UDK 579.695 **doi: 10.15407/biotech8.05.093**

COPPER RESISTANT STRAIN *Candida tropicalis* ROMCU5 INTERACTION WITH SOLUBLE AND INSOLUBLE COPPER COMPOUNDS

Ie. P. Prekrasna
O. B. Tashyrev

Zabolotny Institute of Microbiology and Virology
of the National Academy of Sciences of Ukraine, Kyiv

E-mail: preckrasna@gmail.com

Received 25.06.2015

The focus of the study was interaction of *Candida tropicalis* RomCu5 isolated from highland Ecuador ecosystem with soluble and insoluble copper compounds.

Strain *C. tropicalis* RomCu5 was cultured in a liquid medium of Hiss in the presence of soluble (copper citrate and $CuCl_2$) and insoluble (CuO and $CuCO_3$) copper compounds. The biomass growth was determined by change in optical density of culture liquid, composition of the gas phase was measured on gas chromatograph, redox potential and pH of the culture fluid was defined potentiometrically. The concentration of soluble copper compounds was determined colorimetrically.

Maximal permissible concentration of Cu^{2+} for *C. tropicalis* RomCu5 was 30 000 ppm of Cu^{2+} in form of copper citrate and 500 ppm of Cu^{2+} in form of $CuCl_2$. *C. tropicalis* was metabolically active at super high concentrations of Cu^{2+}, despite the inhibitory effect of Cu^{2+}. *C. tropicalis* immobilized Cu^{2+} in the form of copper citrate and $CuCl_2$ by its accumulation in the biomass. Due to medium acidification *C. tropicalis* dissolved CuO and $CuCO_3$. High resistance of *C. tropicalis* to Cu^{2+} and ability to interact with soluble and insoluble copper compounds makes it biotechnologically perspective.

Key words: yeast, *Candida tropicalis*, copper, resistance to copper, inhibition of metabolism, copper immobilization, copper mobilization.

Nowadays anthropogenic pressure on the environment dramatically increases. Various industries produce and discharge wastes into the environment, such as mining, energy and fuel production, electroplating, electrolysis, leatherworking, photography, etc. One of the main components of the various industrial wastes is heavy metals, such as copper, zinc, cobalt, mercury, chromate, etc. So, the problem of environmental metal pollution drastically becomes more acute.

Microorganisms are plentiful in nature and play vital roles in the geochemical cycling of metals by protonation, chelation, redox and chemical transformation, metal accumulation [1]. Mechanisms of microbial interaction with metals are being exploited in various environmental biotechnologies. Microbial biotechnologies are used for both removing of toxic metals from the industrial wastewater and recovery of heavy metals from low grade ores, dumps, soils, sediments, dumps and industrial wastes [2–5].

Microbial technologies of metal removing or bioleaching have advantages over physical and chemical technologies. For example, chemical precipitation and electrochemical treatment are ineffective and produce large quantity of sludge required to treat with great difficulty. Ion exchange, membrane technologies and activated carbon adsorption process are extremely expensive [2]. Contrary microbial biotechnologies have low operating cost, minimal use of chemicals [2]. So, microbial biotechnologies of both metal removing and bioleaching can be assumed as environment-friendly. Accordingly, search for microorganisms promising for metal biotechnologies is actual area of investigation.

Resistance to toxic metals and ability to interact with toxic metals are the main criteria for microorganisms perspective for metal biotechnologies. In our recent researches high resistant to copper strain *Candida tropicalis* RomCu5 was isolated from highland

Ecuador ecosystem [6]. The strain was able to grow even at 3000 ppm of Cu^{2+} (in form of copper citrate), which by several orders overcomes the inhibiting concentrations of Cu^{2+} for the majority of chemoorganotrophic microorganisms. So the isolated yeast strain corresponds to the first criterion for microorganisms that perspective for metal biotechnologies. The question arises whether it corresponds to the second criterion. That is why the aim of the work is to investigate the interaction of the isolated *C. tropicalis* RomCu5 with soluble and insoluble copper compounds.

Materials and Methods

Cultivation of C. tropicalis RomCu5. *C. tropicalis* RomCu5 was cultured in the presence of 0, 200, 1000 and 3000 ppm of Cu^{2+} in a liquid medium of Hiss (g/l): K_2HPO_4 — 1.0; KH_2PO_4 — 1.0; NH_4Cl — 1.0; glucose — 20.0; dry yeast extract (Serva) — 5.0; distilled water — 1000 ml. Medium of Hiss with 0, 200, 1000 and 3000 ppm of Cu^{2+} was brought in 150 ml flasks. Then suspended in saline (0.9%) *C. tropicalis* RomCu5 was brought to the medium to a final optical density 0.05 units. Flasks were closed with elastic rubber stoppers fixed on the necks of the flasks with aluminum clasps. Microorganisms were cultured at 28 °C. Physiological parameters of growth (optical density, Eh, pH, composition of the gas phase) and concentration of Cu^{2+} in medium were measured every 2 hours of cultivation.

Culture was cultivated on the solid copper containing medium for maximal permissible concentration determining. Copper stock solutions were added to the melted and cooled to 45° nutrient agar (HiMedia Laboratories Pvt. Ltd., USA) and medium poured to the Petri dishes. The suspended in saline (0.9%) microorganisms were inoculated on the surface of the medium and cultivated at 28 °C. Maximal concentration of Cu^{2+} where the growth was observed was accepted as MPC.

Accumulation of Cu^{2+} by C. tropicalis RomCu5. Medium of Hiss (100 ml) and biomass of *C. tropicalis* RomCu5 were brought to 150 ml flasks. Initial optical of density culture liquid was 0.05 units. Flasks were closed with elastic rubber stoppers that were fixed on the necks of the flasks with aluminum clasps. Microorganisms were cultured at 28 °C. When the culture reached mid-log phase of growth (0.7 optical density units) solutions of $CuCl_2$ and copper citrate were brought to the flasks to the final concentration 100 ppm of Cu^{2+}. Values of optical density, Eh, pH, concentration of Cu^{2+} in the culture liquid were measured every 30 min within four hours. The composition of the gas phase in the cultivator was determined hourly.

