THE EFFECT OF SI-IONS ON THE UPTAKE PROCESS OF ZINC AND CHROMIUM BY ARTHROBACTER GLOBIFORMIS 151B

A. Rcheulishvili[1], L. Tugushi[1], E. Ginturi[1], O. Rcheulishvili*[1,2], N. Metreveli[2], M. Gurielidze[3], H.-Y. Holman[4]

Abstract

The main focus of this study was to investigate the uptake of Zn and Cr by chromium-resistant bacteria, as well as the effects of varying concentrations of Si ions on these processes. The research objective *Arthrobacter globiformis* 151B was isolated from basalt samples collected from the area of Kazreti, Georgia, heavily contaminated with Cr(VI). The growth medium contained the research elements with the following concentration: Zn -1 μg/mL, Cr - 7 μg/mL. Bacteria were cultivated for 17, 24, 48, 96, and 144 hours. The concentration of Si ions in the growth medium made up 50, 200, and 800 μg/mL. After the cultivation of bacteria, the cells were sedimented by centrifugation. A bacterial pellet was prepared to measure concentrations of metals (zinc and chromium) using an atomic absorption spectrometer.

Keywords: *Arthrobacter globiformis* 151B, silicium (Si), chromium (Cr), zinc (Zn), atomic absorption spectrometry.

[1] Javakhishvili Tbilisi State University, E. Andronikasvili Institute of Physics, Department of Physics of Biological Systems, Tbilisi, Georgia
[2] Ilia State University, Institute of Biophysics, Tbilisi, Georgia
[3] Agricultural University, S. Durmishidze Institute of Biochemistry & Biotechnology, Laboratory of Nitrogen Fixation & Assimilation Tbilisi, Georgia
[4] University of California, Lawrence Berkeley National Laboratory, Berkeley, California, USA

***Corresponding Authors:** E-Mail: olia.rcheulishvili.1@iliauni.edu.ge

DOI: 10.53555/ecb/2023.12.Si13.211

Introduction

In nature, there are species of metal-resistant bacteria, which have the ability to exist in conditions of high concentrations of heavy metals [1, 2]. *Arthrobacter oxydans*, *Arthrobacter globiformis* 151B, *Arthrobacter sp*. 61B are aerobic bacteria of the *Arthrobacter* genus, they can intensively absorb hexavalent chromium [Cr(VI)] ions from the environment, convert them into the three valence form [Cr(III)], and accumulate it in the cell [3, 4]. Due to this property of bacteria, it is possible to use them for detoxification of an environment polluted with highly toxic Cr(VI).

Silicon (Si) is the eighth most common element in the universe, but it is rarely found in its free form - it is more common in its compounds as silicon dioxide, or silicates, in sands, quartz, quartzites, and flints. Silicon is the second most abundant element in the Earth's crust after oxygen, accounting for 27.7% of the Earth's crust mass.[5, 6] Almost all plants contain it. It is necessary for the elasticity of epithelium and connective tissue, skin, walls of blood vessels, and tendons [7, 8]. Silicon deficiency contributes to the development of atherosclerosis. In a body infected with parasites, the supply of silicon disappears very quickly, due to which it is difficult to heal the wound, the appetite decreases, the blood vessels begin to burst, and the skin itches [8].

Chromium causes various levels of damage to the structure of biopolymers. Cr(VI) undergoes a transformation, during which it forms active intermediate products - Cr(V) and/or Cr(IV), free radicals, and Cr(III) as a final product. Cr(III) is considered less toxic than Cr(VI), although its cationic complexes interact with the negatively charged phosphate group of DNA, which affects DNA replication, and transcription, and causes mutagenesis. Cr(III) also binds to thiol and carboxyl groups of enzymes and causes their structural and functional changes [9, 10, 11].

Zinc, which is a trace element, is found in the form of a divalent cation - Zn(II). Although it is present in small amounts in living organisms, it plays a very important role in vital processes [12]. Zn(II) is associated with the optimal functioning of the living organism's immune system, the functioning of the brain, the nervous system, and the normal condition of hair, skin, bones, and nails. However, zinc is quite toxic at high concentrations; However, the exact molecular mechanisms determining its toxicity are still poorly understood [13, 14]. Studies have shown that Zn(II) ions increase the potential of Cr accumulation by bacterial cells [12, 15].

