ISSN 0206-5657. Вісник Львівського університету. Серія біологічна. 2015. Випуск 70. С. 213–220
Visnyk of the Lviv University. Series Biology. 2015. Issue 70. P. 213–220

УДК: 579.266.4

THE ACTIVITY OF ENZYMES OF GLUTATHIONE ANTIOXIDANT SYSTEM OF *DESULFUROMONAS ACETOXIDANS* IMV B-7384 UNDER THE INFLUENCE OF FERRIC (III) CITRATE

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Desulfuromonas acetoxidans IMV B-7384 bacteria are highly perspective microorganism for construction and development of microbial fuel cell technologies aimed for wastewater remediation from transition metal ions. However, metal ions catalyze formation of reactive oxygen species that could harmfully affect bacterial cells. Activity of glutathione peroxidase, glutathione-S-transferase and glutathione reductase of *D. acetoxidans* IMV B-7384 bacteria was investigated under the influence of different concentrations of ferric (III) citrate. Addition of metal salt into cultural medium caused the increase of activity of all investigated enzymes in comparison with control. The activity of glutathione peroxidase, glutathione-S-transferase and glutathione reductase changed dependently on metal salt concentration and duration of bacteria cultivation. The activity of glutathione peroxidase and glutathione-S-transferase decreased with increasing of duration of bacterial cultivation while activity of glutathione reductase increased under the influence of investigated metal salt. Decreasing of activity of glutathione peroxidase and glutathione-S-transferase could be caused by decreasing of reduced glutathione content in bacterial cells under the influence of ferric (III) citrate. Probably, quantity of reduced glutathione produced by glutathione reductase was not sufficient to maintain the necessary level of this tripeptide in bacterial cells under the influence of ferric (III) citrate. Enhancement of activity of enzymes of glutathione system confirms their necessary role in antioxidant protection of *D. acetoxidans* IMV B-7384 cells.

Keywords: *Desulfuromonas acetoxidans*, glutathione peroxidase, glutathione reductase, glutathione-S-transferase, ferric (III) citrate.

Desulfuromonas acetoxidans IMV B-7384 bacterium isolated and identified at the Department of Microbiology of Ivan Franko National University of Lviv is considered as highly favorable biocatalyst for microbial fuel cell (MFC). MFC supports electric current generation by oxidation of organic matter and transfer of electrons in the processes of dissimilative reduction of 3d-type transition metals, especially ferrous and manganese [16, 17]. Wastewater possesses energetic potential for MFC activity due to contents of high concentrations of biodegradable organic compounds. Application of effluents as the substrate for MFC should allow reduce the cost for their aeration and removal of solids which are the most energy-consuming processes of wastewater treatment. However, wastewater contains various xenobiotics, such as heavy metal ions that could adversely affect bacterial metabolism [16]. Bacteria could enzymatically reduce metal ions in metabolic pathways which are not related to assimilation of these metals. Members of *Deltaproteobacteria* class, in particular *D. acetoxidans,* are able to use S^0, Fe (III) i Mn (IV) as electron acceptors in the process of organic matter oxidation [11, 12]. Relative to *D. acetoxidans* Geobacter metallireducens strain could reduce U (VI), Cr (VI), V (V), Ag (I) and Hg (II) [12]. The main ecological aspect of microbial reduction includes decreasing of solubility of metal compounds and, as a result, lowering their mobility. Microbial reduction of heavy metals could be ap-

