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## ADAPTATION OF *OCHROBACTRUM RHIZOSPHAERAE* IMV B-7956 BACTERIA TO THE INFLUENCE OF COPPER (II) CHLORIDE

*In technologically altered habitats, an increased content of organic compounds, nitrogen, phosphorus, sulfur compounds, antibiotic substances, etc. is found. Therefore, microorganisms that are systematically exposed to various stressors have developed adaptation mechanisms. The strain Ochrobactrum rhizosphaerae IMV B-7956, isolated from the infiltrate lakes of the Lviv solid waste landfill, is resistant to copper, chromium, manganese, and iron in concentrations exceeding the maximum permissible concentrations. The work aimed to study the response of O. rhizosphaerae IMV B-7956 cells to CuCl<sub>2</sub> exposure by detecting changes in the content of lipid peroxidation products, products of the oxidative modification of proteins, activity of antioxidant defense system enzymes, and synthesis of extracellular polymers. Methods. To study the effect of CuCl<sub>2</sub> on prooxidant indicators and the activity of enzymes of the antioxidant defense system, bacteria were pre-incubated in Tris-HCl buffer containing 2–10 mM CuCl<sub>2</sub>. After one hour of incubation, the bacterial cells were washed and cultured for 1, 12, 24, and 48 h in metal-free media. The copper content in the bacterial cells was determined by atomic absorption spectrometry. The content of lipid peroxidation indicators, carbonyl groups in proteins, total low-molecular-weight thiols, enzymatic activity, and the content of exopolysaccharides and extracellular proteins were determined photometrically. Results. Within an hour, O. rhizosphaerae IMV B-7956 bacteria accumulated 2.3–7.8 mg Cu/g of biomass. Under these conditions, an increased content of lipid peroxidation products was detected. During the first hour of growth in bacterial cells, enzymes with catalase and peroxidase activity were activated. During further cultivation, an increase in the activity of other antioxidant defense enzymes was detected. Carbonyl groups in proteins are probably formed due to an increase in the content of lipid peroxidation products, as they are formed later. Within 12–48 h of growth, the copper content in the bacterial cells decreases. This leads to the restoration of growth. Conclu-*

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**sions.** The main damaging effect of  $\text{CuCl}_2$  on bacterial cells was detected during the first 24 h of growth. Activation of the enzymes of the antioxidant system and synthesis of exopolysaccharides are among the adaptations that ensure the survival of bacteria under these conditions.

**Keywords:** heavy metals, oxidative stress, reactive oxygen species, antioxidant enzymes, superoxide dismutase, catalase, glutathione system enzymes.

Bacteria of the genus *Ochrobactrum* inhabit various biotopes. They are usually found in wastewater, soil, and plant rhizosphere (Mahmood et al., 2009; Huber et al., 2010; Whitman et al., 2018). They can be plant or animal symbionts (Imran et al., 2014; Wu et al., 2016; Hu et al., 2020; Mishra et al., 2023). Among the representatives of the genus, there are pathogens of opportunistic infections (Rastogi & Mathur, 2017; Kannan et al., 2022) but there are also species resistant to heavy metals: *Ochrobactrum cytisi* Azn6.2 (Cu, Cd, and Zn) (Rodríguez-Llorente et al., 2010), *Ochrobactrum tritici* 5bv11, *Ochrobactrum* sp. CSCr-3, *Ochrobactrum intermedium* CrT-1, *Ochrobactrum pseudintermedium* ADV31 (highly resistant and able to reduce Cr(VI) strains) (Faisal et al., 2005; He et al., 2009; Morais et al., 2011; Tandon et al., 2020), *Ochrobactrum* MT180101 (resistant to Cu) (Peng et al., 2019b) etc.

A halotolerant strain of *O. rhizosphaerae* IMV B-7956, which is resistant to Cd, Cu, Fe, Cr(VI), Mn, and other ions was isolated from the infiltrates lake of the Lviv solid waste landfill, which contains chlorine compounds, heavy metals, nitrates, oil products, phosphates, sulfates, etc. in concentrations that significantly exceed the maximum permissible concentrations (Malovanyy et al., 2019; Hnatush et al., 2021b).

The development of technologies for bioremediation based on strains isolated from technologically altered habitats is a promising area of study. Microorganisms that are regularly exposed to various stressors, including heavy metal compounds, have developed resistance mechanisms. Microorganisms resistant to heavy metals have been isolated from various extreme environments (Hnatush et al., 2021a; Hnatush et al., 2022; Komplikevych et al., 2023a; Komplikevych

et al., 2023b). The study of bacterial resistance mechanisms is important for understanding the ways of using a strain or microbial community in complex preparations for bioremediation. The adaptation of microorganisms to heavy metal compounds includes the efflux of metals from the cell and the synthesis of extracellular polymeric substances (exopolysaccharides, proteins, etc.) (Nanda et al., 2019; Sharma & Shukla, 2021). Some microorganisms transform metals into less toxic forms by reducing, sequestering, or precipitating them (Tayang & Songachan, 2021; Sharma & Shukla, 2021). One of the methods of bioremediation is the accumulation of metal ions on the surface or inside the cells of microorganisms. For this purpose, live (bioaccumulation) or dead (adsorption) cells are used (Nanda et al., 2019). Metal ions that enter the cells harm biopolymers because they contribute to the formation and distribution of reactive oxygen species (ROS) in the cells (Sachdev et al., 2021; Maslovska et al., 2023).

