.2	2.3±0.2
17:0	1.7±0.2	1.2±0.1	1.6±0.2	1.2±0.1	1.15±0.1	1.55±0.1
16:1	19±1	12±0.5	12.4±0.5	12.8±0.8	11±0.8	7.5±0.5
18:2	0.03±0.005	0.05±0.001	0	0	0	0
18:1cys	0.13±0.02	0.3±0.05	0.55±0.05	0.45±0.05	0.8±0.05	0.6±0.05
18:1trans	29±2	30±2	26±2	27±1.5	28±1.5	23±1.5
17:0cy	12.5±0.5	14±0.8	13±0.8	12.7±0.8	11.5±0.8	17±0.9
19:0cy	0.3±0.05	2.2±0.3	2.1±0.3	2±0.1	1.9±0.1	6±0.5
i15:0	0.11±0.05	0.1±0.005	0.17±0.005	0.22±0.05	0.25±0.05	0.05±0.003
a15:0	0.16±0.05	0.08±0.005	0.15±0.005	0.13±0.05	0.18±0.05	0.9±0.05
i17:0	0.01±0.005	0.75±0.005	0.35±0.005	0.19±0.05	0.32±0.06	0.61±0.08

Note: *12:0 – dodecanoic acid; 14:0 – tetradecanoic acid; 16:0 – hexadecanoic acid; 18:0 – octadecanoic acid; 20:0 – eicosanoic acid; 13:0 – tridecanoic acid; 15:0 – pentadecanoic acid; 17:0 – heptadecanoic acid; 16:1 – cis-9-hexadecenoic acid; 18:2 – cis,cis-9, 12-octadecadienoic acid; 18:1 cis – cis-9-octadecenoic acid; 18:1 trans – trans-9-octadecenoic acid; 17:0cy – cis-9,10-methylenehexadecanoic acid; 19:0cy – cis-9,10-methyleneoctadecanoic acid; i15:0 – iso-12-methyltetradecanoic acid; a15:0 – anteiso-13-methyltetradecanoic acid; i17:0 – iso-15-methylhexadecanoic acid.*

Probably, under the influence of ferric citrate due to free radical damage to cellular macromolecules, the balance between pro- and antioxidants is induced to be shifted towards prooxidants and oxidative stress. One of the adaptive reactions of *D. acetoxidans* IMV B-7384 to the action of free radicals is changes in fatty acid composition, which are aimed at reducing lipid unsaturation and maintaining the level of membrane fluidity.

2. Physiological and biochemical properties of *Chlorobium limicola* IMV K-8 under the influence of copper (II) sulfate

The toxicity of Cu^{2+} ions is due to their participation in the reactions of formation of reactive oxygen species (ROS), which have a detrimental effect on the functioning of bacteria. To study the physiological and biochemical properties of *C. limicola* IMV K-8 under the influence of copper (II) sulfate, salt concentrations that cause inhibition of biomass accumulation by 10–70% were selected (table 3). The accumulation of copper ions in bacterial cells and on their surface has been studied[24]. It is established that due to the influence of copper ions, the processes of lipid peroxidationL and OMP intensify[25].

Table 3

Indicators of free radical damage to proteins and activity of enzymes of the antioxidant defense system *Chlorobium limicola* IMV K-8 under the influence of copper (II) sulfate

Indicators	Concentration of copper (II) sulfate, mM					
	0	**0.05**	**0.1**	**0.125**	**0.25**	**0.5**
1	**2**	**3**	**4**	**5**	**6**	**7**
Biomass, g/l	10.6±0.4	10.0±0.4	9.5±0.4	8.2±0.3	3.9±0.2	3.3±0.2
Carbonyl groups, nmol/mg protein	0.3±0.02	0.2±0.02	0.3±0.03	0.2±0.02	0.2±0.02	0.1±0.01
Diene conjugates, μmol/mg protein	0.3±0.03	0.4±0.03	0.4±0.03	0.5±0.03	0.4±0.03	0.2±0.02
Lipid hydroperoxides, cond. units/g protein	136.5±5	149.6±6	154.4±6	166.4±6	253±11	189±7
TBARS, nmol/mg protein	196.3±8	233±13	286±14	421±20	392±18	367±17
Peroxidase, units of activity/min· mg protein	11.3±0.5	13.1±0.7	13.7±0.7	12.0±0.6	10.5±0,4	8.8±0.3

[24] Segin T., Hnatush S., Maslovska O., Halushka A., Zaritska Y. Biochemical indicators of green photosynthetic bacteria *Chlorobium limicola* response to Cu^{2+} action. The Ukr. Biochem. J. 2020. Vol. 92, № 1. P. 103–112. http://doi.org/10.15407/ubj92.01.103

[25] Сегін Т., Гнатуш С., Горішний М. Процеси ліпопероксидації у клітинах *Chlorobium limicola* IMB K-8 за впливу Cu (II) сульфату. Вісн. Дніпропетр. ун-ту. Біол. Екол. 2016. Вип. 24, № 1. С. 72–78. http://doi.org/10.15421/011608

Continuation table 3

1	2	3	4	5	6	7
SOD, units of activity/min·mg protein	36.5±4	79.7±6	69.2±5	64.9±4.8	42.1±4.2	41.3±4
Glutathione peroxidase, units of activity/mg protein	36.5±4	50.6±5	115.9±8	134±11	170±13	203±14
Glutathione-S-transferase, units of activity/mg protein	36.8±4	38.5±4	59±5.5	69.9±5.7	70.3±5.8	50.3±5
Cu^{2+} content in buffer solution, mM	0	0.001± ±0.0001	0.002± ±0.0001	0.004±0. ±0001	0.003± ±0.0001	0.005± ±0.0001
Cu^{2+} content in cells, mM	0	153,2±6	223±10	225±10	331±12	569±17

Under the influence of copper (II) sulfate in the cells of bacteria *C. limicola* IMV K-8 increases the content of diene conjugates, lipid hydroperoxides and TBARS with increasing concentration of metal salts in the incubation medium. The content of carbonyl groups in bacterial proteins under the influence of copper ions decreased or did not differ significantly compared to the control, which is probably due to the formation of protein aggregates that make it impossible to determine these groups in the reaction with 2,4-dinitrophenylhydrazine. In response to the action of copper ions in the cells of the studied bacteria involved antioxidant enzymes, including SOD, peroxidase and components of the glutathione system – glutathione peroxidase and glutathione-S-transferase. Glutathione reductase activity in *C. limicola* IMV K-8 cells was not detected[26].

