

## EFFECT OF TRANSITION METAL COMPOUNDS ON CATALASE ACTIVITY OF SULFUR-REDUCING BACTERIAL *DESULFUROMONAS ACETOXIDANS* CELLS

O. Vasyliv, S. Hnatush

*Ivan Franko National University of Lviv  
4, Hrushevskiyi St., Lviv 79005, Ukraine  
e-mail: oresta.vasyliv@gmail.com*

*Desulfuromonas acetoxidans* are gram-negative obligatory anaerobic sulfur-reducing bacteria. Aerobes and facultative anaerobes possess specific enzymatic antioxidant system which defends them against toxic and mutagenic compounds or detrimental influence of the oxygen. One of the most crucial components of it is catalase. It's assumed that lack or low activity of this system's enzymes of the obligatory anaerobic microorganisms, such as sulfur- or sulfate-reducing bacteria, causes their high sensitivity to the influence of oxygen or toxic compounds, such as transition metals. The influence of  $\text{FeSO}_4$ ,  $\text{FeCl}_3 \times 6\text{H}_2\text{O}$ ,  $\text{MnO}_2$ ,  $\text{MnCl}_2 \times 4\text{H}_2\text{O}$ ,  $\text{NiCl}_2 \times 6\text{H}_2\text{O}$  and  $\text{CoCl}_2 \times 6\text{H}_2\text{O}$  on catalase activity of sulfur-reducing bacterial *D. acetoxidans* cells has been investigated. The highest activity of investigated enzyme has been observed under the influence of 0.5 mM, 1.5 mM and 2.0 mM of  $\text{NiCl}_2 \times 6\text{H}_2\text{O}$ ,  $\text{FeSO}_4$  and  $\text{CoCl}_2 \times 6\text{H}_2\text{O}$  respectively on the second day, 1.5 mM of  $\text{MnO}_2$  on the third day and 0.5 mM of  $\text{FeCl}_3 \times 6\text{H}_2\text{O}$  and 1.0 mM of  $\text{MnCl}_2 \times 4\text{H}_2\text{O}$  on the fourth day of growth in comparison with control samples.

*Key words:* *Desulfuromonas acetoxidans*, sulfur bacteria, antioxidant defense, catalase, transition metals.

Nowadays applying of ecological biotechnologies is one of the most crucial directions in solution of the problem of different toxic metal ions environmental pollution, which is a result of negative anthropogenic influence. Investigations of effective biological mechanisms of harmful compounds detoxification by different microorganisms is one of the ways of its development [9]. Transition metals, such as Ferrum, Manganese, Nickel, Cobalt, Argentum, Cooper, Cadmium, Zinc, Molybdenum etc are necessary in the micromolar concentrations for normal functionality of microorganisms while their growth and functional activity are inhibited under the influence of millimolar concentrations of these metals [14]. Despite that an exorbitant quantity of bacteria possesses effective defensive mechanisms that protect them against negative toxic xenobiotics influence. It determines their resistance towards high metal ion concentrations in the surrounding environment [15].

Nickel, Ferrum, Manganese and Cobalt are required as trace elements by many bacteria. Thus these metals are under specific investigations. Ferrum ions support effectivity of cell transport systems work, take part in the intracellular energy production and cell division [9]. Nickel and Cobalt are similar to Ferrum by their electrochemical properties. It allows to assume that these elements could substitute iron under its restricted concentrations in the environment. These elements are essential nutrients for selected microorganisms where they participate in a variety of cellular processes. Nickel ion is specifically incorporated into nickel-dependent enzymes, often via complex assembly processes requiring accessory proteins and additional non-protein components, in some cases accompanied by nucleotide triphosphate hydrolysis. To date, nine nickel-containing enzymes are known: urease, Ni-Fe-hydrogenase, carbon monoxide dehydrogenase, acetyl-CoA decarbonylase/synthase, methyl coenzyme M reductase, certain superoxide

dismutases, some glyoxylases, aci-reductone dioxygenase, and methylenediurease [13]. Cobalt is a constituent of vitamin B<sub>12</sub> and other corrinoids that are widespread coenzymes [14]. This metal also possesses a significant role in the regulation of enzymes activity, especially these which are involved in the regulation of aminoacids transamination [7]. It was investigated that Ferrum and Manganese can be used as a final electron acceptors in the processes of anaerobic respiration, such as dissimilative Fe<sup>3+</sup>- and Mn<sup>4+</sup> – reduction that is carried by sulfur-reducing *Desulfuromonas acetoxidans* bacteria.