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plied for remediation of environments polluted with heavy metals and organic compounds [12]. It is known that transition metal ions could catalyze formation of reactive oxygen species (ROS) [14]. Aerobic and facultative anaerobic microorganisms possess effective complex of protective enzymatic and non enzymatic systems for ROS destruction [6, 7]. Important part of microbial antioxidant protection is glutathione antioxidant system. Glutathione system is known to be one of the main reduced glutathione-dependent redox systems that consist of γ-glutamate-cysteine ligase, glutathione synthetase, glutathione reductase, glutathione peroxidase, glutathione-S-transferase and NADPH. The role of this reduction system is either indispensable or dispensable at diverse microbes. Understanding the physiological function of this system in microbial cells resistibility against oxidative damage will help to increase our knowledge about cellular mechanisms of microbial resistance [8]. Functioning of this system in sulfur-reducing microorganisms, in particular *D. acetoxidans,* under the influence of metal ions is not sufficiently investigated. Investigation of changes of biochemical properties of *D. acetoxidans* IMV B-7384 cells under the influence of Fe (III) compounds dependently on their concentration in the medium is important. This knowledge could be useful for optimization of the processes of exoelectrogenesis and understanding the pathways of regulating mechanisms of bacterial metabolism at the process of wastewater remediation.

The aim of this work was to investigate changes of activity of glutathione peroxidase, glutathione-S-transferase and glutathione reductase of *D. acetoxidans* IMV B-7384 cells under the influence of ferric (III) citrate.

Materials and methods

Microbial strain *D. acetoxidans* IMV B-7384, applied in these investigations belongs to the Ukrainian Collection of Microorganisms of D.K. Zabolotny Institute of Microbiology and Virology of NAS of Ukraine [9]. Bacteria have been cultivated in the modified Postgaite C medium during four days [15]. Sodium fumarate (42 mM) was used as electron donor and its acceptor as well. Ferric (III) citrate in concentration 10–20 mM was added to the growth medium of *D. acetoxidans* IMV B-7384 for investigation of its influence on activity of enzymes of bacterial glutathione antioxidant system. It was shown that these concentrations of ferric citrate caused decreasing of biomass by 20–50% [3]. Metal salt was not added to the control. After the second, third and fourth day of bacterial growth cells were disintegrated on the ultrasonic homogenizer at 22 kHz at 4°C and cell-free extracts were obtained [3]. Protein concentration in cell-free extract was determined by Lowry method [13]. Activity of glutathione peroxidase (EC 1.11.1.9) and glutathione reductase (EC 1.8.1.7) was measured as described in [1]. Activity of glutathione-S-transferase (EC 2.5.1.18) was determined by the method indicated in [2]. Statistical calculation of results was carried out by application of the Origin and Excel programs [5]. The crucial statistical indexes were calculated on the basis of direct data, such as arithmetical mean (M) and standard deviation of arithmetical mean (m). Student coefficient was calculated for the estimation of validity of difference between statistical characteristics of five alternative blocks of data. The difference was claimed to be valid under the index of validity $P>0.95$.

Results and discussion

Functioning of glutathione antioxidant system of bacterial cells is investigated not enough. It is known that this part of antioxidant protective system is not presented among all prokaryotic organisms. It is specific for various species and it could be even strain-specific [8]. In the literature, there is no data about functioning of glutathione system in *D. acetoxidans* bacteria. Due to BLASTP program, which is available on NCBI [18] there were identified genes that encode enzymes of glutathione system in the strains that are closely related to *D. acetoxidans* IMV B-7384. Genes encoding glutathione peroxidese and glutathione-S-transferase were detected in

Pelobacter propionicus DSM 2379. Genes encoding glutathione peroxidese, glutathione reductase, glutathione-S-transferase and enzymes of glutathione biosynthesis of Fe (III)-reductors of *Shewanella* genus (*S. baltica* OS195 and *S. putrefaciens* CN-32) were identified also [18].

In our previous work we have investigated the content of reduced glutathione in *D. acetoxidans* IMV B-7384 bacteria under the influence of different concentrations of ferric (III) citrate [3]. It was shown that the content of reduced glutathione in bacterial cells significantly increased and reached to 1.2 mmoles/g of cells under the addition of ferric (III) citrate. Based on received data it was assumed that glutathione system is involved *in* antioxidant protection of *D. acetoxidans* IMV B-7384 bacteria.