Quantity of copper accumulated in the biomass was determined as follows. Microorganisms were cultured in medium of Hiss to mid-log growth phase (0.7 optical density units). Then solutions of $CuCl_2$ and copper citrate were brought to culture liquid to the final concentration 100 ppm of Cu^{2+}. Microorganisms were cultured at 28 °C within four hours. Then biomass was precipitated at 5 000 g for 15 min on a centrifuge. Concentration of Cu^{2+} in the supernatant and the amount of copper accumulated on the surface and inside the cells of *C. tropicalis* RomCu5 were determined. Concentration of Cu^{2+} in the supernatant was determined titrimetically with PAR (4-2-pyrydilazo-resorcinol). To determine the amount of copper accumulated on the surface of cells biomass was washed three times in distilled water by centrifugation and then in solution of citric acid (pH = 4). Copper is stable in a soluble form (Cu^{2+}) in acidic conditions (pH 0–5). Therefore, when the biomass was washing in a solution of citric acid, copper desorbed from the cell surface to solution. Concentration of Cu^{2+} in the supernatant was determined colorimetrically with PAR. To determine the concentration of copper accumulated inside the cells biomass was burned after cell had being washed in a solution of citric acid. Biomass was put in tube of heat-resistant Pyrex glass and burned in the flame. The tubes were cooled and the solution of citric acid (pH = 4) was brought to the tubes. Copper that precipitated on the surface of walls of the tubes after biomass combustion dissolved in citric acid. Then the Cu^{2+} was determined colorimetrically.

Mobilization of insoluble copper compounds by C. tropicalis RomCu5. The one day cultivated culture (1.2 units of optical density) was brought in 150 ml flasks that contained copper carbonate $CuCO_3$ or copper oxide CuO. Flasks were closed with elastic rubber stoppers that were fixed on the necks of the flasks with aluminum clamps. Microorganisms were cultured at 28 °C. Concentration of Cu^{2+} in the culture liquid, was measured every day for five days.

Preparing of Cu^{2+} stock solutions. Stock solutions of copper citrate and $CuCl_2$ contained 20 000 ppm of Cu^{2+}. To prepare copper citrate solution 20 g of $C_6H_6Na_3O_7 \cdot 1½H_2O$ were dissolved in beaker in 50 ml of distilled water. Then 5.34 g of $CuCl_2 \cdot 2H_2O$ were brought to the

beaker with sodium citrate solution, so the dark blue solution of copper citrate was obtained. The solution was put to 100 ml flask and brought up to line with distilled water. To prepare $CuCl_2$ solution 5.34 g of $CuCl_2 \cdot 2H_2O$ were brought to the beaker with distilled water. After the $CuCl_2$ was dissolved the solution was put in 100 ml flask and brought up to the line with distilled water.

Concentration of Cu^{2+} was determined colorimetrically at low Cu^{2+} concentrations and titrimetically at high concentrations. Both methods are based on the ability of 4-2-pyrydilazo-resorcinol (PAR) to form coloured (dark-cherry) complex with copper. To determine Cu^{2+} concentration colorimetrically 0.5 ml of 0.5% aqueous PAR was brought to 3 ml of the sample. Optical density of the solution was determined at the photoelectric colorimeter KFK 2-MP at $\lambda = 490$ nm, the length of optical step = 0.5 cm. Values of optical density linearly depended on the Cu^{2+} concentration in the range of 1–7 ppm of Cu^{2+}.

To determine Cu^{2+} concentration titrimetically 0.1 ml of 0.1% aqueous PAR was brought to 2 ml of sample. The solution was titrated with an aqueous solution of EDTA (2.5 g/l). EDTA breaks down complex of Cu^{2+}-PAR, and at the point of transition causes a sharp change in colour from dark cherry to lemon. Amount of EDTA solution spent on destruction of complex Cu^{2+}-PAR linearly depends on the concentration of Cu^{2+} in the range of 25–3 000 ppm.

Biomass growth of microorganisms in liquid culture was determined by the change of optical density at the photoelectric colorimeter KFK 2-MP at $\lambda = 540$ nm, the length of optical step = 0.5 cm.

Concentration of the gas (O_2, CO_2) was determined by standard method based on the thermal conductivity of the katharometer on gas chromatograph LHM-8-MD. Two steel columns were used. The first one (I) was for the analysis of H_2, O_2, N_2 and CH_4, the second one (II) was for the analysis of CO_2.

Parameters of columns: I — l = 3, m, d = 3 mm, the sorbent 13X (NaX); II — l = 2, m, d = 3 mm, the sorbent Porapak-Q. The temperature of columns was +60 °C, the temperature of evaporator was +75 °C and of detector +60 °C. The detector current was 50 mA. Gas carrier is argon; gas flow rate was 30 ml/min. The concentration of gases (%) was calculated according to the peak areas. Plastic sterile 2.5 ml syringes (company "Bayer") with a rubber seal on the piston were used for gas sampling.

Redox potential (Eh) and pH were determined with the pH-meter-milivoltmeter "pH-121" (or "EV-74") with platinum measuring electrode EPV-1, flow-silver chloride reference electrode EVL-1MZ and combined glass electrode ESL-63-07 (for pH measurement).

Results and Discussion

Copper resistant yeast strain *C. tropicalis* RomCu5 and strain of filamentous fungi were isolated from highland Ecuador ecosystems. Both strains were able to grow at 3 000–30 000 ppm of Cu^{2+} (Fig. 1). Thus, both strains have biotechnological perspectives for copper containing waste water treatment. The study is focused on interaction of *C. tropicalis* RomCu5 with copper compounds, whereas interaction of filamentous fungi strain is the subject of further investigations.

