ЕКСПЕРИМЕНТАЛЬНІ ПРАЦІ

EXPERIMENTAL WORKS

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ACCUMULATION AND EFFLUX OF COPPER AND CADMIUM IONS BY *PSEUDOMONAS AERUGINOSA* STRAINS

The ability of Pseudomonas aeruginosa A17, A03 and C25a strains to accumulate and export copper and cadmium ions has been studied. P. aeruginosa strains have been found to bind up to 65% cadmium ions to the cell surface while A03 and C25a cells bound more than 90% copper ions on the cell surface. The inhibitors chloramphenicol and N-N-dicyclohexylcarbodiimide (DCCD) inhibited metal accumulation and efflux by P. aeruginosa strains. ATP-driven efflux systems are involved in copper and cadmium resistance mechanisms of the studied P. aeruginosa strains.

Key words: Pseudomonas aeruginosa, accumulation, efflux, heavy metals.

The wide distribution of heavy metals in the environment is the result of many human activities, mostly industrial, although agriculture and municipal wastes also contribute. The search for new technologies of detoxification of these pollutants including biotechnological has direct attention to bacteria-metal interactions. By expanding the knowledge on the defense mechanisms of microorganisms against heavy metals it will be possible in future to develop the new methods of heavy metal bioremediation. Many microorganisms are able to bind heavy metal ions either on the cell surface (the cell wall, the capsule) [3, 6, 15] or inside the cell (sequestration) [8]. A vast number of microbes can export metal ions outside the cell by efflux systems [10].

The purpose of this work was to study accumulation and export of cadmium and copper ions by three multiresistant strains *Pseudomonas aeruginosa* and effect of chloramphenicol and N-N-dicyclyhexylcarbodiimide (DCCD) inhibitors on these processes.

Materials and methods

Microorganisms and medium. Three strains *P. aeruginosa* A17, A03 i C25a were used in this study. *P. aeruginosa* A17 has been isolated from cow manure, *P. aeruginosa* A03 — from the field soil, *P. aeruginosa* C25a — from the soil at the territory of the machine-building plant [2]. Bacterial cultures were grown in the minimal mineral medium (M) containing the following (g/l): KH_2PO_4 — 0.5, NH_4NO_3 — 0.5. $MgSO_4$ — 0.1, yeast extract — 0.5, CH_3COONa — 10.

Metal uptake analysis. Copper and cadmium content in the cells was determined by atomic adsorption spectrometry (AAS) using atomic adsorption spectrophotometer Saturn-3 at 324.8 nm and 227.8 nm for copper and cadmium, respectively.

Metal accumulation by bacterial cells was determined as cited in Gelmi et al [7] with some modifications. Bacteria were incubated in the liquid medium M containing 1 g/l Cu^{2+} or 0.5 g/l Cd^{2+} in a rotary shaker (240 rev per min) at 30 °C for 48 h. Cells were centrifuged with 0.9% NaCl solution at 5000 g three times. Then cells were divided into two equal portions. One portion of biomass was dried for 24 h at 80 °C. The rest of biomass was incubated in hypertonic 9% NaCl solution for 12 h to remove metal ions bound to cell wall components. The weight of the dried biomass was measured and after acid digestion metal content was determined by AAS. The amount of metal taken up by the cells was determined on a dry weight basis.

Efflux assay. The efflux assay was performed as described previously [9] with some modifications. To determine export of copper and cadmium ions cells grown for 24 h were harvested by centrifugation (5000 g, 10 min). Cell pellets were washed three times with 0.9% NaCl solution. Cells were added to the medium M containing 1 g/l Cu^{2+} or 0.5 g/l Cd^{2+} and incubated for 1 h at 30 °C. Then cells were divided into two equal portions. One portion was centrifuged and washed three times with 0.9% NaCl solution and dried for 24 h at 80 °C. The other portion of biomass was centrifuged and washed three times with 0.9% NaCl solution and incubated in a metal-free medium for 1 h at 30 °C. Metal content was measured in the dried cells following acid digestion .

Effect of inhibitors. To study the effect of inhibitors on accumulation and efflux of copper and cadmium ions washed cells were pre-incubated for 10—15 min at 30 °C in the liquid medium M containing 100 mg/l chloramphenicol or 200 μM DCCD. The inhibitors were present throughout the assay [9]. The analysis of copper and cadmium content in biomass was conducted as described previously.

Statistical analysis. All the experiments were done as three independent replicates. The values represented are the means plus the standard deviations. Means were compared by the Student t test. A P value of 0.05 was considered significant [1].

