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Heavy metals specific proteomic responses of a highly resistant Arthrobacter globiformis 151B

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A B S T R A C T

The gram positive aerobic bacteria Arthrobacter globiformis 151B is a promising candidate for bioremediation of Cr(VI) and other metals ions because it exhibits resistance against high concentrations of Cr(VI) and other metallic ions. This bacterial species could reduce highly toxic and carcinogenic Cr(VI) into Cr(III). In this study, we investigated tolerance and accumulation of Cr(VI) and Zn(II) on protein level by proteomic approach. Arthrobacter globiformis 151B was grown at 3 following conditions: 1. with Cr (VI); 2. with Zn(II); 3. without Cr(VI) and Zn(II). Bacterial cells were harvested in a time dependent fashion (36, 60 and 120h after the starting of cultivation) and changes in proteome expression was analyzed using two-dimensional gel electrophoresis and liquid chromatography and mass spectrometry (LC-MS/MS) coupled with bioinformatics to identify proteins. Significant changes in protein expression included both up- and downregulation of different groups of proteins. Most remarkable changes were associated with metal-binding proteins and proteins involved in active transport. Parallel experiments with Atomic Absorption Spectroscopy revealed that reduced chromium appears mostly soluble and mainly associated with organics: especially with bacterial proteins. Our results signify that Arthrobacter globiformis 151 B is naturally equipped at the proteomic level correspondingly with the relevant genes, to survive extreme toxic conditions, thus has great potential for bioremediation.

Keywords: Heavy metals, Parallel experiments, Bioinformatics, Bacterial protein, Bacterial cells, Arthrobacter.

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Introduction

Arthrobacter and many other members of the actinobacteria group are common residents of soil and have high tolerance to stressful conditions encountered in the soil environment, including the ability to degrade para-substituted phenolic compounds and humic substances [1]. The particular strain of this family, *Arthrobacter globiformis* 151B is Gram-positive, aerobic bacteria, which can survive in heavy- metal contaminated territory and has high potential for environment remediation [2]. This strain was isolated out of basalt samples from heavy metal contaminated site of Georgia –Kazreti [2]. Our previous studies by ESR spectroscopy has shown that bacteria posses the ability to reduce Cr(VI) into Cr(III), which is less soluble and less

toxic compound [3, 4]. FTIR absorption spectroscopy showed that Arthrobacter oxydans is capable to reduce Cr(VI) and complete uptake of 35 µg/mL of Cr(VI) concentration was achieved in about 10 days after Chromium addition into the medium [5]. We have also shown that Cr(VI) reduction process is very fast, and occurs on bacterial cell wall [2]. This reduction is not influenced by the presence of Zn(II) at concentration of 40 mg/L, but the higher concentrations (200 mg/L) inhibits the reduction of Cr(VI), by preventing the contact of Cr(VI) with bacterial cell wall [3]. The Cr(VI) reduction mechanisms, and the fate of reduced Cr(III) are under study. Some of studies provide evidence that Cr(VI) reduction is an enzymatically catalyzed reaction attributed to soluble proteins for some bacteria [6] and cell membranes for others [7, 8].

Further study of the mechanisms Cr(VI) reduction by *Arthrobacter globiformis* 151B is an important topic, because such bacteria could be effectively used for bioremediation processes. In the present study we have inquired (1) if heavy metal exposure Cr(VI), Zn(II) induces changes in the proteome of *Arthrobacter globiformis* 151B and (2) what is the fate of reduced Cr(III). Experiments were conducted in a time dependent fashion and 2-D electrophoresis/MS and Atomic Absorption Spectroscopy (AAS) approaches were used. As far as we know no such studies were conducted before.

Materials and methods

Bacterial Growth Conditions

Bacterial cells of *Arthrobacter globiformis* 151B 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 without any salt addition; 2. Cells with addition of only Cr(VI); 3. Cells with addition of only Zn(II); Experiments were started at the early stationery phase of bacterial cell growth. Cr(VI) and Zn(II) were added simultaneously to the bacterial cell cultures at this stage as K_2CrO_4 and $ZnSO_4$ respectively. Their (metals) concentration made up 40 mg/l in a nutrient liquid medium. The experimental samples from these groups were taken in a time dependent fashion: 36, 60 and 120 hours after the start of cultivation. Experimental design is provided in figure 1. Bacterial culture growth without or with Cr(VI) and Zn(II) proceeded without medium renewal.

