Journal of Pharmaceutical and Applied Chemistry
An International Journal

http://dx.doi.org/10.18576/jpac/050203

Influence of Na on Zn Uptake by *Arthrobacter Globiformis* 151B

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

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

Received: 2 Feb. 2019, Revised: 1 Mar. 2019, Accepted: 3 Mar. 2019.
Published online: 1 May 2019.

Abstract: The role of Na on the Zn uptake by *Arthrobacter globiformis* 151B has been studied. Arthrobacter, that are known as chrome resistant bacteria, can be used for detoxification of high toxic Cr(VI) in polluted environment. The studied bacterial strain was isolated from basalt samples, taken in Georgia – Kazreti sampling sites that are highly contaminated with Cr(VI). The bacterial strains were cultivated for various period of time (17, 24, 48, 96 and 144 hours). The Na concentration in the growth media was fixed as 2.0, 3.5, 6.5 and 9.5 mg/ml. The cultivated bacterial samples were prepared for further analysis for Zn content. The measurements were conducted using with atomic absorption spectrophotometer. The obtained results suggest that rise of Zn uptake by *Arthrobacter globiformis* 151B is facilitated by high Na concentration in the initial stage of bacterial growth.

Key words: Arthrobacter, *Arthrobacter globiformis* 151B, sodium (Na), zinc (Zn), concentration, biomass.

1 Introduction

Heavy metals that are found with high concentration in nutrient enriched environment belong to systemic toxicants. They can induce multiple organ damage, sometimes even at lower levels of exposure. Increased concentration of the significant and essential elements of Zn, Ni, Cu, etc. have toxic and cancerogenic effect on the living processes. The most of microorganisms in the polluted environment have adopted the different mechanism to avoid the toxic effect of the heavy metals [1–3]. Arthrobacter can detoxify chrome ions by reducing or accumulate them inside the cell. Further studies suggested that reduction of Cr(VI) is progressed on the cell wall [4]. The Zn(II) ion role in this process has also a significant role to increase Cr accumulation and reduction [5].

The latest studies have been demonstrated that many well investigated bacteria loses its physiological activity in the polluted environment. The most microorganism are also unable to adapt the high concentration of metals in the environment. Therefore, the bacteria in our studies was isolated from the polluted soil, mineral rocks, water [6–8]. Arthrobacter *(Arthrobacter oxydans, Arthrobacter globiformis* 151B, and *Arthrobacter oxydans* 61B) are habitants of soil bacteria and have great potential to be involved in the environmental restoration [9]. The most of Arthrobacter are resistant against heavy metals [10–12]. They can uptake various chemical elements such as Cr, Zn, Cu and etc. [13]. The adsorbed chemical elements participate in the biochemical processes of the bacteria. The studies revealed the bacterial strains can also perform reduction of the adsorbed Cr(VI) to Cr(III) [14].

Presumable the intracellular uptake ability of Cr, Cu and Zn by bacteria can be changed by the joint action of these metals with some macroelements like Na, K, Mg, etc.

Our studies address the influence of the most prevalent macroelement sodium (Na) on zinc (Zn) uptake by the Arthrobacter strain. Even though the concentration of microelement zinc is low in the living organism, it has significant function in cell living processes. Zinc is crucial for normal functioning of immune systems, brain and nervous system. Zinc is important for hair, skin and nails, for development of reproduction system.

* Corresponding author E-mail: archeuli@gmail.com

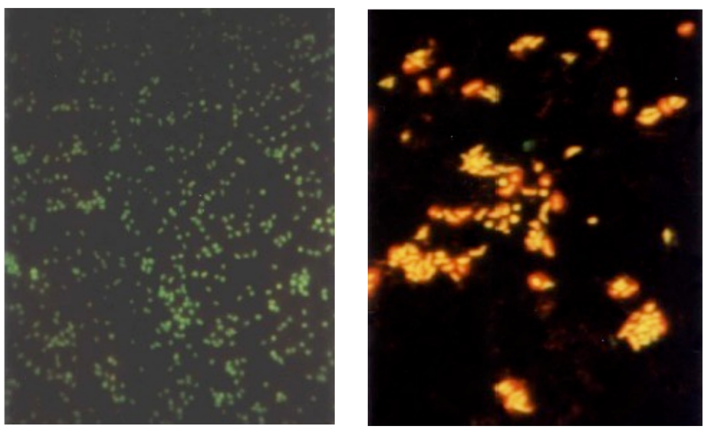
2 Materials and Methods

The studying object Arthrobacter species, *Arthrobacter globiformis* 151B, was isolated from the heavy metal polluted basalts from Kazreti region (Georgia) [15]. Arthrobacter is gram positive, obligates aerobes. Colonies of *Arthrobacter globiformis* 151B are white-cream in their coloration, coccoid, with unusual bulbous protuberances. Its growth phases follow shape of coccus – rod - coccus (Figure 1). After 15–17h of incubation, it is rod, but after 18–24h it is gradually transformed to coccoid shape. It does not produce spores, is immotile and unstable in the acidic environment. The optimal temperature for bacterial growth is 20–28°C.