The main feature of *C. tropicalis* RomCu5 that had caused interest is its high resistance to copper compounds. The maximal permissible concentrations (MPC) of copper for the strain (i.e. the maximal concentrations of copper where the growth of the strain was observed) were 30 000 ppm of Cu^{2+} in the form of copper citrate and 500 ppm of Cu^{2+} in the form of $CuCl_2$. To compare the maximal permissible concentrations of Cu^{2+} were determined for culture from Ukrainian Collection of

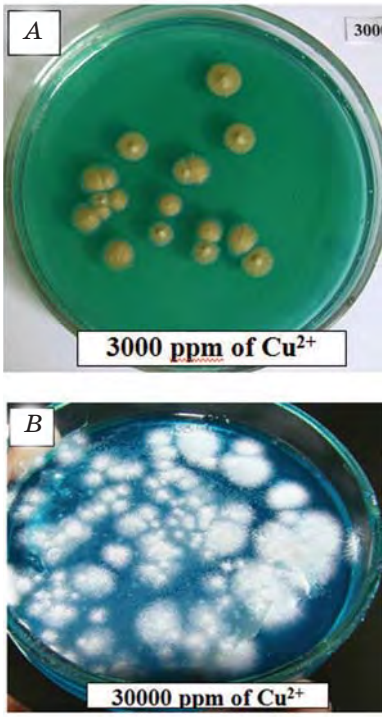


Fig. 1. **Growth of *C. tropicalis* RomCu5 (*A*) and filamentous fungi (*B*) isolated from highland Ecuador ecosystem in the presence of Cu^{2+}**

microorganisms *Saccharomyces cerevisiae* B-176 had never cultivated in the presence of Cu^{2+} before. The MPC for *S. cerevisiae* B-176 were 1 000 ppm of Cu^{2+} in the form of copper citrate and 100 ppm of Cu^{2+} in the form of $CuCl_2$. So, the strain RomCu5 was more resistant to copper by 30 (in form of copper citrate) and by 5 (in form of $CuCl_2$) times than *S. cerevisiae* B-176.

Despite high resistance to copper, growth of *C. tropicalis* RomCu5 was inhibited proportionally to increasing of Cu^{2+} concentration. Copper inhibits the growth of strain and adversely affects its metabolic activity. The growth of the strain began on fourth hour of cultivation at the absence of copper in the medium (Fig. 2, *A*). On the 14th hour of cultivation the strain reached its stationary phase of growth. The maximum optical density was 1.36 units of optical density. The concentration of CO_2 in the gas phase naturally increased with the biomass growth, and the concentration of O_2 decreased. The pH value changed from neutral (7.6) to acidic (5.5). The Eh value did not change significantly during the experiment. As concentration of O_2 during the growth decreased the redox conditions should have changed from oxidizing to reducing and redox potential should have lowered to negative values. The Eh value varied from +260 to +365 mV, which indicates the specific redox pair formation and requires the further investigation.

Culture growth began on the fourth hour of cultivation exactly as in control when of 200 ppm of Cu^{2+} (in the form of copper citrate) was present in the medium (Fig. 2, *B*). However, culture reached stationary phase on the 28th hour of cultivation. That is, culture growth twice slowed down compared with the control in the presence of 200 ppm of Cu^{2+}. The maximum biomass yield did not differ from control and was 1.28 units of optical density. The concentration of CO_2 increased with

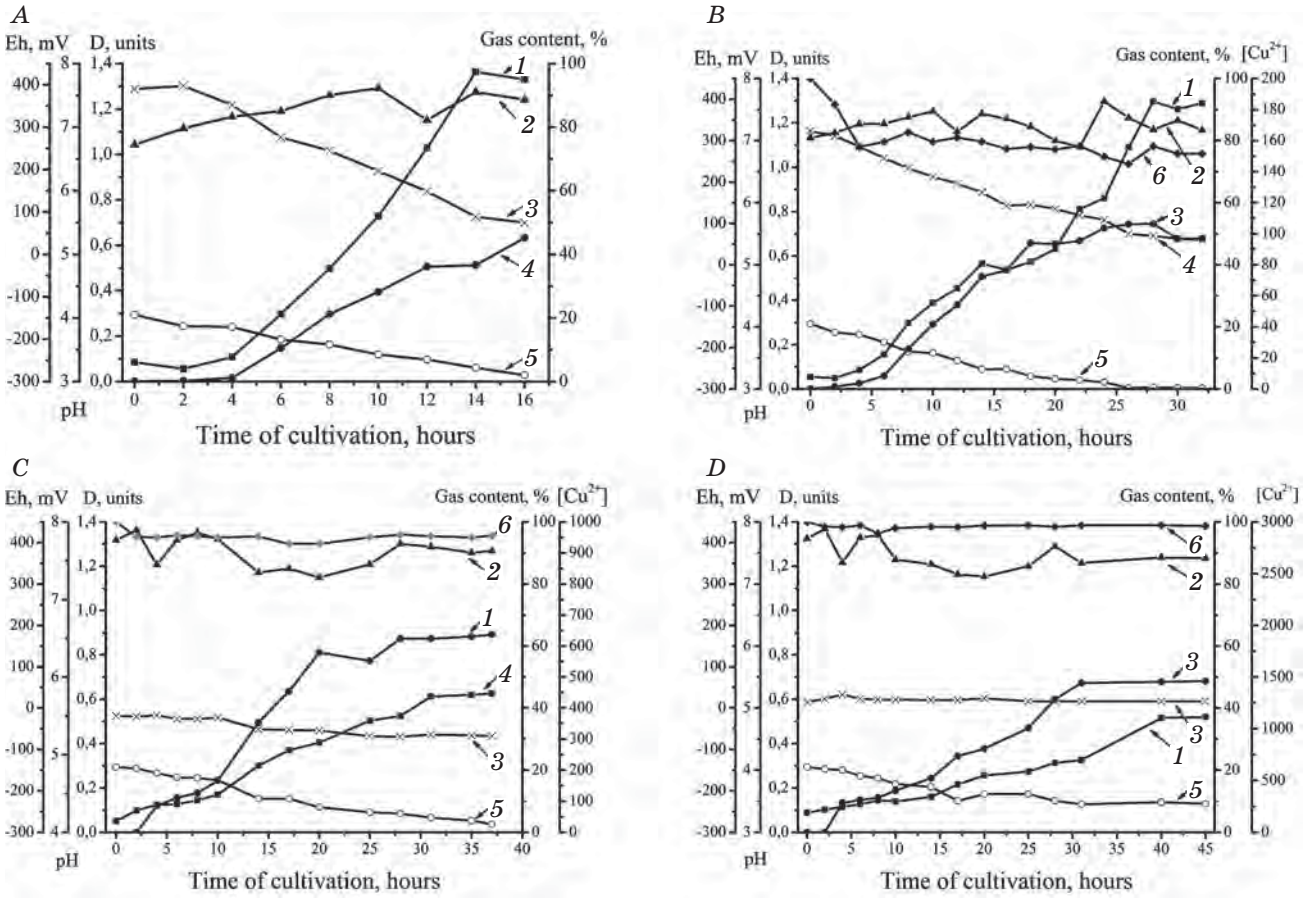


Fig. 2. **Metabolic parameters of *C. tropicalis* RomCu5:**
A — Cultivation at — absence of Cu^{2+} (control); *B* — 200 ppm of Cu^{2+}; *C* — 1000 ppm of Cu^{2+}; *D* — 3000 ppm of Cu^{2+}: *1* — optical density, units (standard deviation (SD) = ± 0.005–0.03); *2* — Eh, mV (SD = ± 9.9–23.6); *3* — pH (SD = ± 0.03–0.05); *4* — CO_2,% (SD = ± 0.05–1.75); *5* — O_2,% (SD = ± 0.06–0.31); *6* — concentration of Cu^{2+}, ppm (SD = ± 1.5–9.8)
In order not to overload the *Fig. 2 P*-values are discussed on the *Fig.3*.