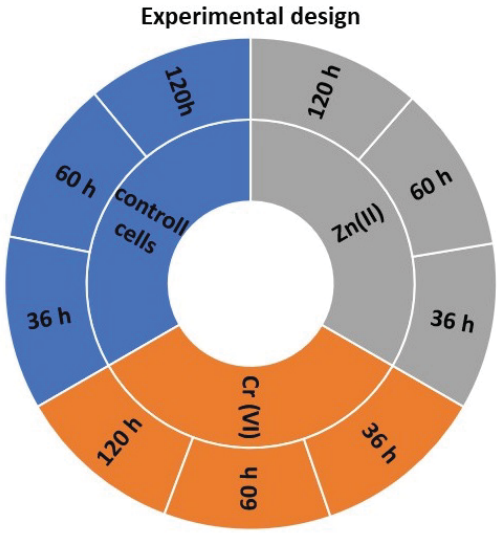

Fig. 1. *The scheme of experimental design*

Evaluation of Cr(VI) and Zn(II) 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 Zn(II) and Cr(VI) was detected by counting of the Colony Forming Units on metal containing agar plates. Cell viability was observed on high (>1000 mg/l) metal concentration conditions.

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 either for 2 - D electrophoresis/MS or Atomic absorption spectroscopy analysis.

For determination the metal accumulation capacity 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 [9], dried cells were ashed in nitric acid, diluted with bidistilled water and analyzed by atomic absorption spectrometry (aas). Analyst 800 was used.

2-D electrophoresis and MS

Sample preparation

Bacterial cell wall lyses and protein extraction- The samples for 2-D electrophoresis and MS analysis

were carried out essentially as described in [10] Bacterial pellets were resuspended in buffer (20 mM Tris-acetate, pH 7.8, 20 mM NaCl, 2 mM EDTA, 100µg/mL lysozyme). Samples were incubated for 30 min at 37°C with intermittent vortexing. 9M Urea, 4% Tween 40, 2% Pharmalyte, 2% Mercaptoethanol, 2% protease inhibitor (bacterial) were added and lysates were centrifuged at 15,000 × *g* for 30 min at 4°C.

Metal accumulation ability by bacterial proteins was determined by atomic absorption spectroscopy, on the portion of extracted proteins.

Protein Quantification. Protein concentration is supernatants was quantified by a micro-BCA kit (Pierce, Thermo Scientific) in quadruplicate. Appropriate buffer controls were used.

Isoelectric focusing

Isoelectric focusing (IEF) strips (linear pH 3–10 and pH 4-7) were rehydrated in 8M urea, 0.5% Triton X-100, 0.5% Pharmalyte 3–10, and 30 mM Destreack reagent overnight. Protein samples (40 µg) were loaded onto rehydrated strips in buffer containing 7M urea, 2M thiourea, 2% Triton X-100, 0.1% ASB-14, 2-mercaptoethanol, 2% Pharmalyte 3–10, bromophenol blue and 2% protease inhibitor. IEF was carried out at 500 V for 3 h and 3,500 V approximately for 18 h.

Equilibration

IEF strips were equilibrated for 15 min in a buffer containing 0.05M Tris-HCl (pH 6.8), 6M urea, 30% glycerol, 3% SDS, and 1% DTT, followed by equilibration in the same buffer with 2.5% DTT iodoacetamide instead of 1% DTT for 15 min.

SDS Electrophoresis

SDS electrophoresis was run on a 1-mm thick, 12.5% polyacrylamide gel at 25°C. For the first 1 hour 10 mA per gel at 80 V was applied and then for 17 h 12 mA per gel at 150 V.

Staining, Scanning, and Analysis

The gels were stained with a silver stain kit (GE-Healthcare), omitting the glutaraldehyde step. Silver-stained gels were scanned with an image scanner (Labscan 6.0. GE Healthcare). Images were digitalized and processed using Image Master 2D platinum 7.0 software. In each series of experiments, the three type of *A. globiformis* 151B samples were analyzed concurrently for each time point. Proteins that exhibited at least 1.5-fold difference between the conditions were selected. The relative intensities of protein spots coinciding by location (isoelectric point and molecular weight) from different experiments were compared by *t*-test. The significantly differentially expressed protein spots ($p < 0.05$) were excised, destained, and stored at −20°C until MS analysis.