Glutathione peroxidase (E.C.1.11.1.9) is one of the key enzymes that catalyzes oxidation of glutathione. The enzyme reduces hydrogen peroxide and unstable organic peroxides derived from polyunsaturated fatty acids [1].

The highest activity of glutathione peroxidase of *D. acetoxidans* IMV B-7384 bacteria, which was grown without addition of ferric (III) citrate, was detected on the third day of cultivation (Fig. 1). With the increasing of cultivation duration the activity of enzyme become decreased. Addition of ferric (III) citrate to the growth medium caused significant increasing of glutathione peroxidase activity. The highest activity of enzyme was observed on the second day of bacterial cultivation under the influence of all investigated concentrations of ferric (III) citrate. With the increasing of cultivation duration under the influence of ferric (III) citrate up to four days the activity of enzyme has been decreased. Activity of glutathione peroxidase also depended on concentration of metal salt in the growth medium. Activity of glutathione peroxidase increased by 12.5 times in comparison with control and equaled 35.5±2.56 mkmoles GSH/min·mg of protein under the influence of 10 mM of ferric (III) citrate on the second day of bacterial growth. Addition of ferric (III) citrate on the second day of growth in concentration 12–20 mM elevates activity of enzyme up to 50–54 mkmoles reduced glutathione/min·mg of protein. On the third and fourth days the enzyme activity was increased under enhancement of metal salt concentration.

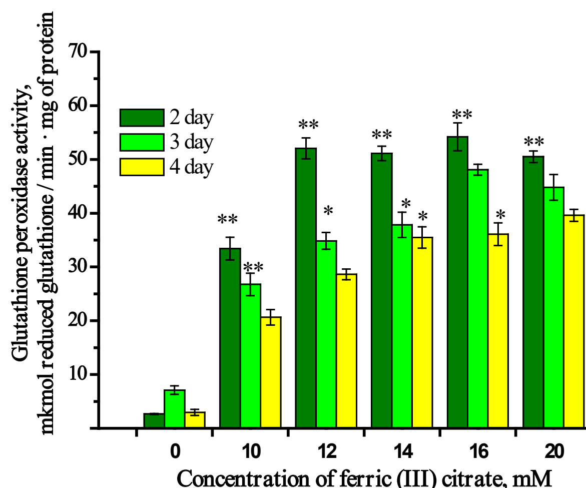

Fig. 1. Activity of glutathione peroxidase of *D. acetoxidans* IMV B-7384 under the influence of ferric (III) citrate (* – $p \geq 0.95$, n=3;** – $p \geq 0.99$, n=3 – in comparison with control).

Glutathione-S-trasferase catalyzes nucleophilic attack by the tripeptide glutathione (GSH) on the electrophilic groups of a wide range of hydrophobic toxic compounds, thus promoting

their excretion from the cell. Glutathione-S-transferase is involved in detoxification of hydrophobic hydroperoxides with high molecular mass: hydroperoxides of polyunsaturated fatty acids, phospholipids and hydroperoxides of mononucleotides [4]. It is opposite to glutathione peroxidase because of the most suitable substrates for this enzyme are hydrophilic hydroperoxides with small molecular mass.

