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Study on the protective role of selenium against cadmium toxicity in lactic acid bacteria: An advanced application of ICP-MS

Iris Liliana Caballero Araúz [a], Scott Afton [b], Kazimierz Wrobel [a,d], Joseph A. Caruso [b,d], J. Felix Gutiérrez Corona [c], Katarzyna Wrobel [a,d,*]

[a] *Instituto de Investigaciones Científicas, Universidad de Guanajuato, L. de Retana No. 5, 36000 Guanajuato, Mexico*
[b] *Department of Chemistry, University of Cincinnati, Cincinnati, OH 45221-0172, USA*
[c] *Instituto de Investigación en Biología Experimental, Facultad de Química, Universidad de Guanajuato, L de Retana No. 5, 36000 Guanajuato, Mexico*
[d] *The University of Cincinnati Metallomics Center of the Ameritas, University of Cincinnati, Cincinnati, OH 45221, USA*

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Abstract

In this work, *Lactobacillus casei rhamnosus* were obtained from the commercial product of fermented milk and possible antagonistic effect of selenium (as sodium selenite) against cadmium toxicity was studied. The bacteria capability to incorporate Se was demonstrated: after 1 week exposure to Se(IV), its total concentration in the freeze-dried biomass was 405 ± 28 μg/g (7.4 ± 0.8 μg/g in control). In the presence of Se(IV) and Cd(II), the bacterial growth and cell viability were improved and lipid peroxidation less marked with respect to bacteria exposed to Cd(II) alone. The distribution of Se and Cd in molecular mass fractions of bacteria extracts was investigated by size exclusion chromatography with diode array and ICP-MS detection. The results obtained suggest that the antagonistic effect of Se is due to lower incorporation of cadmium at a high molecular mass (MM < 600 kDa). Slightly different distribution of elements in the fractions of MM < 40 kDa suggests the formation of new chemical species involving Cd and Se in bacteria exposed to Cd(II) + Se(IV) as compared to those exposed to Cd(II) alone. The study illustrates the high utility of atomic spectrometry to critically inform molecular questions that could be important in the industrial processes based on bacterial activity.

Keywords: Cadmium toxicity; Selenium; Antagonism; *Lactobacillus*; ICP-MS

1. Introduction

The variety of lactic acid bacteria have been widely used in the production of fermented foods and in different industrial processes (mainly production of sugars) [1,2]. A number of studies has aimed the improvement of the industrial and probiotic strains, yet the prevention of heavy metal poisoning has rarely been considered [3–5].

In the living organisms, a metal-induced toxicity has been associated with oxidative damage [6–8]. The role of both, endogenous and exogenous antioxidants in alleviating harmful effects associated with heavy metals exposure has been demonstrated in several experimental systems [9–12]. Within this context, selenium has been classified as an antioxidant and/or a heavy metal antagonist [13]. The protective effect of selenium against mercury toxicity in different biological systems appears to have been the most studied [14–19]. Beneficial effects of selenium in exposure to cadmium and silver have also been observed and several studies focused the elucidation of mechanisms involved [17,20–23] although a full mechanistic explanation has not been realized. In humans, cadmium was shown to disrupt the antioxidant enzyme system, resulting in increased production of oxygen radicals, which causes damage to membranous structures such as mitochondria and the endoplasmic reticulum [21]. It is believed though, that the antioxidant properties of selenium compounds could be involved in the protection mechanism [20].

Better understanding of the processes occurring in living organism requires the use of different analytical methods,

* Corresponding author at: Instituto de Investigaciones Científicas, Universidad de Guanajuato, L. de Retana No. 5, 36000 Guanajuato, Mexico.
Tel.: +52 473 7327555; fax: +52 473 7326252.
E-mail address: katarzyn@quijote.ugto.mx (K. Wrobel).