In-gel Digestion

Excised proteins were reduced with TCEP and alkylated with iodoacetamide. Samples were then treated with acetonitrile, dried, and rehydrated in activated trypsin (Thermo Scientific, Pierce) to begin digestion; proteins were digested at 37°C overnight. After digestion, the supernatant was pipetted into a sample vial and loaded into an autosampler for automated LC-MS/MS analysis.

MS analysis

Mass spectrometry experiments were performed using LTQ Fleet ion trap fitted with nanospray ion sources (ThermoFisher). The separation of peptides was performed by reverse-phase chromatography using EASY-nLC™ 1000 Integrated Ultra High Pressure Nano-HPLC system at a flow rate of 300nL/min and an LC-Packings (Dionex, Sunnyvale, CA) PepMap 100 column (C18, 75µM i.d. × 150mm, 3µM particle size). Peptides were loaded onto an LC-Packings precolumn (Acclaim PepMap 100 C18, 5µM particle size, 100A, 300µM i.d × 5mm) from the autosampler using 0.1% formic acid for 5min at a flow rate of 5µL/min to desalt samples and focus peptides prior to analytical separation. After this period, the six port valve was switched to allow elution of peptides from the precolumn onto the analytical column. Solvent A was water + 0.1% formic acid in water and solvent B was 100% acetonitrile + 0.1% formic acid. The LTQ instrument was operated in a data-dependent manner, in which a survey scan was performed to analyse the m/z values of ions which were eluted from a reverse-phase HPLC column. MS/MS spectra data were analyzed using SEQUEST (Proteome Discoverer 1.4), searching against UniProt UniRef100 Arthrobacter species protein databases.

AAS measurements

The total chromium and total zinc contents in bacterial proteome were measured using an atomic absorption spectrometer (Analyst 800) with an acetylene-air flame. The detection was carried out at 357.9 nm (for chromium) and at 213.8 nm (for zinc). The instrumentation detection limit for Cr measurement was 0.02 µg/ml and for Zn was 0.01 µg/ml.

Results and discussion

2-D gel electrophoresis

We examined the behavior of bacterial proteome under the influence of Zinc(II) and Cr(VI) to compare to control cells, using 2-D gel electrophoresis and mass spectrometry analyses. MS analysis was used to determine the identity of the excised proteins.

The 2-D gel electrophoresis of *Arthrobacter globiformis* 151B protein extracts was carried out initially with two pH gradients: 3.0 – 11.0 and 4.0 – 7.0. The majority of the proteins on the 3.0 – 11.0 pH gradient gels were concentrated between pH 4.0 and 7.0 (Figure 2-A.). Thus, for the better resolution and identification of differentially expressed bands 2-D gel electrophoresis was continued using strips with a pH gradient from 4.0 to 7.0. (Figure 2 - **B. C. D)**. Most significant stable differences were observed at 60h of cultivation and all results provided below represent the differences at this time point.