Bacteria *C. limicola* IMV K-8 are able to carry out anoxygenic photosynthesis, which is an important physiological and biochemical process of photosynthetic bacteria. This process is carried out with the participation of photosynthetic pigments.

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[26] Сегін Т., Гнатуш С., Масловська О., Василів О. Активність ензимів глутатіонової антиоксидантної системи бактерій *Chlorobium limicola* ІМВ К-8 за впливу купрум (ІІ) сульфату. Мікробіологія і біотехнологія. 2018. Вип. 1, № 41. С. 39–47. http://doi.org/10.18524/2307-4663.2018.1(41).117284

In the cells of green photosynthetic bacteria *C. limicola* bacteriochlorophylls ***c*** and ***d***, small amounts of bacteriochlorophyll ***a***, and some carotenoids, namely chlorobactin, isorenieratin, and others, are involved in photosynthesis[27,28]. Bacteriochlorophylls ***a***, ***c***, ***d*** and chlorobactin (table 4), which are the main pigments, were found in cell-free extracts of *C. limicola* IMV K-8. Under the influence of Cu^{2+} ions, the content of all photosynthetic pigments underwent significant changes. Under the influence of all studied concentrations of copper (II) sulfate, except for 0.05 mM, the content of photosynthetic pigments decreased compared to the control. Under the influence of 0.05 mM copper (II) sulfate the content of bacteriochlorophyll ***a***, ***c*** and chlorobactin increased by 2–2.5 times, the content of lycopene – by 5.5–6 times, compared with the control. The significant increase of content of lycopene is due to the fact that this pigment is a precursor of chlorobactin biosynthesis in green photosynthetic bacteria[29].

Table 4

The content of pigments in cell-free extracts of *Chlorobium limicola* IMV K-8 under the influence of copper (II) sulfate

Pigments	Pigments, %				
	Concentration of copper (II) sulfate, mM				
	0	0.05	0.1	0.25	0.5
Bacteriochlorophyll *a*	100	226.1±11.3*	18.4±0.9*	2.7±0.1*	10.6±0.5*
Bacteriochlorophyll *c*	100	240.6±12.0*	50.4±2.5*	4.4±0.2*	10.7±0.5*
Bacteriochlorophyll *d*	100	42.3±2.1*	18.2±0.9*	11.4±0.6*	37.0±1.9*
Chlrobactin	100	240.6±12.0*	50.4±2.5*	4.4±0.2*	10.7±0.5*
Lycopene	100	596±29.8*	26.1±1.3*	0	0

***Note**: * – p ≥ 0,95, n = 3 – probable changes compared to control*

The relationship between reduced and oxidized glutathione is a major determinant of oxidative stress. The content of reduced (GSH) and

[27] Кушкевич І. В., Гнатуш С. О. Пігменти фотосинтезувальних зелених сіркобактерій *Chlorobium limicola* Ya-2002 за впливу солей важких металів. Мікробіологія і біотехнологія. 2010. Вип. 3. С. 61–70.

[28] Thiel V., Tank V., Bryant D. Diversity of chlorophototrophic bacteria revealed in the Omics Era. Annu. Rev. Plant Biol. 2018. Vol. 69, № 16. P. 1–29. https://doi.org/10.1146/annurev-arplant-042817-040500

[29] Maresca J., Romberger S., Bryant D. Isorenieratene biosynthesis in green sulfur bacteria requires the cooperative actions of two carotenoid cyclases. J. Bacteriol. 2008. Vol. 190, № 19. P. 6384–6391. https://doi. org/10.1128/JB.00758-08

oxidized (GSSG) glutathione in cell-free extracts of *C. limicola* IMV K-8 under the influence of different concentrations of copper (II) sulfate was studied (table 5). With increasing concentration of Cu^{2+} ions in the incubation medium, a moderate increase in the content of both reduced and oxidized glutathione was observed, which indicates the participation of this antioxidant in the protection of cells under the influence of copper (II) sulfate. The highest content of reduced and oxidized glutathione was found under the influence of 0.25 mM and 0.5 mM copper (II) sulfate, respectively, compared to the control.

Table 5

The content of reduced (GSH) and oxidized (GSSG) glutathione in cell-free extracts of *Chlorobium limicola* IMV K-8 under the influence of copper (II) sulfate

Concentration of copper (II) sulfate, mM	Content of glutathione, µg/mg of cells	
	GSH	GSSG
0	0.5±0.03	0.47±0.02
0.05	0.72±0.04	0.63±0.03
0.1	0.75±0.04	1.32±0.07*
0.125	1.27±0.06*	1.88±0.09*
0.25	1.89±0.09*	2.35±0.12*
0.5	1.31±0.07*	9.6±0.49*

***Note**: * – p ≥ 0.95, n = 3 – probable changes compared to control*

Cell membranes are one of the main targets of heavy metal ions, so the mechanisms of adaptation to the influence of its components are to maintain an appropriate level of membrane fluidity. Changes in the fatty acid composition of membrane lipids are the most important reactions of the cell during oxidative stress[30].

