

Nickel ion efflux is the main mechanism of resistance in *Escherichia coli* V38

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Nickel resistant *Escherichia coli* V38 was isolated from city sewage sludge and could grow in the presence of 4 mM NiCl₂. When 1 mM or 2 mM NiCl₂ with ⁶³Ni²⁺ was added to the culture at the beginning of logarithmic phase, immediate uptake of ⁶³Ni²⁺ which was followed by slow and delayed efflux were observed; the level of both uptake and delayed efflux were dependent on the NiCl₂ concentration used. The efflux delay corresponded to the duration of growth prolonged lag period (data from [6]). When cultures of *E. coli* resistant V38 and sensitive JM101 strains pregrown overnight in the presence of nontoxic NiCl₂ were washed and suspended in 0°C efflux medium with the same nontoxic nickel concentration and ⁶³Ni²⁺, accumulation of Ni²⁺ by cells of both strains was observed. When the cells were transferred to 37°C only resistant V38 cells effluxed the nickel ions; the amount of nickel in the sensitive cells did not change. Ni²⁺ active efflux needed energy supply, as the efflux was observed in the presence of glucose, and was weak when glucose was exhausted or the cells were dead. Applications to waste waters treatment are discussed.

Key words: nickel ion efflux, *Escherichia coli*, nickel resistance

INTRODUCTION

The existence of various organisms in heavy metal polluted environment demonstrates the presence of mechanism protecting the living systems from toxic activity of metal ions. Various eukaryotic organisms bind metal ions with metallothioneins of phytochelators inside the cell [7], to cell wall structures [9] or to specially secreted glucoproteins [1].

Many microorganisms efflux metal ions, when the concentration inside the cell exceeds the physiological level. This process is energy dependent and is determined by special proteins. For the first time this efflux mechanism was shown in *Staphylococcus aureus* for cadmium [10]. This type of resistance based on ion efflux was proved for Cd²⁺, Co²⁺, Zn²⁺ and Ni²⁺ in *Alcaligenes eutrophus* [4]. Growth experiments in the presence of NiCl₂ showed the prolongation of lag period both in *Alcaligenes eutrophus* N9A [8] and in *Escherichia coli* V38 [6]. In *E. coli* prolongation of lag period was dependent on NiCl₂ concentration [6]. However, the prolongation of lag phase or delay of growth was not observed when the *A. eutrophus* N9A [8] or *E. coli* V38 [6] were pregrown in the presence of subinhibitory Ni²⁺ concentrations. These results indicated that the resistance to nickel was inducible.

Is it possible to use this mechanism of bacterial resistance for treatment of metal-polluted waste waters?

In this paper the data are presented that the main resistance mechanism in *E. coli* V38 is Ni²⁺ efflux; this process is active and needs energy supply. Delay of both the efflux and growth (duration of lag period) are almost in direct dependence on the concentration used. The possibilities to use this mechanism in solving the ecological problems are discussed.

MATERIALS AND METHODS

Bacterial strains: *Escherichia coli* V38 (a strain isolated from Vilnius city sewage sludge) resistant to 4 mM Ni²⁺ [6]; *E. coli* JM101 F' traD36 proAB lacIq ΔlacZ)M15 / supE1 thi & (lac-pro AB) [11], this strain was used as a Ni²⁺ sensitive control. The strains were grown at 37°C in mineral salt medium [6]. To get an agar medium, 1.5% of Bacto-Agar was added to the mineral medium Nutrient Broth was used in control growth experiments. Optical density measurements were made with Perkin Elmer Spectrophotometer 550 at wave length 550 nm

⁶³NiCl₂ (Izotop, Russia) was used for uptake and efflux experiments. The overnight bacterial culture grown with (induction) or without NiCl₂ was har-

vested by centrifugation, and the **cells** were suspended for 20–30 min in cold (0°C) efflux medium containing (in grams per litre of distilled water) 6.06 Tris-HCl, 7.6 NaCl and 1.6 glucose [11]. At this moment NiCl₂ to a definite concentration and ⁶³Ni²⁺ to a trace concentration were added. To measure the amount of ⁶³Ni²⁺ in the **cells**, 0.5 ml samples of the cell suspension were filtered (pore size of filters 0.4 μm, Synpor, Czech Republic) and then washed with buffer (5 mM Tris-HCl, 0.13 M NaCl, 2 mM EDTA). The dried filters were immersed in scintillation liquid, and radioactivity was measured (Counter SL30 Intertechnique). The amount of nickel in the **cells** was calculated from the radioactivity of the cells, as part of ⁶³Ni²⁺ in NiCl₂ solution was known and expressed as μM Ni per mg dry weight of bacteria.