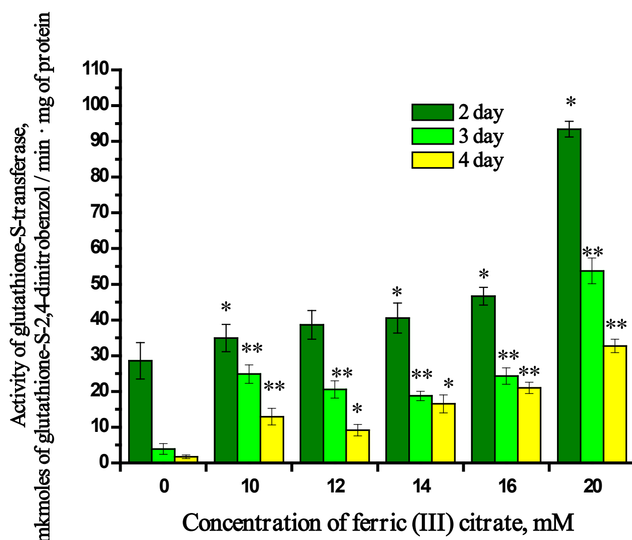

Fig. 2. Activity of glutathione-S-transferase of *D. acetoxidans* IMV B-7384 under the influence of ferric (III) citrate (* – $p \geq 0.95$, n=3;** – $p \geq 0.99$, n=3 – in comparison with control).

Activity of glutathione-S-transferase of *D. acetoxidans* IMV B-7384 grown in the medium without ferric (III) citrate significantly changed during four days of cultivation (Fig. 2). The highest activity of enzyme was observed on the second day of bacteria growth. Activity of glutathione-S-transferase on the third and fourth days considerably decreased in comparison with activity of enzyme on the second day. Addition of ferric (III) citrate to cultural medium caused enhancement of glutathione-S-transferase activity of cell-free extracts of *D. acetoxidans* IMV B-7384. Under addition of 10 mM of ferric (III) citrate on the second, third and fourth days of bacterial growth activity of enzyme increased by 1.1; 6.3 and 7.4 times respectively in comparison with control. Similar changes of enzyme activity were observed under the influence of all investigated concentrations of ferric (III) citrate.

Activity of glutathione-S-transferase also changed dependently on concentration of metal salt in the medium. Activity of enzyme increased with raising of metal salt concentration up to 20 mM on the second day of *D. acetoxidans* IMV B-7384 growth. On the third day of growth activity of glutathione-S-transferase under the influence of ferric (III) citrate in concentrations 10–16 mM remained at the values 20–25 mkmoles glutathione-S-2,4-dinitrobensol/min·mg of protein. Significant raising of enzyme activity on this day was observed under the influence of 20 mM of metal salt. Similar changes of glutathione-S-transferase activity under the addition of ferric (III) citrate were observed on the fourth day.

Biological role of glutathione reductase is to maintain a high level of reduced glutathione and a low level of it's oxidized form in bacterial cell. Since glutathione serves as a redox agent in the cell, the activity of glutathione reductase is very significant. Enzyme functioning in bacterial cell makes it possible to reduce the glutathione synthesis [1].

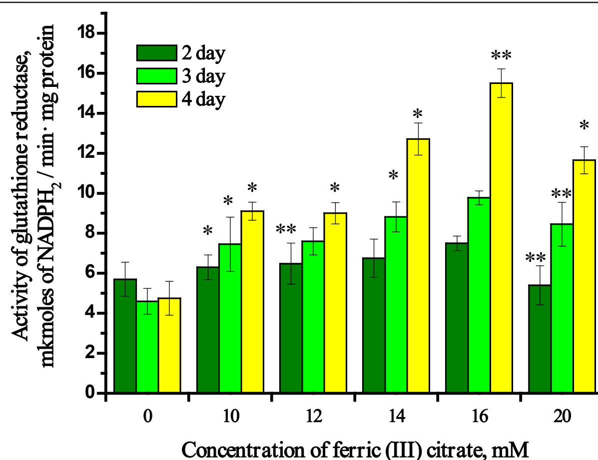

Fig. 3. Activity of glutathione reductase of *D. acetoxidans* IMV B-7384 under the influence of ferric (III) citrate (* – $p \geq 0.95$, n=3;** – $p \geq 0.99$, n=3 – in comparison with control).