biomass growing. The maximal concentration of CO_2 did not vary significantly from control values and was 48.5%. Appropriately, concentration of O_2 decreased as well to 0.28%. The Eh values of culture liquid were slightly higher than in the control (+310 ... +395 mV), which was due to the high redox potential of copper. The concentration of copper decreased from 200 to 170 ppm of Cu^{2+} during the first four hours of cultivation. During further cultivation Cu^{2+} concentration did not change significantly. Such way of interaction very likely indicates the sorption of Cu^{2+} by the yeast biomass.

The growth of yeast's biomass slowed down by 2.1 times comparatively with control in the presence of 1000 ppm of Cu^{2+}. The stationary phase of growth here was reached on 28th hour of cultivation (Fig. 2, *C*). Moreover the biomass yield decreased. The maximal value of the optical density was 0.63 units, which by 2.3 less than control value. As in other variants of experiment the concentration of CO_2 increased in the gas phase during the biomass growth whereas the concentration of O_2 decreased. The value of Eh did not change notably. Concentration of Cu^{2+} in the culture liquid lowered from 1000 to 950 ppm of Cu^{2+} during the first four hours of cultivation.

Culture came to the stationary phase of growth at 40th hour of cultivation in the presence of 3000 ppm of Cu^{2+}, i.e. growth slowed by 2.8 times (Fig. 2, *D*). Maximal biomass yield was by 2.7 times lower in comparison with the control and amounted 0.52 units of optical density. Concentration of Cu^{2+} lowered from 3000 to 2950 ppm of Cu^{2+}.

The growth of *C. tropicalis* RomCu5 was inhibited as the concentration of Cu^{2+} increased. The biomass yield falls down from 1.36 units of optical density in control to 1.28, 0.63 at 0.52 units at 200. 1000 and 3 000 ppm of Cu^{2+} respectively (Fig. 3, *A*; $P \le 0.05$). Thus, the Cu^{2+} concentration and biomass yield have strong negative linear correlation ($r = -0.8$).

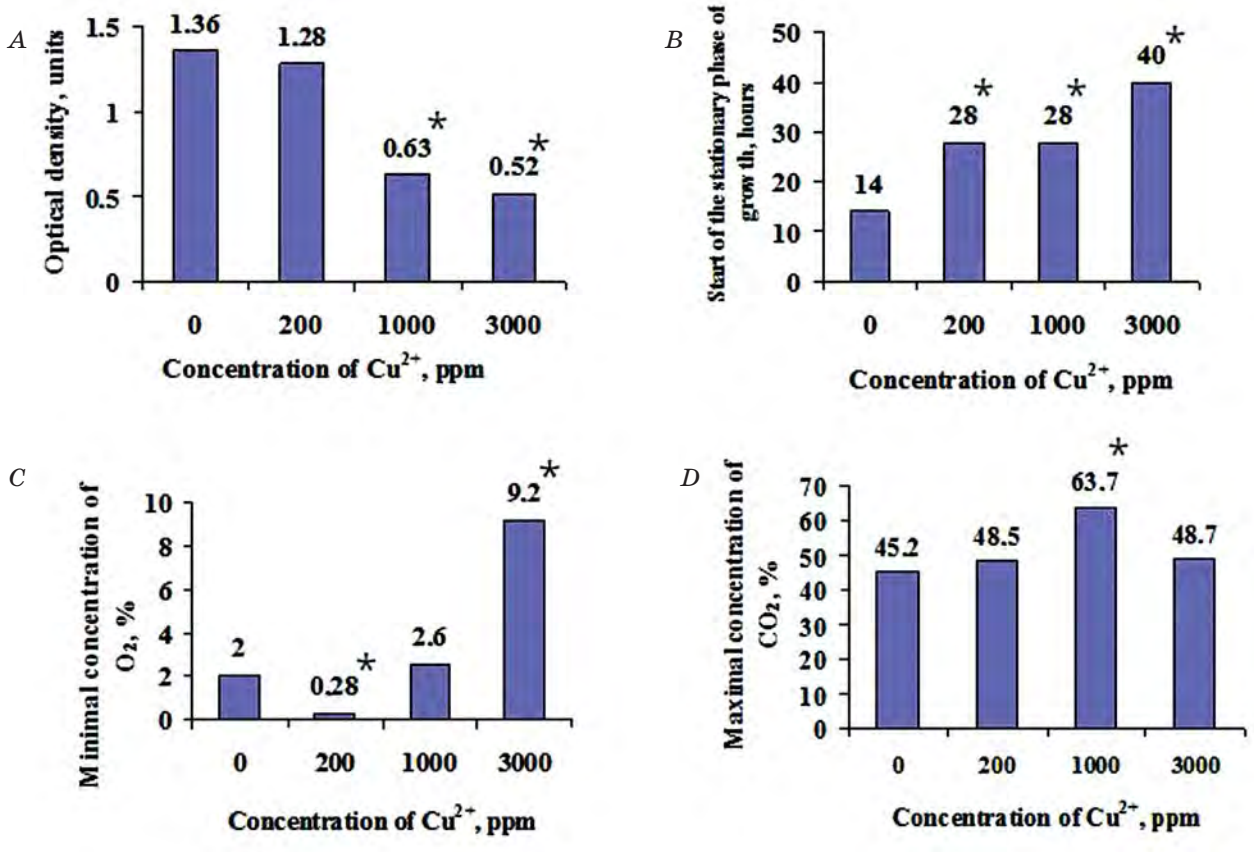


Fig. 3. **Comparison of metabolic parameters of *C. tropicalis* RomCu5 at presence of increasing Cu^{2+} concentration:**
A — optical density, units, $r = -0.8$, $*P \le 0.05$ (comparatively to control, i.e. cultivation of *C. tropicalis* without Cu^{2+}), SD = ± 0.01–0.03; *B* — start of stationary phase of growth, hours, $r = 0.8$, $*P \le 0.05$, SD = 0; *C* — minimal O_2 concentration, %, $r = 0.99$, $*P \le 0.05$, SD = ± 0.06–0.31; *D* — maximal CO_2 concentration, %, $r = 0.09$, $*P \le 0.05$, SD = ± 0.05–1.75

The growth rate slowed down with Cu^{2+} concentration rising. In the control culture reached stationary phase of growth on 14th hour of cultivation (Fig. 3, *B*). At 200 and 1000 ppm of Cu^{2+} culture's growth twice slowed down. That means culture reached stationary phase on 28th hour of cultivation. At 3000 ppm culture slowed down by 2.8 times compared with control ($P \leq 0.05$). The stationary phase of growth began on 40th hour of cultivation.