We studied the influence of sodium (Na), potassium (K), cesium (Cs), calcium (Ca), chromium (Cr), zinc (Zn), and copper (Cu) assimilation by *Arthrobacter globiformis* 151B [16, 17, 18, 19, 20]. We were interested in the effect of silicon ions on the absorption process of zinc and chromium by *Arthrobacter globiformis* 151B.

Materials and Methods

Arthrobacter globiformis 151B was selected as the research objective which was isolated from basalts from the Kazreti region polluted with heavy metals [21]. The colonies of *Arthrobacter globiformis* 151B are white-creamy, smooth, round, bulging, with a shiny surface. The culture does not produce pigment. Grows well on simple synthetic and complex organic nutrient areas. Its development phases are coccus-bacilli-coccus. The 15-17 hour culture represents rods, which subsequently form different shapes. In the period of 18-24 hours, the structures formed by it gradually fragment into cocci. It does not produce spores. It is not characterized by the ability to move. The culture is gram-positive and acid-sensitive. The optimal temperature for culture growth is 20^0C-28^0C.

To implement the purpose of the research, we conducted the following experiment:

Before starting the experiment, culture 151B was transferred from solid agar (TSA agar) to 100 mL of liquid agar (TSB) in 500 mL Erlenmeyer flasks placed in a thermostat on a constant shaker: After 24 h of cultivation, we transferred 10-10 mL of the culture liquid (suspension) to the flasks prepared for the experiment, where there was 90 mL of TSB (i.e., it is 90+10=100 mL of TSB). Cultivation was carried out in 500 mL Erlenmeyer flasks in 100 mL TSB, and the bacteria were incubated at 26^0C.

The growth area contained the research elements with the following concentrations: Zn -1 μg/mL, Cr – 7 μg/mL. In 1 flask we had a pure bacterial sample (control), in 3 flasks we added a solution of Si in the form of sodium silicate (Na2SiO3·9H2O) as follows: in 1 flask we added 1 mL, in the second 4 mL, and in the third 16 mL so that the concentration of Si in the growth, medium made up 50, 200, and 800 μg/mL, respectively. Bacteria were cultivated for 17, 24, 48, 96, and 144 hours. After cultivation, we pelleted the cells by centrifugation (3000 rpm, 10 min, 0^0C), decanted the supernatant, and washed the residual bacterial sediment with sterile, distilled water. The bacterial pellet was washed and centrifuged twice. We dried the obtained biomasses with a low-temperature lyophilizer and weighed their total masses. From the total amount of bacterial pellets, we took the necessary amount for analysis, weighed it (≈30 mg), and placed it in test tubes. In order to convert the samples into a liquid state, we added concentrated nitric acid (1 mL) to the test tubes,

heated them, and after completing the ashing procedure, diluted with bidistillate water up to 10 mL. We analyzed the zinc and chromium content of the obtained samples by atomic absorption spectrometer (Analyst 800). The acetylene-air flame was used.

Results and discussions

The process of assimilation of Zn and Cr by bacteria and the influence of Si ions on this process were studied. We studied a total of 20 samples: 1-5 were controls, in samples 6-10, the concentration of Si in the growth medium was 50 μg/mL, in samples 11-15 it is 200 μg/mL, and in samples 16-20 it is 800 μg/mL in

The atomic absorption spectroscopy measurement results are given in Fig. 2 and 3.