*D. acetoxidans* are gram-negative obligatory anaerobic sulfur-reducing bacteria that inhabit sulfur containing aquatic environments. These bacteria belong to the  $\delta$  - *Proteobacteria* class [10].

Sulfur- and sulfate-reducing bacteria (SRB) have a significant meaning in the maintenance of ecological stability of surrounding environment. They are able to control transfer of metals in aquatic sediments by the hardly soluble metal sulfids creation. The process, which is held by the sulfur cycle reduce stage bacteria, represents an effective mechanism of biological remediation of overconcentrated heavy metals environmental pollution [4]. SRB possess a unique potential of spontaneous removal of metals and sulfates from the surrounding environment during the process of sulfides bioprecipitation.

Reactive oxygen species (ROS) such as superoxide radical, hydrogen peroxide, hydroxyl radical etc are toxic to all living cells. They are produced as a result of prolonged influence of oxygen or toxic xenobiotics, such as heavy metal ions, on the cell. ROS cause amino-acid residues modification, proteins' sulfhydryl groups oxidation, peptides' connections cleavage, release of metal from metaloproteids, nucleic acids depolymerization, point mutations, polysaccharides and unsaturated fatty acids oxidation etc [2, 6]. Aerobs and facultative anaerobs possess an enzymatic antioxidant defensive system that prevents the consequences of this toxic and mutagenic compounds and toxic oxygen influence. One of the most important components of it is catalase (hydrogen peroxide: hydrogen peroxide oxidoreductase). This enzyme causes the two-electron H<sub>2</sub>O<sub>2</sub> cleavage with O<sub>2</sub> and H<sub>2</sub>O production. In this case H<sub>2</sub>O<sub>2</sub> is used as electrons donor. Catalase belongs to the enzymes that support their activity during long time and almost don't need the activation energy. It was investigated that catalase could join four NADFH<sup>+</sup> molecules. As a result it is defended against inactivation and enzyme activity enhances. Catalase hardly influxes inside the cell and loses it's activity in the extracellular surrounding, especially because of high proteolytic enzymes activity [6].

Aerobic microorganisms possess an effective cascade functioning complex of defensive enzymatic and non-enzymatic systems that cleavage reactive oxygen species and synthesize DNA repair enzymes and antioxidant defense regulations [2].

Obligatory anaerobic microorganisms, such as sulfur- and sulfate-reducing bacteria possess an increased sensitivity to the oxygen. This fact is explained by the lack or low activity of antioxidant defensive system enzymes. It was investigated that this bacteria are able to live in the environments that contain oxygen because of possession the alternative defensive mechanism against oxidative stress. It includes:

- specific rubredoxin oxidoreductase that possesses a superoxide dismutase activity, causes an intercellular O<sub>2</sub> - reduction with H<sub>2</sub>O production and also serves as terminal oxidase;
- Rubrerythrin that possesses NADH-peroxidase activity;
- Similar to rubredoxin proteins that are used as intermediate electron donors [4].

Catalase activity has been observed among the several species of sulfate-reducing bacteria of *Desulfovibrio* genus. There are *D. desulfuricans*, *D. vulgaris*, *D. oxyclinae* and *D. gigas* [6].

Despite that, it isn't investigated enough in sulfur-reducing bacteria.

That's the aim of our work was to investigate the influence of different valences transition metals compounds, such as  $\text{FeSO}_4$ ,  $\text{FeCl}_3 \times 6\text{H}_2\text{O}$ ,  $\text{MnO}_2$ ,  $\text{MnCl}_2 \times 4\text{H}_2\text{O}$ ,  $\text{NiCl}_2 \times 6\text{H}_2\text{O}$  and  $\text{CoCl}_2 \times 6\text{H}_2\text{O}$  on catalase activity of bacterial *Desulfuromonas acetoxidans* cells.