The studying samples were processed with the following Experimental procedure:

The bacterial strains were incubated in 100mL liquid nutrient medium – TSB (*Tryptic Soy Broth*), included 2.0mg/ml Na concentration and 1µg/ml Zn. NaCl solution was used to increase Na concentration in some samples up to 3.5, 6.5 and 9.5 mg/ml. The bacterial strains were grown for 17, 24, 48, 96 and 144 hours. After cultivation, the bacterial suspension was centrifuged (3000 rpm, 10 min, 0°C), the pellet was washed with distilled water, was dried with freeze drying at a lyophilisator. 30 µg of the dry samples was liquidized with 1mL concentrated nitric acid, warmed up to complete ashing and was diluted with bidistilled water.

The samples were tested for metal content with atomic absorption spectrophotometer (an acetylene–air flame). The measurements were taken with an atomic absorption spectrometer "Analyst–800".



(b)

Fig. 1: *Arthrobacter oxydans* bacterial cells: (a) growth stationary phase and (b) growth exponential phase. Images are obtained with fluorescence microscopy.

3 Results

The studies addressed Zn assimilation process by the bacteria and Na ions effect on this process. The visualization of this dependence is shown on the Figure 2. The results indicates at low concentration of Na (3.5mg/ml) Zn uptake by the bacteria is slowed down, while high concentration of Na (6.5mg/ml) in the growth media stimulates the Zn uptake by the bacteria. Zn uptake is intensively progressed during first 17h of incubation. After it, Zn is eliminated from the bacterial cell. Zn concentration inside the cell is minimal during the following 7h of incubation. After it Zn concentration increases and reaches the same value gained after first 17h cultivation (≈220µg/g). The maximum value of Zn concentration reached after 17h incubation(400µg/g) with the presence of high Na concentration (of 9.5mg/mL) in the growth media.

Zn concentration is decreased after 24h of cultivation, afterwards the tendency of Zn concentration increases is observed. The samples incubated 96 and 144 hours had same Zn concentration (≈150µg/g) as the sample cultivated for 24h. Additionally, Zn concentration is minimal after 24h incubation for all the concentration of Na.

The dependence of bacterial biomass (M) on cultivation time (T) is presented of Figure 3. The biomass of the bacteria is increased after applying the additional three different values of Na concentration. The biomass concentration is high after 24h incubation, is decreased after 48 hours incubation, but is increased again after 96 and 144hours incubation. The bacterial biomass is ≈190 mg in the sample, when the media contains 6.5mg/ml Na concentration.

The obtained results indicates that Zn concentration during the high bacterial biomass is low. The samples with all Na concentration after 24h incubation have bacterial biomass 103, 160, 132, and 142 mg, while Zn concentrations in the same samples are very low: correspondingly, 56, 97, 85, and 157μg/g. Therefore, the bacterial biomass and Zn concentrations are dependent on each other: when the bacterial biomass is increased, Zn uptake process is slowed down.

The dependence of Zn on Na concentration in the nutrient medium is showed on the Figure 4. As the Figure 4 demonstrates that increase of Na concentration stimulates the Zn assimilation process by *Arthrobacter globiformis* 151B bacteria.

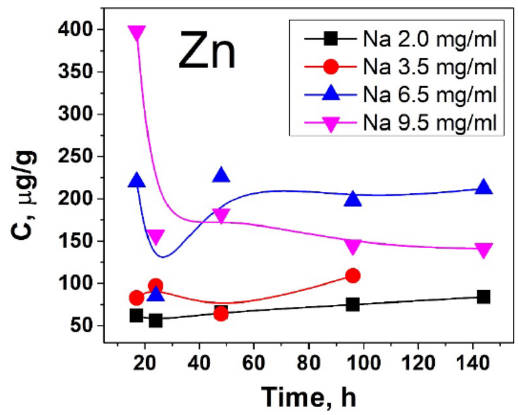


Fig. 2: Dependence between Zn concentration (C) and cultivation time (T) of *Arthrobacter globiformis* 151B bacteria at presence of various Na concentrations in media.

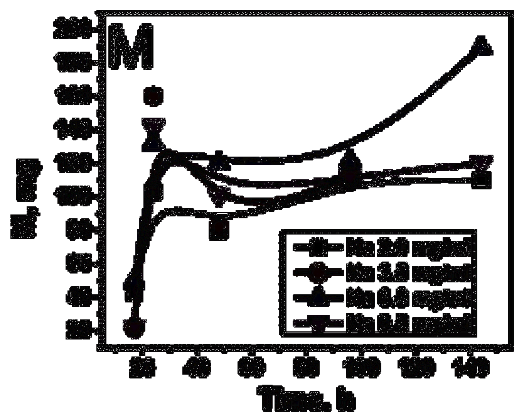


Fig. 3: Dependence of bacterial biomass M (mg) on bacterial cultivation time T (h).