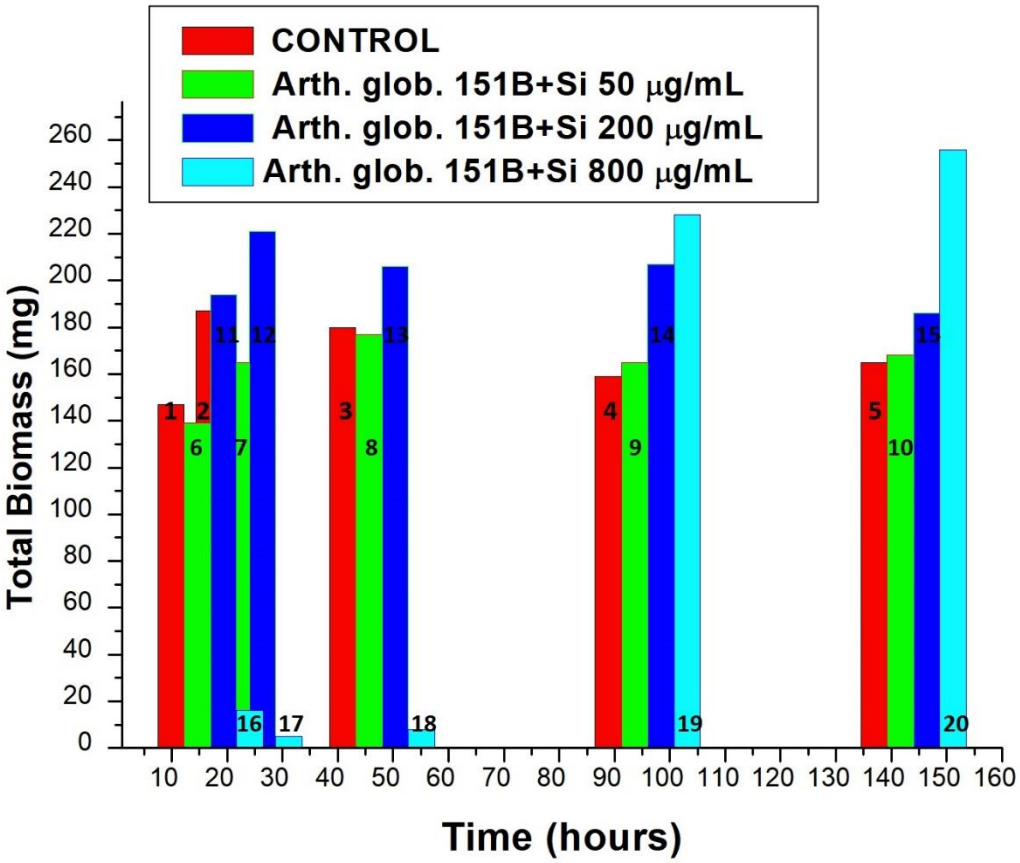

Figure. 1. The Effect of different concentrations of silicium ions on the biomass of bacteria according to time.

According to Fig.1, it can be seen that during the first 48 hours of bacterial growth, their mass is minimal (≈5 mg), at an increased concentration of Si ions in the nutrient area (800 μg/mL). In samples 19 and 20, the bacterial mass increases and reaches a maximum value up to 256 mg. As shown in Fig. 2 we can see that the concentration of Zn in *Arthrobacter globiformis* 151B is minimal ≈at 110 μg/g, in sample 7 (red, round dots), when the concentration of Si in the nutrient medium is 50 μg/mL. At the concentration of 200 μg/mL of silicon in the growth medium, Zn absorption by bacteria increases relatively. The maximum value of ≈378 μg/g is obtained in sample 18 (pink dots, inverted triangles) when the concentration of Si solution in the growth medium is 800 μg/mL.

Figure 3 shows that the concentration of Cr in *Arthrobacter globiformis* 151B is minimal (≈710 μg/g), in sample 7 (red, round dots), when the concentration of Si in the nutrient medium is 50 μg/mL. It should also be noted that at the concentration of 50 and 200 μg/mL of silicon in the nutrient area, the absorption of Cr by bacteria is significantly reduced. In samples 6-15, it varies between ≈770-2032 μg/g, which is lower than the control samples. The maximum value of ≈5033 μg/g concentration of Cr was taken in sample 17 (pink dots, inverted triangles) when the Si concentration in the growth medium was 800 μg/mL.