### MATERIALS AND METHODS

The object of investigations was sulfur-reducing *Desulfuromonas acetoxidans* bacterium that was extracted from Yavoriv sulfur aquatic field (Lviv region), obtained in the pure culture and identified at the Department of Microbiology of Ivan Franko National University of Lviv, Ukraine. Bacteria were cultivated in the modified Postgait C medium [3, 5] during four days under the anaerobic conditions and temperature  $+30^\circ\text{C}$  with addition from 0.5 to 2.5 mM of  $\text{FeSO}_4$ ,  $\text{FeCl}_3 \times 6\text{H}_2\text{O}$ ,  $\text{MnO}_2$ ,  $\text{MnCl}_2 \times 4\text{H}_2\text{O}$ ,  $\text{NiCl}_2 \times 6\text{H}_2\text{O}$  and  $\text{CoCl}_2 \times 6\text{H}_2\text{O}$  into the growth medium. Control samples didn't contain any investigated metal compounds. After the second, third and fourth day of bacterial growth cell-free extracts were obtained by the following technique. Cells were washed by 0.9% NaCl solution and disintegrated on the ultrasonic homogenizer at 22 kHz at  $0^\circ\text{C}$  during five minutes. Cell debris were sedimented by centrifugation at 5635–8800g at  $4^\circ\text{C}$  during 30 minutes. Catalase activity was measured spectrophotometrically with wave length 410 nm by the quantity of cleaved hydrogen peroxide [12]. Reaction mixture contains 2,8 ml of 0,5%  $\text{H}_2\text{O}_2$  solution and 0.1–0.2 ml of cell-free extract, diluted by  $n$  times with concentration 1 mg of protein per 1 ml. Incubation was carried out during 5 minutes. Reaction was stopped by 1 ml of 6%  $(\text{NH}_4)_2\text{MoO}_4$  solution. Probe that contains  $\text{H}_2\text{O}$  instead of cell-free extract solution served as a control.

Enzyme activity was calculated by the formula:

$$A, \frac{\mu\text{mol}}{\text{min} \cdot \text{mg of protein}} = \frac{\Delta E_{410} \cdot 4 \cdot n}{110,6 \cdot t \cdot V \cdot C},$$

where  $\Delta E_{410}$  – difference between  $E_{410}$  of control sample and  $E_{410}$  of probe; 4 – the overall volume of reaction mixture, ml;  $n$  – solution of cell-free extract, times; 110.6 – constant, determined by the calibration curve;  $t$  – time of incubation, minutes,  $V$  – volume of cell-free extract, added to the reaction mixture, ml;  $C$  – protein concentration in the probe, mg/ml.

Protein concentration in the cell-free extract was determined by Lauri method [11].

The crucial statistical indexes were calculated on the base 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 two alternative blocks of data. The difference was claimed to be valid under the index of validity  $P > 0,95$  [1]. Statistical calculation of results were carried out by the Origin and Excel programs.

### RESULTS AND DISCUSSION

Catalase activity of sulfur-reducing *D. acetoxidans* bacteria cell-free extracts has been investigated under the influence of different concentrations of ferrous (II) sulfate, ferrous (III) chloride hexahydrate, manganese (IV) oxide, manganese (II) chloride tetrahydrate, nickel (II) chloride hexahydrate and cobalt (II) chloride hexahydrate during four days of cultivation.

Addition of different  $\text{FeCl}_3 \times 6\text{H}_2\text{O}$  concentrations caused the highest activity of investigated enzyme ( $61,36 \pm 0,77 \mu\text{mol}/\text{min} \times \text{mg}$  of protein) on the fourth day of growth under the influence of 0.5 mM of investigated metal salt hydrate (fig. 1, A). It increased by 2.2 times on the second, by 3.8 times on the third and by 5 times on the fourth days of growth under such influence of ferrous (III) chloride hexahydrate on investigated bacterial cells in comparison with control samples. Increase of  $\text{FeCl}_3 \times 6\text{H}_2\text{O}$  concentration by 2.5 mM in the growth medium caused the catalase activity decrease by 2.5 times on the second day of cultivation and increase by 2.7 and 3.9 times respectively on the third and fourth day of growth in comparison with control samples.

Obviously, increase of duration of ferrous (III) chloride hexahydrate influence on *D. acetoxidans* cells caused the enhance of catalase activity. It is possibly could be explained by reactive oxygen species production, especially of hydrogen sulfide creation under the influence of investigated metal salt. It was investigated that existence of unconjugated Ferrous ions, sulfides and sulfhydryl groups in bacterial growth medium enhances peroxide radicals production that significantly increases bacterial antioxidant defensive system activity [4].

Under the influence of ferrous (II) sulfate the highest catalase activity was observed on the second day of bacterial growth with addition of 1.5 mM of metal salt in the growth medium. It equaled  $28,52 \pm 0,34 \mu\text{mol}/\text{min} \times \text{mg}$  of protein (fig. 1, B). Increase of metal salt concentration in the growth medium from 0.5 to 1.5 mM caused the enhance of catalase activity by 2.1–2.5 times on the second and by 42–80% on the third day of growth respectively in comparison with control samples. Activity of investigated enzyme decreased by 65% under the influence of 0.5 mM of  $\text{FeSO}_4$  and by 3.3 times under the influence of 1.5 mM of investigated Ferrous salt respectively on the fourth day of growth. Catalase activity increased by 2.4 and 1.5 times on the second and third days and decreased by 3 times on the fourth day of growth respectively under the influence of 2.5 mM of  $\text{FeSO}_4$  in comparison with control samples. Possibly, increase of duration of investigated metal salt influence doesn't cause toxic effect on investigated bacterial cells. As a result, catalase activity as one of the mechanisms of antioxidant defensive system decreased.