The **aim** of the work was to study the response of *O. rhizosphaerae* IMV B-7956 cells to  $\text{CuCl}_2$  exposure through the detection of the changes in the content of lipid peroxidation products, products of the oxidative modification of proteins, activity of antioxidant defense system enzymes, and synthesis of extracellular polymers.

**Materials and Methods.** The bacteria *O. rhizosphaerae* IMV B-7956 (GenBank accession number: MZ165353.1) were isolated from the infiltrates of the Lviv solid waste landfill at the Department of Microbiology of the Ivan Franko National University of Lviv (Hnatush et al., 2021b). The bacteria were grown at  $+28 \pm 2$  °C in 500 mL flasks with 100 mL of tryptic soy broth (TSB) (Merck, USA).

To study the effect of copper ions on *O. rhizosphaerae* IMV B-7956 cells, the bacteria were incubated for an hour in 0.05 M Tris-HCl buffer (pH 7.5) with 2, 4, 8, or 10 mM  $\text{CuCl}_2$ . After incubation, the cells were washed and transferred (0.1 g/L) into metal-free TSB and cultured for 48 h. The cells were pretreated with copper chloride because the addition of metal salt directly to the TSB medium, which is optimal for these bacteria, results in the formation of insoluble  $\text{Cu}(\text{OH})_2$ .

The metal concentration in the cells, lipid peroxidation (LPO) products, carbonyl groups in proteins, enzymatic activity, and total low-molecular-weight thiol content were determined after 1, 12, 24, and 48 h of cultivation. The extracellular polymeric substance content was determined after 48 h of bacterial growth.

To determine the copper content in cells, a sample of cells ( $0.45 \pm 0.01$  g) was subjected to acid decomposition in the autoclaves of the START D microwave laboratory system (Milestone, Italy). Copper was measured using atomic absorption spectrometry (Zeeman Atomic Absorption Spectrometer AA240Z Varian with GTA 120 Graphite Tube Atomizer, Australia) (argon flow was 0.3 L/min, the ashing temperature was 800 °C, and the temperature of the atomization stage was 2300 °C). Copper was detected at 327 nm.

To obtain cell-free extracts, cells were disrupted by sonication (22 kHz, 5 min, 0 °C) in 0.05 M potassium phosphate buffer (pH 7.0) with phenylmethylsulfonyl fluoride ( $10^{-5}$  M) and ethylenediaminetetraacetate (EDTA) ( $10^{-5}$  M), and cell debris was precipitated by centrifugation (8000 g, 30 min, 4 °C). Protein concentration was determined by the Bradford method (Bradford, 1976). The processes of LPO were studied by changes in the content of diene conjugates, lipid hydroperoxides, and thiobarbituric acid reactive substances (TBARS) in the cell-free extract of bacteria (described in detail in the Komplikevych et al., 2023a). The content of carbonyl groups in proteins was determined by the reaction with 2,4-dinitrophenylhydrazine (Lushchak

et al., 2004). The enzymatic activity (superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione S-transferase, glutathione reductase) was studied photometrically according to previously described methods (Holovchak et al., 2012). The total low-molecular-weight thiol content was determined in the reaction with Elman's reagent (Hawkins et al., 2009).

The extracellular polymeric substances were extracted using 2% EDTA (Pan et al., 2010). In the resulting supernatant, the content of exopolysaccharides was determined using anthron (Frolund et al., 1996), and extracellular proteins were determined by the Bradford method (Bradford, 1976).

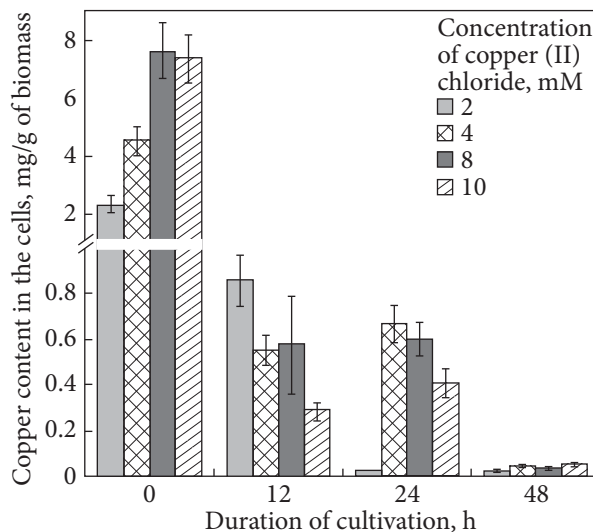
All experiments were performed in three replicates. The results are presented as average values with standard deviation ( $x \pm \text{SD}$ ). The influence of  $\text{CuCl}_2$  on the indicators of interest was estimated using the t-test ( $p$ -value < 0.05). The graphs were constructed using OriginPro 8.5 (OriginLab Corporation, USA, 2010).