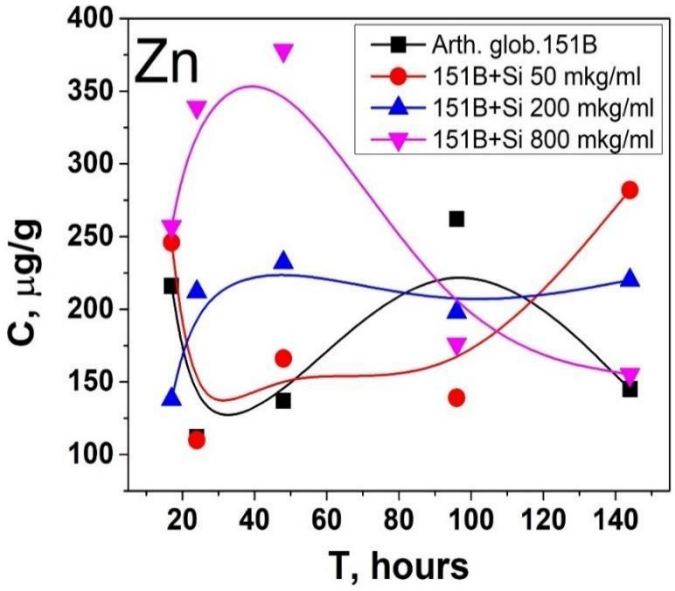

Fig .2.The time score (hr) of Zn accumulation (μg/g) by bacteria

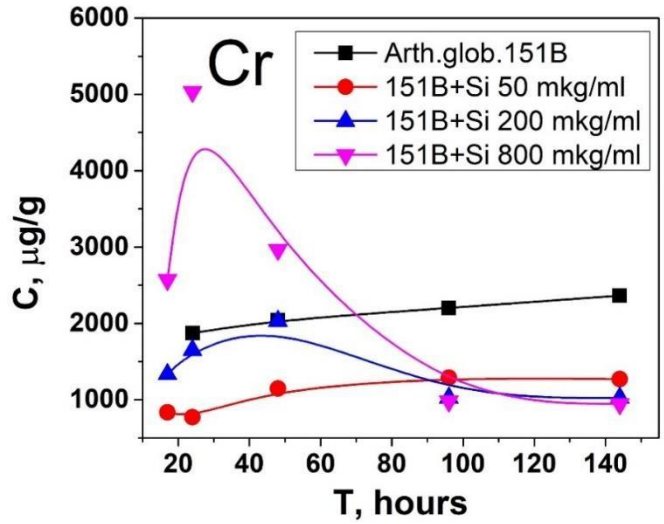

Fig. 3. The time score (hr) of Cr accumulation (μg /g) by bacteria.

Conclusions

Based on the results obtained from the conducted experiment, it can be said, that the increased concentration of Si ions in the nutrient medium promotes the assimilation of Zn and Cr by *Arthrobacter globiformis* 151B during the first 48 hours of bacterial growth. It should be noted that the mentioned bacteria absorb chromium in a much larger amount compared to zinc. The increased concentration of Si ions (800 μg/mL) in the growth medium inhibits the growth and development of *Arthrobacter globiformis* 151B during the first 48 hours. At the next stage of bacterial growth, it helps and significantly increases the biomass of bacteria.

REFERENCES

1. Codd R., Dillon C., Levina A., Lay P.A. Studies on the genotoxicity of chromium: from the test tube to the cell, Coordination Chemistry Reviews, 2001, v. 216-217, 537-582.
2. Rouch DA, Lee BTO, Morby AP, 1995. Understanding cellular responses to toxic agents: A model for mechanism choice in bacterial metal resistance. J. Ind. Microbiol, 14, 132-241.
3. Tsibakhashvili NY, Mosulishvili LM, Kalabegishvili TL, Kirkesali EI, Murusidze IG, Kerkenjia SM, Frontasieva MV, Holman HY, 2008. Biotechnology of Cr(VI) transformation

