



# The use of lactic acid bacteria to reduce mercury bioaccessibility



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## ABSTRACT

Mercury in food is present in either inorganic [Hg(II)] or methylmercury (CH<sub>3</sub>Hg) form. Intestinal absorption of mercury is influenced by interactions with other food components. The use of dietary components to reduce mercury bioavailability has been previously proposed. The aim of this work is to explore the use of lactic acid bacteria to reduce the amount of mercury solubilized after gastrointestinal digestion and available for absorption (bioaccessibility). Ten strains were tested by addition to aqueous solutions containing Hg(II) or CH<sub>3</sub>Hg, or to food samples, and submission of the mixtures to gastrointestinal digestion. All of the strains assayed reduce the soluble fraction from standards of mercury species under gastrointestinal digestion conditions (72–98%). However their effectiveness is lower in food, and reductions in bioaccessibility are only observed with mushrooms (≤68%). It is hypothesized that bioaccessible mercury in seafood forms part of complexes that do not interact with lactic acid bacteria.

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## 1. Introduction

Mercury is a toxic trace element widely distributed in nature. The population may be exposed to it in the work place, however, diet is the main route of exposure for most individuals. Among foodstuffs of animal origin, large marine predatory fish, such as swordfish, shark, bonito or tuna, usually have the highest mercury contents, mainly in the form of methylmercury (CH<sub>3</sub>Hg) (Sahuquillo, Lagarda, Silvestre, & Farré, 2007; Storelli, Stuffer, & Marcotrigiano, 2002). Mercury concentrations are generally lower in products of plant origin, except some mushrooms. High concentrations of mercury have been found in certain edible species such as *Boletus edulis* and *Agaricus arvensis* (Falandysz et al., 2003). In these products, inorganic divalent mercury [Hg(II)] is the predominant species (77–100%) (Pilz et al., 2011; Rieder, Brunner, Horvat, Jacobs, & Frey, 2011).

Methylmercury is considered a possible human carcinogen (Group 2B; IARC, 1993) and a neurotoxic compound, especially during development. Inorganic mercury is a potent nephrotoxic species and produces gastrointestinal and immunological disorders (NTP, 1993). The US Environmental Protection Agency has classified mercuric chloride as a possible carcinogen for humans (Group C; U.S. EPA, 1999). *In vivo* studies have found limited absorption of inorganic mercury salts (2–38%), whereas absorption of CH<sub>3</sub>Hg salts is high (over 80%) (EFSA, 2012). High absorption of the organic form has been confirmed in rats dosed with seafood

products, in which bioavailabilities between 75% and 93% were observed (Yannai & Sachs, 1993).

There is an ongoing debate within the scientific community and agencies responsible for health about the risk associated with the intake of certain seafood products owing to their high mercury contents. Various studies support the idea that maternal mercury exposure through seafood consumption during pregnancy is associated with neuropsychological deficits in children (Grandjean, White, Weihe, & Jørgensen, 2003; Xue, Holzman, Rahbar, Trosko, & Fischer, 2007). On the basis of these data, food safety agencies have issued recommendations to eliminate or diminish the consumption of tuna, bonito, swordfish and pike in susceptible populations such as children and pregnant or breastfeeding women (Health Canada, 2008). However, it has been demonstrated that certain essential nutrients in seafood products, such as selenium, omega-3 fatty acids, iron or vitamin E, may provide beneficial effects on brain development. They may also protect against the development of heart disease (Choi, Cordier, Welhe, & Grandjean, 2008), and therefore have positive effects, despite the possible adverse effects caused by mercury exposure.

These studies suggest the possibility that the diet may protect against mercury toxicity. In addition to affecting the same epidemiological outcomes in opposite directions, the counteraction of dietary compounds could also involve a toxicokinetic interaction mechanism. A previous study conducted by Jadán-Piedra, Sánchez, Vélez, and Devesa (2016) has shown that certain food components can reduce the amount of mercury solubilized during gastrointestinal digestion, and thus the amount of metal absorbed. The authors indicate that the addition of these compounds to the food product,

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or their use as supplements, may be beneficial for susceptible populations or for regular consumers of food matrices with high mercury contents.