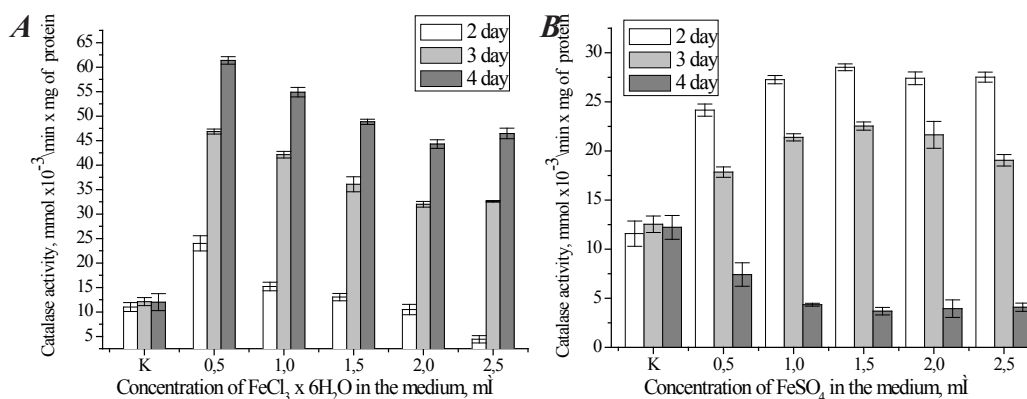


Fig. 1. The influence of (A) ferrous (III) chloride hexahydrate and (B) ferrous (II) sulfate on catalase activity of cell-free *D. acetoxidans* extracts during four days of cultivation.

Obviously,  $\text{Fe}^{2+}$  as a final products of dissimilative  $\text{Fe}^{3+}$ - reduction are necessary for supporting of normal cell functionality of *D. acetoxidans* bacteria [9].

Results of nickel (II) chloride hexahydrate influence on catalase activity of sulfur-reducing *D. acetoxidans* bacteria are presented on the fig. 2, A. The maximal activity of investigated enzyme was observed on the second day of growth under the influence of 0.5 mM of  $\text{NiCl}_2 \times 6\text{H}_2\text{O}$ . It equaled  $30,49 \pm 0,63 \mu\text{mol}/\text{min} \times \text{mg}$  of protein. Catalase activity was higher by 2.7; 2 and 1.7 times respectively on the second, third and fourth days of growth in comparison with control samples under the influence of this metal salt hydrate concentration. Increase of  $\text{NiCl}_2 \times 6\text{H}_2\text{O}$  concentration to 2.5 mM caused the increase of catalase activity by 67 and 32% respectively on the second and third days of growth compared with control samples. On the fourth day of growth it decreased to the control samples level.

Possibly, high concentrations of hydrogen peroxide are produced on the first stages of different concentrations of nickel (II) chloride hexahydrate influence on investigated bacteria.

As a result, high catalase activity was observed during the second day of *D. acetoxidans* bacteria growth under the influence of  $\text{NiCl}_2 \times 6\text{H}_2\text{O}$ .

Addition of different  $\text{CoCl}_2 \times 6\text{H}_2\text{O}$  concentrations into the growth medium caused the highest catalase activity of sulfur-reducing *D. acetoxidans* bacteria under the influence of 2.0 mM of investigated metal salt hydrate on the second day of growth. It equaled  $38,68 \pm 0,59 \mu\text{mol}/\text{min} \times \text{mg}$  of protein (fig. 2, B).