A probable way of adaptation of *C. limicola* IMV K-8 cells to the influence of copper ions is to change the fatty acid composition of lipids, which increases the fluidity of the membrane and probably necessary for more efficient efflux of metal ions from bacterial cells[31]. Under the

[30] Baysse C. Role of membrane structure during stress signaling and adaptation in *Pseudomonas*. *Pseudomonas*: *book* / C. Baysse, F. O'Gara. Springer, 2007. P. 193–224. https://doi.org/10.1007/978-1-4020-6097-7_7

[31] Segin T., Hnatush S., Maslovska O., Vasyliv O. Changes of fatty acid composition of *Chlorobium limicola* IMV K-8 cells under the influence of copper (II) sulfate. Mikrobiol. Z. 2018. Vol. 80, № 3. P. 40–52. https://doi. org/10.15407/microbiolj80.03.040

influence of copper (II) sulfate, an increase in the content of octadecanoic, pentadecanoic, heptadecanoic, hexadecanoic, cis-9-hexadecane, trans-9-octadecenoic, cis-9,10-methylenehexadecanoic and i15: 0 – iso-12-methyl tetradecanoic acid. Under these conditions, the content of tetradecanoic and cis-9-octadecenoic acids decreases (table 6).

Table 6

The fatty acid content of *Chlorobium limicola* IMV K-8 under the influence of copper (II) sulfate

Fatty acids, % from the total area of the peaks	Concentration of copper (II) sulfate, mM					
	0	**0.05**	**0.1**	**0.125**	**0.25**	**0.5**
12:0	0.18±0.01	0.21±0.01	0.19±0.01	0.12±0.01	0.18±0.01	0
14:0	29.53±0.7	24.82±0.7	21.77±0.7	36.19±0.8	23.79±0.7	36.62±1.2
16:0	11.15±1.4	15.77±1.5	18.78±1.5	20.67±1.6	22.54±1.6	14.31±1.5
18:0	1.78±0.3	3.69±0.9	4.34±1.1	1.27±0.3	1.51±0.3	2.09±0.3
15:0	0.94±0.05	1.18±0.06	1.12±0.06	1.11±0.06	2.65±0.7	1.26±0.06
17:0	0.69±0.04	1.85±0.07	1.34±0.06	0.82±0.05	0.89±0.05	0.83±0.05
16:1	23.55±1.7	29.34±1.5	27.64±1.5	28.64±1.5	36.32±1.8	30.72±1.7
18:1cys	0.65±0.04	0.64±0.04	0.37±0.03	0.26±0.02	0.08±0.001	0.78±0.04
18:1trans	5.23±0.9	15.4± 1.5	19.74±1.7	15.18±1.5	8.99±1.4	6.3±1.3
17:0cy	0.46±0.04	0.61±0.04	0.68±0.04	0.45±0.01	1.65±0.2	0.55±0.1
i15:0	0.72±0.04	1.51±0.06	0.82±0.05	0.59±0.03	0.2±0.01	1.01±0.1
a15:0	1.03±0.2	2.36±0.2	1.74±0.2	0.68±0.2	0.55±0,1	1.31±0,2
i16:0	1.08±0.5	1.91±0.6	1.04±0.5	0.66±0.2	0.22±0.01	1.32±0.2
i17:0	0.31±0.03	0.62±0.04	0.29±0.02	0.22±0.02	0	0.38±0.1
14:0 2-OH	0.56±0.03	0	0	0.7±0.04	0.34±0.03	2.11±0.7

Note: *12:0 – dodecanoic acid; 14:0 – tetradecanoic acid; 16:0 – hexadecanoic acid; 18:0 – octadecanoic acid; 15:0 – pentadecanoic acid; 17:0 – heptadecanoic acid; 16:1 – cis-9-hexadecenoic acid; 18:1 cis – cis-9-octadecenoic acid; 18:1 trans – trans-9-octadecenoic acid; 17:0cy – cis-9,10-methylenehexadecanoic acid; i15:0 – iso-12-methyltetradecanoic acid; a15:0 – anteiso-13-methyltetradecanoic acid; i16:0 – iso-14-methyl-pentadecane acid; i17:0 – iso-15-methylhexadecanoic acid; 14:0 2-OH – 2-hydroxytetradecanoic acid.*

Our results suggest that one of the first reactions of adaptation of *C. limicola* IMV K-8 cells under the influence of copper ions is *cis/trans* isomerization of monounsaturated fatty acids, as well as the synthesis of fatty acids with cyclopropane ring. Maintaining the nessesetive level of fluidity of the membrane under these conditions provide fatty acids with a branched carbon chain.

Thus, under the influence of copper (II) sulfate in *C. limicola* IMV K-8 cells increases the content of compounds that are likely to be formed under the influence of free radical compounds, in particular, some products of lipid peroxidation and oxidized glutathione. These conditions also increase the activity of enzymes of the antioxidant defense system and changes in the content of fatty acids in lipids, which are likely to maintain the required level of membrane fluidity under these conditions.

3. Relationships between the processes of free radical damage to lipids and proteins, the accumulation of biomass of bacteria *Chlorobium limicola* IMV K-8 and the concentration of copper (II) sulfate in the environment

Factor analysis was performed to establish the relationship between the processes of free radical damage to lipids and proteins, the accumulation of biomass of bacteria of the sulfur cycle and the concentration of heavy metal compounds in the environment.

In order to determine the structure of the data and to establish relationships between various microbiological and physicochemical parameters under the influence of copper ions we analyzed the main factors. The main objectives of this analysis are to reduce the number of variables and to determine the structural relationships between all studied indicators. Each latent factor is a linear combination of the original variables that are part of it. As a result of the factor analysis, the data were reduced, because in the obtained matrices 35 variables were combined into 6 factors, the eigenvalue of which according to the Kaiser and Cattel criteria was greater than 1 (table 7).

Table 7

Eigenvalues of *Chlorobium limicola* IMV K-8 cells under the influence of different concentrations of copper ions

Value	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	13.31	38.04	13.31	38.04
2	8.01	22.87	21.32	60.91
3	4.16	11.89	25.48	72.8
4	3.09	8.83	28.57	81.63
5	2.89	8.26	31.46	89.89
6	1.80	5.15	33.26	95.04

According to the analysis of the matrix of factor loadings of the studied parameters of *C. limicola* IMV K-8 cells, six latent factors explain 95% of the total data variance (table 7). Such a high value of the total variance indicates the orderliness of the system and is due to the fact that it takes into account a large number of different parameters and factors that are likely to have a significant impact on the functionality of the system.