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preferably based on different physicochemical principles and providing complementary information on the system under study [24]. Historically, the effects of exogenous xenobiotics have been studied by measuring the biological and/or physicochemical parameters that had changed under exposure of the organism. The current analytical approach is much more direct, since it involves the characterization of metallic (or metalloid) chemical species generated under exposure conditions. The combination of suitable pretreatment procedures and separation techniques with element specific detection has proven to be a powerful analytical tool [25,26]. In particular, size exclusion chromatography with detection by inductively coupled plasma mass spectrometry (SEC–ICP-MS) has provided important preliminary information on the pattern of element binding to different molecular mass fractions in biological samples [27–30]. Owing to the multielemental capabilities of ICP-MS, several elements can be monitored simultaneously and the results obtained may suggest possible interactions among these elements [28,31,32]. This technique was used to assess the localization and speciation of selenium and heavy metals in biological extracts [17,18].

Since the capability of lactic bacteria to concentrate selenium from the growth medium had been reported [33], in this work, the effect of this element (as sodium selenite) in *Lactobacillus casei rhamnosus* exposed to Cd(II) was studied. Simple spectrophotometric assays were used to evaluate and compare the effect of bacterial exposure to Cd(II) with that observed in the exposure to Cd(II) + Se(IV). To gain insight into possible interactions between selenium and cadmium in bacteria at molecular levels, the distribution of two elements in molecular mass fractions was investigated by size exclusion chromatography with UV and ICP-MS detection. The results obtained provide consistent evidence of the antagonistic role of selenium against cadmium toxicity in lactic acid bacteria. This application further demonstrates the utility of atomic mass spectrometry as a means of gaining important information on molecular level problems.

2. Materials and methods

2.1. Apparatus

Total selenium concentrations were determined using a model 3110 Perkin-Elmer atomic absorption spectrometer with HGA600 graphite furnace and AS 60 autosampler. The hollow cathode lamp for Se was a Perkin-Elmer Lumina lamp. The instrumental parameters were as follows: spectral line 196.0 nm; slit width 0.7 nm; background correction with deuterium lamp; platform atomization; and peak area measurements. The heating program applied was as follows: (1) drying (130 °C, 25 s ramp, 15 s hold), (2) pyrolysis (1100 °C, 10 s ramp, 10 s hold), (3) cooling step (20 °C, 1 s ramp, 15 s hold) and (4) atomization (2100 °C, maximum power heating, 4 s hold) and cleaning (2700 °C, 1 s ramp, 3 s hold). Matrix modifier was used (2 μg of Pd + 5 μg of Mg, 5 μL), and the volume of sample = 20 μL.

For UV–vis spectrophotometric assays, a Spectronic 3000 Diode Array Milton Roy spectrophotometer was used (resolution 0.35 nm) with User Data 2.1 software for spectral data acquisition, storage and manipulation (Milton Roy Inst. Co.). All data treatment operations were carried out using the GRAMS/386 TM software package, version 3.01A (Galactic Ind. Co., Salem, MA).

Chromatographic separations were accomplished with an Agilent 1100 liquid chromatograph (Agilent Technologies; Palo Alto CA, USA) equipped with a binary HPLC pump, an autosampler, a vacuum de-gasser system, a thermostated column compartment, and a diode array detector. The chromatographic column used was a Superdex 200 10/300 GL SEC (10 mm × 300 mm × 13 μm) (Amersham Pharmacia Biotech AB, Uppsala, Sweden). The HPLC conditions were as follows: 50 mM Tris–HCl (pH 7.5) as a mobile phase; elution at a flow rate 0.65 mL/min; injection volume 100 μL; diode array detection 280 nm.

The inductively coupled plasma mass spectrometer used for specific element detection was an Agilent 7500ce (Agilent Technologies, Tokyo, Japan). The ICP-MS was equipped with a conventional Meinhard nebulizer, a Peltier-cooled spray chamber (2 °C), and an octopole collision/reaction cell with hydrogen gas pressurization (purity of 99.999%). The ICP-MS conditions were as follows: forward power 1500 W; plasma gas flow 15.0 L/min; internal flow 1.0 L/min; carrier gas flow 1.1 L/min; collision gas 3.5 mL/min; quadrupole bias −15.0 V; octopole bias −18.0 V. The isotopes monitored were ^{77}Se, ^{78}Se, ^{80}Se, ^{111}Cd, ^{112}Cd, ^{114}Cd, with a dwell time of 100 ms per isotope.