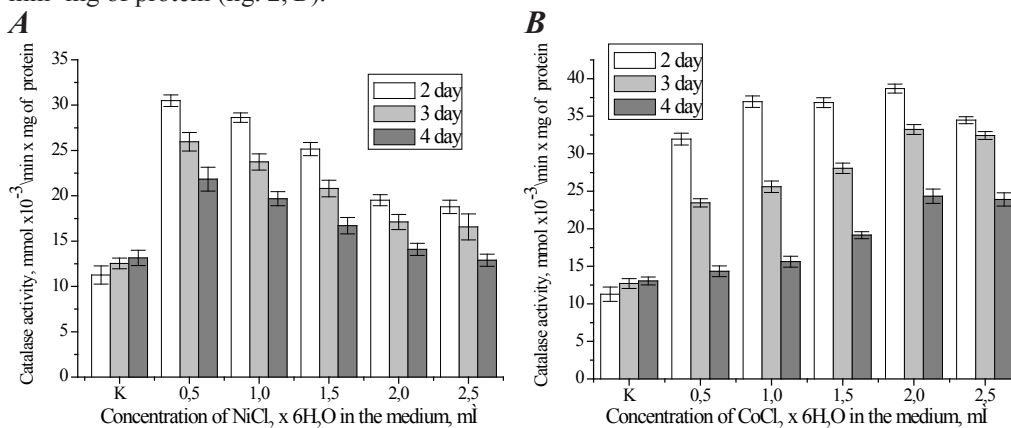


Fig. 2. The influence of (A) nickel (II) chloride hexahydrate and (B) cobalt (II) chloride hexahydrate on catalase activity of cell-free *D. acetoxidans* extracts during four days of cultivation.

Catalase activity increased by 3.4; 2.6 times and by 86% respectively on the second, third and fourth days of growth compared with control samples under the influence of investigated cobalt (II) chloride hexahydrate concentration. Unlike  $\text{NiCl}_2 \times 6\text{H}_2\text{O}$  influence on investigated enzyme activity of *D. acetoxidans*, increase from 0.5 to 2.5 mM of cobalt salt hydrate concentration caused the increase of investigated enzyme activity in comparison with control samples during the whole investigated period of cultivation. It increased by 3, 2.6 and 1.8 times respectively on the second, third and fourth days of growth in comparison with control samples under the influence of 2.5 mM of cobalt (II) chloride hexahydrate.

Obviously, increase of Cobalt ions concentration in the bacterial growth medium causes the intracellular ROS content enhance on the first stages of metal-bacteria interaction. It is caused cell defensive mechanisms activation, especially activation of antioxidant system as a reaction to stress, caused by transition metal long-term exposure. Possibly, high  $\text{Co}^{2+}$  concentrations are more toxic to investigated bacterial cells in comparison with  $\text{Ni}^{2+}$ .

Catalase activity changes of sulfur-reducing *D. acetoxidans* bacteria under the influence of manganese (IV) oxide have been investigated.  $\text{MnO}_2$  and  $\text{FeCl}_3 \times 6\text{H}_2\text{O}$  are strong prooxidants. That's why catalase activity was the most high under the influence of these compounds in comparison with all other investigated transition metals influence. Addition of manganese (IV) oxide into the growth medium caused the maximal activity of investigated enzyme under the influence of 1.5 mM of  $\text{Mn}^{4+}$  on the third day of growth. It equaled  $56,32 \pm 0,67 \mu\text{mol}/\text{min} \times \text{mg}$  of protein (fig. 3, A). Under such conditions investigated enzyme activity increased by 4.6 times in comparison with control samples. Increase of  $\text{MnO}_2$  concentration from 0.5 to 2.5 mM caused the inhibition of catalase activity by 1.4–1.8 times on the second, 4.3–4.1 times on the third and 2.3–2.2 times on the fourth days of cultivation in comparison with control samples. Obviously, existence of  $\text{MnO}_2$  in the cultural medium causes the intensive reactive oxygen species production. It causes an activation of catalase synthesis as the mechanism of cell defense against an oxidative stress.

Decrease of investigated enzyme activity possibly is caused by partial manganese (IV) oxide cleavage and  $Mn^{4+}$  involving in the process of  $Mn^{4+}$  – reduction, which is held by sulfur-reducing *D. acetoxidans* bacteria. In this process they support themselves with the energy and  $Mn^{2+}$  that are needed for bacterial cells normal physiological and biochemical functionality [8, 9].

Under the influence of manganese (II) chloride tetrahydrate the highest catalase activity of sulfur-reducing *D. acetoxidans* bacteria was observed on the fourth day of growth with addition of **1.0 mM of metal salt hydrate in the growth medium. It was  $36,62 \pm 0,70 \mu\text{mol}/\text{min} \times \text{mg}$  of protein** under such growth conditions (fig. 3, B). Investigated enzyme activity increased by 2.9 times under the influence of this concentration of  $MnCl_2 \times 4H_2O$  in comparison with control samples. Increase from 0.5 to 2.5 mM of metal chloride hexahydrate concentration in the growth medium caused the increase of catalase activity by 2.6–2.3, 2.6–2.4 and 2.8–2.7 times respectively on the second, third and fourth days of cultivation in comparison with control samples. Under the influence of  $MnCl_2 \times 4H_2O$  catalase activity was lower in comparison with the influence of  $MnO_2$  that is strong prooxidant.

