Journal of Functional Foods 35 (2017) 466–473

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Food prospects of selenium enriched-*Lactobacillus acidophilus* CRL 636 and *Lactobacillus reuteri* CRL 1101

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ARTICLE INFO

Article history:
Received 23 February 2017
Received in revised form 3 May 2017
Accepted 4 June 2017

Keywords:
Lactobacillus
Lactic acid bacteria
Selenium
Selenocysteine
Nanoparticles
Selenium resistance

ABSTRACT

Selenium, which is present as SeCys in selenoproteins, is involved in cancer prevention, thyroid functioning, and pathogen inhibition. Lactobacilli can biotransform inorganic Se into seleno-amino acids. Growth, Se accumulation and seleno-amino acid formation by *Lactobacillus acidophilus* CRL636 and *L. reuteri* CRL1101 in a Se-supplemented medium were studied. Moreover, survival of Se-enriched strains to different pH values and bile salts was analyzed. *L. acidophilus* CRL636 showed low growth rate in the presence of Se while differences were less evident for *L. reuteri* CRL1101, which displayed higher amounts of intracellular SeCys and SeMet than the CRL636 strain. Interestingly, both lactobacilli could produce Se-nanoparticles. Se-enriched lactobacilli showed lower growth rates than non-Se exposed cells. The adverse effect of bile salts and the ability to survive at pH 4.0 diminished for the Se-enriched *L. reuteri* strain. The studied lactobacilli could be used as Se-enriched probiotics or as a vehicle for manufacturing Se-containing fermented foods.

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1. Introduction

Selenium (Se) is an essential trace element in living systems, found in nature as selenate and selenite. These soluble inorganic Se forms are potentially toxic. Certain microbes, fungi and plants can biotransform Se into elemental Se (Se_0) nanoparticles and into organic forms such as selenocysteine (SeCys), selenomethionine (SeMet) and selenomethylselenocystine (SeMeCys) (Deng et al., 2015; Kieliszek, Błażejak, Gientka, & Bzducha-Wróbel, 2015; Palomo-Siguero, Gutiérrez, Pérez-Conde, & Madrid, 2016). Se deficiencies in human health have been associated to cancer, cardiovascular disease, diabetes, thyroid disorders and male infertility (López-Heras et al., 2014). Se human health beneficial effects are attributed to the presence of SeCys in 25 selenoproteins including thioredoxin reductase, glutathione peroxidase, iodothyronine deiodinases, selenoprotein P, selenoprotein W, and selenoprotein 15 (Palomo, Gutiérrez, Pérez-Conde, Cámara, & Madrid, 2014). SeCys is incorporated into the polypeptide chain in a UGA codon, which commonly signals a translation termination factor, when a *cis* and *trans*-acting element and protein factors decode this codon as the 21st amino acid SeCys (Papp, Lu, Holmgren, & Khanna, 2007). Selenium is incorporated in humans through the diet especially as SeMet, SeCys, selenate or selenite. SeMet can be incorporated into proteins in place of methionine, or can be transformed into SeCys via a *trans*-sulfuration pathway. In turn, SeCys is converted into hydrogen selenide, a key metabolite to SeCys insertion in proteins (Nicastro & Dunn, 2013).

Lactic acid bacteria (LAB) are commonly used as starter cultures for the production of fermented foods; some species from this large group can bind, transport, and store metal ions inside the cell. The use of these bacteria to study their interaction with different ions for their application in functional food production, nutraceuticals and toxic ion removal from drinking water has been studied in the last few years (Mrvčić, Stanzer, Sôlić, & Vesna, 2012). Regarding Se binding and absorption by LAB, several studies on Se-enriched probiotics (Kheradmand et al., 2014; Pophaly, Singh, Kumar, Tomar, & Singh, 2014; Xia, Chen, & Liang, 2007; Yazdi, Mahdavi, Setayesh, Esfandyar, & Shahverdi, 2013; Zhang et al., 2009), Se nanoparticle production (Nagy et al., 2016), Se bioremediation (Pieniz, Okeke, Andreazza, & Brandelli, 2011), and Se-enriched foods (Deng et al., 2015; Palomo et al., 2014) have been conducted. However, growth behavior of LAB in the presence of Se as well as the mechanisms involved in the biotransformation of inorganic Se into organic Se remains unclear. Indeed, publications on Se

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http://dx.doi.org/10.1016/j.jff.2017.06.009
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effect on bacterial phenotype have been conducted mainly in *Lactobacillus reuteri* and only by proteomic approaches (Galano et al., 2013; Lamberti et al., 2011; Mangiapane et al., 2014).

The aim of this work was to analyze if the strains *L. acidophilus* CRL 636 and *L. reuteri* CRL 1101 could be used for producing Se-enriched fermented foods or for being administrated as Se-enriched probiotics. In this study two objectives were followed: firstly, the ability of the strains to grow, accumulate and biotransform Se into Se amino acids and nanoparticles in the presence of selenite was studied. Secondly, the capacity of the Se-enriched strains to survive in physiological conditions such as the gastrointestinal digestion (bile salts and pH 8) and to adhere to the intestine mucosa through hydrophobicity assays was analyzed to have some insights on their possible use as Se-enriched probiotic strains. Moreover, the ability to survive at the low pH (4) values of fermented foods during storage was investigated.

2. Materials and methods

2.1. Microorganisms and growth conditions

The strains *Lactobacillus acidophilus* CRL 636 and *L. reuteri* CRL 1101 used in this work were obtained from the Culture Collection of the Centro de Referencia para Lactobacilos (CERELA), San Miguel de Tucumán, Argentina. Cultures were stored at −20 °C in de Man, Rogosa and Sharpe medium (MRS) broth with 10% (v/v) glycerol. Cells were transferred twice in fresh (MRS) before analysis, and further cultured in the absence (control) and the presence of 5 mg Se/L as Na_2SeO_3 (SIGMA-ALDRICH chemical CO, MO, USA) (Se-MRS) at 37 °C during 24 h. The amount of Se used was the minimum concentration needed to observe changes in microbial growth without causing massive death (data not shown). In addition, this Se value is similar to that used for other lactobacilli (*L. delbrueckii* subsp. *bulgaricus* and *L. reuteri*) strains (Lamberti et al., 2011; Xia et al., 2007).