**Results.** During an hour of incubation of *O. rhizosphaerae* IMV B-7956 bacteria in 0.05 M Tris-HCl buffer (pH 7.5) with  $\text{CuCl}_2$ , 2.3–7.8 mg of Cu/g biomass was accumulated in the cells (Fig. 1). The copper content in the bacterial cells increased with increasing concentration of  $\text{CuCl}_2$  in the buffer. However, at 8–10 mM  $\text{CuCl}_2$  in the buffer, the content of copper in bacterial cells was not significantly different (7.4–7.6 mg of Cu/g biomass).

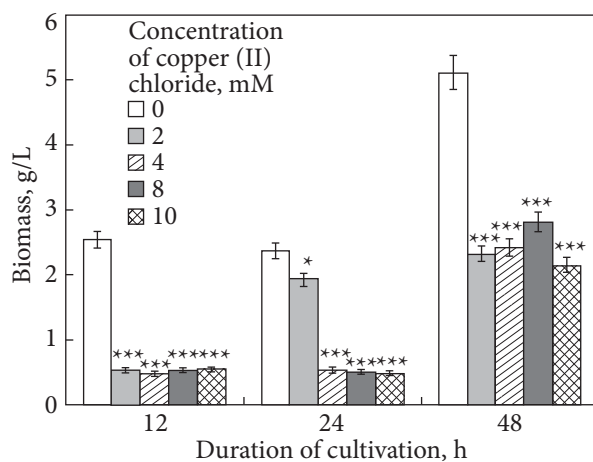
After incubation of bacteria in a buffer with different concentrations of copper (II) chloride, they were grown in metal-free tryptic soy broth. Under these conditions, the copper content in the cells gradually decreased. Under the influence of 4–10 mM  $\text{CuCl}_2$ , during 12–24 h of cultivation, the copper content in the cells remains nearly unchanged and decreases within 48 h of growth. After 48 h of cultivation, 17.9–51.1  $\mu\text{g}$  of Cu/g biomass was detected in *O. rhizosphaerae* IMV B-7956 cells, depending on the metal salt content in the incubation buffer (Fig. 1).

A significant decrease in biomass accumulation (by 79–81 %) of *O. rhizosphaerae* IMV B-7956 bacteria was detected during 12 and 24 h of growth. Further cultivation resulted in a 45–58 % decrease in biomass accumulation compared to the control (Fig. 2).

The copper that entered the cells caused free radical damage to lipids and proteins of *O. rhizosphaerae* IMV B-7956, as evidenced by an increase in the content of LPO and oxidative modification of protein products compared to the control (Fig. 3). During the first hour of bacterial growth, the content of primary LPO products in *O. rhizosphaerae* IMV B-7956 cells increased along with the copper content in the cells (Fig. 3a, b). The content of diene conjugates under these conditions exceeded their content in the control by 1.4–4.7 times, and lipid hydroperoxides — by 1.7–14.8 times. During further cultivation, the content of diene conjugates slightly decreased, but until 12 h of cultivation, it remained high compared to the control and during further growth of *O. rhizosphaerae* IMV B-7956 was lower than in the control. The content of lipid hydroperoxides in *O. rhizosphaerae* IMV B-7956 cells was increased compared to the control up to 24 h of growth. After 24 h of cultivation, the content of lipid hydroperoxides was the highest under the influence of 8 mM of  $\text{CuCl}_2$ , and under the influence of 10 mM of this salt, it decreased but exceeded the content of these products in the control. We assume that under the influence of 10 mM copper (II) chloride, intensive formation of secondary LPO products occurs, which is reflected in the increase in the content of TBARS under the influence of this concentration within 24 h of cultivation. After 48 h of cultivation, the content of lipid hydroperoxides was slightly lower or did not differ from the control. Similar changes occurred in the content of secondary LPO products (Fig. 3c). During the 1–12 h of growth, the content of TBARS in *O. rhizosphaerae* IMV B-7956 cells was higher than in the control and increased with increasing concentration