Several lactic acid bacteria (LAB) have been reported to bind to and remove toxic contaminants from food and water. Successful applications have been described for the removal of toxic trace elements (Halttunen, Salminen, & Tahvonon, 2007), including mercury from aqueous solutions (Kinoshita et al., 2013). This, together with their GRAS (Generally recognized as safe)/QPS (Qualified presumption of safety) status and probiotic qualities suggests that LAB may be ideal organisms for use as a tool to prevent or reduce heavy metal toxicity and intestinal absorption (Monachese, Burton, & Reid, 2012). In fact, this type of intervention has proved effective in rodents exposed orally to cadmium and lead through drinking water (Jama, Mitić-Ćulafić, Kolarević, Đurašević, & Knežević-Vukčević, 2012; Zhai, Narbad, & Chen, 2015); however, the protective effect of LAB for mercury has not been elucidated.

The aim of this study was to seek LAB strains capable of reducing the amount of soluble mercury obtained after gastrointestinal digestion that is available for intestinal absorption (bioaccessibility). A total of 10 LAB strains were evaluated, selected on the basis of previous data showing their ability to bind the main forms of mercury present in food [Hg(II) and CH<sub>3</sub>Hg] (unpublished data). Lactic acid bacteria were added to aqueous solutions of Hg(II) and CH<sub>3</sub>Hg or to food samples, and the mixtures were subjected to a simulated gastrointestinal digestion to determine the ability of these strains to reduce the bioaccessibility of mercury.

## 2. Materials and methods

### 2.1. Reagents and chemicals

The standard solution of Hg(NO<sub>3</sub>)<sub>2</sub> (1000 mg/l, Merck, Spain) and CH<sub>3</sub>HgCl (1000 mg/l) were acquired from Merck (Spain) and Alfa Aesar (Spain) respectively. MRS (de Man, Rogosa and Sharpe) broth (BD Difco) was used for LAB culture. The enzymes and bile salts used for the *in vitro* gastrointestinal digestion were purchased from Sigma (Sigma-Aldrich, Spain): namely, porcine pepsin (enzyme activity, 944 U/mg protein), porcine pancreatin (activity equivalent to 4 × US Pharmacopeia specifications/mg pancreatin) and bile extract (glycine, taurine conjugates and other bile salts).

Other reagents used were: nitric acid (Merck), H<sub>2</sub>O<sub>2</sub> (30% v/v, Prolabo, Spain), SnCl<sub>2</sub> (Scharlab, Scharlau Chemie, Spain), phosphate-buffered saline (PBS, HyClone, Spain), NH<sub>4</sub>HCO<sub>3</sub> (Merck), L-cysteine (L-Cys; Sigma) and bovine serum albumin (BSA; Biowest, Labclininc, Spain).

### 2.2. LAB strains and culture conditions

The strains used in the present study are listed in Table 1. Cells were grown overnight in MRS broth at 30 °C under static conditions. For the assays, cells were washed with phosphate-buffered saline (PBS, HyClone), harvested by centrifugation (4000g, 5 min, 4 °C), resuspended in PBS, and kept in ice until use.

### 2.3. Effect of LAB on bioaccessibility of mercury

The efficiency of the 10 strains of LAB in reducing mercury bioaccessibility was evaluated in aqueous solutions of Hg(II) and CH<sub>3</sub>Hg, prepared from commercial standards of Hg(NO<sub>3</sub>)<sub>2</sub> and CH<sub>3</sub>HgCl, and in fillets of swordfish (*Xiphias gladius*) cooked without additional ingredients.

Cooked food samples (5 g) or standard solutions of mercury species (1 mg/l) with or without the corresponding amount of

**Table 1**

Strains of acid lactic bacteria (LAB) used in this study.

Designation	Strain	Origin
BL299	<i>L. plantarum</i> 299V (DSM <sup>a</sup> 9843)	Human intestine
BL36	<i>L. brevis</i> ATCC <sup>b</sup> 14869	Human intestine
BL17	<i>L. acidophilus</i> ATCC 4356	Human intestine
BL166	<i>L. plantarum</i> WCFS1	Human intestine Kleerebezem et al. (2003)
BL259	<i>L. reuteri</i> BL <sup>c</sup> 259	Rat caecum
BL23	<i>L. casei</i> BL23	Laboratory strain Mazé et al. (2010)
BL377	<i>L. rhamnosus</i> GG (ATCC 53103)	Human intestine
BL5	<i>L. johnsonii</i> ATCC 11506	Human intestine
BL260	<i>L. intestinalis</i> BL <sup>c</sup> 260	Rat caecum
BL327	<i>L. rhamnosus</i> ATCC 9595	Human oral cavity

<sup>a</sup> Deutsche Sammlung von Mikroorganismen und Zellkulturen.