2.2. Reagents and samples

All chemicals were of analytical reagent grade, and deionized water (18.2 MΩ cm) was used throughout (Labconco). For atomic absorption spectrometry, a stock standard solution containing 1000 mg/L of selenium was purchased from Sigma. Working solution corresponding to the highest calibration standard (100 μg/L Se) was prepared daily by appropriate dilution of the stock solution with 0.2 M nitric acid. The solutions of matrix modifiers, $Pd(NO_3)_2$ and $Mg(NO_3)_2$, 10,000 mg/L, were from Perkin-Elmer.

The liquid growth medium was prepared by dissolving 5.5 g of yeast extract, 12.5 g of peptone (from casein), 11 g of dextrose, 1 g of $MgSO_4 \cdot 7H_2O$, 0.25 g of KH_2PO_4, 0.25 g K_2HPO_4, 10 g CH_3COONa and 5 mg of $Fe_2(SO_4)_3 \cdot 7H_2O$ in 1 L of deionized water (pH 6.8 ± 0.1). When required, the medium was solidified with 20 g/L of bacteriological agar. Sterilization was carried out in an autoclave (121 °C, 15 min). The solutions of sodium selenite (1.0 mg/L Se), cadmium chloride (100–800 μgCd/L) or Se(IV) together with Cd(II) were added to the liquid growth medium. These solutions were sterilized by filtration (0.22 μm). The reagents were from Sigma.

To test cell viability, the following Sigma reagents were used: 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), hydrochloric acid and isopropanol. The solution

1 mgMTT/mL was prepared in the liquid growth medium and 40 mM HCl was in isopropanol.

For the assay of malondialdehyde (MDA), the MDA precursor (1,1,3,3-tetraethoxypropane), thiobarbituric acid (TBA), acetic acid and ethyl acetate were from Sigma. Erioglaucine A and aliquat 336 were from J.T. Baker Chemicals Co. and Aldrich, respectively. The stock solution of MDA (5 mM) was prepared as described by Fenaille et al. [34]. The solutions of TBA (0.6% in 4 M acetic acid, pH 2.5), 6.25×10^{-4}% Erioglaucine A, 0.06% aliquat 336 in ethyl acetate were also prepared.

Sodium dodecylsulfate (SDS) from Sigma and Tris (hydroxymethyl) aminomethane hydrochloride (Tris–HCl) from Fisher were used in extracts from the bacteria.

Calibration of the SEC column was performed using a mixture of the following molecular mass standards: thyroglobulin 669 kDa; alcohol dehydrogenase 150 kDa; carbonic anhydrase 30 kDa; and vitamin B_{12} 1.3 kDa.

A Multi-Block heater from Barnstead/Lab-line was used for the acid digestion of samples.

The bacterial culture was obtained from the commercial beverage based on fermented milk. According to the nutritional information given by the producer, the bacterial strain was *L. casei rhamnosus*.

2.3. Bacteria growth

For the isolation of microorganism, the fermented milk beverage was diluted 1:10 with the liquid growth medium and an aliquot (350 μL) was inoculated onto the solid growth medium without Se(IV), or Cd(II) (Petri dishes, 37 °C, 24 h). One isolated colony was streaked in fresh solid medium and incubated in these same conditions to obtain single colonies; one colony was streaked again in fresh solid medium and the procedure was repeated twice, in order to homogenize the cell population so that all the cells in it are derived from a single cell. The pre-inoculum for the cultures in liquid medium was obtained by transferring a colony to the liquid medium (5 mL) and incubating at 37 °C for 24 h.

To obtain the bacteria fortified with selenium, 350 μL of culture was transferred to 50 mL of liquid medium containing 1.5 mg/L Se (as sodium selenite). After incubation (37 °C, 24 h), the bacteria were filtered, washed by centrifugation (9000 × *g*, 5 min) and suspended in a fresh medium (50 mL) containing 2 mg/L Se. Under these conditions, the bacteria were grown during 4 days, changing the medium each 24 h. Finally, the concentration of sodium selenite was increased to 2.5 mg/L Se and the samples were incubated 2 days (the fresh medium each 24 h).