Copper concentration naturally leads to decrease of O_2 consumption (Fig. 3, *C*). Concentration of O_2 in gas phase increases in diapason 200–3 000 ppm of Cu^{2+} (perfect linear positive correlation, $r = 0.99$; $P \leq 0.05$). At 200 ppm of Cu^{2+} it was 0.28%, whereas at 1000 and 3 000 ppm of Cu^{2+} it was 2.6% and 9.2%. Concentration of O_2 at the control is even higher that at 200 ppm of Cu^{2+}, which is likely caused by longer cultivation of culture at 200 ppm of Cu^{2+}. Surprisingly, that CO_2 concentration does not indicate the culture inhibition by Cu^{2+} (zero correlation, $r = 0.1$). Minimal CO_2 concentration was observed in the control (45.2%) and maximal at 1000 ppm of Cu^{2+} (65.7%) (Fig. 3, *D*).

In all cases concentration of Cu^{2+} in the culture liquid decreased for 30–50 ppm at first four hours of cultivation. This apparently indicates the non-specific accumulation of Cu^{2+} by yeast's biomass.

Microbial immobilization of Cu^{2+} is possible due to reduction of Cu^{2+} to Cu(I)↓, precipitation with metabolites, accumulation of biomass. Let us consider the possible mechanisms of copper compounds immobilization by *C. tropicalis* RomCu5. The first possible mechanism is reduction of soluble cation Cu^{2+} to insoluble compound of Cu(I). Fig. 4 shows the reactions of copper transformation in water solution. Out of presented reactions the precipitation of copper by its reduction is possible according to the equation (Fig. 4, reaction 1):

$$2Cu^{2+} + H_2O + 2e = Cu_2O\downarrow + 2H^+$$
(Eh = +430 мВ at pH = 4.6).

According to the thermodynamic prediction of microbial interaction with toxic metals the microbial reduction of toxic metals is possible on the following conditions [8, 9]:

1. The reduction reaction has to be within the zone of thermodynamic stability of water (Fig. 4). Zone of thermodynamic stability of water is limited by two redox reactions *a* and *b* (Fig. 4). Water in reaction *a* is a reductant that is oxidized to O_2. In the reaction *b*, proton of water is an oxidizer that is reduced to H_2. Obviously, microorganisms can carry out only those reactions of energy metabolism that are within the thermodynamic stability of water.

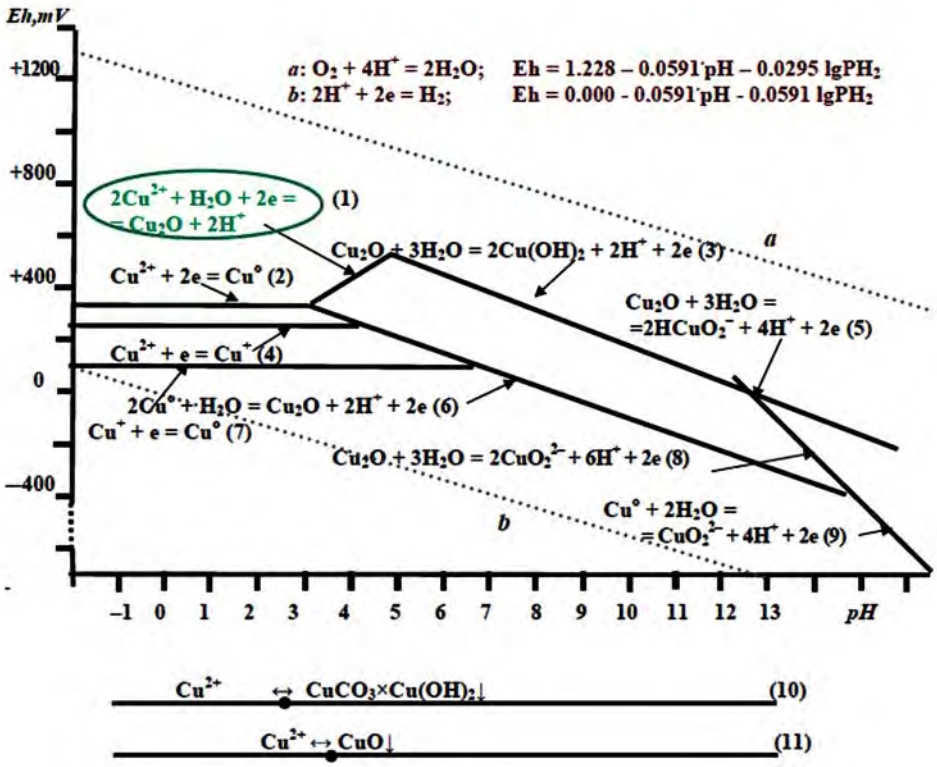


Fig. 4. **Redox states of copper compounds**

2. Nonspecific reduction of acceptor occurs if the difference between the donor (microorganisms) and acceptor (metals) systems at least 100 mV. Larger the difference between the donor and acceptor systems, the more effective reduction of metal is going.

The Fig. 4 shows that the reaction of Cu^{2+} to $Cu_2O\downarrow$ reduction is within the thermodynamic stability of water, which satisfies the first condition. But the second condition is not fulfilled. The calculated Eh value of Cu^{2+} reduction to Cu_2O at 200 ppm (0.003 mol/l) of Cu^{2+} is +348 mV (pH = 5) in accordance with following equation):

$$Eh = 0.203 + 0.0591 \times pH + 0.0591 \times lg\{Cu^{2+}\}.$$

The Eh value created by *C. tropicalis* RomCu5 in control was on average +338 mV. So, the potential difference between the donor and acceptor systems is virtually absent. That is why the reduction of Cu^{2+} to Cu_2O by *C. tropicalis* RomCu5 is impossible.