The highest activity of glutathione reductase of cell-free extracts of *D. acetoxidans* IMV B-7384 strain grown without addition of ferric (III) citrate was observed on the second day of growth (Fig. 3). On the third and fourth days of growth activity of enzyme slightly decreased in comparison with enzyme activity on the second day and its value remained 5.2–5.3 mkmoles of $NADFH_2$/min·mg of protein. Addition of ferric (III) citrate to the cultural medium caused significant increasing of glutathione reductase activity of bacterial cells. Activity of glutathione reductase of cell-free extracts of *D. acetoxidans* IMV B-7384 under the influence of ferric (III) citrate changed in dependence on metal salt concentration and duration of bacterial cultivation. Under the influence of all investigated concentrations of metal salt the highest enzyme activity was observed on the fourth day of growth. On the second day of bacteria growth under addition of 10–16 mM of ferric (III) citrate glutathione reductase activity slightly increased in comparison with control and was 6.1–6.3 mkmoles of $NADPH_2$/min·mg of protein. Addition of 20 mM of metal salt to cultural medium on the second day of growth caused no changes of glutathione reductase activity of cell-free extracts of *D. acetoxidans* IMV B-7384. On the third and fourth days of bacterial growth activity of enzyme increased with raising the concentration of ferric (III) citrate up to 16 mM. Activity of glutathione reductase decreased under the influence of 20 mM of ferric (III) citrate in comparison with the influence of 16 mM of investigated metal salt.

Thus, under addition of ferric (III) citrate into the cultural medium of *D. acetoxidans* IMV B-7384 the activity of all investigated enzymes of glutathione system increased in comparison with control. Enhancement of activity of glutathione enzymes system confirms their necessary role in antioxidant protection of *D. acetoxidans* IMV B-7384 cells. However, activity of glutathione peroxidase and glutathione-S-transferase decreased with increasing of duration of bacterial cultivation at the presence of ferric (III) citrate. Decreasing of these enzymes activity could be caused by diminishment of reduced glutathione content in bacterial cells. In our previous work we have investigated the content of reduced glutathione in cell-free extracts of *D. acetoxidans* IMV B-7384 under the influence of ferric (III) citrate. It was shown that the highest content of reduced glutathione under the influence of all investigated concentrations of metal salt was observed on the second day of bacterial growth. On the third and fourth days content of reduced glutathione significantly decreased in comparison with content of this tripeptide on the second day [3]. Maintaining the respective quantity of reduced glutathione is supported by glutathione reduc-

tase. Activity of glutathione reductase of *D. acetoxidans* IMV B-7384 cell-free extracts increased with enhancement of bacterial cultivation duration under the influence of ferric (III) citrate. Possibly, the reduced glutathione synthesized by glutathione reductase is not sufficient to maintain the necessary level of tripeptide in bacterial cells under the influence of ferric (III) citrate. Also, decreasing of reduced glutathione content in *D. acetoxidans* IMV B-7384 cell-free extracts could be caused by direct interaction of tripeptide with active intermediates of lipoperoxidation [10]. In this case oxidized glutathione is not formed. Thus increasing of glutathione reductase activity under the influence of ferric (III) citrate is not substantial. It is known that biosynthesis of reduced glutathione is more important to maintain the reduced glutathione content in *E. coli*, compared with the reduction of oxidized glutathione back to reduced glutathione by glutathione reductase [8]. Decreasing of reduced glutathione content in *D. acetoxidans* IMV B-7384 cell-free extracts under the influence of ferric (III) citrate also could be caused by damage of enzymes of glutathione synthesis by products of lipid peroxidation.