Growth was monitored by determining the optical density (OD at 600 nm), plate count (cfu/mL), and pH of the cultures.

2.2. Analysis of metabolites

Glucose content, organic acids (lactic, acetic, and formic), and ethanol production were analyzed during fermentation by High Performance Liquid Chromatography (HPLC). HPLC was performed using a Knauer Smartline System HPLC (Berlin, Germany) with a Knauer Smartline RI detector fitted with a Biorad Aminex HPX-87H column (300 × 7.8 mm, Hercules, CA, USA). The operating conditions were the following: 5 mmol/L H_2SO_4 was used as eluent at a flow rate of 0.6 mL/min during 30 min, and an internal temperature of 45 °C. For the quantification of sugars and organic acids, calibration curves for each compound were performed using pure standards at different concentrations. Samples were previously deproteinized by Carrez method and desalted (Ortiz, Fornaguera, Raya, & Mozzi, 2012).

2.3. Determination of total selenium in bacterial cell pellets by ICP-MS

The bacterial cell pellets collected by centrifugation (5000g, 5 min) of 24 h cultures in the presence and absence of Se were washed twice in milliQ water and submitted to acid digestion in a 1000 W microwave oven (MSP microwave oven, CEM, Matthews, NC, USA) with 1 mL of concentrated HNO_3 and 0.5 mL of 30% (v/v) H_2O_2. The resulting solutions were diluted by adding 25 mL of deionized water. Selenium concentration was determined with an Agilent 7700-collision/reaction cell ICP-MS (Agilent Technologies, Santa Clara, CA, USA). Hydrogen gas was employed as collision gas for selenium determination. The equipment measuring conditions are listed in Table 1. A control group of each bacterial species unexposed to Se was performed in parallel.

Table 1
Operation conditions for HPLC coupled to ICP-MS.

Operating conditions	
ICPMS parameters for Se determination	
RF power (W)	1550
Plasma gas flow rate (L/min)	15.0
Ar auxiliary flow rate (L/min)	0.30
Carrier gas flow rate (L/min)	0.75
Nebulizer	Slurry
Spray chamber	Scott
Acquisition mode	Continuos
Isotopes monitored	^{76}Se, ^{77}Se, ^{78}Se, ^{80}Se
Replicates	3
Reaction gas	H_2
Reaction gas (mL H_2/min)	6
AEX chromatographic parameters	
Column	Hamilton PRP-X100 (150 mmx 4.6 mm, 10 μm)
Mobile phases	Ammonium citrate 10 mM, 2% Methanol (pH 5.0)
Mode	Isocratic
Flow rate (mL/min)	1
Injection volume μL	100
RP chromatographic parameters	
Column	Zorbax C8 (250 × 4.60 × 5 μm)
Mobile phases	2% Methanol, 0.1% Trifluoroacetic acid (pH 2.2)
Mode	Isocratic
Flow rate (mL/min)	1
Injection volume (μL)	100 μL

2.4. Determination of selenium species in bacterial cell pellets by HPLC-ICP-MS

Selenium species were determined in bacterial cell pellets by enzymatic hydrolysis followed by HPLC-ICP-MS. With the aim of improving selenium species extraction, the cell pellet obtained as described above was exposed to a two-step enzymatic hydrolysis protocol. For this purpose, 0.05 g of bacterial cell pellet were treated with 500 μL of 10 mg/mL lysozime in TAE buffer (40 mM Tris, 20 mM acetic acid, and 1 mM EDTA, pH 7.0) and incubated during 2.5 h at 37 °C. Afterwards, 500 μL solution of 100 μg/mL of protease type XIV (SIGMA-ALDRICH chemical CO) in TAE Buffer were added to the cell suspension and sonicated with an ultrasonic probe (Sonoplus ultrasonic homogenizer, Bandenlin, Berlin, Germany) during 50 s at 60% of ultrasound amplitude. Finally, the obtained extracts were centrifuged at 7.500g during 15 min and the supernatants were filtered through a 0.22 μm membrane filters and analyzed by HPLC-ICP-MS (Agilent Technologies, Santa Clara, CA, USA) using two different chromatographic columns, a Hamilton PRPX100 (250 × 4.1 mm, 10 μm) column and a C8 Zorbax R_x-C_8 S column, with different retention mechanisms: anion exchange and reversed phase. The experimental conditions are listed in Table 1. Identification of Se species was carried out by matching retention times and by spiking experiments. Stock standard solutions of selenomethionine (SeMet), selenomethylcystine (SeMetCys), and selenocysteine ($SeCys_2$) (SIGMA-ALDRICH chemical CO) were prepared by dissolving them in 3% (v/v) of HCl to obtain a 1000 mg/L stock solution to prepare the standards. Inorganic selenium solution was prepared by dissolving sodium selenite (Na_2SeO_3) and selenate (Na_2SeO_4) in Milli-Q water. Stock solutions were stored at 4 °C, whereas working standard solution were freshly prepared by dilution before use. Se species concentra-

tion was calculated by comparing the area of each species peak with its calibration curve (20–700 μg/L).

2.5. Detection of Se nanoparticles by Transmission Electron Microscopy (TEM)

The presence, size and composition of Se nanoparticles were assessed by TEM (JEOL JEM 2100; USA) equipped with an X-ray energy dispersive spectroscopy (XEDS) microanalysis composition system (Oxford Inca). In the cell pellets of the studied strains cultured in Se-MRS nanoparticles were detected by using a high-resolution transmission electron microscope (JEOL JEM 2100; USA) equipped with an X-ray energy dispersive spectroscopy (XEDS) microanalysis composition system (Oxford Inca). Cells were cultured overnight in 5 mL of MRS supplemented or not with 5 mg Se/L as Na_2SeO_3, centrifuged (5000*g*, 5 min), and resuspended in 1 mL of fresh MRS. Samples were prepared by evaporating a drop of culture onto a 300 mesh lacey carbon copper grid.