RESULTS AND DISCUSSION

In previous paper [6] it was shown that the addition of NiCl₂ to the culture of *E. coli* V3 stopped the growth and this lag period was dependent on the concentration used. We have no data about the processes which occurred in the cells during this prolonged lag period in *E. coli* V38. Some morphological changes were described in *E. coli* B cells caused by Cd²⁺ [2]. However, it was not known what changes occurred in Ni²⁺ uptake during lag period.

To investigate the dynamics of Ni²⁺ uptake, *E. coli* V38 cells were grown in mineral medium, and at the beginning of logarithmic phase the culture was divided into three aliquots, to which 1 mM and 2 mM of NiCl₂ with trace ⁶³Ni²⁺ were added, and one aliquot was as a control. The samples were taken to measure optical density of the culture and radioactivity in the cells. In Fig. 1 data on radioactivity are plotted with the curves of optical density taken from [6]. The ⁶³Ni²⁺ uptake reached maximum in about 10 min, and both the level of uptake and the delay of efflux were dependent on Ni²⁺ concentration. A good correlation is clearly seen between efflux delay and duration of lag period; the following logarithmic growth renewed when efflux was almost finished (Fig. 1).

To investigate the resistance mechanism in *E. coli* V38, the culture was grown overnight in a mineral-salt medium in the presence of nontoxic 0.4 μM NiCl₂. The Ni²⁺ sensitive strain *E. coli* JM101 was also grown under the same conditions. The cells of both cultures were centrifuged and suspended in a 0°C efflux medium. The suspensions were kept in an icebath and 0.4 μM NiCl₂ (with radioactive ⁶³Ni²⁺) was added. After 30 min the suspensions were transferred to 37°C (Fig. 2). At 0°C the cells accumulated ⁶³Ni²⁺, and after they had been transferred to 37°C, the level of radioactivity in the cells sharply decreased during almost 30 min. This cycle was re-

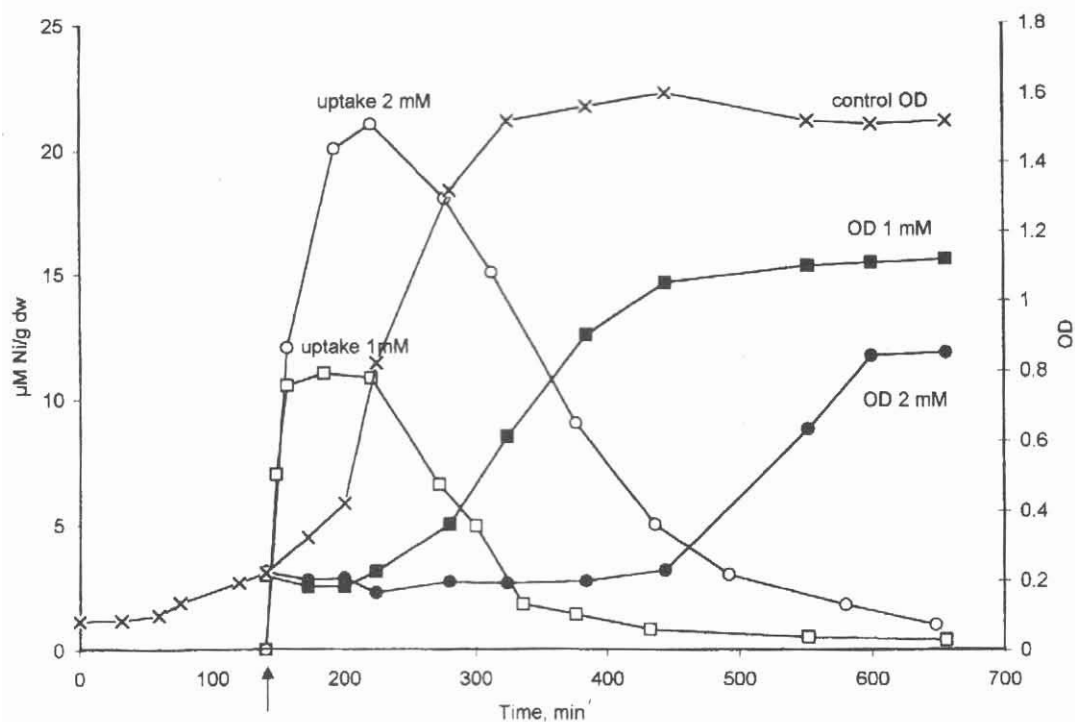


Fig. 1. To *Escherichia coli* V38 growing culture at early logarithmic phase NiCl₂ and ⁶³Ni²⁺ were added (↑). In the presence of both concentrations 1 mM uptake (□) and growth (■) and 2 mM uptake (o) and growth (●) were investigated. The uptake data were plotted on the data of growth from [6]

