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Microbiology

Influence of Na on the Assimilation of Cr(VI), Zn and Cu by the Chromium-Resistant Bacterium *Arthrobacter globiformis* 151B

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ABSTRACT. Here we report the effect of Na ions on the assimilation of Cr(VI), Zn and Cu by the chromium-resistant *Arthrobacter globiformis* 151B. *A. globiformis* 151B cells are known for their ability to assimilate the soluble hexavalent chromium [Cr(VI)] ions from the environment, convert them into the insoluble trivalent form [Cr(III)] which are accumulated inside cells. Thanks to these properties, it is possible to use them to detoxify environment polluted by the highly toxic and mobile Cr(VI). The strain of bacteria under investigation was isolated from basalt samples taken from the sites highly contaminated by Cr(VI) in Kazreti. In this study, solutions of the elements (Cr, Zn and Cu) and of Na were introduced simultaneously into the nutrient media. We measured the influence of Na ions at different concentrations on the assimilation of Cr, Zn and Cu by *A. globiformis* 151B during 17, 24, 48, 96 and 144 hours. The concentration of Na examined was 2 mg/ml, 3.5 mg/ml, 6.5 mg/ml and 9.5 mg/ml. The concentrations of Cr, Zn and Cu accumulated in the cells was measured by atom-absorption spectrometry. *© 2019 Bull. Georg. Natl. Acad. Sci.*

Key words: bacteria (*Arthrobacter globiformis* 151B), biomass, metals, concentration

Heavy metals at high concentrations can be toxic and carcinogenic. It is very important to develop the technologies to remove or reduce the toxicity of heavy metals from the environment. Among the most prospective methods of remediation of polluted environment are the biological technologies based on the use of different microorganisms [1, 2].

Environment polluted by Cr(VI) and other heavy metal compounds is an urgent environmental problem for many countries [3]. In Georgia, many of the heavily contaminated sites are in Kazreti and Zestaphoni [4], where the concentration of Cr in the soil and in water reaches several hundreds of mg/l (the permissible concentration of Cr(VI) in surface waters is less than 0.05 mg/l [3].

Chromium can be extremely toxic or nontoxic depending on its concentration and valence state [5]. In nature usually it exists in trivalent [Cr(III)] or hexavalent forms which have different transport properties. Cr(VI)-compounds are water-soluble, highly mobile and toxic, while Cr(III)-compounds are less water-soluble, less mobile, and therefore less toxic. The genotoxic and carcinogenic action of Cr(VI)-contained material is caused by their ability to penetrate rapidly into cell, as well as by activation of this ability as a result of the intercellular reduction process [6].

Detoxification of Cr(VI) compounds in the environment can be made by reducing Cr(VI) into its Cr(III) form, and as it is known, Cr(III) precipitates, mainly, in the form of $Cr(OH)_3$ or makes a complex with surrounding ligands [7].Various bacterial species (e.g. *Escherichia*, *Pseudomonas*, *Shewanella*, *Desulfovibrio*, *Bacillus* sp.) [8] are able to reduce Cr(VI). However, many are not metal resistant/tolerant. They loss their viability in high concentration of heavy metals. Thus, it is reasonable to isolate endogenous bacteria directly from soil, mineral strata and water contaminated by metals [9-15]. At present, evaluations of bioremediation technologies based on endogenic microorganisms are carried out extensively in many countries [16-18], providing that recently the application of biotechnologies is of high priority in the process of environment reduction in many countries [19]. The efficiency of biotransformation depends on the mechanism of bacteria-metal interaction, thus, for bacteria of any specific species it is necessary to study preliminarily this mechanism in detail.

A majority of the bioremediation research focuses on the applications of gram-negative bacteria; very few concern gram-positive bacteria. Recently it is reported that gram-positive bacteria appeared to be tolerant to higher doses of Cr(VI), as compared to gram-negative bacteria [11]. However, few shed light on the reaction of bacteria to high doses of chromium. The mechanism of origination of chemically active intermediate (Cr(V)/Cr(VI)) products in the process of reducing Cr(VI) by bacteria, practically, is not studied, except for the pioneer researches carried out by Georgian investigators (N. Tsibakhashvili, etc.) [20].

The natural vital medium of bacteria we are interested in, alongside with the elements under investigation (Cr, Zn and Cu), contains the elements (macroelements) that are widely spread in the nature (Na, K, Si …). These elements have an influence on the growth – evolution of bacteria, including the process of assimilation of elements (Cr, Zn and Cu) by bacteria and the biochemical process proceeding in bacteria. It is interesting to study the influence of macroelements on the process of assimilation and distribution of Cr(VI), Zn and Cu in bacteria. Our results presented here make it possible to draw a certain conclusion about the biochemical processes taking place in bacteria, about the mechanisms by which the assimilation of metals, and about the conversion of their compounds are made.