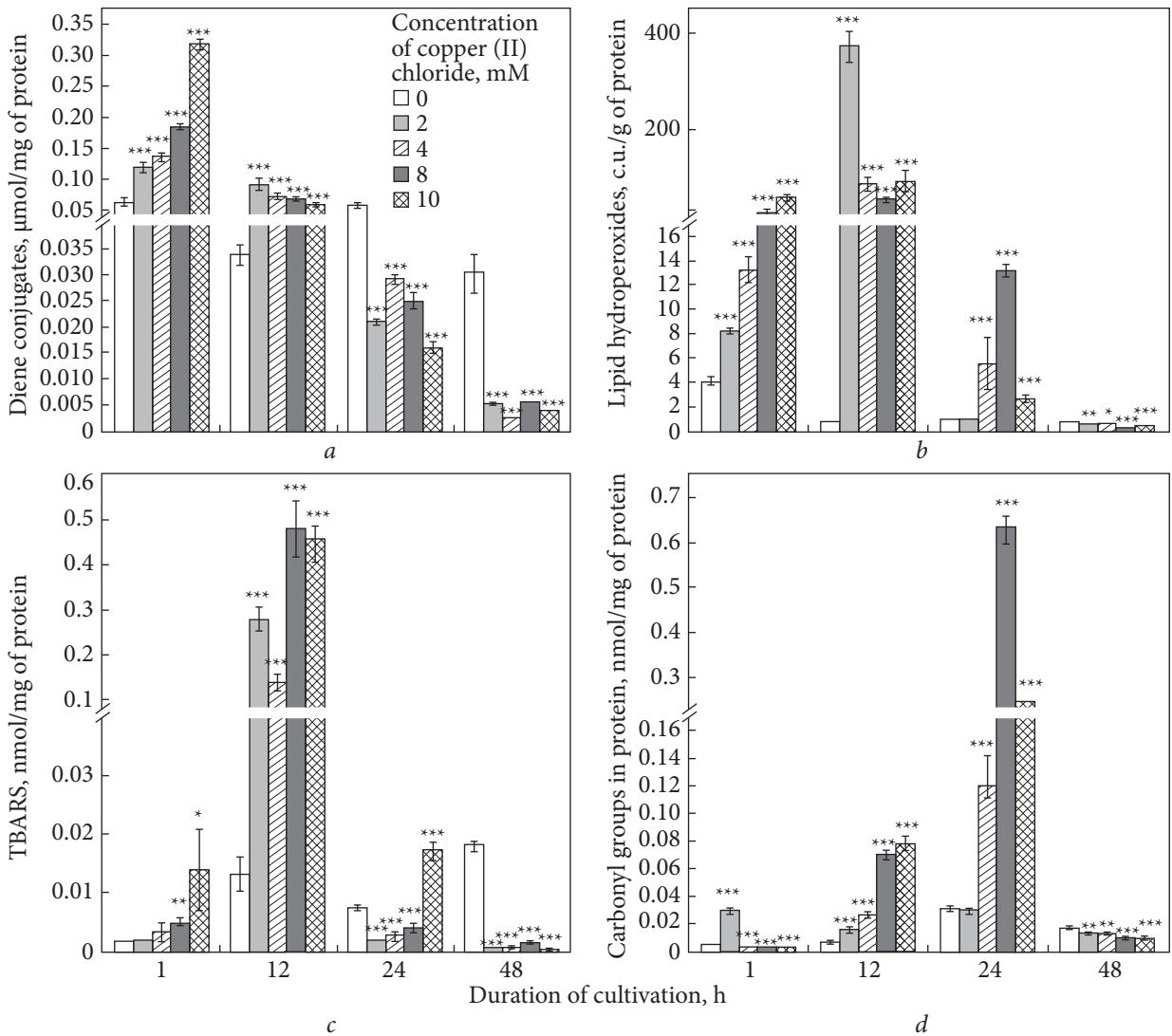
**Fig. 1.** The copper content in *Ochrobactrum rhizosphaerae* IMV B-7956 cells after incubation in Tris-HCl buffer with different concentrations of  $\text{CuCl}_2$  and after growth in metal-free tryptic soy broth



**Fig. 2.** Effect of  $\text{CuCl}_2$  on the biomass accumulation of bacteria *Ochrobactrum rhizosphaerae* IMV B-7956 ( $x \pm \text{SD}$ , \*\*\*  $P \geq 0.999$ )

of copper (II) chloride. During further cultivation, the content of TBARS decreased.

The highest content of carbonyl groups in bacterial proteins was observed after 24 h of cultivation of *O. rhizosphaerae* IMV B-7956 in both the control and after the  $\text{CuCl}_2$  exposure (Fig. 3d). During the first hour of culture growth, an increase in the



**Fig. 3.** The content of diene conjugates (a), lipid hydroperoxides (b), thiobarbituric acid reactive substances (c), and carbonyl groups in proteins (d) of *Ochrobactrum rhizosphaerae* IMV B-7956 bacteria under the influence of  $\text{CuCl}_2$  ( $x \pm \text{SD}$ , \*  $P \geq 0.95$ , \*\*  $P \geq 0.99$ , \*\*\*  $P \geq 0.999$ )

carbonyl group in protein content compared to the control was detected only under the influence of 2 mM  $\text{CuCl}_2$ . Under the influence of 4–10 mM  $\text{CuCl}_2$ , the content of carbonyl groups in the proteins of bacteria increased during 12–24 h of cultivation. After further cultivation, the content of carbonyl groups in the proteins of *O. rhizosphaerae* IMV B-7956 did not exceed that in the control.