2.6. Effect of Se on bacterial membrane hydrophobicity and adhesion

For the assessment of the degree of hydrophobicity the Microbial Adhesion to Hydrocarbons (MATH) method was employed (Ocaña, Bru, de Ruiz Holgado, & Nader-Macias, 1999). Cells were grown in MRS and Se-MRS as described before during 24 h. Pellets (from 5 mL culture) were washed and resuspended in saline solution to obtain an OD_{600} of 0.6. Cell suspension (3.6 mL) was mixed vigorously with 0.6 mL of xylene for 2 min. The aqueous layer was carefully removed and transferred to clean tubes and the absorbance was measured as before. The percentage of hydrophobicity was obtained from the following calculation:

$$\%\ \text{hydrophobicity}: \frac{OD_{before} - OD_{after}}{OD_{before}} \times 100$$

The hydrophobicity of the cells was classified in three groups: high (100–71%), middle (70–36%) and low (35–0%).

2.7. Resistance of Se-enriched strains to different stress conditions

L. acidophilus CRL 636 and *L. reuteri* CRL 1101, previously grown in MRS and Se-MRS at 37 °C during 24 h were further cultured in MRS under different cultures conditions: (i) pH values of 4.0 and 8.0; (ii) the presence of 0.3% (w/v) of bile salts (SIGMA-ALDRICH chemical CO); (iii) MRS-Se. Then, cells were allowed to grow at 37 °C during 24 h and growth was monitored by determining the OD_{600}.

2.8. Statistics

Studies were carried out in triplicate of independent assays and results were expressed as means with standard deviations. TEM images were done in duplicate and images were selected from independent assays of each strain. Chromatograms were performed for independent assays for each strain using several dilutions.

3. Results

3.1. Effect of Se on cell growth and behavior

Both *Lactobacillus* strains were able to grow in the presence of 5 mg Se/L as selenite, being *L. reuteri* CRL 1101 more tolerant to this metalloid than *L. acidophilus* CRL 636. When selenium was added, this latter strain grew only 0.3 log cfu/mL during the first 4 h of incubation reaching a maximum OD_{600} of 0.49 at 24 h while cell viability started to decline after 8 h of incubation reaching a final cell count of 5.3 log cfu/mL. In the absence of Se a higher cell count (8.3 log cfu/mL) at 24 h was observed. The pH started to decline since the first 4 h of incubation being the decrease less pronounced in Se-MRS (Fig. 1a). As consequence of the scarce growth of *L. acidophilus* CRL 636 in Se-MRS, lower glucose consumption (1.5 g/L) was observed respect to the control sample (7 g/L), being lactic acid (3.8 g/L) the main organic acid produced under the control conditions. Acetic acid, as sodium acetate was consumed (0.2 g/L) by the strain during the first 4 h of incubation while after this period acetic acid was produced (0.4 g/L at 24 h) as fermentation metabolite (Fig. 1b). In the presence of Se, acetate was consumed (0.2 g/L) during the first 8 h of incubation showing an increase of 0.1 g/L at 24 h (Fig. 1b). In contrast to the *L. acidophilus* strain, a slight difference in the growth of *L. reuteri* CRL 1101 in MRS and Se-MRS were observed as only a decrease of 1 log cfu/mL in the presence of Se was detected comparing to the control. Accordingly, pH values during growth were similar although some differences were observed during the first 6 h of incubation being the acidification capacity lower with Se (Fig. 1c). *L. reuteri* CRL 1101 consumed glucose (2.8 g/L) more rapidly in MRS than in Se-MRS during the first 4 h of incubation; accordingly, more lactic acid was released (0.5 g/L). At the end of the fermentation (24 h) almost all glucose was consumed in both samples although more lactic (0.5 g/L) and acetic (0.2 g/L) acids were observed in Se-MRS (Fig. 1d).

3.2. Selenium content in bacterial cell pellets determined by ICP-MS

The concentration of Se in the bacterial pellet exposed to 5 mg Se/L as sodium selenite was determined by ICP-MS. The results obtained suggested that Se accumulation is highly dependent on the LAB strain used. *L. acidophilus* CRL 636 accumulated only 0.7% of the added Se during the first 6 h incubation, while 41.2% of Se was found in the cell pellet after 24 h. On the other hand, 58.1% of Se was found in the intracellular cell digest of *L. reuteri* CRL 1101 at 6 h incubation while an increase up to 78.6% was determined at 24 h.

3.3. Selenium species determination in bacterial cell pellets by HPLC-ICP-MS

Once Se accumulation was confirmed, Se species in the bacterial cell pellets were assessed by HPLC-ICP-MS by using two separation columns, anionic exchange and reversed-phase. Fig. 2a–c shows the chromatographic profiles of Se standards (Fig. 2a) and the Se content present in the enzymatically hydrolysed bacterial cell pellets for *L. acidophilus* CRL 636 (Fig. 2b) and *L. reuteri* CRL 1101 (Fig. 2c) by anion-exchange LC-ICP-MS. The results evidenced that 44 and 59% of the Se found in the cell pellets of *L. acidophilus* CRL 636 and *L. reuteri* CRL 1101 were transformed into Se-amino acids, mainly into $SeCys_2$ and SeMet, respectively. In this respect, 0.235 mg/L of $SeCys_2$ and 0.683 mg/L of SeMet were found in *L. acidophilus* CRL 636 cell extracts, while higher amount of both Se species were determined in *L. reuteri* CRL 1101 (1.258 and 1.042 mg/L of $SeCys_2$ and SeMet, respectively). Taking into account that it has been suggested that $SeCys_2$ can co-elute with the oxidized form of SeMet in the PRP-X100 column; the same samples were injected in a reversed-phase chromatography column to confirm the presence of $SeCys_2$. The oxidized form of SeMet was not observed in the C_8 chromatograms obtained for a mixture of Se standards and Se species from enzymatically hydrolysed bacterial pellets confirming that the peak eluting at 2.3 min in the PRP-X100 column corresponds to $SeCys_2$ (Fig. 2d and e).