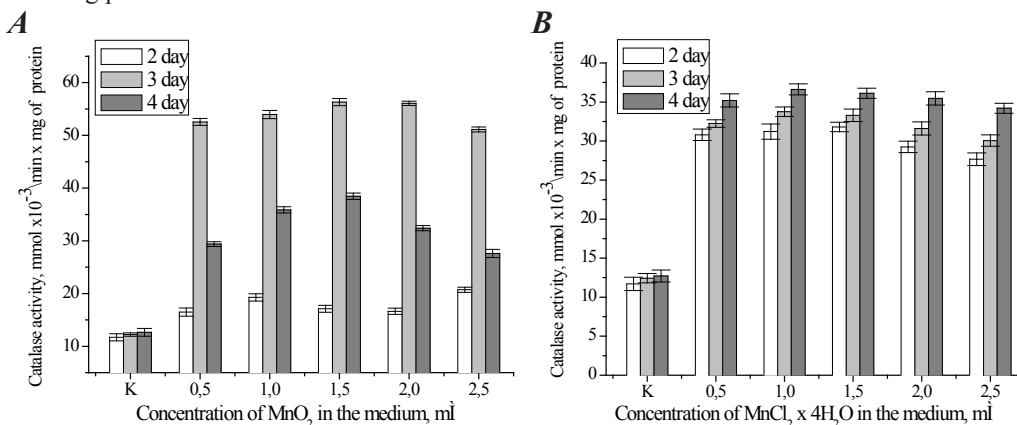


Fig. 3. The influence of (A) manganese (IV) oxide and (B) manganese (II) chloride hexahydrate on catalase activity of cell-free *D. acetoxidans* extracts during four days of cultivation.

Catalase activity of *D. acetoxidans* bacteria almost didn't change during four days of bacterial growth under the influence of different manganese (II) chloride hexahydrate concentrations. Possibly it could be explained by the fact that increase of  $MnCl_2 \times 4H_2O$  concentration and duration of it's influence cause the toxic effect on cell's enzyme functionality, activity of reparation systems, microbial transport systems etc. Possibly, catalase activity increases as one of cell defensive mechanisms against negative influence of reactive oxygen species which are produced because of  $MnCl_2 \times 4H_2O$  long-term exposure on the cells of investigated bacteria.

Thus, the highest catalase activity of sulfur-reducing *D. acetoxidans* bacteria was observed under the influence of 1.5 mM of  $MnO_2$  and 0.5 mM of  $FeCl_3 \times 6H_2O$  respectively on the third and fourth days of cultivation in comparison with control samples. Under such conditions it was higher by 2 times in comparison with the influence of other investigated transition metal compounds. Under the influence of  $NiCl_2 \times 6H_2O$ ,  $FeSO_4$  and  $CoCl_2 \times 6H_2O$  the highest catalase activity was observed on the second day of growth with addition of 0.5 mM, 1.5 mM and 2.0 mM of investigated metal salts respectively. Under the influence of  $MnCl_2 \times 4H_2O$  the maximal activity of investigated enzyme was observed with addition of 1.0 mM of metal salt hydrate into the growth medium. Obviously, ferrous (III) chloride hexahydrate and manganese (IV) oxide that are strong oxidants, served as prooxidants towards sulfur-reducing bacterial *D. acetoxidans* cells.

As a result, the activity of their antioxidant defensive system, in particular catalase activity, was maximal under such influence. Effect of other investigated transition metal compounds caused lower investigated enzyme activity. Possibly, it shows that  $\text{NiCl}_2 \times 6\text{H}_2\text{O}$ ,  $\text{FeSO}_4$  and  $\text{CoCl}_2 \times 6\text{H}_2\text{O}$  possess less toxic effect toward the cells of investigated bacteria in comparison with  $\text{FeCl}_3 \times 6\text{H}_2\text{O}$  та  $\text{MnO}_2$ .