For the experiments on the effect of selenium on cadmium toxicity, the aliquots (350 μL) of pre-inoculum were transferred to a series of tubes containing 5 mL of liquid medium and different concentrations of Se(IV), Cd(II) or both. The tubes with cells were incubated at 37 °C for up to 10 h. During the growth, several samples were taken at different times from inoculation. The set of cultures obtained were as follows: (1) blank, not containing either element; (2) bacteria exposed to Cd(II) (100, 400 and 800 μgCd/L in medium); (3) bacteria exposed to Se(IV) (1 mg/L Se in medium); (4) bacteria exposed to Se(IV) and Cd(II) at the concentrations used for individual element.

2.4. Spectrophotometric measurements

The growth of bacteria was evaluated by measuring the absorbance (at 600 nm) of cultures at different times from inoculation.

The MTT assay was carried out as described elsewhere [35,36]. In brief, the 250 μL aliquots of cultures (1)–(4) were taken at 2.5 h from the inoculation and mixed with 200 μL of MTT solution (Eppendorf tubes). The samples were kept at 37 °C for 4 h and 400 μL of hydrochloric acid in isopropanol were added. After agitation (vortex 2 min), they were left for 10 min to complete the reaction, centrifuged (2000 × *g*, 10 min) and the absorbance of supernatant was measured at 570 nm against the blank (this same sample, but without MTT).

A micro-spectrophotometric assay for malondialdehyde, previously developed in our laboratory was applied [37]. This procedure is based on the formation of MDA–TBA adduct with the use of Eriogaucine A as internal standard (IS) to enhance the analytical performance. The procedure was as follows: at 2.5 h from inoculation the aliquots (20 μL) of the cultures were taken and mixed with 200 μL of TBA and 200 μL of IS. The samples were heated (95 °C, 45 min), cooled and the extraction was carried out with 40 μL of aliquot 336 (0.06%) in 300 μL of ethyl acetate. Once centrifuged (2000 × *g*, 10 min), the absorption spectra of the organic phase was taken (450–700 nm) against aliquot 336 in ethyl acetate. The spectra were smoothed (21 experimental points) and the first derivative spectra were obtained ($\Delta\lambda = 9$ nm) using the Savitzky–Golay algorithm [38]. The analytical signal was defined as the ratio between first derivative absorbances at 543.1 (MDA–TBA adduct) and 644.4 nm (IS). MDA quantification was accomplished by external calibration (0–5 μM MDA).

For each spectrophotometric measurement, three sample aliquots were taken and the mean result with respective relative standard deviation were evaluated.

2.5. Analysis of selenium and cadmium

For these analyses, each culture was obtained in triplicate and, after filtering (Whatman No 1 paper) and washing with deionized water, it was pooled. The biomass obtained in each case was freeze-dried. The samples were placed in liquid nitrogen and cell homogenization was performed by mortar grinding.

For total Se determination in Se-fortified bacteria, the freeze-dried and homogenized biomass (100–200 mg) was placed in a glass tube, and 1 mL of concentrated nitric acid was added (three replicates). The tubes were heated in the stainless steel heating block: at 65 °C for 60 min and then at 120–140 °C for 60 min. Once cooled to room temperature, 0.2 mL of hydrogen peroxide was added to each tube, and the mixture was left for 30 min to complete reaction. The final volume was brought to 10 mL, and Se was determined by ETAAS [39].

For SEC–UV–ICP-MS analysis, the following procedure was carried out: 15 mg of freeze-dried and homogenized biomass

was extracted with 750 μL of 5% SDS in Tris–HCl (50 mM, pH 7.5) by vortexing (5 min) and, then ultrasonication (30 min). After centrifugation (2000 × *g*, 15 min), the supernatant was filtered (0.22 μm) and injected onto the SEC–UV–ICP-MS for analysis.

3. Results

Several sources of lactic bacteria were used, including laboratory strain and pharmaceutical formulation of *Lactobacillus acidophilus* as well as six different commercial products based on fermented milk. In each case, different growth media were examined, varying the source of peptone (vegetal, animal or casein), the composition and concentrations of inorganic salts and pH. In these experiments, the characteristic red color of elemental selenium appeared after inoculation of bacteria except the culture of *L. casei rhamnosus*, which was selected for further experiments. It should be stressed that the reduction of selenite to Se^0 had not been caused by any of growth media tested and this effect was observed only in the presence of bacteria. To investigate the incorporation of selenium to bacteria, the concentration of sodium selenite in medium had been gradually increased from 1.5 to 2.5 mg/L Se over 1 week thus minimizing the growth inhibition observed also by other authors [40–42]. Total selenium determined in the selenized bacteria was 405 ± 28 μgSe/g (freeze-dried biomass) and in the control culture 7.4 ± 0.8 μgSe/g.