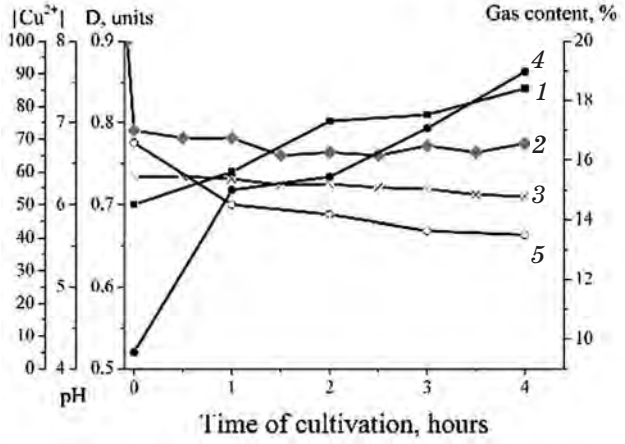
Copper can form insoluble compounds with such microbial metabolites as H_2S, CO_2, oxalate, etc. During the growth of *C. tropicalis* RomCu5 in the presence of Cu^{2+} there was no precipitates formation. The $CuCO_3\downarrow$ formation was not observed as well despite the strain actively produced CO_2 (Fig. 2). Carbon dioxide dissolves in water in neutral and alkaline conditions with formation of HCO_3^- and CO_3^{2-} that precipitate divalent metals as metals carbonates. *C. tropicalis* RomCu5 acidifies the medium up to pH=5.0–5.5, which prevents the formation of insoluble $CuCO_3\downarrow$.

Accumulation of metals in microbial biomass is provided by non-specific sorption on microbial cells, binding of metals with functional groups of cellular polymers, active transport into the cell [2]. *C. tropicalis* RomCu5 was shown to accumulate copper in biomass. Fig. 5 shows the dynamic of Cu^{2+} accumulation by biomass of *C. tropicalis* RomCu5 during cultivation in Hiss medium.

Since the microbial interaction with metals may vary depending on the type of metal compound, two types of copper compounds were used — $CuCl_2$ and copper citrate. When strain was cultivated in the presence of copper citrate concentration of Cu^{2+} in medium decreased from 100 to 85 ppm, i.e. for 15% (Fig. 5, *A*). During the experiment the metabolic activity of yeast was observed. During four hours of cultivation optical density increased from 0.7 to 0.85 units and CO_2 concentration increased from 9.5% to 19%, whereas O_2 concentration fall from 17% to 13.5%.

Culture accumulated copper(II) more efficiently in the presence of Cu^{2+} in form of $CuCl_2$ (Fig. 5, *B*). Thus, the concentration of Cu^{2+} in medium decreased from 100 to 55 ppm, i. e. for 45%. Instead, copper chloride inhibited the growth of yeast strain. Optical density of the medium decreased from 0.7 to 0.6 units. Both synthesis of CO_2 and O_2 consumption were absent.

A



B

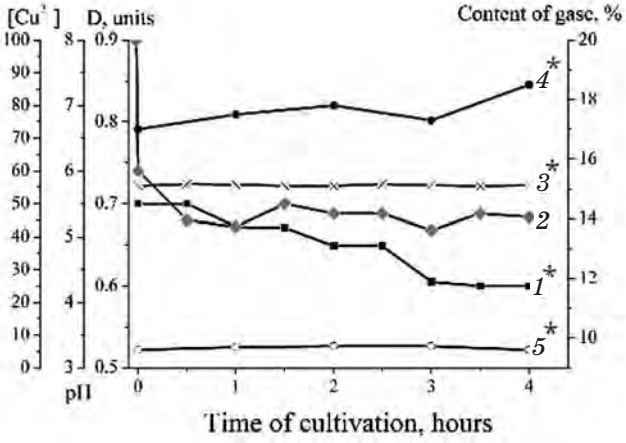


Fig. 5. **Accumulation of copper in the form of copper citrate (*A*) and $CuCl_2$ (*B*) by *C. tropicalis* RomCu5 and it metabolic parameters (Hiss medium):**

1 — optical density, units, SD = ± 0.01–0.04, *except the initial point $P \leq 0.05$ (comparing to control, i.e. cultivation of *C. tropicalis* without Cu^{2+}); *2* — concentration of Cu^{2+}, ppm, SD = ± 1.1–1.7; *3* — pH, SD = ± 0.01–0.05, *$P \leq 0.05$ (for all points); *4* — CO_2,%, SD = ± 0.3–0.5, *except the initial point $P \leq 0.05$; *5* — O_2,%, SD = ± 0.12–0.54, *except the initial point $P \leq 0.05$

Thus, the accumulation of Cu^{2+} in the form of $CuCl_2$ is more efficient compared with copper citrate (Fig. 6). Yeast biomass accumulated 14 out of 100 ppm of Cu^{2+} in the form of copper citrate. Of these, 1 ppm of Cu^{2+} was desorbed from the cell surface by solution of citric acid, and 13 ppm contained inside the cells. In the supernatant 86 ppm of Cu^{2+} remained. Overall, in 1 g of yeast ADM 50 ppm of Cu^{2+} were accumulated. Biomass accumulated 46 ppm of Cu^{2+} when the $CuCl_2$ was used. Of these, 36 ppm of Cu^{2+} was desorbed from the surface of cells walls and 10 ppm of Cu^{2+} was inside the cells. In this case, the yeast accumulated 164 ppm of Cu^{2+} in 1 g of ADM.