The first latent factor (table 8) included indicators of oxidative modification of proteins, activity of some enzymes, namely glutathione peroxidase and the content of oxidized glutathione, the content of some pigments (bchl ***c***, chlorobactin), the content of copper ions, the content of some fatty acids (18:1cis, 18:1trans, 12:0, 14:0, 14:0 2-OH) and bacterial biomass. The variance of the factor is 26.9%, that show the high importance of these indicators in the system. A direct relationship has been established between the activity of enzymes of the antioxidant defense system and the concentration of copper ions in the medium. An inverse relationship has been established with the above indicators and biomass, which confirms that with increasing metal concentration, the accumulation of *C. limicola* IMB K-8 biomass decreases.

Table 8

Matrix of factor loadings of the studied parameters of *Chlorobium limicola* IMB K-8 bacteria under the influence of different concentrations of copper ions

Variable		**Factor loadings**					
		F1	**F2**	**F3**	**F4**	**F5**	**F6**
1		**2**	**3**	**4**	**5**	**6**	**7**
Lipid peroxi–dation indicators	Lipid hydroperoxydes	–	–	–	–0.85	–	–
	TBARS	–	–	–	–	–0.9	–
	Diene conjugates	–	–	–	–0.89	–	–
OMP	Carbonyl groups in proteins	–0.65	–	0.42	–	0.48	–
Activity of enzymes of antioxidant defense system	Glutathione transferase	–	–	–	–0.46	–0.56	–
	Glutathione peroxidase	0.68	–	–	–	–	–
	GSH	–	–	–	–	–0.77	0.59
	GSSH	0.99	–	–	–	–	–
	Superoxide dismutase	–	–	–	0.69	–0.54	–
	Peroxidase	–	–	–	–0.94	–	–
Pigments	bchl *a*	–	0.89	–	–	–	–
	bchl *c*	–0.45	0.87	–	–	–	–
	bchl *d*	–	–	0.66	–	0.56	0.45
	chlorobactin	–0.45	0.87	–	–	–	–
	lycp	–	0.94	–	–	–	–

Continuation table 8

1		2	3	4	5	6	7
Cu^{2+} concentration	in buffer before incubation	0.91	–	–	–	–	–
	in buffer after incubation	0.9	–	–	–	–	–
	in cells	0.91	–	–	–	–	–
	on cell surface	0.85	–0.45	–	–	–	–
Fatty acids	16:1cis	–	–	–0.91	–	–	–
	18:1cis	0.49	0.44	0.43	–	0.55	–
	18:1trans	–0.48	–	–	–	–0.65	–0.54
	15:0	–	–	–0.94	–	–	–
	17:0	–	0.7	–	–	–	–0.59
	12:0	–0,89	–	–	–	–	–
	14:0	0.63	–	0.45	–	–	0.58
	16:0	–	–	–0.62	–	–0.68	–
	18:0	–	–	–	–	–	–0.92
	i15:0	–	0.82	–	–	–	–0.42
	a15:0	–	0.66	–	–	–	–0.72
	i16:0	–	0.77	–	–	–	–0.41
	i 17:0	–	0.86	–	–	–	–
	17:0cy	–	–	–0.93	–	–	–
	14:0 2–OH	0.95	–	–	–	–	–
	Biomass	–0.75	–	0.58	–	–	–
	Expl. Var	*9.43*	*7.49*	*5.18*	*3.21*	*4.40*	*3.55*
	Prp. Totl, %, %	*26.9*	*21.4*	*14.8*	*9.2*	*12.6*	*10.1*

The second latent factor, with a variance of 21.4%, included bacteriochlorophyll ***a***, bacteriochlorophyll ***c***, chlorobactin, lycopene, and some fatty acids with direct links. The concentration of Cu^{2+} on the cell surface, which is associated with both inverse variables, was also included in the second factor. The third latent factor included indicators of fatty acid content (16:1cis, 18:1cis, 15:0, 14:0, 16:0, 17:0cy), OMP, bacteriochlorophyll ***d*** content and biomass accumulation, which are directly related, except for certain fatty acids. The total variance of this factor is 14.8%.

The composition of the fourth latent factor, the variance of which is 9.2% included indicators of lipid peroxidation and glutathione transferase and peroxidase activity, which are directly related. This factor also included superoxide dismutase activity, which is inversible associated with the above variables.

The fifth latent factor (variance is 12.6%) included TBARS, OMP, activity of some enzymes, content of bacteriochlorophyll ***d*** and content of three fatty acids. Indicators of lipid peroxidation and enzymes, OMP and pigments are directly related. The sixth latent factor included the largest amount of fatty acids, compared with other factors, as well as the content of GSH and bacteriochlorophyll ***d***. The total variance is 10.1%.

Based on the analysis of the main factors, it was found that the accumulation of copper ions on the surface and in *C. limicola* cells leads to a decrease in biomass, because the factor loadings of these variables belong to the first factor and are strongly inversely connected (fig. 1). Under the influence of copper ions a significant increase in the content of oxidized glutathione is characterized by a more important damaging effect, which indicates a violation of the redox potential in the cell, oxidative modification of proteins and changes in photosynthetic pigments. These variables belong to the first factor, and their factor loads are significant. With the increase of Cu^{2+} ions in the cells of the studied bacteria, the activity of enzymes of the antioxidant defense system also increases, which indicates that superoxide dismutase, glutathione peroxidase, etc. are involved in the protection of bacteria from the effects of ROS. The redox system of glutathione maintains the intracellular redox status of the cell and neutralizes ROS under oxidative stress. Important reactions of adaptation of the studied bacteria are the increase in glutathione peroxidase activity and changes in the fatty acid composition of lipids, which lead to increased membrane fluidity.