Results and discussion

The ability of three multiply metal resistant strains *P. aeruginosa* A17, A03 i C25a to accumulate and export copper and cadmium ions has been assessed (Fig. 1). Following hypertonic solution treatment there was a considerable decrease in copper and cadmium content in bacterial cells. There was 2–3 fold loss of cell-bound cadmium and the resultant metal content was similar for A17, A03 and C25a cells: 25.1, 35.6 and 24.9 mg Cd/g dry cells, respectively. Copper content in A03 and C25a cells decreased 10-fold but there was only 37% loss of cell-bound copper in A17 cells.

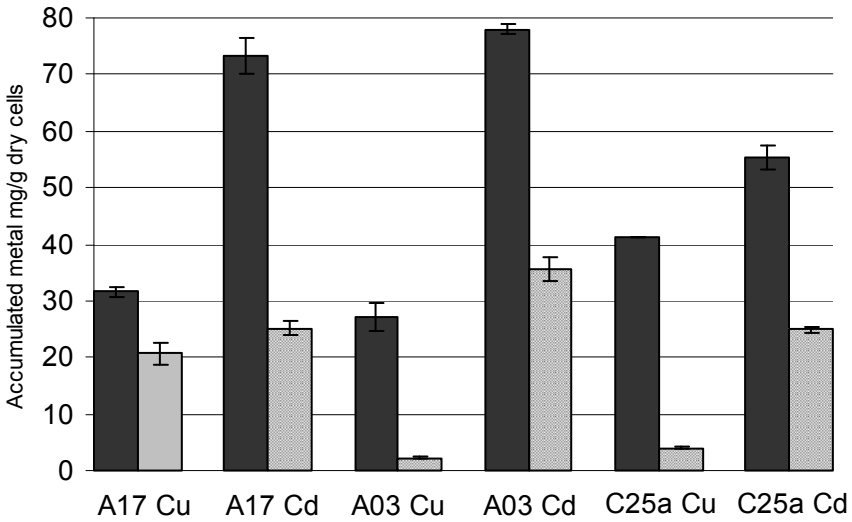

Fig. 1. Accumulation of copper and cadmium by *P. aeruginosa* A17, A03 and C25a
Copper and cadmium content in cells was determined before (black colour) and after (grey colour) hypertonic solution treatment.

As we can conclude, 55–65% cadmium accumulated by A17, A03 and C25a cells was absorbed on the cell surface of the given strains while the percentage of surface–bound copper exceeded 90% of accumulated metal. The ability of strains *P. aeruginosa* A17, A03 and C25a to accumulate copper and cadmium is similar to that reported by other authors [12, 13]. In contrast, some strains *Pseudomonas* are reported to possess both higher [5] and lower metal-binding capacity [4].

The export (efflux) of copper and cadmium ions from A17, A03 and C25a cells has been studied (Fig. 2). After 1 h incubation of A17, A03 and C25a cells in the metal-free medium there was a 80.2, 96.6 and 94% loss of accumulated cadmium, respectively. The efflux of accumulated copper was somewhat slower – 70.3, 47.4 and 38.3%, respectively. Thus it could be concluded that the systems of active transport of metal ions from cells, i.e. the efflux systems, are involved in the resistance mechanisms of A17, A03 and C25a strains against copper and cadmium.

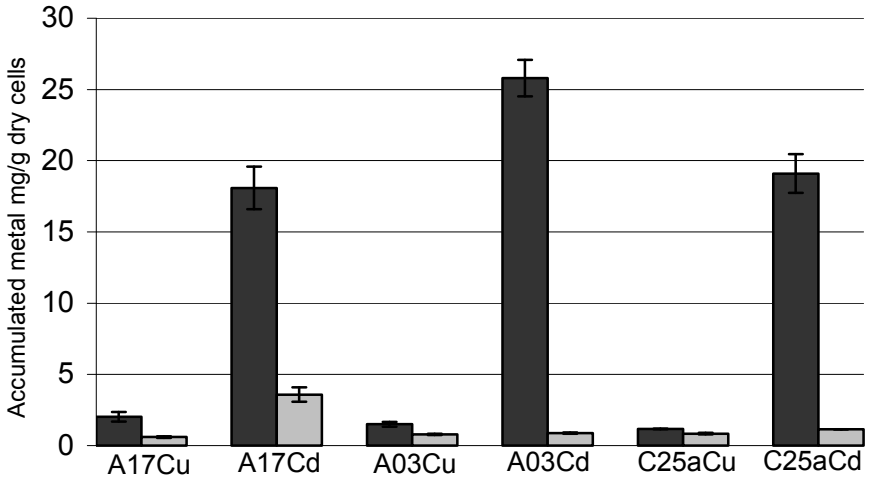

Fig. 2. Copper and cadmium efflux from cells of *P. aeruginosa* A17, A03 and C25a strains
Metal content was determined before (black colour) and after (grey colour) cell incubation in the metal-free medium.