As Fig. 6 shows culture accumulated copper(II) on the surface of the cell in form of $CuCl_2$ by 36 times more efficiently than in form of copper citrate. Chelating of Cu^{2+} with citrate leads to the complex compound forming whose size is in several times bigger than $CuCl_2$. Therefore copper citrate is accumulated less effectively by the cells. In the case strain accumulates Cu^{2+} without loosing its biological activities. Copper chloride has a smaller size, which contributes to its accumulation by non-specific transport systems. Moreover, the size of ionic radius of Cu^{2+} coincides with ionic radii of metals necessary for microbial metabolic processes such as Fe^{2+}, Mg^{2+} [10]. The ionic radii of Cu^{2+} is 0.08 nm, whereas of Fe^{2+}, Mg^{2+} is 0.08 and 0.072 nm. So, Cu^{2+} replaces Mg^{2+} and Fe^{2+} in cell walls by ion exchange. Moreover the Cu^{2+} has positive charge whereas the cell surface has negative one, which causes their electrostatic interaction. So, the bulk of copper accumulates on the surface of the cell. That influences on biosynthetic, transport and energetic functions of the cell membranes. Unsurprisingly, that copper in form of $CuCl_2$ strongly inhibits growth of *C. tropicalis* RomCu5.

Microbial mobilization of insoluble metal compounds is possible by pH value lowering and release into the medium organic compounds (organic and fatty acids etc.) that are metal chelators [11, 12].

Insoluble copper compounds, as CuO, $CuCO_3$, are mobilized at pH value lower than 4,6 (Fig. 4, reactions 10 and 11). *C. tropicalis* RomCu5 lowered pH of culture liquid to 5.0. so it was assumed to mobilize insoluble copper compounds (CuO and $CuCO_3$). Active yeast culture that lowered pH to 5.5 was added to CuO and $CuCO_3$. The concentration of soluble copper compounds in the medium as CuO and $CuCO_3$ dissolved increased proportionally to the time of cultivation (Fig. 7). The maximum concentration of Cu^{2+} in both variants of the experiment was observed on the 5 day and cultivation (43 and 42 ppm of Cu^{2+}).

Candida tropicalis RomCu5 resistant to ultra-high concentrations of copper and able to interact with soluble and insoluble copper compounds was isolated from highland ecosystem of Ecuador. The strain was shown to be metabolically active in the presence of Cu^{2+} in super high concentrations, though Cu^{2+} negatively affected strains` metabolic parameters. Strain is able to immobilize Cu^{2+} in form of copper citrate and $CuCl_2$ by accumulation in biomass. High level of copper accumulation in the biomass of *C. tropicalis* RomCu5 makes it perspective for biotechnologies of copper containing wastewater treatment. As copper accumulation occurs in the

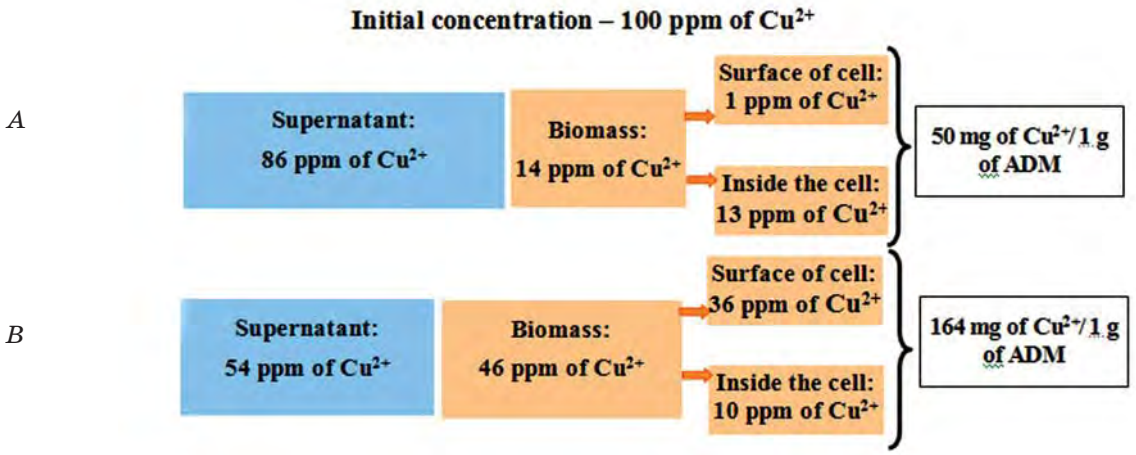


Fig. 6. **Accumulation rate of copper in the form of copper citrate (*A*) and $CuCl_2$ (*B*) by *C. tropicalis* RomCu5 and redistribution of accumulated copper in the yeast cell. ADM — absolutely dry mass**

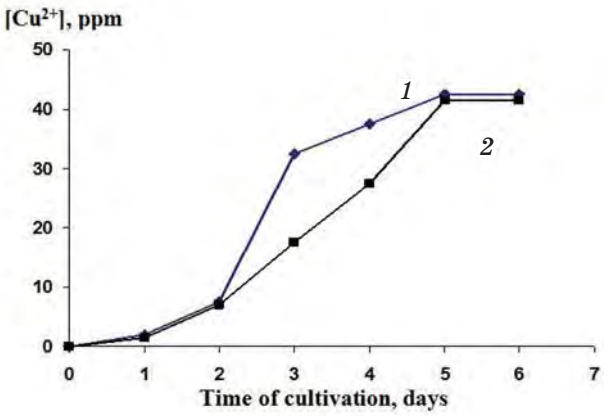


Fig. 7. **Mobilization of insoluble copper compounds by *C. tropicalis* RomCu5:**
1 — CuO; *2* — $CuCO_3$; SD = ± 0.6–1.5; SD = ± 0.6–1.1

result of non-specific processes based on stereochemical analogy, physical and chemical sorption *C. tropicalis* RomCu5 can be assumed to be effective in wastewater treatment from other toxic metals (Co^{2+}, Ni^{2+}, Zn^{2+}, etc). This question requires further researches. Strain mobilizes insoluble compounds of copper (CuO and $CuCO_3$) by lowering the pH. Due to this ability *C. tropicalis* RomCu5 can be used in biotechnologies of copper leaching from low-grade ores and dumps.