Using the same growth conditions as above, the tolerance of bacteria to increasing concentrations of Cd(II) in medium was examined and the total growth inhibition was observed at concentration 1 mg/L Cd. As described in the Procedures, several cultures were then obtained, in the presence of Cd(II) (100, 250, 400, 600 and 800 μgCd/L), in the presence of Cd(II) and Se(IV) (1 mg/L Se), in the presence of Se(IV) alone (1 mg/L Se) and one control culture without any of two elements. The aliquots were taken from cultures at different times from inoculation and the absorbance was measured (OD at 600 nm). The growth inhibition was observed in all cultures exposed to Cd(II); the effect was more pronounced at higher cadmium concentrations and between 2 and 8 h from the inoculation. Apparently, the presence of 1 mg/L Se in medium did not affect the culture growth. As an example in Fig. 1 the results obtained at two levels of Cd(II) in medium and during 8 h of growth are presented. Each absorbance value was obtained as the mean from three replicates (for three aliquots taken from the culture, the relative standard deviation was never higher than 1.5%).

The MTT test of cell viability was applied, taking the culture samples at 2.5 h from the inoculation. The cell growth at this time can be deduced from Fig. 1. Obviously, the results of MTT test were affected by the quantity of biomass present in culture, which was different depending on the exposure conditions (Fig. 1). To avoid such influence, before performing MTT test, the optical density of the culture was measured (A_{600nm}) and then, the absorbance of reduced MTT (A_{570nm}) was normalized with respect to this optical density (A_{570nm}/A_{600nm}). The results obtained are presented in Fig. 2.

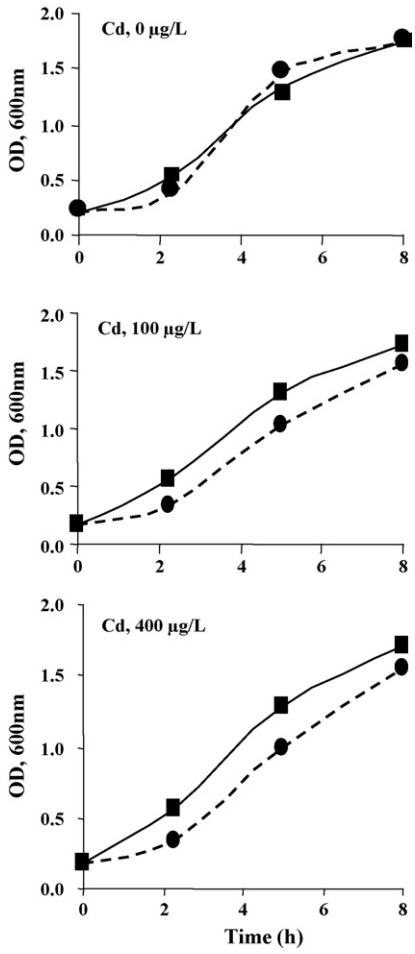

Fig. 1. Absorbance of bacteria cultures exposed to (●) different concentrations of Cd(II) and (■) Cd(II) + 1 mg/L Se(IV), during 8 h of growth (for three aliquots taken from the culture, the relative standard deviation did not exceed 1.5%).

A micro-spectrophotometric assay for malondialdehyde was used to evaluate the oxidative stress in bacteria cultures [37]. It can be observed in Fig. 3 that different MDA levels were found in cultures exposed to Cd(II) relative to those exposed to Cd(II) and Se(IV). The MDA concentration had been increas-

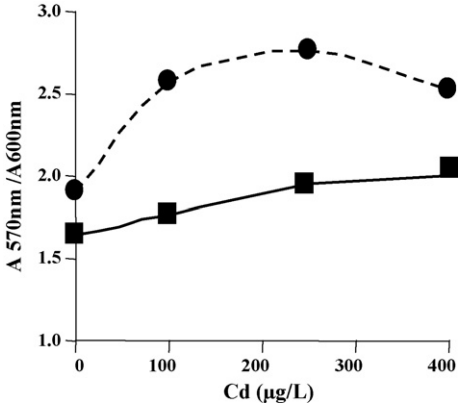

Fig. 2. Effect of the bacteria exposure to (●) Cd(II) and to (■) Cd(II) + 1 mg/L Se(IV) on their capacity for MTT reduction after 2.5 h of exposition (for three aliquots taken from the culture, the relative standard deviation did not exceed 5%).

