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Zn and Cr (VI) Uptake by Bacteria *Arthrobacter globiformis* 151B from the Potassium-Rich Nutrient Medium

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ABSTRACT. ***Arthrobacter globiformis* 151B is a chromium-resistant bacterium having the ability to maintain viability in the habitat of a large number of Cr(VI) ions. *Arthrobacter globiformis* 151B bacteria can absorb hexavalent chromium [Cr(VI)] ions from the environment, convert them into trivalent form [Cr(III)] and accumulate them. Because of such properties of *Arthrobacter globiformis* 151B bacteria, they can be used for detoxification of the environment polluted by highly toxic Cr(VI). *Arthrobacter globiformis* 151B bacteria can absorb the ions of different metals from the nutrient medium. Macroelements (K, Na, Ca, Si) of the nutrient medium can affect the absorption of metal ions. The process of Cr(VI) and Zn assimilation by chromium-resistant bacteria (*Arthrobacter globiformis* 151B) and the influence of high concentration of K ions on the mentioned process are studied in the paper. The strain of the bacteria under investigation was isolated from basalt samples taken from the sites highly contaminated with Cr (VI) in Kazreti. Introducing the solutions of Cr, Zn and K into the nutrient medium, we studied the influence of different concentrations of K-ions on the process of Cr and Zn assimilation by bacteria in different cultivation periods of bacteria (17 h, 24 h, 48h, 96h, 144h). The concentration of K added to nutrient medium was 0.6 mg/ml, 2.0 mg/ml and 5.0 mg/ml. After cultivation of the bacteria the precipitation of cells was carried out by centrifuging and the obtained bacterial pellet was prepared for determining the content of metals (Cr and Zn) in the cell by the use of atom-absorption spectrometry.** *© 2019 Bull. Georg. Natl. Acad. Sci.*

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

Metals at the excess concentration have toxic and carcinogenic properties. Besides Cr, Zn and Cu, the natural medium of bacteria under study contains the elements (macroelements) widely spread in the nature (Na, K, Si, etc). Those elements have an influence on the growth of bacteria, including Cr and Zn assimilation and the biochemical processes proceeding in the bacteria.

Potassium and sodium can loosen the cell membranes making them more permeable for salts.

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The environment pollution by the materials containing Cr(VI) is a topical problem for many countries [1]. One of the most prospective methods for remediation of polluted environment is the biotechnological method based on the use of different microorganisms [2].

Chromium can be extremely toxic or nontoxic depending on its concentration and valence state [3]. In nature usually it is met in trivalent [Cr(III)] and hexavalent forms, which have different transport properties. Cr (VI) compounds are toxic and well soluble in water, while Cr (III) compounds are relatively harmless and less soluble in water. Detoxification of Cr(VI) in the environment can be carried out by its conversion into trivalent form. As is known, trivalent chromium precipitates, mainly, in the form of $Cr(OH)_3$ or makes a complex with surrounding ligands [4]. The recent researches proved that many of the well-studied bacterial species are not metal resistant. They lose viability in coexistence with high concentration of heavy metals. Thus, it is reasonable to isolate the bacteria under investigation directly from the soil, mineral strata and water contaminated by means of metals [5-7]. The use of biotechnology is of paramount importance in the process of environmental restoration in many countries [8]. 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 the mentioned mechanism in detail.

It is to study the influence of macroelement – Potassium (K) on the process of assimilation and distribution of Cr(VI) and Zn in bacteria. In living organisms, potassium is especially important for cell nutrition, exchange reactions and water-salt balance. Potassium has the ability to loosen cell walls, making them more permeable for salts. K ions are important activators of enzymes inside the cell.

The experimental material, obtained as a result of the proposed investigation, makes it possible to draw a certain conclusion about the biochemical processes taking place in bacteria and about the mechanisms by means of which the assimilation of metals and the conversion of their compounds occurs.

Materials and Methods

The object of investigation is *Arthrobcter globiformis* 151B bacteria. As is known, the bacteria of Arthrobacter family are aerobic gram-positive bacteria living in soil [9]. 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 [10, 11], they have a high potential of remediation of chromium-contaminated environment. Georgian investigators studied the distribution of Cr(VI)-resistant microorganisms in basalt rocks, taken from ecologically contaminated regions of Georgia (Kazreti, Zestaphony) [12]. The object of investigation are the bacterial strains isolated from Kazreti basalts.

For studying the influence of K on the process of assimilation of Cr(VI), Zn and other elements by *Arthrobcter globiformis* 151B, we cultivated bacteria in 500 ml Erlenmaier flasks in 100 ml TSB broth. In addition, we introduced K solution in the form of KCl into some samples (flasks). The concentration of K, added to the nutrient medium, was 0.6 mg/ml, 2.0 mg/ml and 5.0 mg/ml K. In five samples, we additionally introduced a solution of Cr (VI), the final concentration of which in the nutrient medium was 40 μg/ml.