It has been shown that DCCD mostly inhibited both accumulation and efflux of copper and cadmium by A17, A03 and C25a cells, however each strain reacted differently to DCCD treatment. DCCD treatment resulted in 2-fold inhibition of copper accumulation by A17 and C25a strains: in the presence of DCCD A17 and C25a cells accumulated 0.9 and 0.5 mg Cu/g dry cells, respectively (Fig. 3) comparing to 2.0 and 1.2 mg Cu/g dry cells by non-treated cells (Fig. 1).

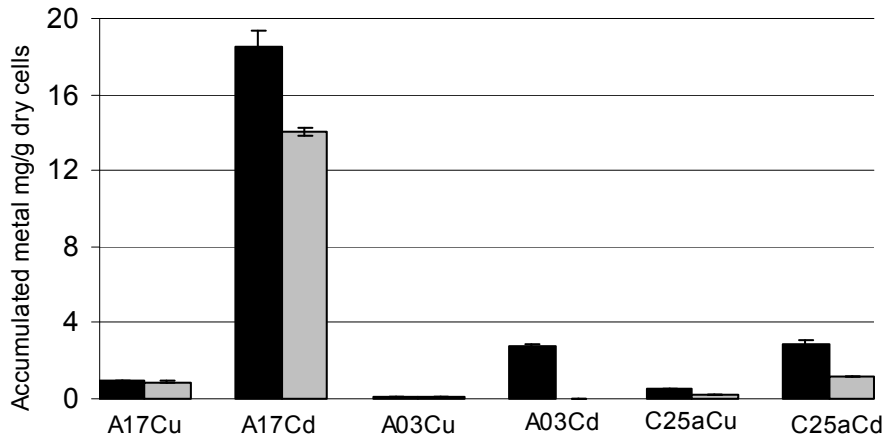

Fig. 3. Effect of DCCD on copper and cadmium efflux by *P. aeruginosa* A17, A03 and C25a strains
Metal content was determined before (black colour) and after (grey colour) cell incubation in the metal-free medium.

DCCD treatment resulted in an almost complete inhibition of copper accumulation by A03 strain. Copper efflux by A17 and A03 cells was completely inhibited. DCCD inhibited cadmium accumulation by A03 and C25a cells. However cells of the strain A17 treated with DCCD retained the same binding capacity as non-treated cells — 18.5 and 18.1 mg Cd/g dry cells, respectively. DCCD also affected cadmium efflux by A17, A03 and C25 cells: cadmium export by A17 and C25a cells comprised 33.9 and 58.9%, respectively, while by non-treated cells — 80.2 and 93.1%, respectively. Cadmium efflux by A03 cells was completely inhibited by DCCD.

Chloramphenicol, similarly fashion to DCCD, inhibited copper accumulation by A17, A03 and C25a cells: the capacity to accumulate copper comprised 23.7, 14.3 and 37.9%, respectively, of that of non-treated cells (Fig. 4). Chloramphenicol also negatively affected copper efflux by A17 and C25a cells.

The chloramphenicol effect on accumulation and efflux of copper and cadmium ions by A17, A03 and C25a cells was less obvious. Chloramphenicol-treated cells of A17 and A03 strains accumulated 36.3 and 57.2% less cadmium, respectively, than the control cells, chloramphenicol had no noticeable effect on copper accumulation by C25a cells. Cadmium efflux by A03 and C25a cells has also been inhibited by chloramphenicol.

The inhibitory effect of DCCD and chloramphenicol on accumulation and efflux of copper and cadmium by strains *P. aeruginosa* A7, A03 and C25a has been shown.