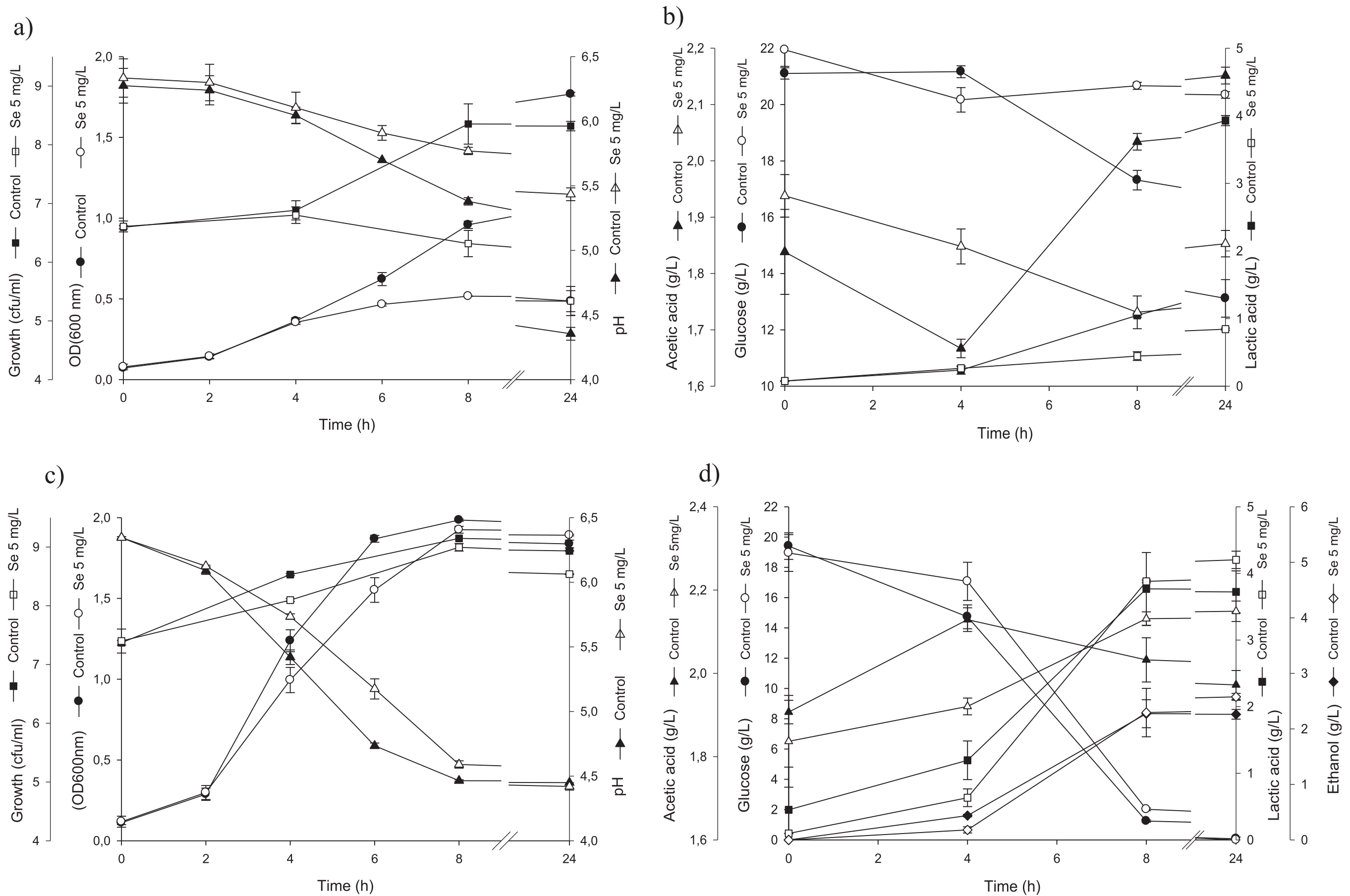

Fig. 1. Growth (OD_{600nm}), viable cell count (cfu/mL) and pH of (a) *L. acidophilus* CRL 636 and (c) *L. reuteri* CRL 1101, and glucose consumption and organic acids release of (b), *L. acidophilus* CRL 636 and (d) *L. reuteri* CRL 1101 grown in MRS and Se-MRS incubated at 37 °C during 24 h.

3.4. Detection of Se nanoparticles by TEM

Due to the finding that the culture media turned red in the presence of selenite indicating the reduction of the added Selenite (grey) into elemental Se (red), the studied strains were observed by TEM to detect the formation of Se nanoparticles (Fig. 3). In fact, both strains produced nanoparticles with sizes between 25 and 370 nm as determined by TEM analysis. The composition of these particles was further confirmed as Se by the X-ray energy dispersive spectroscopy (XEDS) microanalysis. Microscope images revealed that cell lyses occurred in both strains as efflux of cytoplasmic material was observed.

3.5. Effect of Se on bacterial membrane hydrophobicity and adhesion

Cell surface hydrophobicity and adhesion, which are important properties for probiotic strains, were evaluated with both lactobacilli in the presence of Se. *L. acidophilus* CRL 636 showed low hydrophobicity (27.3%) in MRS, which was increased up to 56.0% in the presence of Se. On the other hand, no differences were observed for *L. reuteri* CRL 1101, which showed low hydrophobicity value (27.4%) independently of the presence of Se.

