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Influence of Ca on the assimilation of Cr and Zn by the chromium resistant bacterium *ARTHROBACTER GLOBIFORMIS* 151B

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ABSTRACT

The process of assimilation of Cr(VI) and Zn by chromium-resistant bacteria (Arthrobacter globiformis 151B) and the influence of high-concentration **Ca** ions on this process have been studied in the article. The bacteria are known for their property to assimilate intensively the hexavalent chromium [Cr(VI)] ions from the environment, to convert them into trivalent form [Cr(III)] and to accumulate it in cell. Thanks to these properties, it is possible to use them for detoxification of the environment polluted by highly toxic Cr(VI). The strain of bacteria under investigation was isolated from basalt samples, taken from the places highly contaminated by Cr(VI) in Kazreti. The solutions of the studied elements (Cr and Zn) and of **Ca** were introduced simultaneously into the nutrient medium. We studied the influence of different concentrations of **Ca** ions during different period of time of bacteria cultivation (17h, 24h, 48h, 96h, 144h) on the process of assimilation of Cr and Zn by bacteria. Ca concentration in nutrient medium made up 100 mcg/ml, 400 mcg/ml and 1600 mcg/ml.For determination of the content of Cr and Zn in the cell, after the cultivation of bacteria the precipitation of cells by centrifuge and the preparation of the obtained bacterial pellet for the analysis were carried out. The content of metals was measured by atom-absorption spectrometry.

Keywords: Bacteria (*Arthrobacter globiformis* 151B), Biomass, Metals, Concentration, Cultivation, Detoxification.

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Introduction

Metals at the excess concentrations have toxic and carcinogenic properties. It is very important to develop the technologies by means of which it is possible to remove the toxic 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].

The pollution of the environment by the materials containing Cr(VI) is an urgent problem for many countries [3]. Chromium can be extremely toxic or nontoxic depending on its concentration and valence state [4]. In nature usually it is met in trivalent [Cr(III)] and hexavalent forms which have different transport properties. Cr(VI)-contained materials are well water-soluble and toxic compounds, while Cr(III)-contained materials are less water-soluble and relatively harmless. 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 [5].

Detoxification of Cr(VI), that appeared in the environment, can be made by its conversion into trivalent form, and as it is known, trivalent chromium precipitates, mainly, in the form of $Cr(OH)_3$ or makes

a complex with surrounding ligands [6]. The recent researches proved that Many of the well-studied bacterial spaces, Are not metal resistant/tolerant. They loss their viability in co-existence of high concentration of heavy metals. Thus, it is reasonable to isolate the bacteria under investigation directly from soil, mineral strata and water contaminated by metals [7-11]. At present, the testing of technologies based on endogenic microorganisms is carried out intensively in many countries [12-14], providing that recently the application of biotechnologies is of high priority in the process of environment reduction in many countries [15]. 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.

The natural vital medium of bacteria we are interested in, contains, alongside with the elements under investigation (Cr and Zn) as well the elements (macroelements) that are widely spread in the nature (Na, K, Si, Ca). These elements have an influence on the growth – evolution of bacteria, including the process of assimilation of elements Cr and Zn by bacteria and the biochemical process proceeding in bacteria.

It is interesting to study the influence of Caon the process of assimilation and distribution of Cr, and Zn in bacteria. Ca ions are important activators of enzymes inside the cell.

Calcium is the key intracellular metabolic regulator. Ion Ca^{2+} functions as the most important intracellular factor (secondary mediator) controlling the processes of control of cell functions.Calcium is also important for the functioning of cell membranes.

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 which the assimilation of metals and the conversion of their compounds are made.

Objectives and Methods

For the object of investigation we chose the bacteria of *Arthrobcterglobiformis* 151B. As is known, the genus *Arthrobacter* is aerobic, gram-positive bacteria, living in the soil. *Arthrobacter* belong to the class *Actinobacteria*, order – *Actinomycetales*. Among the reductive bacteria, the interest to the bacteria of this genus is great as, according to the existing data [16, 17] 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, Zestaphony) [18]. The object of investigation is bacterial strains isolated from Kazreti basalts.