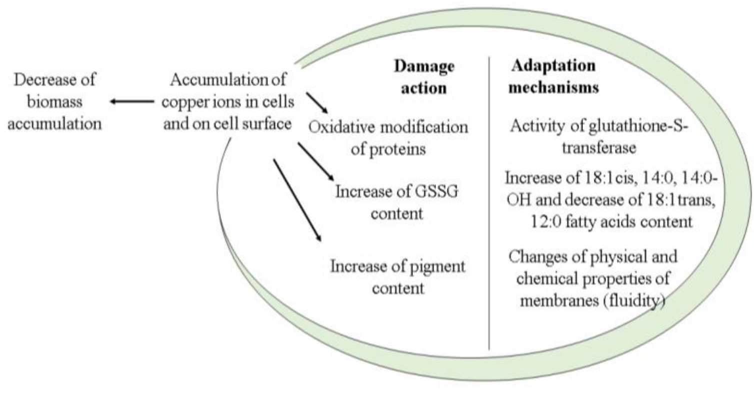

Fig. 1. Hypothetical mechanism of action of copper (II) sulfate and adaptation reaction of *Chlorobium limicola* IMV K-8

The structure of lipids is modified in response to the effect of ROS in bacterial cells, as indicated by changes in the fatty acid unsaturation index, the degree of cis-trans isomerization of double bonds, the ratio of branched/unbranched fatty acids. The chain lengths of the fatty acid residue of lipids also change. It is assumed that free radical processes that cause intensification of lipid peroxidation processes have less damaging effect on *C. limicola* IMV K-8 cells compared to oxidative modification of proteins, as the corresponding changes were included in factors four and five, the eigenvalue of which is much lower than own variance of the 1st factor. SOD and peroxidase activity are also likely to be less important adaptation reactions compared to glutathione peroxidase activity and changes in lipid fatty acid composition, as these variables were also included in factors four and five.

4. Relationships between the processes of free radical damage to lipids and proteins, the accumulation of biomass of bacteria *Desulfuromonas acetoxidans* IMV B-7384 and the concentration of ferric citrate

In order to determine the structure of the data and to establish relationships between physiological and biochemical parameters of *D. acetoxidans* IMV B-7384 under the influence of different concentrations of ferric citrate an analysis of the main factors was conducted. For factor analysis were used indicators of lipid peroxidation (content of diene conjugates, lipid hydroperoxides, TBARS)[32] and OMP[33], activity of ATP-hydrolases[34], activity of enzymes of the antioxidant defense system (SOD, catalase, glutathione peroxidase, glutathione-S-transferase, glutathione reductase activity)[35], the content of reduced glutathione, fatty acids

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[32] Масловська О., Гнатуш С. Інтенсивність процесів перекисного окиснення ліпідів і показники системи антиоксидантного захисту клітин *Desulfuromonas acetoxidans* ІМВ В-7384 за впливу ферум (ІІІ) цитрату. Вісник Львівського університету. Серія біологічна. 2014. Вип. 64. С. 270–278.

[33] Maslovska O., Hnatush S. Oxidative modification of proteins and specific superoxide dismutase activity of *Desulfuromonas acetoxidans* IMV B-7384 under the influence of ferric (III) citrate. Мікробіологія і біотехнологія. 2015. Т. 30, № 2. С. 34–40. https://doi.org/10.18524/2307-4663.2015.2(30).48072

[34] Масловська О., Гнатуш С. Вплив ферум (ІІІ) цитрату на АТФ-гідролази *Desulfuromonas acetoxidans* ІМВ В-7384. Вісник Дніпропетровського університету. Біологія. Екологія. 2013. Т. 21, № 1. С. 3–8.

[35] Maslovska O., Hnatush S., Katernyak S. The activity of enzymes of glutathione antioxidant system of *Desulfuromonas acetoxidans* IMV B-7384 under the influence of ferric (III) citrate. Вісник Львівського університету. Серія біологічна. 2015. Вип. 70. С. 213–220.

composition[36] of *D. acetoxidans* IMV B-7384 under the influence of ferric citrate in concentrations that cause a decrease in biomass accumulation by 10–50%. As a result of the factor analysis, the data were reduced, because in the obtained matrices 33 variables were combined into 6 factors, the variance of which according to the Kaiser and Cattel criteria was greater 1.

According to the analysis of the matrix of factor loadings of the studied parameters of *D. acetoxidans* IMV B-7384 cells, six latent factors explain almost 88% of the total data variance (table 9). This value of the total variance indicates the orderliness of the system and is due to the fact that a sufficient number of different parameters and factors are taken into account, which are likely to have a significant impact on the functionality of the system. Other parameters, such as the accumulation of iron ions in cells or on the cell surface of microorganisms, may not have been taken into account and free radical DNA damage has not been studied. Factors that may also affect the functional status of *D. acetoxidans* IMV B-7384 cells under these conditions are damage to proteins and DNA by lipid peroxidation products, such as the formation of malonic dialdehyde complexes with DNA or proteins.

Table 9

Eigenvalues of *Desulfuromonas acetoxidans* IMV B-7384 cell factors under the influence of different concentrations of iron ions

Value	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	9.50	28.79	9.50	28.79
2	6.91	20.95	16.41	49.74
3	5.02	15.21	21.43	64.95
4	3.38	10.25	24.82	75.20
5	2.37	7.19	27.19	82.40
6	1.77	5.38	28.96	87.77

The first latent factor (table 10) included the content of Fe^{3+} ions in the culture medium, the activity of some enzymes of the antioxidant defense system, in particular, glutathione-S-transferase, glutathione peroxidase, catalase and glutathione reductase activities, as well as the content of some fatty acids. The variance of the factor is 22.85%, which emphasizes the high importance of these indicators in the system. There is a direct

[36] Масловська О., Гнатуш С., Галушка А. Зміни жирнокислотного складу клітин *Desulfuromonas acetoxidans* ІМВ В-7384 за впливу ферум цитрату. Біологічні Студії / Studia Biologica. 2014. Т. 8, № 3–4. С. 87–98.

relationship between the activity of enzymes in the antioxidant defense system, the content of iron ions and fatty acids.

The second latent factor, the variance of which is 19.85 %, included all the studied indicators of lipid peroxidation, OMP processes and some enzymes of the antioxidant defense system, between which direct links have been established. Glutathione-S-transferase and catalase activities, which are associated with the above variables inverse relationships, were also included in the second factor.