3.6. Resistance of Se-enriched strains to different stress culture conditions

The ability of the strains to resist different pH values and the presence of bile salts after being cultured in MRS and Se-MRS was studied (Fig. 4). No effect on *L. acidophilus* CRL 636 growth when bile salts were added to MRS or when the initial pH value was 8.0 was observed; in contrast, no growth was detected at pH 4.0 (Fig. 4a). When the strain was previously grown in Se-MRS (Se-enriched), the ability of selenized cells of *L. acidophilus* CRL 636 to resist bile salts was diminished reaching a final OD_{600nm} of 0.6 (2.3 fold lower than in MRS) after 24 h of incubation. To analyze if this effect was due to the absence of Se in the challenging assay, Se-enriched *L. acidophilus* CRL 636 was inoculated again in Se-MRS. Results indicated that the strain could no adapt to the presence of Se, as a deleterious effect was observed since no growth was detected (Fig. 4b).

Growth of *L. reuteri* CRL 1101 was affected mainly when it was submitted to low pH (4.0) showing a decrease in its growth rate respect to the control (0.63 h^{-1} and 0.32 h^{-1}, respectively). In the presence of bile salts a decrease of 1.14 times in the growth rate was also observed (Fig. 4c). Se-enriched *L. reuteri* CRL 1101 showed a decrease (1.3 fold) in its cell growth rate and an increase (3 h) in the lag phase when grown in MRS while the lag phase was prolonged even more in Se-MRS, observing an increase in the OD only at 24 h (Fig. 4d). On the other hand, no significant differences were observed in cell growth when the initial culture pH was 8.0, or when bile salts were added to Se-enriched *L. reuteri* CRL 1101 comparing to cells grown in MRS. Interestingly, when the initial pH was 4.0, no growth was observed until 24 h incubation (Fig. 4d).

4. Discussion

LAB are usually used for the production of numerous fermented foods worldwide since they are able to improve food safety, the

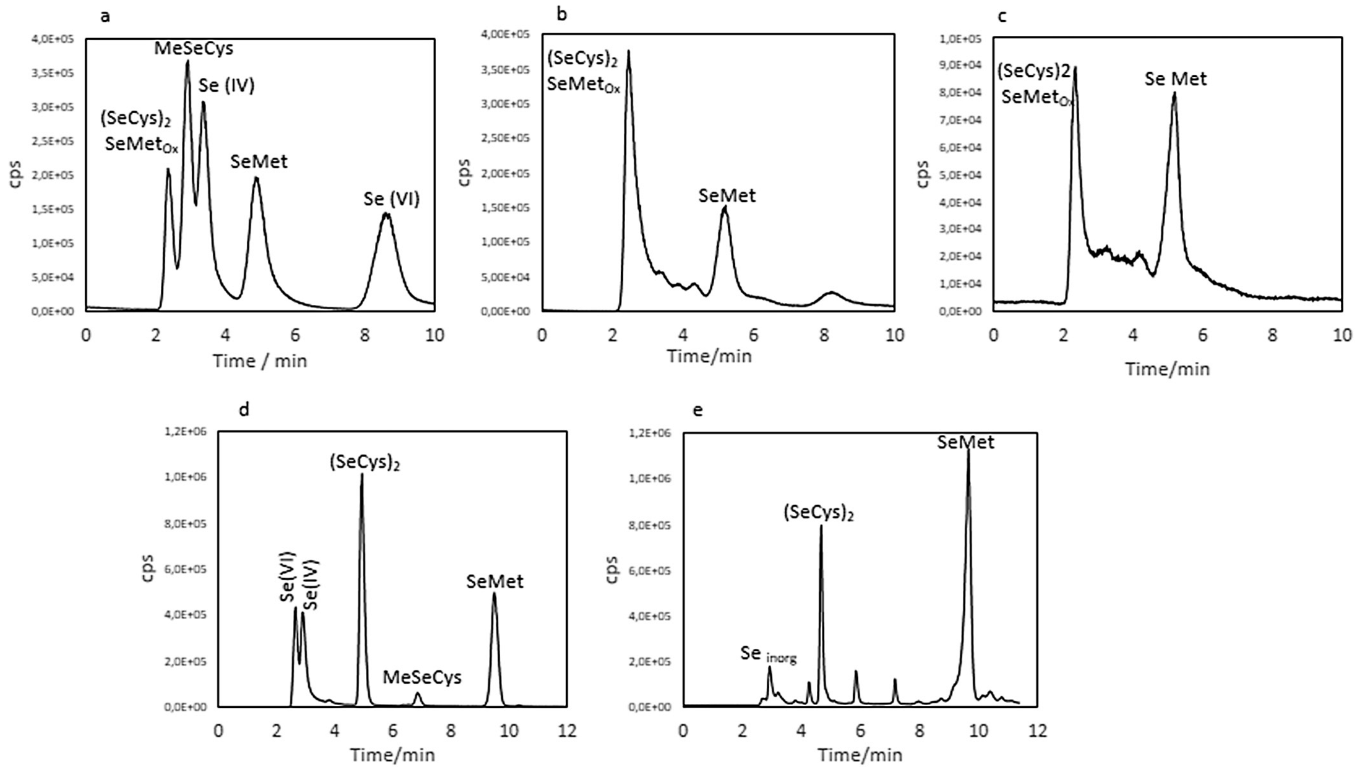

Fig. 2. Chromatographic profiles obtained by anion exchange LC-ICP-MS at ^{78}Se isotope, showing the separation of (a) mixture of Se standards containing 150 μg/L of each Se-species; and Se species from enzymatic cell pellet extracts of (b) *L. acidophilus* CRL 636, (c) *L. reuteri* CRL 1101 using a PRPX100 column, and (d) mixture of Se standards containing 150 μg/L of each Se-species and (e) *L. acidophilus* CRL 636 using a C_8 column. Strains were cultured in MRS in the presence of 5 mg Se/L of Se (IV) at 37 °C during 24 h.