Materials and Methods

We chose the bacteria of *Arthrobacter globiformis* 151B as our model system. As is known [21], the bacteria of Arthrobacter family are aerobic gram-positive bacteria living in soil. They belong to Arthrobacteria class, type– Actinomycetales. Among the reductive bacteria, the interest to the bacteria of this family is great as, according to the existing data [22, 23] they have a high potential of remediation of chromium-contaminated environment. The Georgian investigators studied the distribution of Cr(VI) – resistant microorganisms in basalt rocks taken from ecologically the most contaminated regions of Georgia (Kazreti, Zestaphoni) [24]. From the chosen basalts 157 endolythic bacteria resistant to Cr(VI) were singled out, among which 33 appeared to have the ability to remediate high concentrations of Cr(VI) (about 1000 mg/l). The object of investigation is bacterial strains isolated from Kazreti basalts.

For studying the influence of Na (widely spread in the nature element) on the process of assimilation of Cr(VI), Cu, Zn and other elements by *Arthrobacter globiformis* 151B, we cultivated

bacteria in 500 ml Erlenmeyer flasks in 100 ml TSB broth. We additionally introduced Na solution in the form of NaCl into some samples (flasks); thus the concentration of Na in the nutrient medium was 2 mg/ml, 3.5 mg/ml, 6.5 mg/ml and 9.5 mg/ml. Into the same samples we additionally introduced Cr (VI) solution and thus, the concentration of Cr(VI) in the nutrient medium did not contain the studied elements of the following concentration: Cr – 7 mkg/ml, Zn – 1 mkg/ml, Cu – 0.06 mkg/ml.

The cultivation of bacteria proceeded during 17, 24, 48, 96 and 144 hours. After cultivation we carried out the precipitation by centrifugation (3000 rpm, 10 min., 0^0C), we discarded supernatants while washed the remained bacterial pellet in sterile distillated water. Bacterial pellet (precipitation) contains organelles, membranes. We dried the biomasses by means of low-temperature lyophilizer and weighted them (the whole masses). From the total quantity of bacterial pellet we took the amount necessary for analyses, weighted it (~30 mg) and put it into test tubes. In order to convert the samples into a liquid state, we added the concentrated nitric acid (1 ml) into the test tubes, heated it and after a complete ashing dissolved it by bidistillate to a certain volume. The analysis of the obtained samples on the content of metals was made by atom-absorption spectrometer (Analyst 800) (acetylene –air flame). We studied the process of assimilation of Cr(VI), Cu and Zn by bacteria and the influence of Na ions of this process.

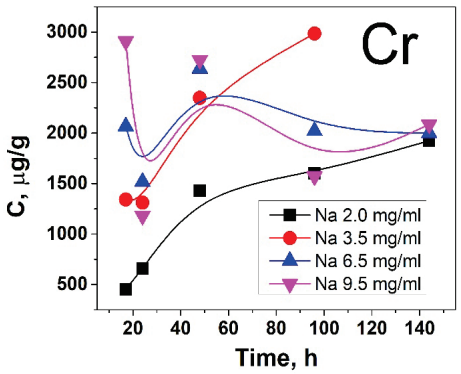

Fig. 1. Dependence of Cr concentration (C – mkg/gr) in bacteria on the time of growth – evolution of bacteria T(h). Concentration of Na in nutrient medium was 2 mg/ml, 3.5 mg/ml, 6.5 mg/ml and 9.5 mg/ml.

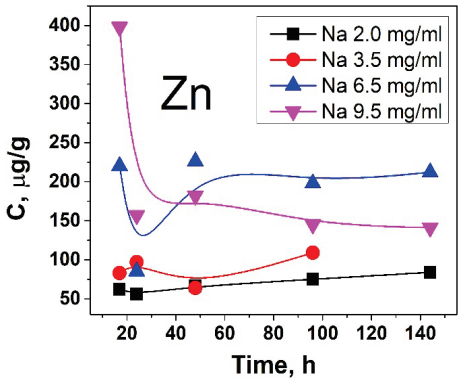

Fig.2. Dependence of Zn concentration in bacteria (C – mkg/gr) on the time of growth – evolution of bacteria T(h).

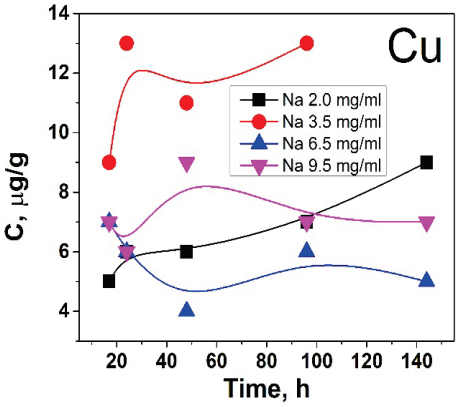

Fig.3. Dependence of Cu concentration (C) in bacteria on the time of growth - evolution of bacteria T(h).

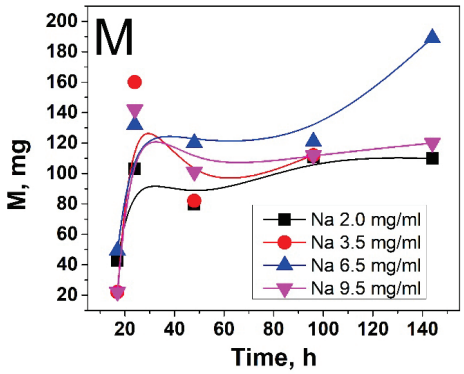

Fig.4. Dependence of bacteria masses M(mg) on the time of growth - evolution of bacteria T(h).