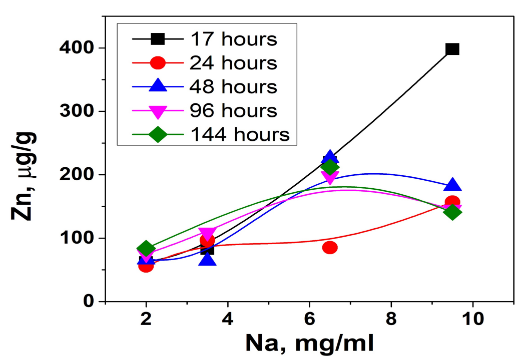


Fig. 4: Dependence of Zn on Na concentration in growth media. Na concentration in media is 2.0, 3.5, 6.5, and 9.5mg/ml.

3 Conclusions

The experimental data indicates Na content has a significant role in Zn uptake. The increase of Na concentration in the growth media stimulates Zn uptake by *Arthrobacter globiformis* 151B, especially in the initial stage of bacterial growth.

Acknowledgement: This work was funded by Grant STCU#6316/SRNSF#STCU–2016–09 from the Science and Technology Centre in Ukrainian (STCU) and Shota Rustaveli National Science Foundation of Georgia (SRNSF). Paper was presented at the 5th International Conference “Nanotechnologies”, November 19–22, 2018, Tbilisi, Georgia (Nano – 2018).

References

[1] D. H. Nies. Efflux-mediated heavy metal resistance in prokaryotes. FEMS Microbiol. Rev., **27**, 313-339.
[2] D. H. Nies. Microbial heavy-metal resistance. Appl. Microbiol. & Biotechnol., **51**, 730-750, 1999.
[3] D. H. Nies, S. Silver. Ion efflux systems involved in bacterial metal resistances. J. Bacteriol., **14**, 189-199, 1995.
[4] R. Codd, C. Dillon, A. Levina, P. A. Lay. Studies on the genotoxicity of chromium: from the test tube to the cell. Coord. Chem. Rev., **216/217**, 537-582, 2001.
[5] N. Y. Tsibakhashvili, T. L. Kalabegishvili, A. N. Rcheulishvili, E. N. Ginturi, L. G. Lomidze, O. A. Rcheulishvili, D. N. Gvarjaladze, H.-Y. Holman. Effect of Zn(II) on the reduction and accumulation of Cr(VI) by Arthrobacter species. J. Ind. Microbiol. & Biotechnol., **38**, 1803-1808, 2011.
[6] M. Megraharaj, S. Avudainayagam, R. Neidu. Toxicity of hexavalent chromium and its reduction by bacteria isolated from soil contaminated with tannery waste. Curr. Microbiol., **47**, 51-54, 2003.
[7] S. P. Kamaludeen, M. Megharaj, N. Sethunathan, R. Naidu. Chromium–microorganism interactions in soils: Remediation implications. Rev. Environ. Contam. & Toxicol., **178**, 93-164, 2003.
[8] U. Badar, N. Ahmad, A. J. Beswick, P. Pattenspipitpaisal, L. E. Macaskie. Reduction of chromate by microorganisms isolated from metal contaminated sites of Karachi, Pakistan. Biotechnol. Lett., 22, 829-836, 2000.
[9] O. Rcheulishvili. Chrome Uptake and Detoxication by Environmental Arthrobacter Bacteria and the Role of Various Metals on These Processes (PhD Thesis), 2014, Tbilisi, Ilia State Univ.
[10] S. P. Marthur, E. X. Paul. Microbial utilization of soil humic acids. Can. J. Microbiol., **13**, 573-580,1967.
[11] Y. Suzuki, J. Banfild. Resistance to accumulation of uranium by bacteria from a uranium- contaminated site. Geomicro-Biol. J.,**21**, 113-121, 2004.
[12] Y. Yamada, H. Motoi, S. Kinoshita, N. Takada, H. Okoda. Oxidative degradation of squalene by Arthrobacter species. Appl. Microbiol., **29**, 400-404,1975.
[13] R. Choudhury, S. Srivastava. Zinc resistance mechanism in bacteria. Curr. Sci., **81**, 768-775, 2001.
[14] N. Y. Tsibakhashvili, L. M. Mosulishvili, T. L. Kalabegishvili, E. I. Kirkesali, I. G. Murusidze, S. M. Kerkenjia, M. V. Frontasieva, H.-Y. Holman. Biotechnology of Cr(VI) transformation into Cr(III) complexes. J. Radioanal. Nucl. Chem., **278**, 565-569, 2008.
[15] N. Y. Tsibakhashvili, L. M. Mosulishvili, T. L. Kalabegishvili, D. T. Pataraya, M. A. Gurielidze, G. S. Nadareishvili, H.-Y. Holman. Chromate-resistant and reducing-microorganisms in Georgia basalts: Their distribution and characterization. Fresenius Environ. Bull., **11**, 35, 2002.