Table 10

Matrix of factor loadings of the studied indicators of *Desulfuromonas acetoxidans* IMV B-7384 under the influence of ferric citrate

Variables		Factor loadings					
		F1	**F2**	**F3**	**F4**	**F5**	**F6**
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
	Concentration of Fe^{3+}	0.765	–	–0.482	–	–	–
Lipid peroxidation indicators	Lipid hydroperoxides	–	0.898	–	–	–	–
	TBARS	–	0.886	–	–	–	–
	Diene conjugates	–	0.913	–	–	–	–
OMP	Carbonyl groups in proteins	–	0.830	–	–	–	–
ATP-hydrolase activity	ATP–hydrolase	–	–	–	–	–	–0.819
	Mg^{2+}–ATP–hydrolase	–	–	–	–	–	–0.852
	Azide–sensitive ATP–hydrolase	–	–	–	–	–	–0.766
Activity of enzymes of antioxidant defense system	Glutathione transferase	0.540	–0.480	–0.601	–	–	–
	Glutathione peroxidase	0.889	–	–	–	–	–
	Glutathione reductase	0.645	0.622	–	–	–	–
	GSH	–	0.750	–	–	–	–
	Superoxide dismutase	–	0.603	–	–	–0.408	–0.451
	Catalase	0.677	–0.483	–0.470	–	–	–
Fatty acids	18:1cis	–	–	–	–	–0.596	–
	18:1trans	–	–	0.700	–	0.488	–
	18:2	–	–	–	–	0.596	–0.588
	15:0	–	–	–	0.919	–	–
	17:0	–	–	–	0.926	–	–
	12:0	0.849	–	–	–	–	–
	14:0	0.834	–	0.414	–	–	–

Continuation table 10

1	2	3	4	5	6	7	8
	16:0	–	–	–	–	0.908	–
	20:0	–0.701	–	–	0.469	–	–
	i15:0	0.431	–	0.802	–	–	–
	a15:0	–	–	0.709	0.568	–	–
	i17:0	0.481	–	–0.516	–	–	–0.448
	17:0cy	–	–	–0.831	–	–	–
	19:0cy	0.469	–	–0.854	–	–	–
Biomass		–	–	0.809	–	–	–
Expl. Var		*7.54*	*6.549*	*5.441*	*2.904*	*2.804*	*3.725*
Prp. Totl, %		*22.850*	*19.850*	*16.490*	*8.800*	*8.500*	*11.290*

The third latent factor includes the accumulation of bacterial biomass and some enzymes of the antioxidant defense system, which are linked by inverse bonds. The total variance of this factor, which also includes the largest amount of fatty acids, is 16.49 %.

The composition of the fourth latent factor, the variance of which is 8.8%, included only the content of some fatty acids, which are interconnected by direct links. The fifth factor included SOD activity, which is associated with the content of fatty acids, that are also part of this factor, inversely related. The sixth latent factor (dispersion is 11.29 %) included ATP-hydrolase activity, some fatty acids and SOD activity. All indicators are directly related.

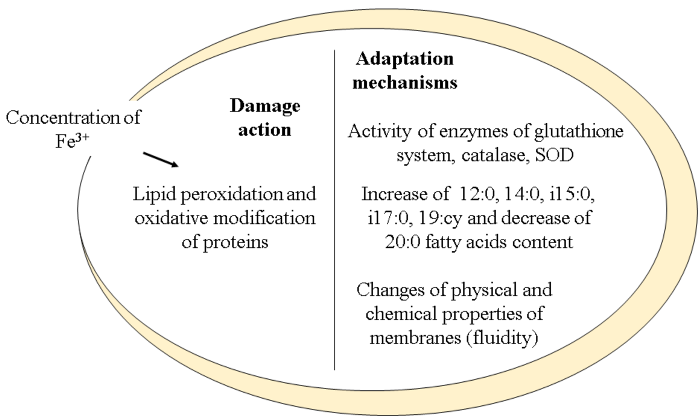

Fig. 2. Hypothetical mechanism of action of ferric citrate and adaptation reaction of *Desulfuromonas acetoxidans* IMV B-7384

Based on the analysis of the main factors, it was found that with increasing concentration of ferric citrate in the medium of bacteria *D. acetoxidans* IMV B-7384 cultivation and lipid peroxidation and OMP, and the duration of cultivation of microorganisms increases the activity of antioxidant enzymes, indicating that superoxide dismutase, catalase, components of the glutathione defense system, in particular, glutathione peroxidase, glutathione-S-transferase, glutathione reductase, etc. are involved in the protection of bacteria from the effects of ROS.

Under the influence of ferric citrate, an important damaging effect is characterized by a significant increase in the products of lipid peroxidation – lipid hydroperoxides, diene conjugates, TBARS and OMP. These variables belong to the second factor, and their factor loadings are significant. Important reactions of adaptation of the studied bacteria are the increase in glutathione-S-transferase activity and changes in the fatty acid composition of lipids, which lead to changes in the physicochemical properties of the membrane, in particular, increase its fluidity. The fluidity of the cytoplasmic membrane also changes under the influence of high concentrations of ferric citrate, which is reflected in the reduction of ATP-hydrolase activities. It is assumed that the activity of these enzymes is reduced due to the direct interaction of ATP-hydrolases with ROS or lipid peroxidation products, which can adversely affect both the synthesis of the enzyme and its activity.

CONCLUSIONS

Factor analysis was used to establish the relationship between the processes of free radical damage to lipids and proteins, the accumulation of biomass of bacteria *C. limicola* IMV K-8 and *D. acetoxidans* IMV B-7384 and the concentration of heavy metal compounds in the environment, in particular, Cu^{2+} and Fe^{3+} ions, adaptation mechanisms of *C. limicola* cells IMV K-8 and *D. acetoxidans* IMV B-7384, respectively.

