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Reduction and uptake of Cr(VI) by Arthrobacter oxydans and uptake of Cr(III) by Arthrobacter sp. 61B under the influence of potassium and magnesium

O. Rcheulishvili[a,b*], N. Metreveli[a], A. Rcheulishvili[b], L. Tugushi[b], M. Gurielidze[c]

[a]Ilia State University; 3/5, Kakutsa Cholokashvili Ave., Tbilisi, 0162, Georgia

[b]Andronikashvili Institute of Physics of Iv. Javakhishvili Tbilisi State University; 6, Tamarashvili Str., Tbilisi, 0177, Georgia

[c]Agricultural University of Georgia; 240, David Agmashenebeli Alley, Tbilisi, 0159, Georgia

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A B S T R A C T

Some bacteria from Arthrobacter genera have great potential for bioremediation. Two strains of Arthrobacter, one isolated from Georgia, contaminated Kazreti region and second- from Columbia basalt rocks of the contaminated site of the US, exhibit resistance against high concentrations of Cr(VI) and other metallic ions. They can reduce highly toxic and carcinogenic Cr(VI) into Cr(III).

We investigated the behavior of bacteria with following metals: K+, Cr(VI) and Mg(II), their uptake process by living, aerobic Arthrobacter oxydans and influence of K+ and Mg(II) on the uptake process of Cr(III) by Arthrobacter sp. 61B. Metal accumulation ability by bacteria, was analyzed using Atomic Absorption Spectroscopy.

Keywords: Cr(VI) reduction, Atomic absorption spectroscopy, Alkali ions, Arthrobacter sp. 61B, Arthrobacter oxydans, Metal accumulation.

*Corresponding author: Olia Rcheulishvili; E-Mail address: olia.rcheulishvili.1@iliauni.edu.ge

Introduction

Studying metal-microorganism interactions is one of the most actual issues for environment protection biotechnologists. Microbes' behavior against some metals is of great importance for developing the proper strategy of restoring the contaminated environment, or can be used to develop the method which allows to clean the environment. Some bacteria of Arthrobacter genera have great potential for bioremediation, as they exhibit resistance against heavy metals, can transform aromatic compounds and can reduce toxic Cr(VI) into less toxic Cr(III) form [1, 2]. According to its toxicity, Cr(VI) takes the 3rd place between the six most acute environmental pollutants, after lead and mercury [3]. Its toxic action is due to Cr(VI)'s capability to easily penetrate cell membranes both in eukaryotic or prokaryotic cells [4]. Different non-specific reductants, such as glutathione reductase, cysteine, NADH or other components of the cell: carbohydrates, teichoic, lipoteichoic acids can reduce Cr(VI) by its subsequent Cr(V/IV/III) products [2, 4]. Magnesium Mg(II) takes 11th place between in body existing elements. Mg(II) is essential for all types of living cells and are co-factor of about 300 important enzymes. Mg(II) ions participate in DNA, RNA or Glutathione synthesis. Mg(II) is essential for energy producing processes: oxidative phosphorylation and glycolysis [5]. The potassium (K) ions are among the most important metal ions in living cells. Natural habitats are often characterized by the coexistence of Cr, Mg and K. Coexistence of some metals can cause or synergic or negative effect upon the accumulation/uptake capabilities by different bacteria. Living prokaryotic/eukaryotic cells have separate transport mechanisms and uptake capabilities for different ions. Against some toxic agents, several resistance mechanisms are known: (1) Changes on bacterial membrane or shell, as a result the barrier for metal ions penetration in cell is increased. Metals' ions (2) Sequestration inside and outside of cell; (3) Driving out from cell using transportation mechanisms; (4) Enzymatic detoxification and (5) weakening the sensitivity of cellular targets [5]. Sequestration or transport mechanisms of different ions, through biological cell membrane are mostly associated with integral membrane proteins: ionic channels. Ionic channel selectivity and transport mechanisms directly depend on ion's hydration energy, atomic radius or valency [6]. Soil microorganisms are influenced by many different metals concomitant action. K^+, Cr(VI) or Mg^{2+} differ according their valency and atomic radius. They characterize with high solubility in biological fluids. Their salts frequently high solubility in water is due to large entropies of solution while heats of solution often are endothermic [7].