into Cr(III) complexes. J. radioanal. Nucl. Chem. 278:565-569.
4. Suzuki Y, Banfild J, 2004. Resistance to accumulation of uranium by bacteria from a uranium- contaminated site. Geomicro-biol. J. 21:113-121.
5. Martin KR. Silicon: the health benefits of a metalloid. Met Ions Life Sci. 2013;13:451-73. doi: 10.1007/978-94-007-7500-8_14. PMID: 24470100.
6. Jurkić LM, Cepanec I, Pavelić SK, Pavelić K. Biological and therapeutic effects of ortho-silicic acid and some ortho-silicic acid-releasing compounds: New perspectives for therapy. Nutr Metab (Lond). 2013 Jan 8;10(1):2. doi: 10.1186/1743-7075-10-2. PMID: 23298332; PMCID: PMC3546016.
7. Pielesz A, Biniás D, Machnicka A, Bobínski R, Laane HM, Tjon J, Ulman-Włodarz I, Waksmánska W. Stabilized Ortho-Silicic Acid as a Modifier of Tissue-Preliminary Comparative Biomaterial Studies. J Biomed Nanotechnol. 2020 Apr 1;16(4):538-547. doi: 10.1166/jbn.2020.2912. PMID: 32970985.
8. Araújo LA, Addor F, Campos PM. Use of silicon for skin and hair care: an approach of chemical forms available and efficacy. An Bras Dermatol. 2016 May-Jun;91(3):331-5. doi: 10.1590/abd1806-4841.20163986. PMID: 27438201; PMCID: PMC4938278.
9. Nino Asatiani, Tamar Kartvelishvili, Nelly Sapojnikova, Marina Abuladze, Lali Asanishvili, Mariam Osepashvili, "Effect of the Simultaneous Action of Zinc and Chromium on Arthrobacter spp." Water Air Soil Pollut (2018) 229: 395
10. Megraharaj M., Avudainayagam S., Neidu R. "Toxicity of hexavalent chromium and its reduction by bacteria isolated from soil contaminated with tannery waste", Curr. Microbiol. 2003, 47, 51-54.
11. Kamaludeen S.P. Megharaj M., Sethunathan N., Naidu R. "Chromium-microorganism interactions in soils: remediation implications". Rev. Environ. Contam. Toxicol. 2003, 178, 93-164.
12. Nelly Y. Tsibakhashvili, Tamaz L. Kalabegishvili, Alex N. Rcheulishvili, Etery N. Gintury, levan G. Lomidze, David N. Gvarjaladze, Olia A. Rcheulishvili, H.-Y. N. Holman. "Effect of Zn(II) on the reduction and accumulation of Cr(VI) by Arthrobacter species", J. Ind Microbiol. & Biotechnol., 38, 1803-1808, 2011.
13. McDevitt CA, Ogunniyi AD, Valkov E, Lawrence MC, Kobe B, et al. (2011) "A Molecular Mechanism for Bacterial Susceptibility to Zinc". PLoS Pathog 7(11): e1002357.
14. Choudhury R, Srivastava S (2001) Zinc resistance mechanism in bacteria. Curr. Sci. 81:768-775.
15. O. Rcheulishvili, L. Tsverava, A. Rcheulishvilia, M. Gurielidzed, R. Solomonia, N. Metreveli, N. Jojua, H-Y Holman; Heavy metals specific proteomic responses of a highly resistant *Arthrobacter globiformis* 151B; Annals of Agrarian Science 17 (2019) 218 – 229
16. A. Rcheulishvili, E. Ginturi, L. Tugushi, M. Gurielidze, N. Rcheulishvili and H.-Y. Holman. "INFLUENCE OF SODIUM ON ASSIMILATION PROCESS OF Cr(VI) AND Cu BY ARTHROBACTER GLOBIFORMIS 151B CHROMIUM-RESISTANT BACTERIUM", European Chemical Bulletin, 2019, 8(4), 128-131. https://www.researchgate.net/publication/333499095
17. Alexandre Rcheulishvili, Etery Gintury, Lela Tugushi, Manana Gurielidze, Hoi-Ying Holman. "Zn and Cr(VI) Uptake by Bacteria Arthrobacter globiformis 151B from the Potassium-Rich Nutrient Medium". BULLETIN OF THE NATIONAL ACADEMY OF SCIENCES, vol. 13, no. 3, pp. 90-95, 2019. http://science.org.ge/bnas/vol-13-3.html
18. A. Rcheulishvili, M. Gurielidze, E. Ginturi, L. Tugushi, Hoi-Ying Holman. "INFLUENCE OF Ca ON THE ASSIMILATION OF Cr AND Zn BY THE CHROMIUM RESISTANT BACTERIUM ARTHROBACTER GLOBIFORMIS 151B". Journal Annals of Agrarian Sciences, vol. 18 No. 3 (2020), 365-368. https://journals.org.ge/index.php/aans/issue/view/20/24
19. Alexandre N. Rcheulishvili, Manana A. Gurielidze, Etery N. Gintury & Lela S. Tugushi. „Assimilation of Cu by the Chromium Resistant Bacterium Arthrobacter Globiformis 151B in the Presence of Ca in Growth Media". Global Journal of Science Frontier Research: A Physics and Space Science; Volume 20 Issue 9 Version 1.0, 2020, pp. 61-64. https://globaljournals.org/GJSFR_Volume20/E-Journal_GJSFR_(A)_Vol_20_Issue_9.pdf
20. Rcheulishvili O, Metreveli N, Solomonia R, Tsverava L, Holman HY. Bioremediation potential of hexavalent chromium-resistant Arthrobacter globiformis 151B: study of the uptake of cesium and other alkali ions. Int Microbiol. 2022 Nov;25(4):745-758. doi: 10.1007/s10123-022-00258-5. Epub 2022 Jun 29. PMID: 35768673.

21. Tsibakhashvili N.Y., L. M. Mosulishvili, T. L. Kalabegishvili, D. T. Pataraya, M. A.Gurielidze, G. S. Nadareishvili, H-Y. N. Holman. (2002). Chromate-resistant and reducing-microorganisms in Georgia basalts: their distribution and characterization. Fresenius Environmental Bulletin. 11(7):352-361.