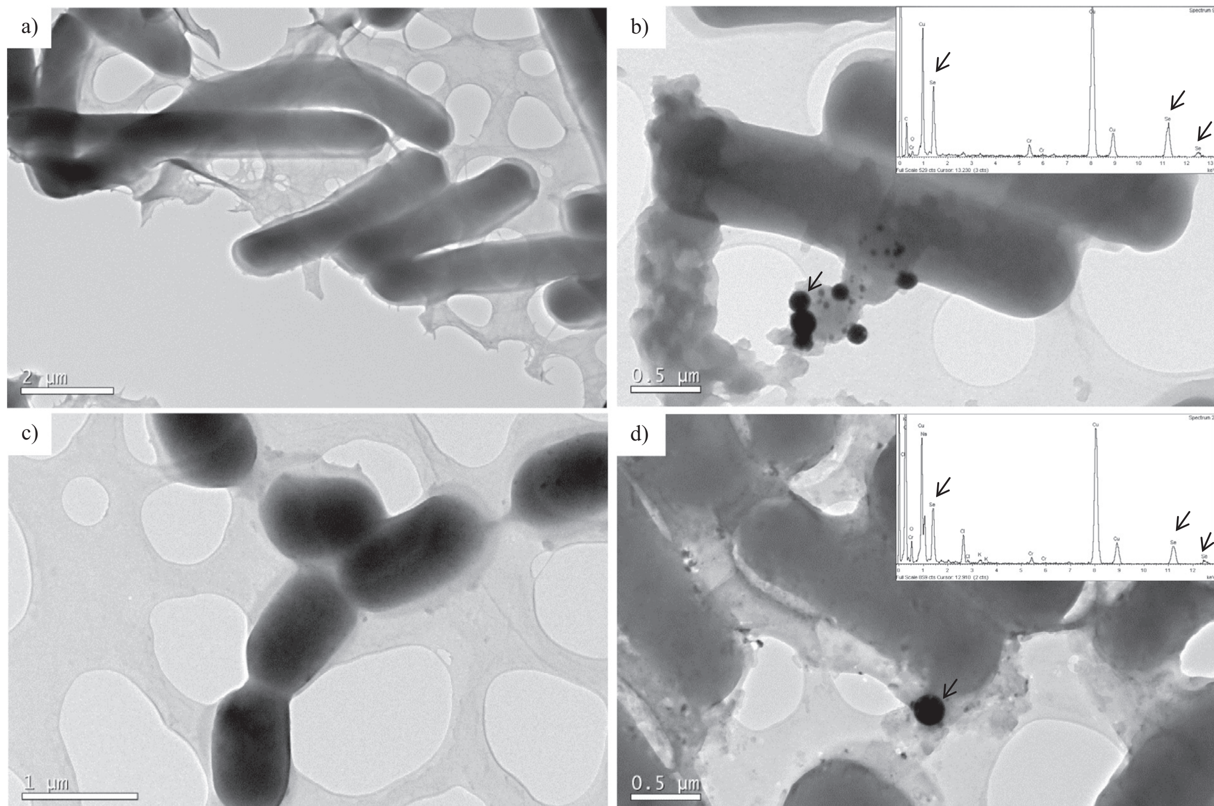

Fig. 3. TEM images and X-ray energy dispersive spectroscopy (XEDS) microanalysis obtained for samples of *L. acidophilus* CRL 636 grown in (a) MRS (control) and (b) MRS-Se, and *L. reuteri* CRL 1101 in (c) MRS (control) and (d) MRS-Se at 37 °C during 24 h. Arrows in images indicate the presence of nanoparticles. Arrows in XEDS spectra indicate the Se emission peaks consisting of SeLα, SeKα and SeKβ at 1.4, 11.22 and 12.49 keV, respectively.