The present data revealed the uptake and reduction potential of Cr(VI) by Arthrobacter oxydans, with concomitant action of K and Mg(II). And the uptake capability of Cr(VI) at presence of 400 μg/l Mg(II) or K ions by bacteria of Arthrobacter sp. 61B.

Objects and Methods

Bacterial Growth Conditions, experimental design and sample preparation.

All chemicals were ACS- reagent grade and purchased from Sigma.

Experiment A. Bacterial cells of Arthrobacter oxydans were grown aerobically in 250-mL Erlenmeyer flasks as a 100-ml suspension in the medium at 21˚C. The cells were grown with a constant shaking (at a speed of 100rpm). The nutrition medium contained Cr(VI) in the form of K_2CrO_4 (70 mg/L), $MgSO_4 \cdot 7H_2O$ (0.2g/L), K_2HPO_4 (2g/L), ammonium citrate (1g/L), glucose (1g/L), yeast extract (1g/L), and 1.0 L of distilled water. The experimental samples from these groups were taken in a time dependent fashion: 1, 7, 11, 15, 19, 22, 29 days after the start of cultivation.

Experiment B. Bacterial cells of Arthrobacter sp. 61B were grown aerobically in 250-mL Erlenmeyer flasks as a 100-ml suspension in TSB broth (Sigma) at 21˚C. The cells were grown with a constant shaking (at a speed of 100rpm). The experiments were carried out on the following groups of bacteria: 1. Cells with addition of 7.1 μg/ml Cr(III); 2. Cells with addition of 7.1 μg/ml Cr(III) + 400 μg/ml Mg(II); 3. Cells with addition of 7.1 μg/ml Cr(III)+ 400 μg/ml of K; Elements Cr(III), Mg(II) and K were added simultaneously to the bacterial cell cultures at this stage as $CrCl_3$, KCl, $MgCl_2$ respectively. The experimental samples from these groups were taken in a time dependent fashion: 16, 24, 48, 96, 144 hours after the start of cultivation. Bacterial culture growth, proceeded without medium renewal.

Evaluation of Cr(VI) resistance

Culture growth was monitored by measuring optical density at 490 and 590nm. The viability was detected by cell growth on agar plates with a cell suspension dilution. Bacterial resistance against Cr(VI) was detected by counting of the Colony Forming Units on metal containing agar plates and measuring the weight of bacterial cells biomass before AAS measurements.

After growing, bacterial cells were harvested from the nutrient medium by centrifugation (3,000g, 15 min, 4˚C), rinsed twice in a Phosphate buffered saline (PBS) and samples prepared atomic absorption spectroscopy (AAS) analysis.

AAS measurements

For the determination of the concentrations of Cr, K and Mg(II) by bacteria itself, wet biomass of bacterial pellet (after centrifugation and washing procedures) was placed in an adsorption-condensation lyophilizer and dried following the procedure reported in [8, 9]. Dried cells were ashed in nitric acid, diluted with bi-distilled water and analysed by AAS method in an acetylene air flame. Analyst 800 (Perkin Elmer) was used. The detection was carried out at 357.9 nm for Cr, at 766.49 nm for K and at 285.2 nm for Mg(II).

The determination of concentration of reduced Cr(III)

The reduction of Cr(VI) to Cr(III) was measured by AAS method, according to the method described in US patent # 7148068 [10]. The method is disclosed for determining the concentration of trivalent chromium Cr(III) in a sample. The addition of perchloric acid has been found to increase the atomic chromium spectrometric signal due to Cr(III), while leaving the signal due to hexavalent chromium Cr(VI) unchanged. This enabled determination of the Cr(III) concentration without pre-concentration or pre-separation from chromium of other valences.

Results and Discussion

To examine the reaction between Cr(VI) and Arthrobacter oxydans, together with K and Mg(II), AAS measurements were carried out. The data of the behavior of K, Cr(VI), and Mg(II) uptake by living Arthrobacter oxydans differs with different metal reactions (Fig.1). The Mg(II) concentration in Arthrobacter oxydans remained almost constant with reaction time. The behaviour of K and Cr differs during the reaction time.