СПИСОК ВИКОРИСТАНОЇ ЛІТЕРАТУРИ

1. *Головчак Н. П., Тарновська А. В., Коцюмбас Г. І., Санагурський Д. І.* Процеси перекисного окиснення ліпідів у живих організмах. Львів: ЛНУ імені Івана Франка, 2012. 250 с.
2. *Мартинчик А. Н., Бондарев Г. И.* Активность глутатионредуктазы и глутатион-S-трансферазы в печени крыс в зависимости от содержания восстановленного глутатиона // Вопр. мед. химии. 1986. Т. 32. № 2. С. 39–43.
3. *Масловська О. Д., Гнатуш С. О.* Інтенсивність процесів перекисного окиснення ліпідів та показники системи антиоксидантного захисту клітин *Desulfuromonas* acetoxidans ІМВ В-7384 за впливу ферум (ІІІ) цитрату // Вісн. Львів. ун-ту. Сер. біол. 2014. Вип. 64. С. 270–278.
4. *Меньшикова Е. Б., Ланкин В. З., Зенков Н. К.* и др. Окислительный стресс. Прооксиданты и антиоксиданты. М.: Слово, 2006. 556 с.
5. *Лакин Г. Ф.* Биометрия. М.: Высшая школа, 1990. 352 с.
6. *Brioukhanov A., Netrusov A.* Aerotolerance of strictly anaerobic microorganisms and factors of defense against oxidative stress: a review // Appl. Biochem. Microbiol. 2007. Vol. 43. N 6. P. 635–652.
7. *Fareleira P., Santos B.* Response of a strict anaerobe to oxygen: survival strategies in *Desulfovibrio gigas* // Microbiol. 2003. Vol. 149. P. 1513–1522.
8. *Fu R., Chen J., Li Y.* The function of the glutathione / glutathione peroxidase system in the oxidative stress resistance systems of microbial cells // Chinese J. Biotechnol. 2007. Vol. 23. N 5. P. 770–775.
9. *Gudz S., Hnatush S., Moroz O.* et al. Certificate of deposit strain of bacteria *Desulfuromonas acetoxidans* Ya-2006 in the Depository of Institute of Microbiology and Virology. Zabolotny NAS of Ukraine on granting registration number IMV B-7384 of 10 April 2013.
10. *Gueraud F., Atalay M., Bresgen N.* et. al. Chemistry and biochemistry of lipid peroxidation products // Free Radic. Res. 2010. Vol. 44. N 10. P. 1098–1124.
11. *Lovely D., Holmes D., Nevin K.* Dissimilatory Fe (III) and Mn (IV) reduction // Adv. Microb. Physiol. 2011. Vol. 59. P. 1–100.
12. *Lovley D., Ueki T., Zhang T. Geobacter*: the microbe electric's physiology, ecology, and practical applications // Adv. Microb. Physiol. 2004. Vol. 49. P. 219–286.
13. *Lowry O., Rosenbrougn N. I., Farr A. L., Randall R. I.* Protein measurement, with the Folin phenol reagent // J. Biol. Chem. 1951. N 193. P. 265–275.

14. *Papanikolaou G., Pantopoulos K.* Iron metabolism and toxicity // Toxicol. Appl. Pharmacol. 2005. Vol. 202. P. 199–211.
15. *Postgate J. R.* The sulfate-reducing bacteria. C.: Cambridge University Press, 1984. 208 p.
16. *Vasyliv O., Bilyy O., Ferensovich Ja., Hnatush S.* Electric current generation by sulfur-reducing bacteria in microbial-anode fuel cell // Proc. SPIE (San-Diego, USA, 2012). N 8472. P. 84720Z-1–7.
17. *Zhang E., Xu W., Diao G., Shuang C.* Electricity generation from acetate and glucose by sedimentary bacterium attached to elecrode in microbial-anode fuel cells // J. Power Sources. 2006. N 161. P. 820–825.
18. www.ncbi.nlm.nih.gov

Стаття: надійшла до редакції 30.04.15

доопрацьована 26.10.15

прийнята до друку 27.10.15

АКТИВНІСТЬ ФЕРМЕНТІВ ГЛУТАТІОНОВОЇ АНТИОКСИДАНТНОЇ СИСТЕМИ БАКТЕРІЙ *DESULFUROMONAS ACETOXIDANS* IMB B-7384 ЗА ВПЛИВУ ФЕРУМ (III) ЦИТРАТУ