Under the influence of copper (II) chloride, an increase in catalase and SOD activity was detected

(Fig. 4). Within the first hour of cultivation, catalase activity increased by 5.8–62.2 times compared to the control. During further cultivation for up to 24 h, the catalase activity of *O. rhizosphaerae* IMV B-7956 increased with increasing concentration of  $\text{CuCl}_2$  in the incubation buffer. During 48 h of cultivation, catalase activity decreased to or below the control level (Fig. 4a). Under the influence of 2 mM  $\text{CuCl}_2$ , the SOD activity of *O. rhizosphaerae* IMV B-7956 increased

3.1-fold during the first hour of growth. Under the influence of higher concentrations of copper (II) chloride, SOD activity decreased during the first hour of cultivation with an increase in the concentration of copper in the cells. The highest SOD activity (939.8—2266.9 units of activity/mg protein) under the influence of  $\text{CuCl}_2$  was detected after 12 h of cultivation. With further cultivation, the SOD activity in *O. rhizosphaerae* IMV B-7956 cells decreased (Fig. 4b).

During an hour of cultivation, the glutathione peroxidase and glutathione S-transferase activity of *O. rhizosphaerae* IMV B-7956 incubated with 2 and 4 mM  $\text{CuCl}_2$  increased (Fig. 5a, b). In this period of cultivation, the glutathione reductase activity of cells incubated with 4 mM  $\text{CuCl}_2$  increased (Fig. 5c), while the activity of the enzymes of the glutathione antioxidant system decreased with increasing concentration of  $\text{CuCl}_2$  in the incubation buffer.

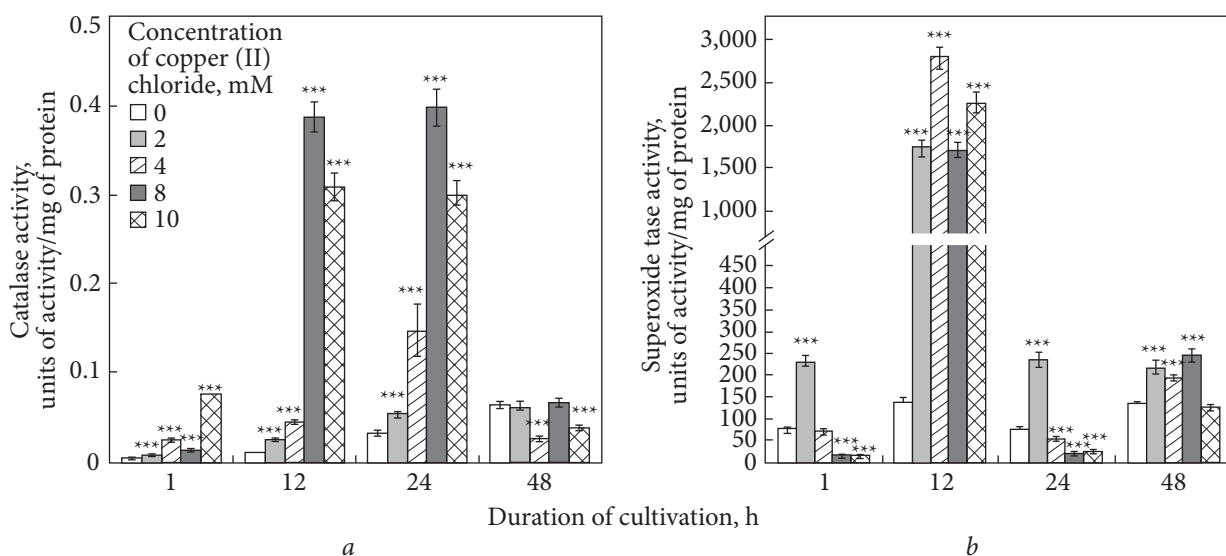
During 12 h of cultivation of *O. rhizosphaerae* IMV B-7956, the activity of these enzymes increased: glutathione peroxidase activity — by 1.4—5.6 times, glutathione S-transferase activity — by 3.4—19.1 times, glutathione reductase

activity — by 2.0—7.6 times, compared to the control. During 48 h of cultivation, glutathione peroxidase activity decreased to the control level. Glutathione S-transferase activity was 1.2—1.7 times lower than in the control. Glutathione reductase activity was higher than in the control during the entire cultivation period.