The Cr(III) and Cr in Arthrobacter oxydans increased with Cr(VI) reduction time. Bacteria is able to reduce Cr(VI) into Cr(III) and its reduction

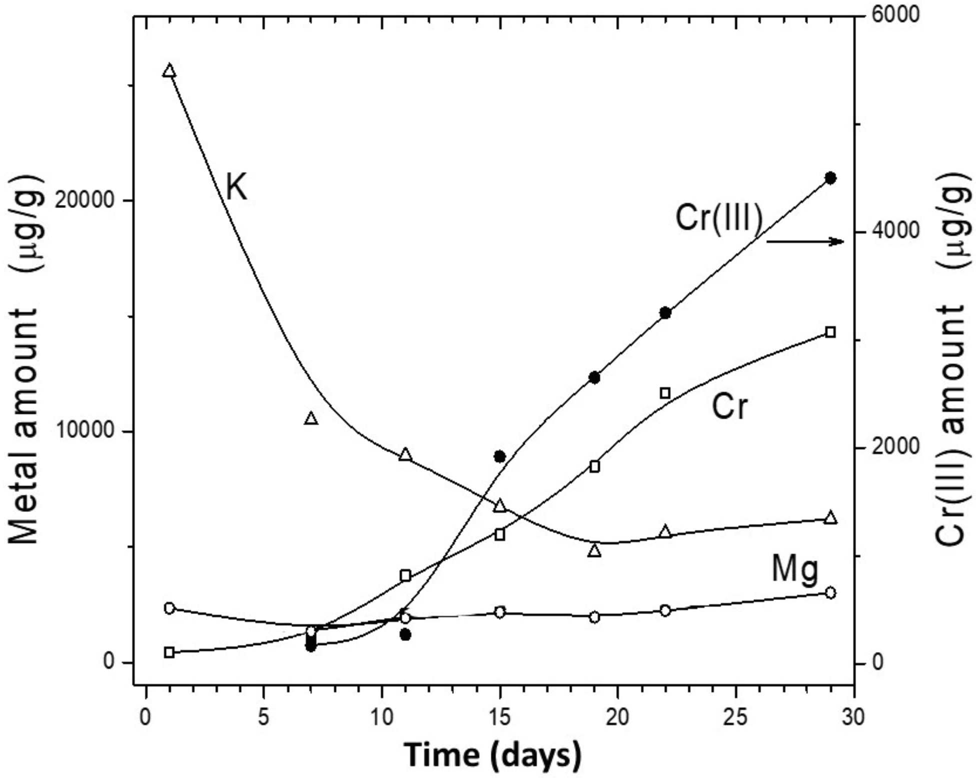

Fig.1. *Uptake of K, Cr, and Mg(II) by Arthrobacter oxydans grown in the nutrient medium containing Cr(VI) (70μg/ml in the form of K_2CrO_4), $MgSO_4{\cdot}7H_2O$ (0.2g/l), and K_2HPO_4 (2g/l). X axis denotes the time of metal action on bacterial cell cultures. Y axis on left denotes the concentration on Mg(II), total Cr and K in the cells of Arthrobacter oxydans. Y axis on right part of figure denotes the concentration of Cr(III). Cr(III) in the samples appears after 7 days innoculation of bacterial cells with Cr(VI)*

starts from 1st day. And Cr(III) concentration reaches its maximum level when reaction time equals at 25 th day. In contrast, the concentration of K in Arthrobacter oxydans is decreased with Cr(VI) reaction time. The rates of Cr(III) and Cr uptake by cells increased when the K concentration was low. Initially the cellular uptake of Cr(III) and Cr were slow. They both increased suddenly, when reaction time was about 11th days as the amount of K in cells approaching its minimum. Cr(III) content by AAS was measured according to the method described here [10]. As K level inside the bacterial cells, is very high in the beginning of cultivation, we suggest that its initial high concentration is bacterias defensive reaction against elevated concentration of toxic Cr(VI) (Fig. 1). In the second experiment we decided to observe the uptake process of Cr(III) by Arthrobacter sp.61B from growth medium under the influence of 400 μg/ml K and Mg(II) (Fig. 2a and 2b)