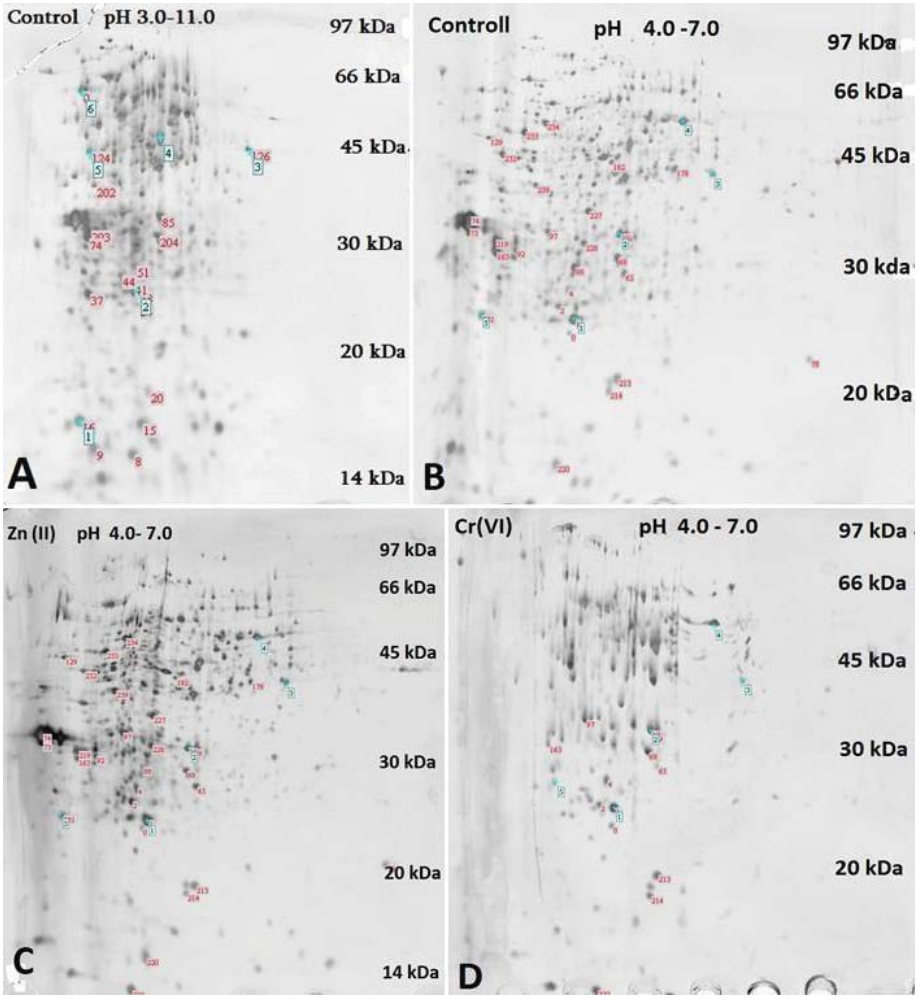

Fig. 2. *Represantitive images of silver stained 2-D gel electrophoresis gels of Arthrobacter globiformis 151 B, protein extracts 60 hour of growth. 2A: protein extract from control cells from 2-D strips with pH linear gradient 3.0 - 11.0; 2 B, C, D representative images of 2-D electrophoresis gels on strips with pH linear gradient 4.0 - 7.0; B - protein extract from control cells; C - protein extract from Zn(II) treated cells; D - protein extract from Cr(VI) treated cells.*

The cultivation of *Arthrobacter globiformis* 151 B with Cr(VI) results in statistically significant differential expression of 41 proteins as compared to untreated, control cells, whereas addition of Zn (II) induces the differential expression of 24 proteins (Table 1A-B).

Differently expressed proteins are involved in a variety of activities and functions. Function of 1st group proteins, expression of which is increased with metal treated cells, mostly relate to stress response reactions and maintenance of the cellular ion homeostasis. It is widely accepted that, these proteins also participate in protein translation processes, activate transcriptional factors and phosphorylate components of signal transduction systems. They can also participate in Zinc and Ferric ion binding processes and act as chaperones. (Table 1 A, B). Function of 2nd group proteins, expression of which decrease in Zn(II) and Cr(VI) treated cells, are mostly related to cellular energetic processes. These energetic processes are associated with cellular active transport. Functions of proteins from 2nd group mostly relate to the trans-membrane transport activity, ATP binding and ATP-ase activity, GTP-ase activity, NADP binding activities. (Table 1. A-B). Availability of Zn to biomolecules is tightly regulated by binding proteins, metallothioneins (MTs) and Zn transporters [19]. Cysteine synthase is increasing in Zn treated cells (see table 1B second row). It is interesting to note that, MTs are cysteine rich proteins that bind up to seven Zn ions with picomolar affinity and carry the labile Zn fraction to cellular compartments. MTs are induced during oxidative stress, scavenge reactive oxygen species (ROS) and reduce heavy metal intoxication [12, 11]. Thus the increase in Cystein synthase could stabilize the necessary conditions for MTs homeostasis. Chromium and Zinc concomitant action causes the strongest oxidative stress in the case of A. globiformis that is demonstrated by the increased activity of superoxide dismutase (SOD) and catalase [13].