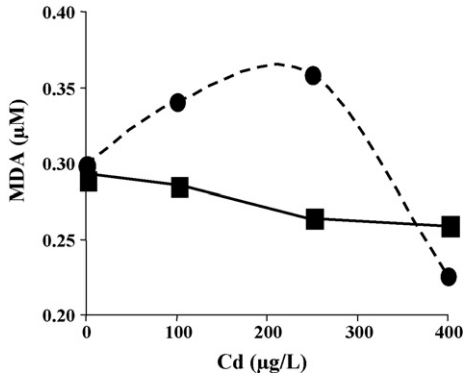

Fig. 3. The MDA levels in the bacteria cultures exposed for 2.5 h to different concentrations of cadmium: (●) Cd(II) alone and to (■) Cd(II) + 1 mg/L Se(IV) (the MDA concentration refers to the volume of culture taken for analysis. For three aliquots taken from the culture, the relative standard deviation did not exceed 5%).

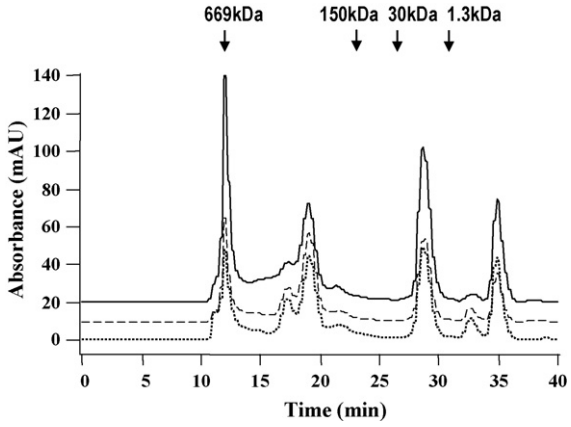

Fig. 4. Typical SEC–UV chromatograms of the bacteria extracts grown in the presence of (—) Se(IV), (· · ·) Cd(II) and (– – –) Se(IV) + Cd(II). The elution of molecular mass standards is marked with arrows (bacteria growth, extraction and instrumental conditions given in the procedures).

ing proportionally to Cd(II) concentration in the growth medium (up to 250 μg/L Cd). At higher cadmium levels in medium, MDA decreased. Apparently, the production of MDA in bacterial cells exposed to Cd(II) was higher as compared to the cultures obtained in the presence of Cd(II) and Se(IV) (Fig. 3). The increase of MDA level in the presence of Cd(II) was only about 20% with respect to the control. However, at this concentration level, the variance coefficient for the micro-spectrophotometric assay was shown to be lower than 5% (due to the use of internal standard) [37].

The distribution of cadmium and selenium in molecular mass fractions, obtained from SDS–Tris/HCl extracts, was investigated by size exclusion chromatography. Diode array spectrophotometric detection provided the elution profile of organic compounds absorbing at 280 nm and ICP-MS detection was used to obtain the elution profiles of cadmium and selenium. For these experiments, the SDS–Tris/HCl extracts were obtained from the freeze-dried and homogenized biomass of the following cultures: (i) bacteria grown in the presence of Se(IV) (1 mg/L Se); (ii) bacteria exposed to Cd(II) (400 μg/L Cd in medium); (iii) bacteria grown in the presence of both elements (growth time 5 h). Typical chromatograms obtained with diode array detection are presented in Fig. 4 (for better comparison of the graphs, they have been offset on *y*-axis). Four molecular mass fractions can be observed: first eluting at a void volume (MM < 600 kDa), second at the MM > 150 kDa, third at the MM < 30 kDa and fourth in the region of low molecular mass (MM > 1.3 kDa). In Fig. 5a, the SEC–ICP-MS chromatograms of cadmium are given. In the bacterial extracts exposed to Cd(II) and to Cd(II) + Se(VI), the bulk elution of cadmium occurred at the void volume, indicating element binding to SDS-soluble compounds of high molecular mass. It can be observed that, in the presence of selenium, the incorporation of cadmium to this MM fraction decreased significantly. On the other hand, the formation of two minor and lower molecular mass fractions containing cadmium can be observed in the extract from bacteria grown in Cd(II) + Se(IV) (between 18 and 23 min). The SEC–ICP-MS chromatograms for ^{78}Se are shown in Fig. 5b. The broad peak for selenium elution in the region of MM > 150 kDa and two minor selenium fractions in the low molecular mass range can be observed. Similar elution profile of selenium was obtained in bacterial extract exposed to Cd(II) + Se(IV). However, the abundance of selenium in high molecular mass fraction was significantly lower and the contribution of selenium in the low molecular mass region increased as compared to bacteria exposed to Se(IV) alone (Fig. 5b). It should be noted that