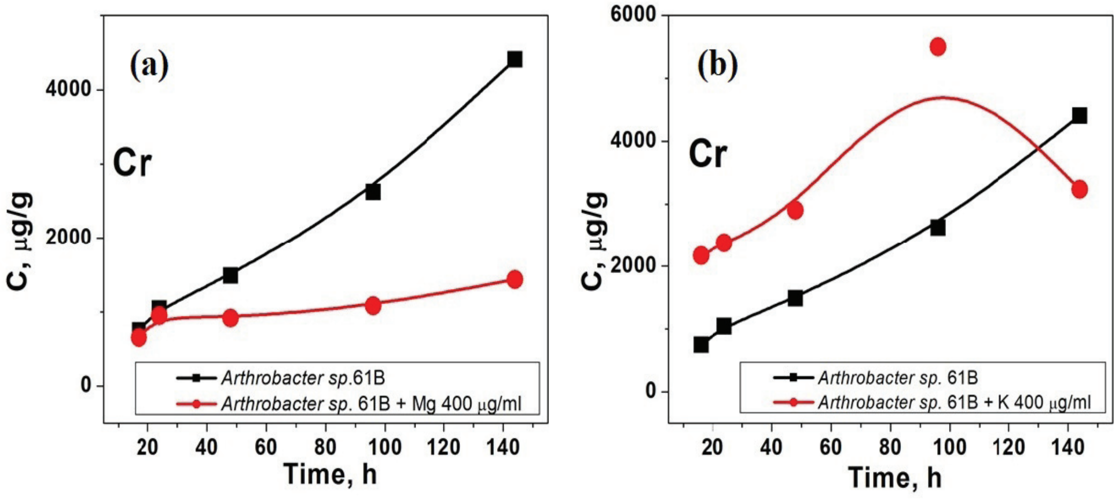

Fig. 2. *Uptake of Cr(III) by Arthrobacter sp. 61B when 400 µg/ml Mg(II) is added in growth medium (a) and when 400 µg/ml K^+ is added in growth medium (b). The growth medium contains 7.1 µg/ml Cr(III)*

It is revealed, that addition of Mg(II) ions, decreases Cr(III) uptake capability by bacteria and after its exposure with Mg(II) restricts the increase of Cr(III) accumulation by cells. Arthrobacter sp.61B without addition of Mg(II) salt, effectively accumulates Cr(III), and its concentration increases with time. Opposite figure is obtained under the influence of 400 µg/ml of K action in the growth medium: at every time point about 1000µg/g higher is the Cr(III) concentration in K treated cells. We can suggest that, K helps in Cr(III) accumulation. The exception is last, 140h time point, when Cr(III) concentration is lower in K treated cells than in control one. Following tendencies are observed on the biomasses of bacteria under the K action: Biomasses of the cells which were grown with or without 400 µg/ml of K do not differ from each other. And 400 µg/ml of Mg(II) increases biomass of Arthrobacter sp.61B at 96h and 144h.

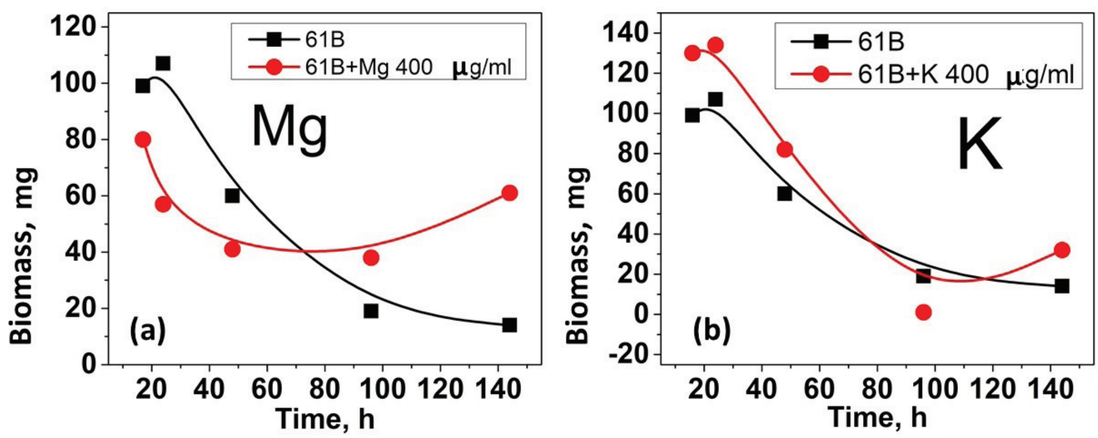

Fig. 3. *The influence of Mg(II) (a) and the influence of K (b) on the biomasses of Arthrobacter sp. 61B*