The nutrient medium (itself) also contained elements in the following concentrations: K- 0.6 mg/ml, Cr - 7 μg/ml, Zn - 1 μg/ml. Thus, in those 5 samples, the total concentration of Cr in the nutrient medium was 47 μg/ml. The cultivation of bacteria proceeded during 17 h, 24 h, 48 h, 96 h and 144 h. After cultivation we carried out precipitation by centrifuging (3000 rpm, 10 min., 0^0C). The supernatants were poured out and the remained bacterial pellet was washed in sterile distillated water. We dried the obtained biomasses by 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 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 Cr(VI) and Zn assimilation by bacteria and the influence of K ions of this process.

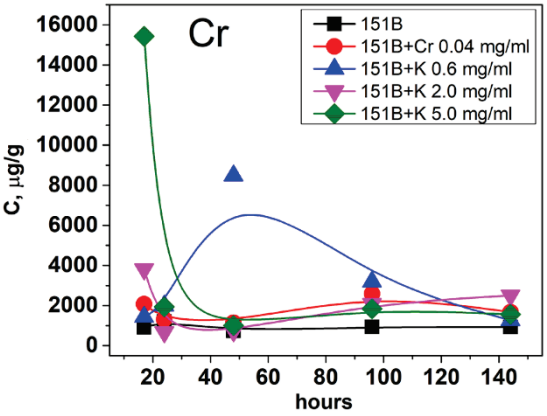

Fig.1. Dependence of Cr concentration (C – µg/g) in bacteria on the time of growth of bacteria T(h). Concentration of K, added in nutrient medium, was 0.6 mg/ml, 2.0 mg/ml and 5.0 mg/ml.

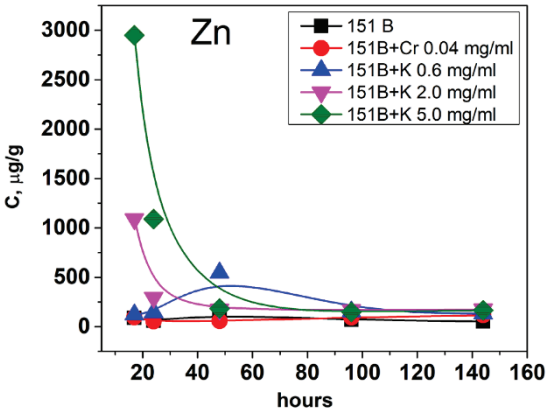

Fig.2. Dependence of Zn concentration in bacteria (C – µg/g) on the time of growth of bacteria T(h). Concentration of K, added in nutrient medium, was 0.6 mg/ml, 2.0 mg/ml and 5.0 mg/ml.

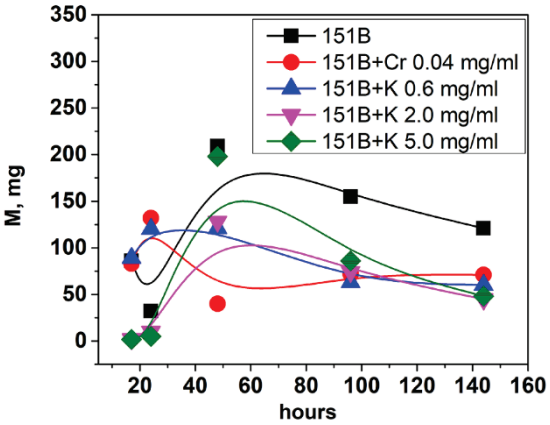

Fig.3. Dependence of bacteria masses M(mg) on the growth of bacteria T(h). Concentration of K in nutrient medium: 0.6 mg/ml, 2.0 mg/ml and 5.0 mg/ml.

Results and Discussion

During the 17-hour cultivation of bacteria, the concentration of Cr was the same (≈1000 µg/g) in the bacteria of the nutrient medium added by 0.6 mg/ml solution of K and in the bacteria of the nutrient medium without K. In the bacteria of the nutrient medium added by 0.6 mg/ml solution of K, the concentration level of Cr increased in 48 hours and then started decrease (96h, or 144h). When the nutrient medium was added by 2.0 mg/ml solution of K, then Cr content was 4 times more and when the nutrient medium was added by 5.0 mg/ml solution of K, then Cr content increased up to 15000 µg/g. It means that the high concentration of K helps the intensive absorption of Cr ions in bacteria. The more K is added to the nutrient medium, the more the concentration of Cr is in bacteria (Fig.1).

During 24-hour cultivation of bacteria, Cr ions were not intensively absorbed in bacteria. After adding K-solution to the nutrient medium in concentration of 0 mg/ml, 0.6 mg/ml, 2.0 mg/ml and 5.0 mg/ml, the concentration of Cr was (approximately) the same in bacteria. When the nutrient medium was added by 0.6 mg/ml of K, the concentration of Cr increased reaching its maximum (8000 µg/g) on the 2nd day. Then the concentration of Cr decreased and in 144-hour cultivation it became the same as in case of other concentrations of K.