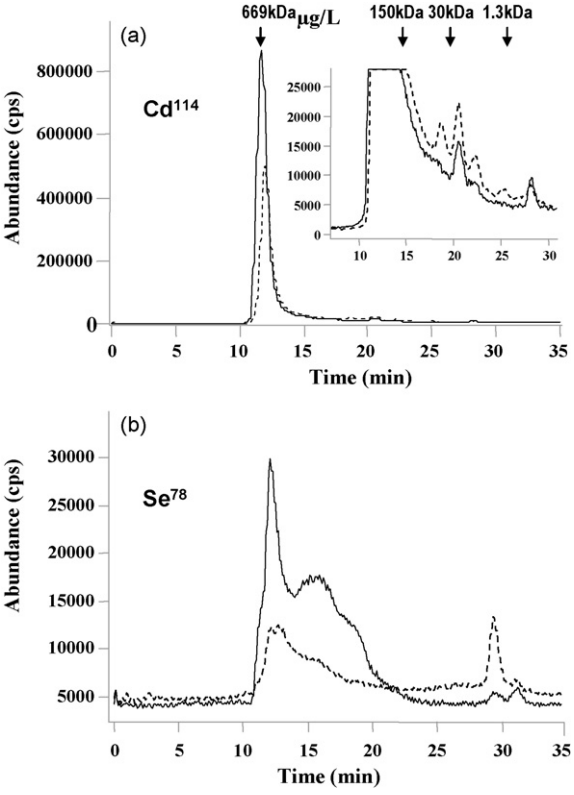

Fig. 5. Typical SEC–ICP-MS chromatograms of the bacteria extracts: (a) (—) bacteria grown in the presence of Cd(II); (– – –) bacteria grown in the presence of Cd(II) + Se(IV) (^{114}Cd monitored). (b) (—) Bacteria grown in the presence of Se(IV); (– – –) bacteria grown in the presence of Cd(II) + Se(IV) (^{78}Se monitored). Bacteria growth extraction and instrumental conditions given in the procedures.

bacteria exposed only to Cd(II) contained minimum selenium levels.

While comparing the elution profiles of cadmium and selenium obtained by SEC–ICP-MS, slightly different distribution of these elements can be observed in the fractions of MM < 40 kDa, depending on the exposure conditions. In particular, the elution profiles of two elements after 18 min of chromatograms were different, while for bacteria exposed to Cd(II) as referred to those exposed to Cd(II) + Se(IV) (Fig. 5). However, any co-elution (possibly indicating the formation of species containing both elements) was not found.

4. Discussion

Even though the capacity of lactic bacteria to incorporate selenium from the growth medium had already been reported [33], our preliminary results were not optimistic. The principal difficulty observed was the massive reduction of sodium selenite in bacteria cultures, which seems to be in agreement with the high reduction potential of some lactobacilli [43]. As the result of systematic experiments that involved different bacteria sources and different growth conditions, the culture of *L. casei rhamnosus* obtained from a commercial beverage was selected for the study. Total selenium determination in the bacteria exposed to sodium selenite and in control bacteria confirmed the incorporation of element to bacteria observed by other authors [33].