The results show that due to the accumulation of copper ions in the cells of *C. limicola* IMV K-8, protein molecules are significantly damaged, which affects the processes of cell metabolism. Significant accumulation of oxidized glutathione indicates a violation of the redox potential of the cell and, probably, the occurrence of oxidative or carbonyl stress. Under these conditions, the processes of pigment synthesis also change, which affects one of the main physiological and biochemical processes of *C. limicola* IMV K-8 – photosynthesis. Glutathione peroxidase is an important enzyme of the antioxidant defense system of *C. limicola* IMV K-8 under the influence of copper ions. Due to the significant increase in glutathione peroxidase activity of *C. limicola* IMV K-8 under the influence of copper ions, lipid peroxidation processes show less damaging effect on *C. limicola* IMV K-8 cells, compared to the processes of oxidative modification of proteins

Glutathione peroxidase, glutathione-S-transferase, glutathione reductase, reduced glutathione, superoxide dismutase and catalase play important roles in providing antioxidant protection of *D. acetoxidans* IMV B-7384 cells under the influence of the iron ions, as these indicators are included to first and second factor. The synthesis of saturated fatty acids, branched carboxylic fatty acids and cyclopropane ring fatty acids are important adaptation reactions of *D. acetoxidans* IMV B-7384 under the influence of iron ions, as the relevant variables are also included in the first and second factors.

The obtained results are important for understanding the ways of regulation of bacterial metabolism under stressful conditions.

SUMMARY

Deteriorating environment has a negative impact on the health and quality of life of Ukrainians. Of particular importance are the issues of analysis of the environmental situation, which are a necessary condition for timely detection and monitoring of environmental problems and identify ways to prevent and overcome them. Pollution of the environment with heavy metal compounds has reached a significant scale. Multiple complementary models of the impact of chemical pollutants on the physiological and biochemical properties of microorganisms and prediction of their adaptive potential to stressors will be valuable not only to solve pollution problems through new ecobiotechnologies, but also to scientifically substantiate conservation and sustainable use of natural resources. Factor analysis allows to establish the relationship between the processes of free radical damage of lipids and proteins, the accumulation of biomass of bacteria in the sulfur cycle and the concentration of heavy metal compounds in the environment. The analysis of the main factors allows to determine the structure of the data and to establish the relationship between prooxidant-antioxidant parameters of the studied bacteria. Factor analysis has shown that the oxidative modification of proteins induced by copper (II) sulfate, changes in the ratio of reduced and oxidized glutathione and the content of photosynthetic pigments are important factors affecting the viability of *C. limicola* IMV K-8 cells. The content of iron ions in the culture medium and the processes of free radical damage to lipids and proteins induced by them affect the functional state

of *D. acetoxidans* IMV B-7384 cells. Free radical processes that intensify lipid peroxidation have less detrimental effect on *C. limicola* IMV K-8 cells than oxidative modification of proteins because the variables of indicators of lipid peroxidation are included into the fourth and fifth factors. Increased glutathione S-transferase activity and changes in the fatty acid composition of membrane lipids, which are aimed at efficient pumping of metal ions from the cell, are more important for the adaptation of *C. limicola* IMV K-8 cells to Cu^{2+}, as the relevant variables included the first and second factors . Superoxide dismutase and peroxidase activities are less important for the adaptation of *C. limicola* IMV K-8, as these variables are included in the fourth and fifth factors.

References

1. Segin T., Hnatush S., Maslovska O., Komplikevych S. Synthesis of glycogen by *Chlorobium limicola* IMV K-8 during growth in wastewater. Visnyk of Lviv University. Biological series. 2020. Vol. 83. P. 67–73.
2. Гудзь С. П., Гнатуш С. О., Мороз О. М., Перетятко Т. Б., Василів О. М. Свідоцтво про депонування штаму бактерій *Desulfuromonas acetoxidans* Ya-2006 у Депозитарії Інституту мікробіології і вірусології ім. Д. К. Заболотного НАН України з наданням реєстраційного номеру IМВ В-7384 від 10 квітня 2013 року.
3. Чайка О. М., Перетятко Т. Б., Гудзь С. П. Сірковідновлювальні бактерії водойм Язівського сіркового родовища. Науковий вісник Ужгородського університету Серія Біологія. 2010. Вип. 28. С. 52–55.
4. Мороз О., Яворська Г., Муравель Н., Клим І. Відновлення феруму (III) сульфатвідновлювальними та сірковідновлювальними бактеріями. Біологічні Студії / Studia Biologica. 2012. Т. 6, № 2. С. 161–172.
5. Hnatush S. O., Maslovska O. D., Segin T. B., Vasyliv O. M., Kovalchuk M. M., Malovanyy M. S. Waste water treatment by exoelectrogenic bacteria, which were isolated from technogenically transformed territories. Ecological Questions. 2020. Vol. 31, № 1. P. 35–44. http://doi. org/10.12775/EQ.2020.005
6. Badalamenti J. P., Torres C. I., Krajmalnik-Brown R. Coupling dark metabolism to electricity generation using photosynthetic cocultures. Biotechnology and Bioengineering. 2013. Vol. 9999. P. 1–9. http://doi. org/10.1002/bit.25011
7. Madigan M. T. The *Chlorobiaceae*, *Chloroflexaceae*, and *Heliobacteriaceae*. *Modern Topics in the Phototrophic Prokaryotes* /

M. T. Madigan, N.A.V. Schaaf, W.M. Sattley; (eds) Hallenbeck P. Cham.: Springer, 2017. P. 139–161. https://doi.org/10.1007/978-3-319-46261-5_4

8. Мороз О. М., Гуль Н.П., Галушка А. А., Звір Г. І., Борсукевич Б. М. Використання різних акцепторів електронів бактеріями *Desulfuromonas sp*., виділеними з озера Яворівське. Вісн. Львів. ун-ту. Сер. біол. 2014. Вип. 65. С. 322–334.