Table 1 A. *The list of statistically significantly differentially expressed proteins between Cr(VI) treated bacteria and control groups (all changes are more than 1.5 fold). Proteins 27-41 are virtually absent in Cr(VI) treated cells. * -indicates for those proteins which are also significantly differentially expressed in Zn(II) vs Control comparisons (see also Table 1B)*

##	Proteins	Direction of changes	Molecular/biological functions
1	Succinate--CoA ligase	↑	ATP binding, metal ion binding, succinate-CoA ligase (ADP-forming) activity
2	Peptidyl-prolyl cis-trans isomerase	↑	peptide binding, peptidyl-prolyl cis-trans isomerase activity, unfolded protein binding (chaperone function)
3	2'-5' RNA ligase	↑	Ligase activity
4	Phage shock protein A (PspA) family protein	↑	Not given
5	Alanine dehydrogenase	↑	alanine dehydrogenase activity
6	Probable xylitol oxidase	↑	D-arabinono-1,4-lactone oxidase activity, flavin adenine dinucleotide binding
7	UncharacterizedN-acetyltransferase BWQ92_22005	↑	Proton donor and acceptor
8	50S ribosomal protein L25-	↑ *	Structural constituent of ribosome, 5s rna binding-participates In translation

9	Cysteine synthase	↑ *	Cysteine synthase activity
10	DNAstarvation/stationary phase protection protein	↑ *	Ferric ion binding, oxidoreductase activity, oxidizing metal ions, cellular ion homeostasis, response to stress
11	Chorismate mutase OS=Arthrobacter sp	↑ *	Prephenate dehydratase (pheA) L-phenylalanine biosynthetic process
12	GntR family transcriptional regulator	↑ *	catalytic activity, DNA binding, DNA binding transcription factor activity, pyridoxal phosphate binding
13	Response regulator receiver protein	↑ *	phosphorelay signal transduction system, regulation of transcription, DNA-templated, protein-glutamate methylesterase activity, chemotaxis
14	Putative periplasmic membrane protein	↑ *	metalloendopeptidase activity, zinc ion binding, chaperone-mediated protein folding
15	LSU ribosomal protein L25P	↑ *	Ribonucleoprotein, Ribosomal protein
16	Uncharacterized protein	↑ *	ATP-ase activity, transmembrane transport, Lipase activity, lipid metabolic process, outer membrane component,
17	Avirulence D protein (AvrD)	↑ *	Non predicted
18	Chlorite dismutase	↓	Non predicted
19	Oxidoreductase(electrons transfer from donors to acceptors)	↓	choline: oxygen 1-oxidoreductase activity
20	ABC transporter substrate-binding protein	↓	ATP-ase activity, ATP binding, trans-membrane transporter activity
21	Ketol-acid reductoisomerase (NADP(+))	↓	Ketol-acid reductoisomerase activity, metal ion binding, NADP binding, isoleucine biosynthetic process, valine biosynthetic process, Branched-Chain Amino acid biosynthesis, Mg-binding
22	Sugar ABC transporter substrate-binding protein	↓	ATP-binding cassette (ABC) transporter complex
23	Amino acid ABC transporter substrate-binding protein, PAAT family	↓	Ionotropic glutamate receptor activity, nitrogen compound transport,
24	Nicotinate phosphoribosyltransferase	↓	Transferase activity, transferring glycosyl groups, nucleoside metabolic process