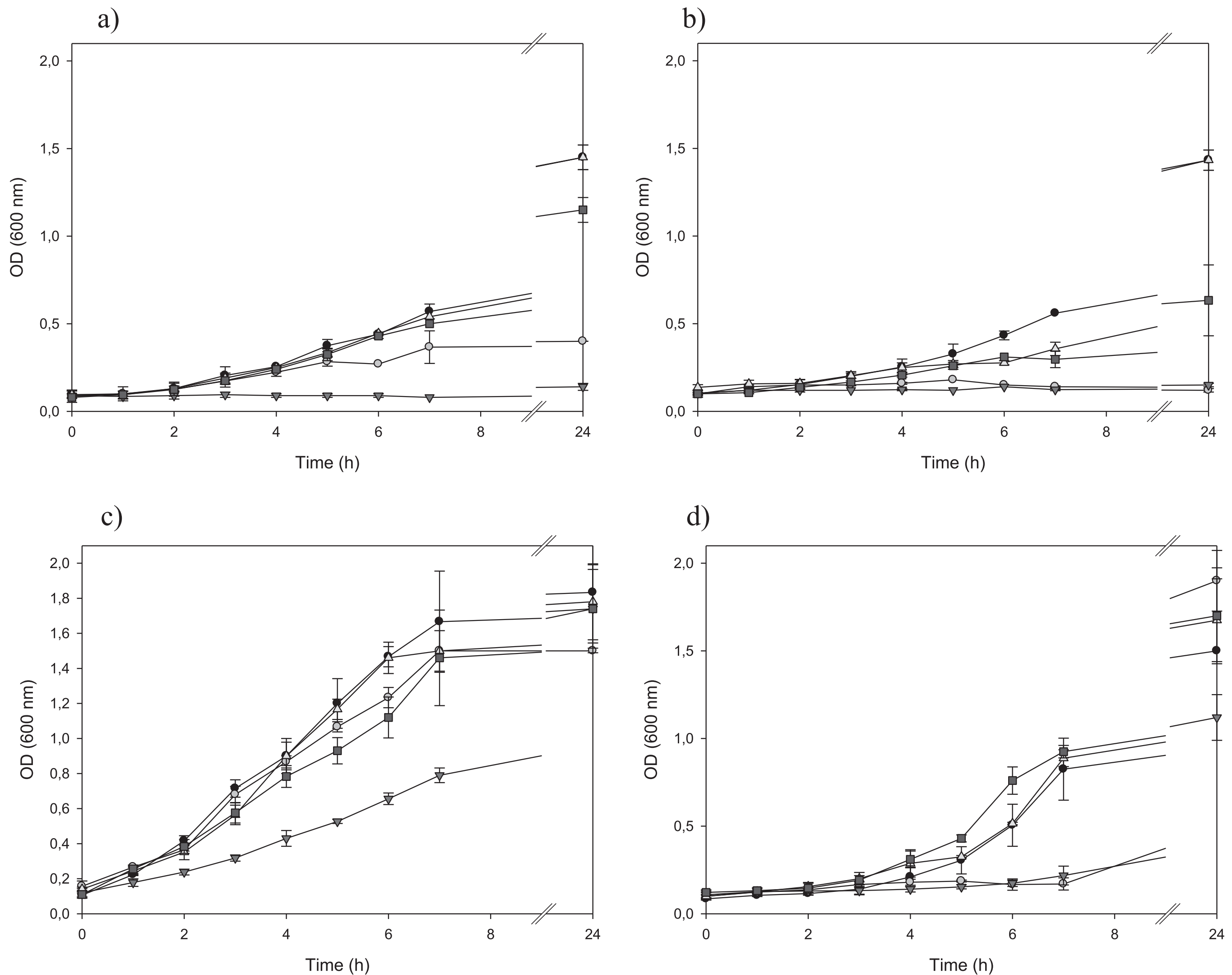

Fig. 4. Growth ($OD_{600\ nm}$) of *L. acidophilus* CRL 636 (a) control, (b) previously grown in presence of 5 mg Se/L and *L. reuteri* CRL 1101 (c) control and (d) previously grown in presence of 5 mg Se/L grown in MRS at 37 °C during 24 h. The strains were grown subsequently in MRS •, MRS with Se 5 mg/L ○, MRS pH 4 ▽, MRS pH 8 △, MRS with bile salts ■.

nutritional value, flavor and texture of the food products (Hugenholtz et al., 2002). Functional foods can be classified into those foods containing probiotics, prebiotics or biogenics, all of them affecting by different means the host health directly in the intestine as probiotics or indirectly by releasing different bioactive components in the fermented food (Mitsuoka, 2014). In general, strains from the species *L. acidophilus, L. rhamnosus, L. reuteri, L. casei, L. plantarum, L. delbrueckii,* and *L. helveticus* are considered potentially probiotics and are commercially available for their use in the food industry (Florou-Paneri, Christaki, & Bonos, 2013; Lebeer, Vanderleyden, & De Keersmaecker, 2008). Functional LAB are able to transform raw materials increasing their bioavailability and reducing toxic components and anti-nutritive factors (Tamang, Shin, Jung, & Chae, 2016).

Selenium is considered an essential nutrient for human health with a recommended daily intake of 55 μg. Moreover, it has been claimed that higher intakes of Se (up to 200 μg per day) can protect the human body against free radicals, which can cause degenerative and age-related diseases as well as certain types of cancer (Alzate, Pérez-Conde, Gutiérrez, & Cámara, 2010). Se health benefits are dependent on the form in which they are consumed (Alzate et al., 2010; Palomo et al., 2014). Indeed, it has been claimed that the most bio-available forms of Se for human body are its organic forms. Some LAB are able to transform inorganic forms of Se, such as selenite and selenate, into its organic forms (i.e. SeMet, SeCys, SeMeCys).

In this work we studied the ability of two potentially probiotic *Lactobacillus* strains (usually used in fermented food products) to grow in the presence of 5 mg Se/L as selenite. Although, both lactobacilli could grow in or resist the presence of 5 mg Se/L of Se, great differences in the growth behavior of the strains were observed. *L. reuteri* CRL 1101 was the most resistant strain showing only 1 log cfu/mL decrease in cell viability after 24 h incubation in Se-MRS. In agreement with our data, Lamberti et al. (2011) reported that 4.38 mg Se/L were needed to observe the same decrease in the cell viability of the strain *L. reuteri* Lb2 BM. However and to the best of our knowledge, no reports on *L. acidophilus* growth rate in the presence of Se are reported although the ability of strains of this species to form Se nanoparticles in the presence of 315 mg/L and 20 mg Se/L as Na_2SeO_3 was recently published by Radhika and Gayathri (2015) and Visha et al. (2015), respectively. These authors characterized *L. acidophilus* Se nanoparticles by different methods but no information on *L. acidophilus* growth behavior or on their ability to produce organic forms of Se was reported.

In this work, a scarce growth in the presence of Se was observed for *L. acidophilus* CRL 636, consistent with the low pH decrease and glucose consumption. Interestingly, differences were observed on the acetic acid profile during fermentation; this acid was released after 8 h incubation in MRS while it was consumed in the presence of Se. Similarly, *L. reuteri* CRL 1101 released acetic acid during the first 4 h of incubation when grown in MRS while this acid was produced in Se-MRS only after 8 h. In addition, a slight increase in the ethanol production was observed after 24 h in the presence of Se. Mangiapane et al. (2014) reported an over-expression of the enzyme acetaldehyde-CoA/alcohol dehydrogenase, which converts acetate into ethanol in *L. reuteri* Lb2 BM when grown in MRS supplemented with Se with respect to the control sample by applying a proteomic approach.