For studying the influence of **Ca** on the process of assimilation of Cr(VI) and Zn by Arthrobcterglobiformis 151B, we cultivated bacteria in 500 ml Erlenmaier flasks in 100 ml TSB liquid medium. We additionally introduced Ca solution in the form of $CaCl_2$ into some samples (flasks), thus, the concentration of Ca, added in the nutrient medium, was100 mcg/ml, 400 mcg/ml and 1600 mcg/ml.

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 also (itself) contained elements of the following concentrations: **Na-3.5mg/ml, K-0.6mg/ml, Ca-25μg/ml,Cr - 7 μg/ml, Zn - 1 μg/ml.**Thus, in these 5 samples, the total concentration of Cr in the nutrient medium was 47 μg/ml. The cultivation of bacteria proceeded during 17h, 24h, 48h, 96h and 144h. After cultivation we carried out the precipitation by centrifuge (3000 rpm, 10 min., 0^0C), we poured out supernatants and the remained bacterial pellet 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 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) and Zn by bacteria and the influence of Ca ions of this process.

Results and Discussion

The results of measurement are given in Fig. 1, 2 and 3.

The content of Cr in bacteria, after 144 hours of cultivation is the same for all concentrations of Ca, added to the nutrient medium.

The content of Cr in bacteria does not undergo significant changes during the entire period of bacteria cultivation, when 0 mg/ml and 1.6 mg/ml Ca are added to the nutrient medium.

Adding 0.4 mg/ml Ca to the nutrient medium increases the penetration of Cr in bacteria within 17 hours of cultivation.

After 17 hours of cultivation, Cr is gradually removed from bacteria. After 144 hours, the Cr content becomes equal when the Ca content in the nutrient medium was 0%/ml, 0.1 mg/ml and 1.6 mg/ml. When the Ca content in food is 0.1 mg/ml, there is a tendency for the Cr content in bacteria to increase at the beginning of cultivation.

The addition of Ca in the nutrient medium causes an increase in the zinc content of the bacteria during the entire cultivation period. For various concentrations of Ca added in the nutrient medium, the highest Zn content in bacteria is observed under 17-hour cultivation with the addition of up to 0.4 mg / ml Ca in the nutrient medium. For this Ca concentration in the food environment, in the next period of cultivation, the concentration of Zn in bacteria gradually decreases. When the concentration of Ca in the medium is 1.6 mg/ml (maximum), we have the maximum concentration of Zn in bacteria after 24 hours of cultivation. During the period after cultivation, the concentration of Zn in bacteria gradually decreases. After 144 hours of cultivation, Zn concentrations are equal in those bacteria that grow in the nutrient medium with Ca. When we did not add Ca in the medium, the concentration of Zn in bacteria is 2 times less.

The addition of Ca in the nutrient medium leads to a sharp decrease in the bacterial mass. The exception is when the Ca concentration in the nutrient medium is 1.6 mg/ml.

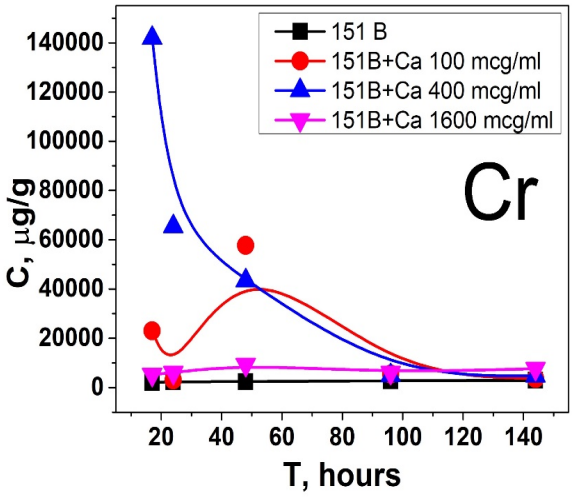

Fig 1. *The effect of Ca on the process of uptake Cr by bacteria (Arthrobacter globiformis 151B). T(hours)- The growth time of bacteria. The Ca concentration in the nutrient medium is 100 mcg/ml, 400 mcg/ml and 1600 mcg/ml.*