### CONCLUSIONS

It was shown that sulfur-reducing *Desulfuromonas acetoxidans* bacteria possess antioxidant defensive system activity, in particular catalase functioning, under the derimental influence of different concentrations of transition metals, despite that there are obligatory anaerobic microorganisms. The influence of such transition metal compounds as  $\text{FeSO}_4$ ,  $\text{FeCl}_3 \times 6\text{H}_2\text{O}$ ,  $\text{MnO}_2$ ,  $\text{MnCl}_2 \times 4\text{H}_2\text{O}$ ,  $\text{NiCl}_2 \times 6\text{H}_2\text{O}$  and  $\text{CoCl}_2 \times 6\text{H}_2\text{O}$  on catalase activity of investigated bacteria has been investigated. The maximal activity of investigated enzyme has been observed under the influence of 0.5 mM, 1.5 mM and 2.0 mM of  $\text{NiCl}_2 \times 6\text{H}_2\text{O}$ ,  $\text{FeSO}_4$  and  $\text{CoCl}_2 \times 6\text{H}_2\text{O}$  respectively on the second day of growth, 1.5 mM of  $\text{MnO}_2$  on the third day of growth and 0.5 mM of  $\text{FeCl}_3 \times 6\text{H}_2\text{O}$  and 1.0 mM of  $\text{MnCl}_2 \times 4\text{H}_2\text{O}$  on the fourth day of bacterial cultivation in comparison with control samples. Under the influence of different concentrations of ferrous (II) chloride hexahydrate and manganese (IV) oxide catalase activity was higher by 2 times in comparison with the influence of other investigated transition metal compounds.

### REFERENCES

1. Лакін Г. Ф. Биометрия. М.: Высшая шк., 1990. 352 с.
2. Меньшикова Е. Б., Ланкин В. З., Зенков Н. К. и др. Окислительный стресс. Прооксиданты и антиоксиданты. М.: Слово, 2006. 556 с.
3. Розанова Е. П. Методы культивирования и идентификации анаэробных бактерий, восстанавливающих серу и ее окисленные соединения / Институт микробиологии АН СССР. М., 1979. С. 123–136.
4. Barton L., Hamilton W. Sulfate-Reducing Bacteria. Environmental and Engineered Systems. New York, USA: Cambridge University Press, 2007. 558 p.
5. Biebl H., Pfennig N. Growth of sulfate-reducing bacteria with sulfur as electron acceptor // Arch. Microbiol. 1977. Vol. 112. P. 115–117.
6. Brioukhanov A. L., Thauer R. K., Netrusov A. I. Catalase and superoxide dismutase in the cells of strictly anaerobic microorganisms // Microbiol. 2002. Vol. 71. N 3. P. 281–285.
7. Cavet S. J., Borrelly G. M., Robinson N. J. Zn, Cu, Co in cyanobacteria: selective control of metal availability // FEMS Microbiol. Rev. 2003. Vol. 27. P. 165–181.
8. Dworkin M., Falkow S., Rosenberg E. et al. The Prokaryotes: Proteobacteria: Alpha and Beta Subclasses. 3rd edition. Minneapolis, USA: Springer, 2006. Vol. 5. 964 p.
9. Eric E., Derek R. Dissimilatory Fe (III) - reduction by the marine microorganism *Desulfuromonas acetoxidans* // Appl. Environ. Microbiol. 1993. Vol. 59. N 3. P. 734–742.
10. Garity G., Winters M., Searles D. Taxonomic Outline of the Procariotic Genera Bergey's Manual of Systematic Bacteriology. 2nd edition. Michigan, USA: Springer-Verlag, 2001. 41 p.
11. Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J. Protein determination with the Folin phenol reagent // J. Biol. Chem. 1951. Vol. 193. P. 265–275.
12. Luck H. Catalase // Methods in enzymatic analysis / H.-U. Bergmeyer ed. London: Academic Press, 1963. P. 855–894.
13. Murlooney S. B., Hausinger R. P. Nickel uptake and utilization by microorganisms // FEMS Microbiol. Rev. 2003. Vol. 27. P. 239–261.

14. Schmidt T., Schlegel H. Nickel and cobalt resistance of various bacteria isolated from soil and highly polluted domestic and industrial wastes // FEMS Microbiol. Ecol. 1989. Vol. 62. P. 315–328.
15. Silver S., Phung T. Bacterial heavy metal resistance: New Surprises // Annu. Rev. Microbiol. 1996. Vol. 50. P. 753–789.