It is known that Se can be accumulated in bacterial cells as selenoproteins or as Se nanoparticles when sodium selenite or selenate are added to the culture media (Deng et al., 2015). Se accumulation has been determined indirectly by spectrophotometric measurements in cell supernatants of *L. bulgaricus* (Xia et al., 2007), by atomic absorption analysis of digested cell pellets of *L. casei*, *Enteroccoccus faecium, L. brevis, L. rhamnosus*, and *L. fermentum* (Andreoni, Luischi, Cavalca, Erba, & Ciappellano, 2000; Deng et al., 2015; Pusztahelyi, Kovács, Pócsi, & Prokisch, 2015), or by ICP-MS of digested cells of *L. delbrueckii* subsp. *bulgaricus* (Palomo-Siguero et al., 2016). In our work, we confirmed that *L. acidophilus* CRL 636 and *L. reuteri* CRL 1101 are able to accumulate Se intracellularly and that the accumulating percentages increase with the incubation time, being *L. reuteri* CRL 1101 capable of accumulating 78% of the added Se after 24 h of incubation. Similarly, Palomo-Siguero et al. (2016) found that a *L. delbrueckii* subsp. *bulgaricus* strain could accumulate 60% of the added Se (10 mg/L) after 24 h incubation and that SeCys and SeMet were produced, although a high amount of remaining Na_2SeO_3 was observed in the cell pellet by using a PRP-X100 column coupled to ICP-MS. Interestingly, the presence of SeCys and SeMet was confirmed in both *L. acidophilus* CRL 636 and *L. reuteri* CRL 1101 cells while no Na_2SeO_3 was detected in the cell pellets. Zhang et al. (2009) reported that *Bifidobacterium animalis* 01 could accumulate Se in the protein fraction of its cell pellet although only SeMet was found after digestion and analysis with LC–ESI–MS–MS. In addition, both of our studied strains could produce Se nanoparticles as observed by TEM. The production of Se nanoparticles by LAB has been studied previously (Eszenyi, Sztrik, Babka, & Prokisch, 2011; Sasidharan & Balakrishnaraja, 2014; Visha et al., 2015) showing also nanoparticle sizes between 40 and 500 nm depending on the strain used. Se nanoparticles are known to be less toxic than inorganic Se forms and their capacity to diminish cancer symptoms have been studied *in vivo* and *in vitro* (López-Heras et al., 2014; Yazdi et al., 2013). In this respect, it has been reported that a Se nanoparticle-enriched *L. plantarum* strain could be used as an immunomodulating factor elevating INF, TNF and IL-2 levels as well as Natural Killer cell activity in a murine model (Yazdi, Mahdavi, Kheradmand, & Shahverdi, 2012). Moreover, it has been observed that Se nanoparticle-enriched *L. brevis* is able to inhibit liver metastasis in metastatic mouse breast cancer (Yazdi et al., 2013).

The ability of Se-enriched lactobacilli to survive under the conditions of the gastrointestinal tract and food storage was analyzed to evaluate their possible utilization as probiotic strains in a fermented food product. Usually at the end of food fermentations, food matrices reach pH values close to 4.0, this value being stable during food shelf life. Microbial growth is a non-desirable attribute during food storage as a post-acidification can affect the flavor of the end-product. The cell growth of our strains was detrimentally affected at low pH, this effect being more noticeable when *L. reuteri* CRL 1101 was previously grown in the presence of Se while *L. acidophilus* CRL 636 could not grow at pH 4 with or without the addition of Se. In contrast, Pusztahelyi et al. (2015) showed that cell growth of *L. casei* and *Enterococcus faecium* strains was not affected in the presence of Se and at pH 4.0 comparing to the control.

Other attributes such as survival at the intestine pH value (8.0) and in the presence of bile salts are necessary for probiotic strains to exert their beneficial effects in the host. *L. reuteri* CRL 1101 showed a lower growth rate in the presence of bile salts respect to the control MRS while this difference was not observed for the Se-enriched cells. Also, Mangiapane et al. (2014) observed a higher tolerance to bile salts when grown *L. reuteri* Lb2 BM in the presence of Se. On the other hand, *L. acidophilus* strains are known to have high bile salt resistance (Ruiz, Margolles, & Sánchez, 2013); indeed, bile salts did not affect growth of *L. acidophilus* CRL 636 in MRS while a negative effect of bile salts in selenized cells was observed.

The capacity of bacteria to adhere to epithelial cells is one of the main criteria for probiotics selection (Duary, Rajput, Batish, & Grover, 2011). Taking into account that cell damage caused by the presence of Se was observed by TEM and that an effect on bacterial cell surface could have occurred, cell hydrophobicity and adhesion properties were studied. *L. acidophilus* CRL 636 increased 29% its adhesion and hydrophobicity capacities while no differences were observed for *L. reuteri* CRL 1101 in the presence of Se. By using a proteomic approach, Mangiapane et al. (2014) reported that the elongation factor Tu (EF-Tu) was up-regulated in *L. reuteri* Lb2 BM. This protein was also found to have adhesion properties in probiotic *Bifidobacterium animalis* subsp. *lactis* strains (Gilad, Svensson, Viborg, Stuer-Lauridsen, & Jacobsen, 2011).

5. Conclusions

The studied strains could grow in Se-MRS, produce Se-nanoparticles and accumulate Se intracellularly partly as SeMet and SeCys, which are themost bioavailable Se forms. Se-enriched cells of both strains were not able to grow under adverse culture conditions such as pH 4.0, which could prevent post acidification of the fermented product during storage. Moreover, Se-enriched cells of *L. reuteri* CRL 1101 could resist the presence of bile salts. Our findings suggest that these strains could be administrated directly as probiotics or be used for formulating fermented products containing Se-enriched bacteria and/or nanoparticles displaying health benefits for the host.

Acknowledgments

We acknowledge the financial support of PIP 003 and FONCyT (PICT2438-2012) from Argentina M. Pescuma was recipient of a fellowship from CONICET, Argentina, for an external internship at Departamento de Química Analítica, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, Ciudad Universitaria, 28040 Madrid, Spain. The UCM group wants to thank the Spanish Commission of Science and Technology (CTQ2014-54801-C2-1-R) and the Community of Madrid/FEDER programme (S2013/ABI-3028, AVANSECAL-CM).

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