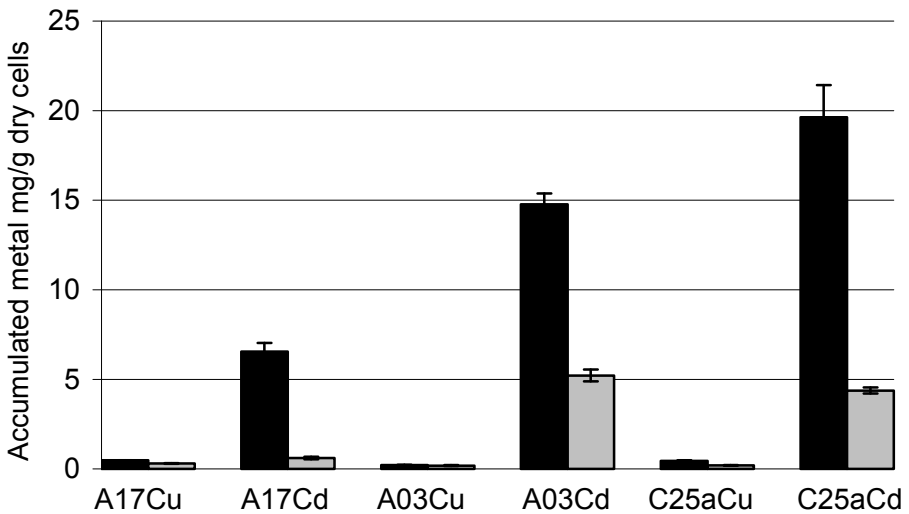

Fig. 4. Effect of chloramphenicol on copper and cadmium efflux by *P. aeruginosa* A17, A03 and C25a strains

Metal content was determined before (black colour) and after (grey colour) cell incubation in the metal-free medium.

As DCCD is a specific inhibitor of ATPase activity in the cell [11] it can be concluded that export of the given metal ions by the studied strains is ATPase-dependent. The involvement of ATPase—driven efflux systems has been shown for a number of bacteria belonging to different taxonomical groups [10, 16] including metal-resistant pseudomonads [9, 14].

However it should be noted that efflux is not the only the system involved in copper and cadmium resistance of strains *P. aeruginosa* A17, A03 and C25a, as the considerable amount of metal and cadmium is absorbed on the cell surface of *P. aeruginosa* strains. The obtained data had led to the conclusion that metal resistance of the studied strains is determined by two different mechanisms — sorption processes on the cell surface and ATP-dependent efflux system.

Conclusions

The ability of three multiresistant *P. aeruginosa* strains to accumulate copper and cadmium ions has been studied. It has been shown that copper and cadmium were mostly bound on the cell surface of the studied strains and were easily removed after hypertonic solution treatment. ATP-driven efflux systems have been involved in copper and cadmium resistance of *P. aeruginosa* strains.

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АКУМУЛЯЦІЯ ТА ЕФЛЮКС ІОНІВ МІДІ ТА КАДМІЮ ШТАМАМИ *PSEUDOMONAS AERUGINOSA*

Реферат

Досліджена здатність штамів *Pseudomonas aeruginosa* до акумуляції та ефлюксу іонів міді та кадмію. Показано, що до 65% іонів кадмію були зв'язані з поверхнею клітин штамів *P. aeruginosa*, більш ніж 90% іонів міді акумулювали на поверхні клітин штами А03 та С25а. Інгібітори хлорамфенікол та N-N-дициклогексилкарбодіімід (ДЦКД) пригнічували акумуляцію та ефлюкс іонів міді та кадмію штамами *P. aeruginosa*. В механізмі стійкості штамів *P. aeruginosa* до іонів міді та кадмію задіяні АТФ-залежні системи ефлюксу.

Ключові слова: *Pseudomonas aeruginosa*, акумуляція, ефлюкс, важкі метали.

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АККУМУЛЯЦИЯ И ЭФФЛЮКС ИОНОВ МЕДИ И КАДМИЯ ШТАММАМИ *PSEUDOMONAS AERUGINOSA*

Реферат

Изучены аккумуляция и эффлюкс ионов меди и кадмия штаммами *Pseudomonas aeruginosa*. Показано, что до 65% ионов кадмия были связаны с поверхностью клеток штаммов *P. aeruginosa*, и более 90% ионов меди аккумулировали на поверхности клеток штаммы А03 и С25а. Ингибиторы хлорамфеникол и дициклогексилкарбодиимид (ДЦКД) ингибировали аккумуляцию и эффлюкс меди и кадмия штаммами *P. aeruginosa*. АТФ-зависимые системы эффлюкса принимают участие в механизме устойчивости штаммом *P. aeruginosa* к меди и кадмию.

Ключевые слова: *Pseudomonas aeruginosa*, аккумуляция, эффлюкс, тяжелые металлы.