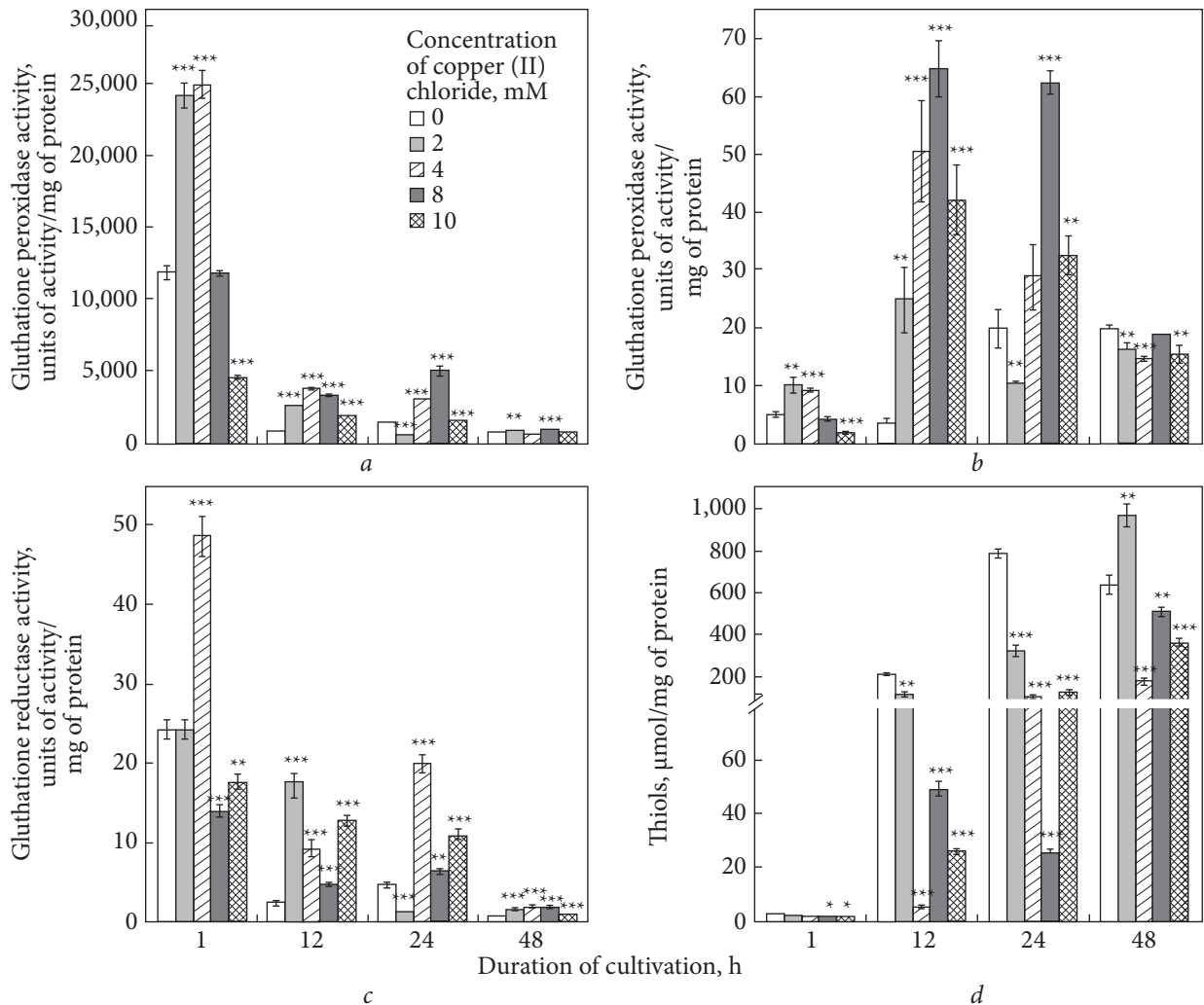
The total content of low-molecular-weight thiols during the first hour of bacterial growth was very low and did not differ from that of the control. During 24 h of cultivation, the content of low-molecular-weight thiols in bacterial cells pre-incubated in the buffer with  $\text{CuCl}_2$  was lower compared to the control (Fig. 5d).

An increase in the concentration of exopolysaccharides in the medium after 48 h of growth of *O. rhizosphaerae* IMV B-7956, pre-incubated in Tris-HCl buffer with 8—10 mM  $\text{CuCl}_2$ , was detected (Fig. 6a). No statistically significant changes in the concentration of extracellular proteins in the culture medium after 48 h of growth of *O. rhizosphaerae* IMV B-7956 were detected (Fig. 6b).

**Discussion.** Neutral and slightly alkaline pH is optimal for the biosorption of Cu(II) by bac-



**Fig. 4.** Catalase (a) and superoxide dismutase (b) activity of cell-free extract of *Ochrobactrum rhizosphaerae* IMV B-7956 under the influence of  $\text{CuCl}_2$  ( $x \pm \text{SD}$ , \*\*\*  $P \geq 0.999$ )