23	Amino acid ABC transporter substrate-binding protein, PAAT family	↓	Ionotropic glutamate receptor activity, nitrogen compound transport,
24	Nicotinate phosphoribosyltransferase	↓	Transferase activity, transferring glycosyl groups, nucleoside metabolic process
25	MarR family transcriptional regulator	↓	DNA-binding, DNA- binding transcription factor activity, transcription DNA templated
26	Elongation factor G	↓	GTP-ase activity, GTP binding
27	Putative tricarboxylic transport membrane protein	↓	Integral component of membrane, outer membrane- bounded periplasmic space
28	SPG23_c14, whole genome shotgun sequence	↓	DNA binding, phosphoreley signal transduction system, regulation of transcription, DNA-templated,
29	tRNA pseudouridine synthase A	↓	tRNA processing, Isomerase, RNA binding, tRNA pseudouridine synthase activity,
30	Putative tricarboxylic transport membrane protein	↓	Integral component of membrane, outer membrane-bounded periplasmic space
31	C4-dicarboxylate ABC transporter substrate-binding protein	↓	ATP-binding, sequence specific DNA binding, transcription factor binding, phosphoreley signal transduction system, regulation of transcription, DNA-templated,
32	Citrate synthase	↓	Citrate (Si)-synthase activity, tricarboxilic acid cycle
33	Carbohydrate ABC transporter substrate-binding protein, CUT1 family	↓	Integral component of membrane
34	Transaldolase	↓	Sedoheptulose-7-phosphate: D-glyceraldehyde-3-phosphate glyceronetransferaze activity, carbohydrate metabolic process, penthose phosphate shunt
35	ATP synthase subunit beta	↓	ATP binding, proton-transporting ATP synthase activity, rotational mechanism, ATP synthesis coupled proton transport
36	ATP-binding transport protein NatA	↓	ATP ase activity, ATP binding
37	Type I restriction-modification enzyme subunit S	↓	Not given

38	Methionine aminopeptidase	↓	DNA binding, metal ion (divalent metal cations) binding, metalloaminopeptidase activity, positive regulation of transcription, DNA template, protein initiator methionine removal, regulation of DNA replication,
39	ATP synthase gamma chain	↓	ATP binding, proton transporting ATP synthase activity,
40	Signal transduction protein	↓	ATP binding, hydrolase activity, phosphoreley sensor kinase activity, integral component of plasma membrane
41	Nitroimidazol reductase NimA,	↓	cofactor binding

Table 1 B. *The list of statistically significantly expressed proteins between Zn(II) treated bacteria and control group (all changes are more than 1.5 fold)*

##	Proteins	Direction of changes	Molecular/biological functions
1	50S ribosomal protein L25-	↑ *	Structural constituent of ribosome, 5s rna binding-participates In translation
2	Cysteine synthase	↑ *	Cysteine synthase activity
3	DNAstarvation/stationary phase protection protein	↑ *	Ferric ion binding, oxidoreductase activity, oxidizing metal ions, cellular ion homeostasis, response to stress
4	Chorismate mutase OS=Arthrobacter sp	↑ *	Prephenate dehydratase (pheA) L-phenylalanine biosynthetic process
5	GntR family transcriptional regulator	↑ *	catalytic activity, DNA binding, DNA binding transcription factor activity, pyridoxal phosphate binding
6	Response regulator receiver protein	↑ *	phosphorelay signal transduction system, regulation of transcription, DNA-templated, protein-glutamate methylesterase activity, chemotaxis
7	Putative periplasmic membrane protein	↑ *	metalloendopeptidase activity, zinc ion binding, chaperone-mediated protein folding
8	LSU ribosomal protein L25P	↑ *	Ribonucleoprotein, Ribosomal protein
9	Uncharacterized protein	↑ *	ATP-ase activity, transmembrane tranbsport, Lipase activity, lipid metabolic process, outer membrane component,
10	Avirulence D protein (AvrD)	↑ *	Non predicted