9. Gadd G. M. Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology. 2010. Vol. 156. P. 609–643. https://doi.org/10.1099/mic.0.037143-0

10. Briffa J., Sinagra E., Blundell R. Heavy metal pollution in the environment and their toxicological effects on humans. Heliyon. 2020. Vol. 6. Iss. 9. P. e04691. https://doi.org/10.1016/j.heliyon.2020.e04691

11. Mathivanan K., Chandirika J.U., Vinothkanna A., Yin H., Liu X., Meng D. Bacterial adaptive strategies to cope with metal toxicity in the contaminated environment – A review. Ecotoxicol. Environ. Saf. 2021. Vol. 226. P. 112863. https://doi.org/10.1016/j.ecoenv.2021.112863

12. Ezraty B., Gennaris A., Barras F., Collet J. Oxidative stress, protein damage and repair in bacteria. Nat. Rev. Microbiol. 2017. Vol. 15, № 7. P. 385–396. https://doi.org/10.1038/nrmicro.2017.26

13. Скороход І., Курдиш І. Низькомолекулярні антиоксиданти мікроорганізмів. Мікробіологічний журнал. 2014. Вип. 76. С. 48–59. http://nbuv.gov.ua/UJRN/MicroBiol_2014_76_3_10

14. Staerck C., Gastebois A., Vandeputte P., Calenda A., Larcher G., Gillmann L., Papon N., Bouchara J.-F., Fleury V. Microbial antioxidant defense enzymes. Rev. Microb. Pathog. 2017. Vol. 110. P. 56–65. https://doi.org/10.1016/j.micpath.2017.06.015

15. Eisen J., Nelson K. The complete genome sequence of *Chlorobium tepidum* TLS, a photosynthetic, anaerobic, green-sulfur bacterium. Proc. Natl. Acad. Sci. USA. 2002. Vol. 99. P. 9509–9514. https://doi.org/10.1073/pnas.132181499

16. Baysse C. Role of membrane structure during stress signaling and adaptation in *Pseudomonas*. *Pseudomonas*: *book* / C. Baysse, F. O'Gara. Springer, 2007. P. 193–224. https://doi.org/10.1007/978-1-4020-6097-7_7

17. Marley W.W. Determining parallel analysis criteria. Journal of modern applied statistical methods. 2006. Vol. 5, № 2. P. 344–346. https://doi.org/10.22237/jmasm/1162354020

18. Bradley J. M., Svistunenko D. A., Wilson M. T., Hemmings A. M., Moore G. R., Le Brun N. E. Bacterial iron detoxification at the molecular level. J. Biol. Chem. 2020. Vol. 295. Iss. 51. P. 17602–17623. https://doi.org/10.1074/jbc.REV120.007746

19. Масловська О., Гнатуш С. Інтенсивність процесів перекисного окиснення ліпідів і показники системи антиоксидантного захисту клітин *Desulfuromonas acetoxidans* ІМВ В-7384 за впливу ферум (ІІІ) цитрату. Вісник Львівського університету. Серія біологічна. 2014. Вип. 64. С. 270–278.

20. Maslovska O., Hnatush S. Oxidative modification of proteins and specific superoxide dismutase activity of *Desulfuromonas acetoxidans* IMV B-7384 under the influence of ferric (III) citrate. Мікробіологія і біотехнологія. 2015. Т. 30, № 2. С. 34–40. https://doi.org/10.18524/2307-4663.2015.2(30).48072

21. Maslovska O., Hnatush S., Katernyak S. The activity of enzymes of glutathione antioxidant system of *Desulfuromonas acetoxidans* IMV B-7384 under the influence of ferric (III) citrate. Вісник Львівського університету. Серія біологічна. 2015. Вип. 70. С. 213–220.

22. Масловська О., Гнатуш С., Галушка А. Зміни жирнокислотного складу клітин *Desulfuromonas acetoxidans* ІМВ В-7384 за впливу ферум цитрату. Біологічні Студії / Studia Biologica. 2014. Т. 8, № 3–4. С. 87–98.

23. Масловська О., Гнатуш С. Вплив ферум (ІІІ) цитрату на АТФ-гідролази *Desulfuromonas acetoxidans* ІМВ В-7384. Вісник Дніпропетровського університету. Біологія. Екологія. 2013. Т. 21, № 1. С. 3–8.

24. Segin T., Hnatush S., Maslovska O., Halushka A., Zaritska Y. Biochemical indicators of green photosynthetic bacteria *Chlorobium limicola* response to Cu^{2+} action. The Ukr. Biochem. J. 2020. Vol. 92, № 1. P. 103–112. http://doi.org/10.15407/ubj92.01.103

25. Сегін Т., Гнатуш С., Горішний М. Процеси ліпопероксидації у клітинах *Chlorobium limicola* ІМВ К-8 за впливу Cu (II) сульфату. Вісн. Дніпропетр. ун-ту. Біол. Екол. 2016. Вип. 24, № 1. С. 72–78. http://doi.org/10.15421/011608

26. Сегін Т., Гнатуш С., Масловська О., Василів О. Активність ензимів глутатіонової антиоксидантної системи бактерій *Chlorobium limicola* ІМВ К-8 за впливу купрум (ІІ) сульфату. Мікробіологія і біотехнологія. 2018. Вип. 1, № 41. С. 39–47. http://doi.org/10.18524/2307-4663.2018.1(41).117284

27. Кушкевич І., Гнатуш С. Пігменти фотосинтезувальних зелених сіркобактерій *Chlorobium limicola* Ya-2002 за впливу солей важких металів. Мікробіологія і біотехнологія. 2010. Вип. 3. С. 61–70.

28. Thiel V., Tank V., Bryant D. Diversity of chlorophototrophic bacteria revealed in the Omics Era. Annu. Rev. Plant Biol. 2018. Vol. 69, № 16. P. 1–29. https://doi.org/10.1146/annurev-arplant-042817-040500

29. Maresca J., Romberger S., Bryant D. Isorenieratene biosynthesis in green sulfur bacteria requires the cooperative actions of two carotenoid cyclases. J. Bacteriol. 2008. Vol. 190, № 19. P. 6384–6391. https://doi. org/10.1128/JB.00758-08

30. Segin T., Hnatush S., Maslovska O., Vasyliv O. Changes of fatty acid composition of *Chlorobium limicola* IMV K-8 cells under the influence of copper (II) sulfate. Mikrobiol. Z. 2018. Vol. 80, № 3. P. 40–52. https://doi. org/10.15407/microbiolj80.03.040

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