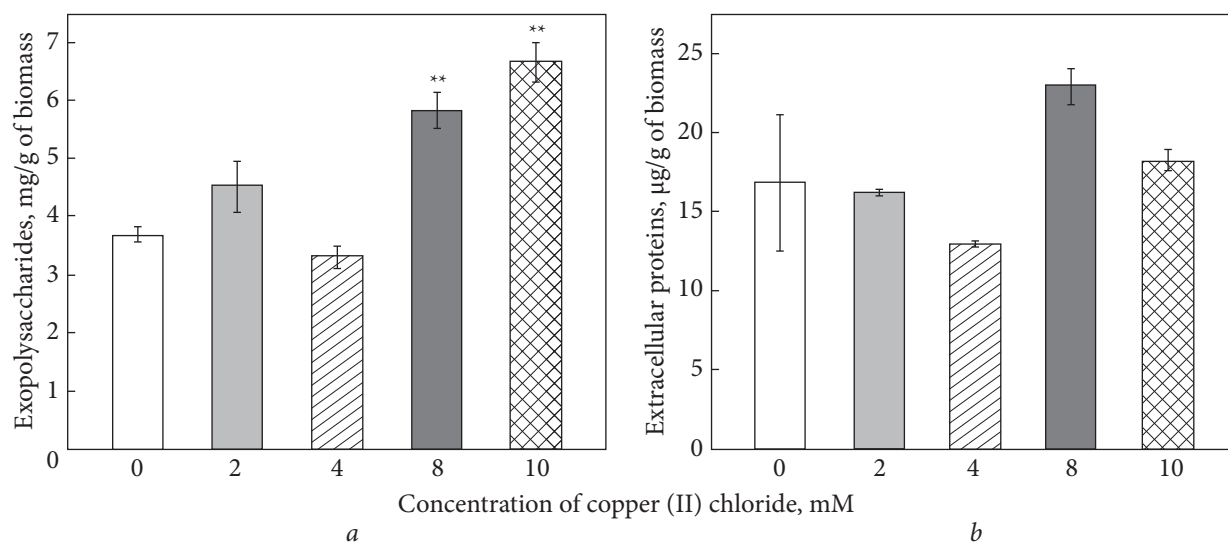
**Fig. 5.** Glutathione peroxidase (a), glutathione S-transferase (b), and glutathione reductase (c) activity, and total low-molecular-weight thiols content (d) in *Ochrobactrum rhizosphaerae* IMV B-7956 cells under the influence of  $\text{CuCl}_2$  ( $x \pm \text{SD}$ , \*  $P \geq 0.95$ , \*\*  $P \geq 0.99$ , \*\*\*  $P \geq 0.999$ )

teria of the genus *Ochrobactrum* (Chen et al., 2015). During the incubation of *O. rhizosphaerae* IMV B-7956 in 0.05 M Tris-HCl buffer (pH 7.5) containing 2–10 mM  $\text{CuCl}_2$ , the cells accumulated up to  $7.6 \pm 0.9$  mg Cu/g biomass. In a study of Chen et al. (2015), bacteria *Ochrobactrum* sp. PHE-OCH accumulated about 3.2 mg Cu/g of biomass during 48 h of growth in a medium with 2.94 mM Cu(II). Interestingly, at 8–10 mM  $\text{CuCl}_2$  in the buffer, we did not find a linear increase in the copper content in the cells of *O.*

*rhizosphaerae* IMV B-7956 as it was under the influence of lower concentrations of the metal salt (Fig. 1). Similar results were obtained in the work of (Peng et al., 2019a). At an initial copper concentration of 2 mg/L, cells of *Ochrobactrum* MT180101 adsorbed 85.5 % of copper from the solution within the first hour of cultivation. With an increase of copper concentration in the solution above 10 mg/L, the adsorption capacity of *Ochrobactrum* MT180101 cells differed slightly and ranged between 6–8 mg Cu/g of biomass

(Peng et al., 2019a). Probably, under the influence of these  $\text{CuCl}_2$  concentrations, several mechanisms compensate for the penetration of copper into cells. It is known that the penetration of copper ions into the cell is prevented by the binding of metal ions to cell wall components, the production of chelating compounds, transformation to less toxic forms, and efflux from the cell (Koh & Henderson, 2015; Peng et al., 2019b). Some siderophores, such as yersiniabactin in complex with copper ions, exhibit SOD activity outside the cell (Koh & Henderson, 2015). A significant effect of copper on biomass accumulation was detected within 24 h of culture growth (Fig. 2). With further cultivation, the growth processes were restored. These changes correlate with a decrease in the content of copper in the cells of *O. rhizosphaerae* IMV B-7956 during cultivation. The resistance of the studied strain to copper ions is correlated with the resistance to this metal of other representatives of this genus: *Ochrobactrum anthropi* DE2010 tolerates up to 10 mM copper (Villagrasa et al., 2021) and *Ochrobactrum pseudogrignonense* GGUPV1 — up to 50 mM copper (Chaturvedi & Verma, 2015). The content of LPO products increased immediately after the

entry of copper into the cells and remained high compared to the control during 24 h of culture growth (Fig. 3a—c). The oxidative modification of proteins in *O. rhizosphaerae* IMV B-7956 cells is a secondary process because the increase in carbonyl groups in proteins occurred after 12 h of bacterial growth (Fig. 3d). Likely, the formation of carbonyl groups in *O. rhizosphaerae* IMV B-7956 proteins takes place as a result of scavenging ROS, LPO products, etc. It is known that carbonyl groups can be introduced into proteins by secondary reactions of nucleophilic Cys, His, and Lys residues with aldehydes formed during LPO (Dalle-Donne et al., 2003). During the first hour of growth of *O. rhizosphaerae* IMV B-7956 incubated with  $\text{CuCl}_2$ , there was an increase in catalase and, slightly, in glutathione peroxidase activities (Fig. 4a, 5a). The SOD and glutathione reductase activities increased after 12 h of growth (Fig. 4b, 5c). According to the results of the study, organic and inorganic peroxides are likely to be formed in high concentrations in *O. rhizosphaerae* IMV B-7956 cells under the influence of copper. Therefore, catalases and peroxidases are activated immediately after the entry of copper into the cells. The *katA* gene encoding a monofunc-