11	Ribonucleoside-diphosphate reductase subunit beta	↑	metal ion binding, ribonucleoside-diphosphate reductase activity, thioredoxin disulfide as acceptor, Binds 2 iron ions per subunit
12	Acylpyruvate hydrolase	↑	hydrolase activity
13	Zn-dependent hydrolase	↑	metal ion binding, metalloendopeptidase activity, ribonuclease P activity
14	Succinyl-CoA ligase [ADP-forming] subunit alpha	↓	ATP binding,metal ion binding, succinate-CoA ligase (ADP-forming) activity, ATP + succinate + CoA = ADP + phosphate + succinyl-CoA. Binds 1 Mg^{2+} ion per subunit
15	Short-chain dehydrogenase/reductase SDR	↓	oxidoreductase activity aspartic-type endopeptidase activity
16	Superoxide dismutase	↓	metal ion binding, superoxide dismutase activity,Binds 1 Fe cation per subunit, 2 superoxide + 2 H^+ = O_2 + H_2O_2
17	ABC transporter permease	↓	antibiotic transmembrane transporter activity, ATPase activity,ATP binding, efflux transmembrane transporter activity, Confers resistance against macrolides
18	Ribosome-recycling factor	↓	translation, translational termination, Responsible for the release of ribosomes from messenger RNA at the termination of protein biosynthesis
19	LacI family transcriptional regulator	↓	alanine racemase activity, DNA binding, regulation of transcription, DNA-templated, transcription, DNA-template
20	ATP-dependent DNA helicase RecG	↓	ATP binding, ATP-dependent DNA helicase activity, nucleic acid binding, DNA recombination, DNA repair
21	D-isomer specific 2-hydroxyacid dehydrogenase, NAD-binding protein	↓	4-phosphoerythronate dehydrogenase activity, NAD binding, protein dimerization activity
22	LysR family transcriptional regulator	↓	DNA binding, DNA binding transcription factor activity
23	Alpha-hydroxy-acid oxidizing enzyme	↓	(R-pantolactone dehydrogenase (flavin) activity, FMN binding, oxydoreductase, Flavoprotein, FMN,
24	Uncharacterized lipoprotein YufN	↓	Component of cellular membrane,

Bacterial defensive mechanisms against high Cr(VI) concentrations, are shown in different ways: a) Chromate efflux by the ChrA transporter, which is the membrane potential driven energy-dependent process; b) Chromate reduction to Cr(III) carried out by chromate reductases, enzymes belonging to the widespread NAD(P)H-dependent flavoprotein family; c) Other mechanisms of bacterial resistance to chromate involve the expression of components of the machinery for repair of DNA damage, and systems related to the homeostasis of iron and sulfur [14].

Zinc is an essential trace element for most organisms; Zn^{2+} ion performs numerous structural, regulatory, and catalytic roles in a range of proteins. However, this nutrient can neither be synthesized nor degraded and individual cells need to be able to maintain steady levels of Zinc in the face of near-zero or excessively high environmental concentrations [15]. It is becoming increasingly apparent that the ability of commensal organisms to adapt to the host environment depends upon the ability to withstand large fluxes in Zinc availability that are produced by the host [15, 11]. Zn in bacteria is primarily used as a metalloenzyme cofactor with a total concentration in the 0.1–1.0 мм range [16, 17, 18]. Zn is incorporated into about 10% of all human proteins, and well over 300 enzymes are known to require Zn(II) for catalytic or structural functions [19, 20]. Metallic homeostasis of the cell, should be defended according the special metal sensors, which should correctly distinguish between the inorganic elements [21]. These metal sensor proteins transcriptionally regulate the expression of genes that encode metal transporters and other resistance proteins [22, 23, 24]. Almost half of all enzymes must associate with a particular metal to function [21]. Zinc fluxes are involved in regulating a wide variety of cellular functions, including host immune activation [25]. In most bacteria, the zinc uptake repressor (Zur) controls the expression of a small number of genes required to adapt to conditions of severe zinc depletion [20]. When the intracellular Zinc concentration is far below a critical threshold, $[metal]_{free} < 1/K_{metal}$, the zinc-free form of Zur has low affinity for the DNA operator, which overlaps the promoter, thus allowing unfettered access by RNA polymerase to transcribe the genes encoding a high affinity Zn uptake system(s) [20]. In addition, genes encoding the efflux system are transcriptionally repressed by the apo form of the Zn efflux repressor, ZntR in Escherichia coli, under these conditions. As levels of bioavailable Zn rise to $[metal]_{free} > 1/K_{metal}$ (Zinc-replete conditions), the Zinc-bound form of Zur binds tightly to the operator site, preventing transcription [15].

AAS Analyses

AAS measurements have revealed that portion of Zinc and Chromium, which are accumulated by *A.globiformis* 151B cells, are bound to bacterial proteins. These experiments indicated two different behavior tendency between Chromium and Zinc in the proteome of *Arthrobacter globiformis 151 B.* Concentration of Zinc in bacterial proteins is increasing after time and reaches maximal level after 120 hour growth, when bacterial medium contains 40 mg/l Zn(II). (Fig. 3 A). But behavior of Chromium in proteome quite differs from Zinc's: Chromium content in proteins is decreasing after time. If it was maximal at 35 hour of bacterial growth phase, after 120 hour, Chromium content in proteome is decreased significantly (Fig 3 B).