Once the capability of lactic bacteria to incorporate selenium from the growth medium was demonstrated, these same experimental conditions were used to investigate the effect of selenium on cadmium toxicity. As shown by absorbance measurements (Fig. 1), a better growth was observed in the presence of both elements, as compared to bacteria exposed only to Cd(II), which suggest the protective role of selenium.

To obtain more direct evidence of any selenium effect, the MTT test of cell viability was applied. In principle, the reduction of MTT has been ascribed to the cell respiration processes [35,36]. Thus, the concentration of the reduced form of reagent (absorbance at 570 nm) is supposed to be directly proportional to the number of viable cells in the sample. Interestingly, the bacteria exposed to Cd(II) showed better capacity for MTT reduction as compared to bacteria exposed to cadmium and selenium (Fig. 2), contradictory to the effect expected. However, in various biological systems, enhanced lipid peroxidation has been proposed as the primary mechanism for cadmium toxicity [13,44,45]. If so, reactive oxygen species generated in the oxidative stress would contribute to the reduction of MTT and this effect has already been reported [46]. It was assumed though that the increased reduction capacity of bacteria observed at Cd(II) concentrations up to 250 μg/L Cd, had been due to the increased oxidative stress. At higher concentrations of Cd(II), the reduction capacity decreased because of the growth inhibition. As to the results obtained for the cultures grown in the presence of Se(IV) and Cd(II), less marked differences between $A_{(570nm)}/A_{(600nm)}$ was observed over the entire range of Cd(II) concentration, confirming the assumption on the protective (antagonistic) effect of selenium.

Malondialdehyde is a widely accepted biomarker of lipid peroxidation. The results presented in Fig. 3 confirm the increased oxidative stress in bacteria grown in the presence of Cd(II). The decay of MDA level at higher cadmium levels (over 250 μg/L Cd) likely was due to lower number of viable cells present in the cultures. Another possibility is the neutralization of lipid peroxidation products by their binding to aminoacids, which has been proposed as a defensive mechanism activated at mitochondrial level in oxidative stress [47]. The results of MDA assay in bacteria exposed to Cd(II) + Se(IV) provide further evidence on the protective role of selenium. In other words, selenium seems to act as the antagonist against cadmium toxicity, which is manifest in lactic bacteria by the growth inhibition and the increased lipid peroxidation.

To gain an insight into possible interactions between selenium and cadmium in bacteria at molecular level, the distribution of two elements in molecular mass fractions of bacteria extracts was investigated. It should be noted that the elution profiles (280 nm) were similar at different exposure conditions, indicating that none of the two elements caused significant modifications in the organic structures of bacterial extracts (Fig. 4). The SEC–ICP-MS results indicated the bulk incorporation of both, selenium and cadmium to high molecular mass, SDS-soluble compounds, independent of the exposure conditions. However, in cell exposure to Cd(II) + Se(IV), both of them were incorporated at lower rates as compared to bacteria grown in the presence of only one element. These results indicate that the protective role of selenium seems to be in terms of diminished incorporation of cadmium, in agreement with the first part of this study. In particular, if the cadmium uptake is lower, its toxic effects related with oxidative stress would be less pronounced. Our results suggest that the interaction between cadmium and selenium might occur extracellularly. It seems possible that high reduction potential of bacteria cause reduction of selenium (Se^{2-}) in the liquid medium with subsequent covalent binding of cadmium, thus hindering the uptake of both elements by bacteria. On the other hand, the difference in elution profiles of cadmium and selenium observed in the fractions of MM < 40 kDa, seems to suggest slight modifications at molecular level, depending on the exposure conditions. Further work is needed for identification of chemical species eluting after 18 min of SEC chromatograms.

5. Conclusion

Several complementary analytical procedures were applied in this work to gain an insight into the possible effect of selenium Se(IV) in cultures exposed *in vivo* to Cd(II). The experimental evidences are provided on the protective role of selenium against cadmium toxicity, manifested by the inhibition of growth and the increased lipid peroxidation. The results of size exclusion chromatography coupled to ICP-MS detection suggest that this protective effect is due to lower uptake of cadmium in the presence of selenium. Furthermore, the modification of low molecular mass structures as a result of different exposure conditions is suggested. The study illustrates the high utility of atomic spectrometry to critically inform molecular questions that could

be important in the industrial processes based on bacterial activity.

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