**Fig. 6.** Content of exopolysaccharides (a) and extracellular proteins (b) in the growth medium of *Ochrobactrum rhizosphaerae* IMV B-7956 under the influence of  $\text{CuCl}_2$  ( $x \pm \text{SD}$ , \*\*  $P \geq 0.99$ )



tional catalase (DelVecchio et al., 2002; Paulsen et al., 2002), as well as genes encoding DyP-type peroxidases (IR196\_RS09260 GenBank), thioredoxin-dependent thiol peroxidase (Paulsen et al., 2002; Chain et al., 2005; Minogue et al., 2014), and peroxiredoxin (IR196\_RS04305 GenBank, BME\_RS13010 GenBank) are found in the genome of *Ochrobactrum* representatives. An increase in the activity of all studied enzymes under the influence of  $\text{CuCl}_2$  during the cultivation of *O. rhizosphaerae* IMV B-7956 was revealed, hence their role is important for the neutralization of the excess of free radicals, LPO products and, as a result, the restoration of growth processes. No increase in the content of low-molecular-weight thiols was detected, despite the acti-

vation of glutathione reductase activity (Fig. 5d). The increase in the content of exopolysaccharides in the cultural medium of *O. rhizosphaerae* IMV B-7956 after 48 h of cultivation is probably due to the extracellular binding of copper ions by these polymers (Fig. 6).

Thus,  $\text{CuCl}_2$  at concentrations of 2–10 mM, being accumulated in the cells of *O. rhizosphaerae* IMV B-7956 bacteria, causes free radical damage to macromolecules. The main damaging effect was detected during the first 24 h of growth. The activation of antioxidant system enzymes and the synthesis of exopolysaccharides are among the adaptations that ensure the survival of *O. rhizosphaerae* IMV B-7956 bacteria under the influence of copper.

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#### АДАПТАЦІЯ БАКТЕРІЙ *OSCHROBACTRUM RHIZOSPHERAE* IMB B-7956 ДО ВПЛИВУ КУПРУМУ (II) ХЛОРИДУ

У техногенно змінених біотопах виявляють підвищений вміст органічних сполук, сполук нітрогену, фосфору, сульфуру, антибіотичних речовин тощо. Тому мікроорганізми, які систематично піддаються впливу різних стресових чинників, виробили механізми адаптації. Штам *Ochrobactrum rhizosphaerae* IMB B-7956, виділений із озер інфільтратів Львівського полігону твердих побутових відходів, є стійким до купруму, хрому, мангану, феруму в концентраціях, які перевищують гранично допустимі. **Метою** роботи було дослідити відповідь клітин бактерій *O. rhizosphaerae* IMB B-7956 за впливу  $\text{CuCl}_2$ , виявляючи зміни вмісту продуктів перекисного окиснення ліпідів, окисної модифікації білків, активність ензимів антиоксидантної системи захисту та синтез позаклітинних полімерів. **Методи.** Для дослідження впливу  $\text{CuCl}_2$  на прооксидантні показники та активність ензимів антиоксидантної системи захисту, бактерії попередньо інкубували у трис-НСІ буфері із  $\text{CuCl}_2$  у концентраціях 2—10 мМ. Після години інкубування клітини бактерій відмивали та культивували у середовищі без солі металу упродовж 1, 12, 24 та 48 годин. Вміст купруму в клітинах бактерій визначали методом атомно-абсорбційної спектроскопії. Вміст показників перекисного окиснення ліпідів, карбонільних груп у білках, загальний вміст тіолів, ензиматичну активність та вміст екзополісахаридів та позаклітинних білків визначали фотометрично. **Результати.** Упродовж години бактерії *O. rhizosphaerae* IMB B-7956 нагромаджували 2,3—7,8 мг  $\text{Cu}$ /г біомаси. За цих умов виявлено підвищений вміст продуктів перекисного окиснення ліпідів. Упродовж першої години росту в клітинах бактерій активуються ензими із каталазою та пероксидазою активністю. За подальшого культивування виявлено зростання активності й інших ензимів антиоксидантного захисту. Карбонільні групи у білках, ймовірно, утворюються внаслідок збільшення вмісту продуктів перекисного окиснення ліпідів, оскільки утворюються пізніше. Упродовж 12—48 годин росту вміст купруму в клітинах бактерій суттєво знижується. Це приводить до відновлення ростових процесів. **Висновки.** Основна шкідлива дія  $\text{CuCl}_2$  на клітини бактерій виявлена упродовж перших 24 годин росту. Активування ензимів антиоксидантної системи, синтез екзополісахаридів є одними із адаптацій, які забезпечують виживання бактерій за цих умов.

**Ключові слова:** важкі метали, оксидативний стрес, активні форми кисню, антиоксидантні ензими, супероксиддисмутаза, каталаза, ферменти глутатіонової системи.