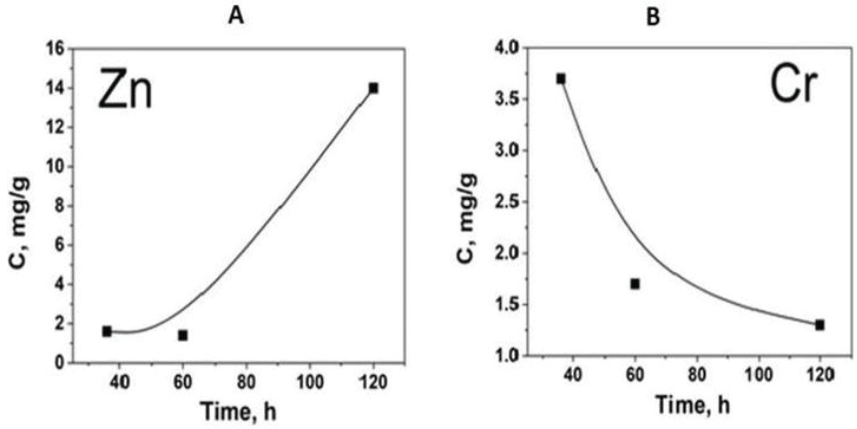

Fig. 3. *The dependence of the concentration of Zn(II) and Cr (mg/g) in proteins from the time T (h) of cultivation of bacteria Arthrobacter globiformis 151 B.*

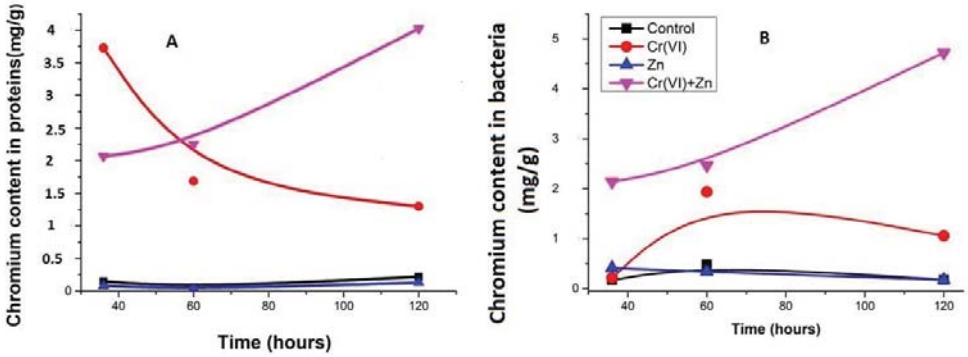

Fig. 4. *Chromium accumulation capacity in proteins (A) and in the whole bacteria itself (B). Influence of Zinc(II) ions on Chromium accumulation capacity in bacterial proteins (A) and in bacteria (B).*

In this experiment we examined Zn(II) and Cr(VI) joint action on the chromium accumulation capacity, on both: protein or bacterial levels. Samples were made according to abovementioned design: same concentration of Cr(VI) and Zn(II) were added, but together (joint). Experiment reveal, that Zinc(II) ions, on given concentration (40 mg/l), enhances bacterial ability for Chromium accumulation Fig. 4 B. Zinc also increases Chromium content in bacterial proteins (reduced Chromium-protein binding) Fig. 3A. and the concentration of proteins with bound chromium-increased after time (for 120 hour). Zinc action enhances Chromium accumulation in bacteria by two metals simultaneous presence. Without Zinc, chromium content in proteome tends to decrease after time. And maximal content is observed at early stage of cultivation-36 hour. For 120 hour Cr content in proteins is significantly decreased.

Conclusion

Remarkable changes in bacterial proteome were associated with metal-binding proteins and proteins involved in active transport. Experiments with atomic absorption spectroscopy revealed that reduced chromium appears mostly associated with organics: especially with bacterial proteins. Zinc(II) ions enhances bacterial ability for Chromium accumulation. Zinc(II) also increases reduced Chromium-protein connections and their content increases after time (for 120 hour).

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Conflicts of Interest

None declared.

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