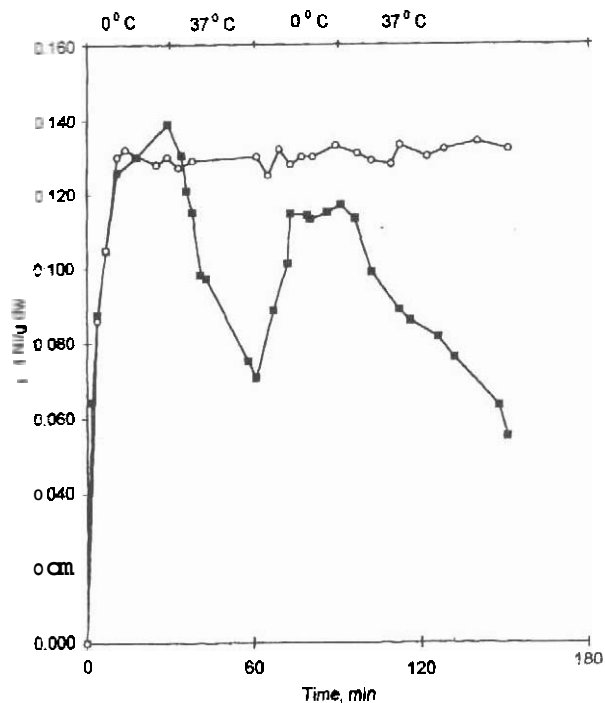


Fig. 2. Uptake at 0°C and efflux at 37°C of 0.4 μM NiCl_2 & $^{63}\text{Ni}^{2+}$ by resistant strain *E. coli* V38 cells (■) or sensitive *E. coli* JM101 cells (○). The temperature was changed every 30 min as indicated at top

peated (in Fig. 2, the transfer to 0°C for 30 min and return to 37°C was repeated twice, and the accumulation at 0°C and the efflux at 37°C were observed). The cells of sensitive control culture *E. coli* JM101 accumulated $^{63}\text{Ni}^{2+}$ in cold at the same level as did the resistant cells, but after transfer at 37°C, the level of $^{63}\text{Ni}^{2+}$ in the cells did not change at least for 1 h (Fig. 2). The data showed that the resistant *E. coli* V38 accumulated $^{63}\text{Ni}^{2+}$ at 0°C and actively effluxed the ions at 37°C. Similar results were observed in experiments with *A. eutrophus* resistance to Co^{2+} , Zn^{2+} and Cd^{2+} . The cells accumulated metal ions at 4°C and effluxed at 37°C [5]. It is possible to suggest that many microorganisms have evolved the uptake-efflux resistance mechanism for bivalent cations.

In polluted environment or under experimental conditions where metal (Ni^{2+}) concentration is toxic, metal ions could enter the cell using magnesium uptake system and could be effluxed by cation-proton antiporter system [3, 5]. These processes are active and energy-dependent [10].

Investigations of the uptake-efflux system in *E. coli* V38 dependence on energy source were carried out. The resistant cells were grown overnight in mineral medium in the presence of 0.05 mM NiCl_2 expecting a complete glucose exhaustion during growth. The cells were centrifuged and suspended in cold uptake-efflux medium containing 0.1 mM NiCl_2 and $^{63}\text{Ni}^{2+}$ and kept in an icebath for 15 min. The cells were transferred to a 37°C waterbath, but before this 0.1% glucose had been added to only one aliquot. As a control, an aliquot of the culture heated at 100°C for 3 min was used. The data are presented in Fig. 3. An active uptake and the following efflux were seen in the cells of the aliquot to which glucose was added. A low uptake and slow efflux were observed in the cells of the aliquot to which glucose was not added – the registered weak uptake-efflux could be attributed to the use of residual energy source in the cells. The control-heated cells showed neither Ni accumulation nor a following efflux. The data allow a suggestion that the nickel resistance in *E. coli* V38 is determined mainly by the energy-dependent uptake-efflux mechanism.