In the next period of cultivation the concentration of Cr increased slowly to the end of the experiment except for the bacteria of the nutrient medium added by 0.6 mg/ml K solution.

If K was not added to the nutrient medium, the concentration of Cr practically did not change during the whole period of bacteria cultivation and it was ≈1 mg/g.

As for the concentration of Zn, its concentration was the same in17-hour, 24-hour, 96-hour and 144-hour cultivations (Fig.2) if K was not added to the nutrient medium or 0.6 mg/ml was added.

During 48-hour cultivation, a slight increase of Zn concentration was observed when 0.6 mg/ml K solution was added to the nutrient medium. During 17-hour cultivation, a fast and intensive absorption of Zn was observed when 2.0 mg/ml or 5.0 mg/ml K solution was added to the nutrient medium.

During 17-hour cultivation, when the concentration of K added to the nutrient medium was 5.0 mg/ml, the level of Zn content in the bacteria was 3000 μg/g. The results obtained show that the addition of different concentrations of K helps fast and intensive absorption of Zn ions in bacteria in case of 17-hour and 24-hour cultivations. Besides, the higher the concentration of K in the environment, the higher the concentration of Zn in bacteria. The same is observed in case of Cr.

In case of 96-hour and 144-hour cultivations, the concentration of Zn in bacteria was practically the same when different concentrations of K (0 mg/ml, 0.6 mg/ml, 2.0 mg/ml and 5.0 mg/ml) was added to the medium.

According to the results obtained, it can be concluded that the more K is added to the nutrient medium, the better the Zn and Cr ions are absorbed into bacterial cells. Later the bacteria get used to the new (stressful) conditions and start regulation of ions.

As for the bacteria masses (Fig. 3) the high concentrations of K (2.0 mg/ml and 5.0 mg/ml) avert the growth of bacteria (17-hour and 24-hour cultivations). The bacteria get used to the stress conditions (adaptation) and after that they start growing fast (48-hour cultivation). Then their masses decrease (96- or 144-hour cultivation), but their masses are greater than those of 17-hour and 24-hour cultivations.

During the first 17 hour of cultivation the growth of the bacteria was very intensive (0 and 0.6mg/ml K was added to the medium). In 24-hour cultivation bacterial masses decreased only in case the nutrient medium was not added by K solution. Then the bacteria started growing fast (48-hour cultivation) and then their masses decreased slowly (96, 144 hours), but their masses were greater than those of the bacteria in the nutrient medium added by K solution. The mass increased sharply (maximum) and then the mass decreased slowly. In that period of time (48, 96 or 144 hours) the bacteria mass was more in case of applying other concentrations of K.

In case of addition 0.6 mg/ml K the speed of bacteria growth was the same as in case when K was not added to the nutrient medium (17-hour cultivation). Addition of 0.6 mg/ml solution of K caused the increase of the masses in 24-hour cultivation (in opposite to the case when K solution was not added to the nutrient medium). In 48-hour cultivation the bacteria masses remained the same and then they decreased but not so sharply. After that the masses decreased (96-hour and144-hour cultivations) but they were less, than those of the bacteria from the medium, where K solution was not added. In case of 144-hour cultivation the bacterial masses were the same, except the case when K solution was not added to the medium. In that case the bacteria masses were greater.

The work is dedicated to the memory of Mrs. Nelly Tsibakhashvili.

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მიკრობიოლოგია

Cr(VI) და Zn-ის შეთვისება კალიუმით გამდიდრებული საკვები არიდან *Arthrobacter globiformis* 151B მიერ

ა. რჩეულიშვილი*, ე. გინტური*, ლ. ტუღუში*, მ. გურიელიძე**, ჰ. ჰოლმანი§

**ბიოლოგიური სისტემების ფიზიკის განყოფილება, ივანე ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტის ელეფთერ ანდრონიკაშვილის ფიზიკის ინსტიტუტი; თბილისი, საქართველო*
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მოცემულ შრომაში შესწავლილ იქნა Cr(VI) და Zn-ის შეთვისების პროცესი ქრომრეზისტენტული ბაქტერიების მიერ (Arthrobacter globiformis 151B) და ამ პროცესებზე მაღალი კონცენტრაციის K-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: K – 0.6 მგ/მლ, Cr – 7 მკგ/მლ, Zn - 1 მკგ/მლ. შესწავლილ იქნა ბაქტერიების მიერ Cr და Zn-ის შეთვისების პროცესზე K-ის იონების სხვადასხვა კონცენტრაციის გავლენა, ბაქტერიის კულტივირების სხვადასხვა დროის განმავლობაში (17, 24, 48, 96 და 144 სთ). საკვებ გარემოში დამატებული K-ის კონცენტრაცია შეადგენდა 0.6 მგ/მლ, 2.0 მგ/მლ და 5.0 მგ/მლ. უჯრედში მეტალების შემცველობის (Cr, Zn) 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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