Results and Discussion

As shown in Fig.1 Cr (40 mkg/ml) added into the nutrient medium causes an abrupt increase of the content of Cr accumulated by bacteria during 4 days of their growth and exposure to Cr. In the nutrient medium with 7 mkg/ml and 40 mkg/ml contents of Cr, on the 6-th day of cultivation an equalization of Cr content takes place. In the samples taken after 17-hour cultivation, together with the increase of the concentration of added Na the content of Cr is increasing as well. In the medium containing Na, after 24-hour cultivation the content of Cr in bacteria decreases and makes about one and the same value. The decrease of Cr content coincides with the increase of bacteria biomass (Fig. 4). In bacteria grown during 2 days the increase of Cr is observed, coinciding with the decrease of bacteria biomass.

In the samples taken after 17-hour cultivation of bacteria, the content of Zn increases with the increase of Na concentration added into the nutrient medium. After 24-hour cultivation the content of Zn in bacterial cells decreased as compared to the 17-hour bacterial culture and its value is higher for the bacteria incubated in the medium containing Na. In 48-hour bacterial cells the increase of Zn content is observed with the increase of the content of Na (6.5 mg/ml and 9.5 mg/ml) in the nutrient medium, coinciding with the decrease of bacterial biomass. The contents of Zn in bacteria do not much differ from each other when the concentration of Na in the medium is 3.5 mg/ml and 2.0 mg/ml. In the medium containing Na, in case of 48-hour cultivation of bacteria, the content of Zn is more (Fig. 2).

In the nutrient medium containing Na, after 17-hour cultivation the content of Cu increases in the bacterial cells, but in 24-hour cultures it decreases (Fig.3).

As it is seen from the results obtained (Fig.4), bacteria evolves rapidly during 17-24 hours, besides, Na added into nutrient medium favors the growth-development of bacteria. After 48-hour cultivation, in case of existence of different concentrations of Na in the nutrient medium, the bacterial biomass is decreased. For further period (4 and 6 days) a slow increase of the bacterial biomass is observed. It can be said that Na added into the nutrient medium slightly favors the growth of bacteria during the whole period of its growth-evolution.

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მიკრობიოლოგია

Na-ის გავლენა Cr(VI), Zn და Cu-ის შეთვისების პროცესზე Arthrobacter globiformis 151B – ქრომრეზისტენტული ბაქტერიების მიერ

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მოცემულ შრომაში შესწავლილ იქნა Cr(VI), Zn და Cu-ის შეთვისების პროცესი ქრომრეზისტენტული ბაქტერიების მიერ (*Arthrobacter globiformis* 151B) და ამ პროცესებზე მაღალი კონცენტრაციის Na-ის იონების გავლენა. ბაქტერიები ცნობილია იმით, რომ გარემოდან ინტენსიურად ითვისებენ ექვსვალენტიანი ქრომის [Cr(VI)] იონებს, გარდაქმნიან მათ სამვალენტიან ფორმაში [Cr(III)] და ახდენენ მის აკუმულაციას უჯრედში. ბაქტერიების ამ თვისების გამო შესაძლებელია მათი გამოყენება მაღალტოქსიკური Cr(VI)-ით დაჭუჭყიანებული გარემოს დეტოქსიკაციისათვის. გამოსაკვლევი ბაქტერიების შტამი გამოყოფილ იქნა ბაზალტის ნიმუშებიდან, რომლებიც აღებული იყო კაზრეთის, Cr(VI)-ით ძლიერ დაჭუჭყიანებული ადგილებიდან. საკვები არე შეიცავდა საკვლევ ელემენტებს შემდეგი კონცენტრაციით: Cr – 7მკგ/მლ, Zn – 1მკგ/მლ, Cu – 0,06მკგ/მლ. შესწავლილ იქნა ბაქტერიების მიერ Cr, Zn და Cu-ის შეთვისების პროცესზე Na-ის იონების სხვადასხვა კონცენტრაციის გავლენა, ბაქტერიის კულტივირების სხვადასხვა დროის განმავლობაში (17 სთ, 24 სთ, 2, 4 და 6 დღე-ღამე). საკვებ გარემოში Na-ის კონცენტრაცია შეადგენდა 2 მგ/მლ, 3,5 მგ/მლ, 6,5 მგ/მლ და 9,5 მგ/მლ. უჯრედში მეტალების შემცველობის (Cr, Zn და Cu) განსაზღვრის მიზნით, ბაქტერიის კულტივირების შემდეგ მოხდა უჯრედების დალექვა ცენტრიფუგირებით და მიღებული ბაქტერიული ნალექის მომზადება ანალიზისთვის. მეტალების შემცველობა გაზომილ იქნა ატომურ-აბსორბციული სპექტრომეტრის დახმარებით.

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