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Бактерії *Desulfuromonas acetoxidans* IMB B-7384 є перспективними мікроорганізмами для конструювання мікробних паливних елементів і розроблення біотехнологій для очищення стічних вод від іонів металів зі змінною валентністю. Однак іони металів стимулюють утворення активних метаболітів оксигену, які можуть згубно впливати на клітини бактерій. Досліджено активність глутатіонпероксидази, глутатіон-S-трансферази та глутатіонредуктази у бактерій *D. acetoxidans* IMB B-7384 за впливу різних концентрацій ферум цитрату. За внесення солі металу в середовище для вирощування бактерій спостерігали зростання активності усіх досліджених ферментів, порівняно з контролем. Активність глутатіонпероксидази, глутатіон-S-трансферази та глутатіонредуктази змінювалася залежно від концентрації солі металу і тривалості вирощування бактерій. Активність глутатіонпероксидази та глутатіон-S-трансферази знижувалася зі збільшенням тривалості культивування бактерій, у той час як активність глутатіонредуктази за впливу солі металу зростала зі збільшенням тривалості вирощування. Зниження активності глутатіонпероксидази та глутатіон-S-трансферази може бути зумовлене зниженням вмісту відновленого глутатіону у клітинах бактерій. Можливо, кількості відновленого глутатіону, які утворилися глутатіонредуктазою, були недостатні для підтримання необхідного рівня трипептиду у клітинах бактерій за впливу ферум (III) цитрату. Зростання активностей ферментів глутатіонової системи свідчить про їхню важливу роль в антиоксидантному захисті клітин *D. acetoxidans* IMB B-7384.

Ключові слова: Desulfuromonas acetoxidans, глутатіонпероксидаза, глутатіонредуктаза, глутатіон-S-трансфераза, ферум (III) цитрат.

ISSN 0206-5657. Вісник Львівського університету. Серія біологічна. 2015. Випуск 70

АКТИВНОСТЬ ФЕРМЕНТОВ ГЛУТАТИОНОВОЙ АНТИОКСИДАНТНОЙ СИСТЕМЫ БАКТЕРИЙ *DESULFUROMONAS ACETOXIDANS* ИМВ В-7384 ПРИ ВЛИЯНИИ ФЕРУМ (III) ЦИТРАТА

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Бактерии *Desulfuromonas acetoxidans* ИМВ В-7384 – это перспективные микроорганизмы для конструирования микробных топливных элементов и разработки биотехнологий очистки сточных вод от ионов металлов с переменной валентностью. Однако ионы металлов стимулируют образование активных метаболитов кислорода, которые могут пагубно воздействовать на клетки бактерий. Исследована активность глутатионпероксидазы, глутатион-S-трансферазы и глутатионредуктазы у бактерий *Desulfuromonas acetoxidans* ИМВ В-7384 при воздействии различных концентраций феррум (III) цитрата. При внесении соли металла в среду для выращивания бактерий наблюдали рост активности всех исследуемых ферментов по сравнению с контролем. Активность глутатионпероксидазы, глутатион-S-трансферазы и глутатионредуктазы менялась в зависимости от концентрации соли металла и времени выращивания бактерий. Активность глутатионпероксидазы и глутатион-S-трансферазы снижалась с увеличением времени культивирования бактерий, в то время как активность глутатионредуктазы при воздействии соли металла возрастала с увеличением времени выращивания. Рост активностей ферментов глутатионовой системы свидетельствует об их важной роли в антиоксидантной защите клеток *D. acetoxidans* ИМВ В-7384. Снижение активности глутатионпероксидазы и глутатион-S-трансферазы может быть обусловлено снижением содержания восстановленного глутатиона в клетках бактерий. Возможно, количества восстановленного глутатиона, которые образовались глутатионредуктазой, были недостаточны для поддержания необходимого уровня трипептида в клетках бактерий при воздействии феррум (III) цитрата.

Ключевые слова: *Desulfuromonas acetoxidans*, глутатионпероксидаза, глутатион-S-трансфераза, глутатионредуктаза, феррум (III) цитрат.