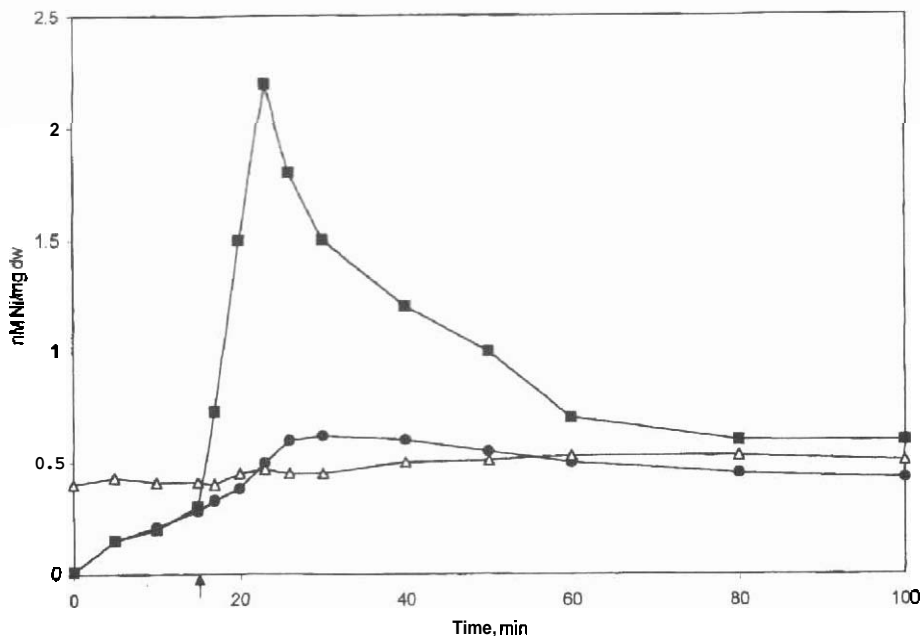


Fig. 3. Glucose effect on nickel ion uptake and efflux by resistant strain *Escherichia coli* V38. The culture overnight induced with 0.05 mM NiCl_2 was centrifuged, cells were suspended in 0°C uptake-efflux medium for 15 min in the presence of 0.1 mM NiCl_2 and $^{63}\text{Ni}^{2+}$. To one aliquot of suspension 0.1% glucose was added at the moment of transfer to 37°C (↑) (■); to the second aliquot glucose was not added (●) and the cells of the third aliquot were heated for 3 min at 100°C (A)

The presented data show the possibilities of application in wastewaters treatment: the management of metal uptake-efflux by changing the temperature and energy supply indicates the ways for further investigations.

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Escherichia coli V38 YRA ATSPARI NIKELIUI DĖL JONŲ IŠMETIMO

Santrauka

Nikelui atspari *Escherichia coli* V38 buvo išskirta iš miesto nuotekų dumblo ir augo esant 4 mM $NiCl_2$. Įdėjus 1 arba 2 mM $NiCl_2$ kartu su $^{63}Ni^{2+}$ į augančią kultūrą logaritminės fazės pradžioje, $^{63}Ni^{2+}$ tuoj pat buvo paėmamas iš ląstelių ir po to lėtai ir uždelstai išmetamas; paėmimo lygis ir uždelstas išmetimas priklausė nuo naudotos $NiCl_2$ koncentracijos. Išmetimo uždelsimas atitiko pailgėjusio lag periodo trukmę (duomenys paimti iš [6]). *E. coli* abiejų kamienų – atsparaus V38 ir jautraus JM101 – ląstelių pauginus per naktį terpeje su nenuodinga $NiCl_2$ koncentracija ir suspendavus 0°C išmetimo terpeje su tokiu pačiu Ni kiekiu pridejus $^{63}Ni^{2+}$, buvo rastas Ni^{2+} kaupimasis abiejų kamienų ląstelėse. Perkibus ląsteles į 37°C, tik atsparios ląstelės išmetė Ni^{2+} , o jautriosios jo kiekis nesikeitė. Ni^{2+} -aktyvus išmetimas reikalauja energijos šaltinio, nes išmetimas buvo matomas tik esant gliukozei terpeje; silpnas išmetimas buvo matomas terpeje be gliukozės ir jo visai nebuvo tiriant negyvas ląsteles. Aptariamos atsparių bakterijų panaudojimo užteršto vandens valymui galimybės.

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ВЫБРОС ИОНОВ КАК ОСНОВА РЕЗИСТЕНТНОСТИ *Escherichia coli* V38 К НИКЕЛЮ

Резюме

Устойчивый к никелю штамм *Escherichia coli* V38 был выделен из ила городских сточных вод и мог расти при наличии 4 mM хлорида никеля. При добавлении к среде с культурой *E. coli* V38 в начале логарифмической фазы 1 mM или 2 mM $NaCl_2$ совместно с $^{63}Ni^{2+}$ ионы никеля были сразу же захвачены клетками и после этого постепенно и с задержкой выброшены. Степень захвата и задержка выброса зависели от концентрации хлорида никеля. Задержка выброса соответствовала продлению лаг-периода роста (данные взяты из [6]). При культивировании в течение 20 ч устойчивого штамма V38 и чувствительного штамма JM101 в среде с нетоксичной концентрацией хлорида никеля и при суспендировании при 0°C в среде выброса с такой же концентрацией никеля (и радиоактивного никеля) накопление никеля было отмечено в клетках обоих штаммов. При переносе клеток в среду с температурой 37°C только устойчивые клетки выбрасывали ионы никеля, в то время как в чувствительных количество никеля не изменялось. Для активного выброса никеля требуется источник энергии, так как выброс наблюдался только при наличии в среде глюкозы. Неживые клетки никель не выбрасывали. На основе полученных данных в статье дискутируется возможность использования устойчивых бактерий при очистке воды от металлов.