Стаття: надійшла до редакції 30.06.11

доопрацьована 01.10.11

прийнята до друку 18.10.11

### ВПЛИВ СПОЛУК ПЕРЕХІДНИХ МЕТАЛІВ НА АКТИВНІСТЬ КАТАЛАЗИ У КЛІТИНАХ СІРКОВІДНОВЛЮВАЛЬНИХ БАКТЕРІЙ *DESULFUROMONAS ACETOXIDANS*

О. Васи́лів, С. Гнатуш

Львівський національний університет імені Івана Франка  
вул. Грушевського, 4, Львів 79005, Україна  
e-mail: oresta.vasyliv@gmail.com

*Desulfuromonas acetoxidans* – грамнегативні облигатно анаеробні сірководновлювальні бактерії. Для захисту від токсичних і мутагенних сполук й усунення токсичного впливу кисню в аеробів та факультативних анаеробів існує ензиматична система антиоксидантного захисту, однією з найважливіших складових якої є каталаза. Припускають, що відсутність чи низька активність ферментів даної системи в облигатно анаеробних мікроорганізмів, зокрема сірко- та сульфатредуючих бактерій, зумовлює їхню підвищену чутливість до кисню та дії токсичних сполук, зокрема перехідних металів. Досліджено вплив  $\text{FeSO}_4$ ,  $\text{FeCl}_3 \times 6\text{H}_2\text{O}$ ,  $\text{MnO}_2$ ,  $\text{MnCl}_2 \times 4\text{H}_2\text{O}$ ,  $\text{NiCl}_2 \times 6\text{H}_2\text{O}$  та  $\text{CoCl}_2 \times 6\text{H}_2\text{O}$  на активність каталази сірководновлювальних бактерій *D. acetoxidans*. Виявлено максимальну активність досліджуваного ферменту за впливу 0,5 мМ, 1,5 мМ та 2,0 мМ відповідно  $\text{NiCl}_2 \times 6\text{H}_2\text{O}$ ,  $\text{FeSO}_4$  та  $\text{CoCl}_2 \times 6\text{H}_2\text{O}$  на другу добу, 1,5 мМ  $\text{MnO}_2$  на третю добу та 0,5 мМ  $\text{FeCl}_3 \times 6\text{H}_2\text{O}$  і 1,0 мМ  $\text{MnCl}_2 \times 4\text{H}_2\text{O}$  на четверту добу культивування, порівняно з контролем.

**Ключові слова:** *Desulfuromonas acetoxidans*, сіркобактерії, антиоксидантний захист, каталаза, перехідні метали.



**ВЛИЯНИЕ СОЕДИНЕНИЙ ПЕРЕХОДНЫХ МЕТАЛЛОВ НА АКТИВНОСТЬ  
КАТАЛАЗЫ В КЛЕТКАХ СЕРОВОССТАНАВЛИВАЮЩИХ БАКТЕРИЙ  
*DESULFUROMONAS ACETOXIDANS***

**О. Василю, С. Гнатуш**

*Львовский национальный университет имени Ивана Франко  
ул. Грушевского, 4, Львов 79005, Украина  
e-mail: oresta.vasylyv@gmail.com*

*Desulfuromonas acetoxidans* – грамотрицательные облигатно анаэробные серовосстанавливающие бактерии. Для защиты от токсических и мутагенных соединений и устранения токсического влияния кислорода у аэробов и факультативных анаэробов служит ферментативная система антиоксидантной защиты, важнейшей частью которой является каталаза. Предполагается, что отсутствие или низкая активность ферментов этой системы у облигатно анаэробных микроорганизмов, в частности серо- и сульфатредуцирующих бактерий, обуславливает их повышенную чувствительность к кислороду и действию токсических соединений, в частности переходных металлов. Исследовано влияние  $\text{FeSO}_4$ ,  $\text{FeCl}_3 \times 6\text{H}_2\text{O}$ ,  $\text{MnO}_2$ ,  $\text{MnCl}_2 \times 4\text{H}_2\text{O}$ ,  $\text{NiCl}_2 \times 6\text{H}_2\text{O}$  и  $\text{CoCl}_2 \times 6\text{H}_2\text{O}$  на активность каталазы серовосстанавливающих бактерий *D. acetoxidans*. Зафиксирована максимальная активность исследуемого фермента при влиянии 0,5 мМ, 1,5 мМ и 2,0 мМ соответственно  $\text{NiCl}_2 \times 6\text{H}_2\text{O}$ ,  $\text{FeSO}_4$  и  $\text{CoCl}_2 \times 6\text{H}_2\text{O}$  на вторые сутки, 1,5 мМ  $\text{MnO}_2$  на третьи сутки, 0,5 мМ  $\text{FeCl}_3 \times 6\text{H}_2\text{O}$  и 1,0 мМ  $\text{MnCl}_2 \times 4\text{H}_2\text{O}$  на четвертые сутки культивирования по сравнению с контролем.

*Ключевые слова:* *Desulfuromonas acetoxidans*, серобактерии, антиоксидантная защита, каталаза, переходные металлы.