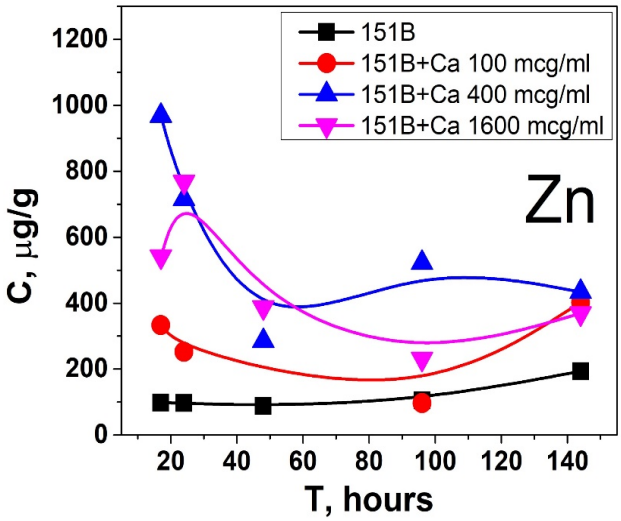

Fig 2. *The effect of Ca on the process of uptake Zn by bacteria (Arthrobacter globiformis 151B). T(hours)- The growth time of bacteria. The Ca concentration in the nutrient medium is 100 mcg/ml, 400 mcg/ml and 1600 mcg/ml*

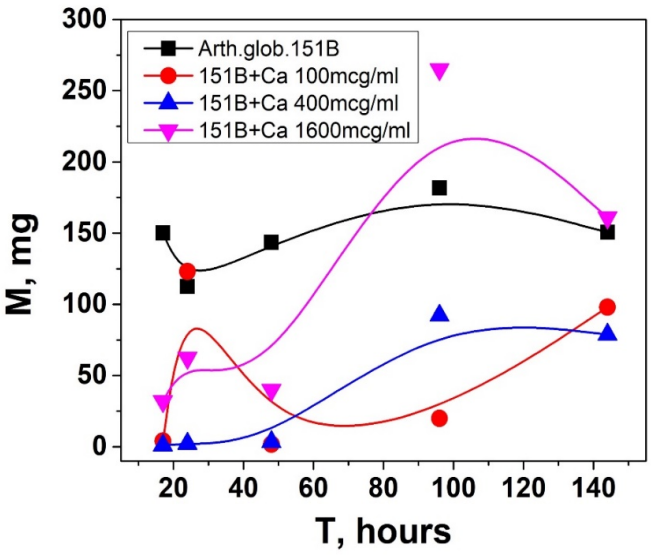

Fig 3. *The Ca concentration in the nutrient medium is 100 mcg/ml, 400 mcg/ml and 1600 mcg/ml.*

Conclusion

The addition of Ca in the nutrient medium increases the content of Cr in bacteria in the initial stage of cultivation, when the concentration of Ca in the nutrient medium is 100 and 400 µg/g. After cultivation, the Cr content in bacteria is almost the same for different concentrations of Ca in the environment.

The addition of Ca promotes the penetration of Zn into bacteria at various concentrations of Ca in the environment. At various concentrations of Ca

in the environment, the content of Zn in bacteria is higher during the entire cultivation period.

The addition of Ca in the nutrient medium slows the growth of bacteria, at various concentrations of Ca. An exception is the case when 1.6 mg/ml Ca is added to the nutrient medium. The influence of Ca is pronounced at the initial stage of the cultivation of bacteria. In the next cultivation period, the bacterial biomass increases and approaches the biomass value when bacteria are cultured without addition of Ca.

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