REFERENCES

1. *Gadd G. M.* Microbial influence on metal mobility and application for bioremediation. *Geoderma*. 2004, V. 122, P. 109–119. doi:10.1016/j.geoderma. 2004.01.002.
2. *Kotrba P., Machkova M., Macek T.* Microbial Biosorption of Metals. *Springer*. 2011, 329 p. doi:10.1007/978-94-007-0443-5.
3. *Vera M., Schippers A., Sand W.* Progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation—part A. *Appl. Microbiol. Biotechnol*. 2013, 97 (17), 7529–7541. doi:10.1007/s00253-013-4954-2.
4. *Lloyd J. R., Lovley D. R.* Microbial detoxification of metals and radionuclides. *Current Opinion in Biotechnology*. 2001, V. 12, P. 248–253.
5. *Mishra D., Ha Rhee Y.* Microbial Leaching of Metals from Solid Industrial Wastes. *J. Microbiol.* 2014, 52 (1), 1–7. doi:10.1007/s12275-014-3532-3.
6. *Tashyrev O. B., Prekrasna Ie. P., Tashyreva H. O., Belikova O. Yu.* Resistance of microbial communities from Ecuador ecosystems to representative toxic metals — CrO_4^{2-}, Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+}. *Mikrobiol. Zh.* 2015, 77 (4), 44–61.
7. *Tashyrev O., Prekrasna Ie.* Express method for redox potential and pH measuring in microbial cultures. *Inter. J. Bioautomation.* 2014, 18 (3), 217–230.
8. *Tashyrev A. B., Galinker E. V., Andreyuk E. I.* Thermodynamic prediction of redox interaction of microorganisms with metals-oxidants (Hg^{2+}, CrO_4^{2-} и Cu^{2+}). *Dopovidi Natsionalnoi Academii Nauk*. 2008, V. 4, P. 166–172. (In Russian).
9. *Tashyrev A. B.* Theoretical aspects of microbial interaction with metals. Reduction transformation of metals. *Mikrobiol. Zh.* 1994, V. 6, P. 76–87. (In Russian).
10. *Tashyrev A. B.* Theoretical aspects of microbial interaction with metals. Microbial accumulation of metals due to their stereochemical analogy with macroelements. *Mikrobiol. Zh.* 1994, V. 6, P. 89–97. (In Russian).
11. *Birch L., Bachofen R.* Complexing agents from microorganisms. *Experientia.* 1990. 46 (8), 827–834.
12. *Gadd G. M.* Metals, minerals and microbes: geomicrobiology and bioremediation. *Microbiology*. 2010, V. 156, P. 609–643. doi: 10.1099/mic.0.037143-0.

ВЗАЄМОДІЯ МІДЬРЕЗИСТЕНТНОГО ШТАМУ *Candida tropicalis* RomCu5 ІЗ РОЗЧИННИМИ І НЕРОЗЧИННИМИ СПОЛУКАМИ МІДІ

Є. П. Прекрасна
О. Б. Таширев

Інститут мікробіології і вірусології
ім. Д. К. Заболотного НАН України,
Київ

E-mail: preckrasna@gmail.com

Досліджено взаємодію *Candida tropicalis* RomCu5, ізольованого з високогірної екосистеми Еквадору, з розчинними та нерозчинними сполуками міді.

Штам *C. tropicalis* RomCu5 культивували в рідкому середовищі Гісса у присутності розчинних (цитрат міді та $CuCl_2$) та нерозчинних (CuO та $CuCO_3$) сполук міді. Приріст біомаси штаму визначали за зміною оптичної густини, склад газової фази — на газовому хроматографі, редокс-потенціал та pH культуральної рідини — потенціометрично. Концентрацію розчинних сполук міді оцінювали колориметрично.

Максимально допустима концентрація Cu^{2+} для *C. tropicalis* RomCu5 становила 30 000 мг/л Cu^{2+} у формі цитрату міді та 500 мг/л Cu^{2+} у формі $CuCl_2$. *C. tropicalis* RomCu5 був метаболічно активним за вмісту Cu^{2+} у надвисоких концентраціях, незважаючи на інгібуючу дію Cu^{2+}. *C. tropicalis* RomCu5 іммобілізував Cu^{2+} у формі цитрату міді та $CuCl_2$ за рахунок акумуляції в біомасі. *C. tropicalis* RomCu5 розчиняв CuO та $CuCO_3$ внаслідок закислення середовища. Висока стійкість *C. tropicalis* RomCu5 до Cu^{2+} і його здатність взаємодіяти з розчинними та нерозчинними сполуками міді робить його перспективним для використання у біотехнологіях очищення стічних вод від металів та видобутку міді з бідних руд та відвалів.

Ключові слова: дріжджі, *Candida tropicalis*, мідь, стійкість до міді, пригнічення метаболізму, іммобілізація міді, мобілізація міді.

ВЗАИМОДЕЙСТВИЕ МЕДЬРЕЗИСТЕНТНОГО ШТАММА *Candida tropicalis* RomCu5 С РАСТВОРИМЫМИ И НЕРАСТВОРИМЫМИ СОЕДИНЕНИЯМИ МЕДИ

Е. П. Прекрасная
А. Б. Таширев

Институт микробиологии и вирусологии
им. Д. К. Заболотного НАН Украины,
Киев

E-mail: preckrasna@gmail.com

Изучено взаимодействие *Candida tropicalis* RomCu5, изолированного из высокогорной экосистемы Эквадора, с растворимыми и нерастворимыми соединениями меди.

Штамм *C. tropicalis* RomCu5 культивировали в жидкой среде Гисса в присутствии растворимых (цитрат меди и $CuCl_2$) и нерастворимых (CuO и $CuCO_3$) соединений меди. Прирост биомассы штамма определяли по изменению оптической плотности, состав газовой фазы — на газовом хроматографе, редокс-потенциал и pH культуральной жидкости — потенциометрически. Концентрацию растворимых соединений меди оценивали колориметрически.

Максимально допустимая концентрация Cu^{2+} для *C. tropicalis* RomCu5 составляла 30 000 мг/л Cu^{2+} в форме цитрата меди и 500 мг/л Cu^{2+} в форме $CuCl_2$. *C. tropicalis* RomCu5 был метаболически активным в присутствии Cu^{2+} в сверхвысоких концентрациях, несмотря на ингибирующее действие Cu^{2+}. *C. tropicalis* RomCu5 иммобилизовал Cu^{2+} в форме цитрата меди и $CuCl_2$ за счет аккумуляции в биомассе. *C. tropicalis* RomCu5 растворял CuO и $CuCO_3$ вследствие закисления среды. Высокая устойчивость *C. tropicalis* RomCu5 к Cu^{2+} и его способность взаимодействовать с растворимыми и нерастворимыми соединениями меди делает его перспективным для использования в биотехнологиях очистки сточных вод от металлов и добычи меди из бедных руд и отвалов.

Ключевые слова: дрожжи, *Candida tropicalis*, медь, устойчивость к меди, угнетение метаболизма, иммобилизация меди, мобилизация меди.