Conclusion

In bacteria isolated from polluted basalts in Georgia Arthrobacter sp. ٦١B and Arthrobacter oxydans, which is isolated from Columbia basalt rocks originated from a highly contaminated site in the USA we studied: (1) the uptake process of Cr(VI) and Cr(III) with the presence of K and Mg(II). Experiments revealed that the content of Cr(III) in the bacteria of Arthrobacter oxydans increases with the time of growth. Hence, Arthrobacter oxydans can be used for effectively removing toxic Cr(VI) from the environment. The uptake of Cr(III) from growth medium by Arthrobacter sp. 61B was enhanced by the presence of 400 μg/ml K. Mg(II) ions with the same concentrations affected the accumulation of Cr(III) by Arthrobacter sp. 61B. It is shown that the uptake of Cr(VI), its accumulation and reduction to Cr(III) form by bacteria of Arthrobacter oxydans continues during the entire period of the experiment without saturation. According to the data, Arthrobacter oxydans can be used for effectively removing toxic Cr(VI) from the environment. Besides, it was shown that the uptake of K reaches the maximum quantity in the beginnig of experiment after one day, bacteria begin its' efflux from the cell.

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Conflict of Interests

The authors declare no conflict of interest.

References

[1] 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 (2011) 1803-1808.

[2] N. Asatiani, M. abuladze, T. Kartvelishvili, N. Bakradze, N. Sapojnikova, N. Tsibakhashvili, L.Tabatadze, L. Lejava, L. Asanishvili, H.Y. Holman, Effect of Cr(VI) action on Arthrobacter Oxydans Curr Microbiol 49 (2004) 321-326.

[3] A. J.D. McCartor, D. B. A. Becker, World's worst pollution problems, Blacksmith Institute; New York & Green Cross; Switzerland, 2010.

[4] R. Codd, C.T. Dillon, A. Levina, P.A., Lay Studies on the genotoxicity of chromium: From test tube to the cell. Coordin Chem Rev (2001) 216–217: 537–582

[5] A.C. Ross, B. Caballero, R.J. Cousins, K.L. Tucker, T.R. Ziegler; Modern Nutrition in Health and Disease. 11th ed. (2012) 2012:159-75

[6] D. Naranjo, H. Moldenhauer, M. Pincuntureo, I.Díaz-Franulic, Pore size matters for potassium channel conductance; J Gen Physiol. 2016 Oct; 148(4): 277–291. doi: 10.1085/jgp.201611625

[7] J. Mahler, I. Persson, A study of the hydration of the Alkali Metal Ions in Aqueous Solution; Inorg Chem. 2012 Jan 2;51(1):425-38. doi: 10.1021/ic2018693. Epub 2011 Dec 14.

[8] T. Kalabegishvili, I. Murusidze, E. Kirkesali, A. Rcheulishvili, E. Ginturi, N. Kuchava, N. Bagdavadze, E. Gelagutashvili, M. V. Frontasyeva, I. Zinicovscaia, S.S. Pavlov, A.Y. Dmitriev, Gold and silver nanoparticles in Spirulina platensis biomass for medical application, Ecol. Chem. Eng. S. 20 (2013) 621–631. https://doi.org/10.2478/eces-2013-0043.

[9] O. Rcheulishvili, L. Tsverava, A. Rcheulishvili, M. Gurielidze, R. Solomonia, N. Metreveli, N. Jojua, H.-Y. Holman, Heavy metals specific proteomic responses of highly resistant Arthrobacter globiformis 151B, Annals of Agrarian Science, 17 (3) (2019), 218-229.

[10] A.N. Rcheulishvili, N.Ya. Tsibakhashvili. "Method of Trivalent Chromium Concentration Determination by Atomic Spectrometry". U.S. Patent Docket No: S-101100, Customer Number 31971 (2003). United States Patent 7148068. (2006)