<sup>b</sup> American Type Strain Culture Collection.

<sup>c</sup> Our laboratory collection.

the LAB strain [final OD<sub>595</sub> (optical density at 595 nm) 4] were weighed, and deionized water was added to a final volume of 50 ml. This mixture was submitted to the *in vitro* gastrointestinal digestion following the protocol described by Jadán-Piedra, Clemente, Devesa, and Vélez (2016), with slight modifications. Briefly, the pH of the mixture was adjusted to 2.0 with 6 M HCl, and a solution of pepsin (0.1 g of pepsin/ml dissolved in 0.1 M HCl) was added to a final concentration of 0.2 mg/ml. The mixture was incubated at 37 °C for 2 h with constant shaking.

The digest was then submitted to the intestinal digestion step. The pH was increased to 6.5 by adding 1 M of NH<sub>4</sub>HCO<sub>3</sub>, and a solution of pancreatin and bile extract was added (0.004 g/ml of pancreatin and 0.025 g/ml of bile extract in 0.1 M of NH<sub>4</sub>HCO<sub>3</sub>) to obtain final concentrations of 25 µg of pancreatin/ml and 150 µg of bile extract/ml. The mixture was incubated at 37 °C for 2 h with constant shaking.

After digestion, the samples were transferred to tubes and centrifuged (15300g, 4 °C, 30 min). The total concentrations of mercury were quantified in the soluble fraction (bioaccessible fraction) and the cellular pellet obtained. Bioaccessibility was determined by means of the following equation:

$$\text{Bioaccessibility} = [A/B] \times 100$$

where A is the concentration of mercury in the bioaccessible fraction after application of the simulated digestion and B is the concentration of mercury in the cooked product or in the standard solution.

### 2.4. Study of factors influencing the effect of LAB on bioaccessibility of mercury from food

Various factors that may influence the effect of LAB on the bioaccessibility of mercury were studied, using *Lactobacillus casei* BL23. The factors analysed are described below.

#### 2.4.1. Effect of the food matrix

Fillets of yellowfin tuna (*Thunnus albacares*) and mushrooms of the species *Boletus edulis* were analysed. The samples were cooked without the addition of other ingredients and subsequently homogenized. Bacteria were then added (final OD<sub>595</sub> 4), and the mixture was subjected to the process of gastrointestinal digestion described in Section 2.3. The data obtained were compared with those obtained from samples without added bacteria.

#### 2.4.2. Effect of cooking

To determine whether the cooking process could affect retention of mercury by the bacteria, the bioaccessibility of raw and cooked swordfish samples submitted to the simulated digestion with or without *L. casei* BL23 (final OD<sub>595</sub> 4) were analysed, following the procedure described in Section 2.3.

#### 2.4.3. Effect of the gastric and intestinal steps of the digestion

The effect of the steps of the digestive process on retention of mercury by LAB was evaluated. This assay was conducted with cooked samples of swordfish and mushroom. After the addition of *L. casei* BL23 (final OD<sub>595</sub> 4), part of the sample was submitted only to the gastric step and the rest was submitted to the complete gastrointestinal digestion. In both cases the protocol described in Section 2.3 was followed.

#### 2.4.4. Effect of bacterial concentration

Three OD<sub>595</sub> values (7, 10 and 15) were assayed. For this study, cooked samples of swordfish and mushroom were used, to which the corresponding amount of *L. casei* BL23 was added and then the whole mix was submitted to the gastrointestinal digestion process described in Section 2.3.

#### 2.4.5. Effect of the presence of l-Cys and BSA

Two different approaches were assayed. First, cells of *L. casei* BL23 were suspended (OD<sub>595</sub> 4) in solutions of Hg(II) or CH<sub>3</sub>Hg (1 mg/l) prepared in PBS supplemented with l-Cys (1 and 5 mg/l) or with BSA (0.1, 0.5, 1 and 5 mg/l) and the mixture was incubated for 1 h. After the incubation the solutions were centrifuged at 4000g for 10 min at 4 °C. The pellet was recovered to determine the amount of mercury retained by the bacterial cells.

In a second assay, the solutions of Hg(II) or CH<sub>3</sub>Hg (1 mg/l), supplemented with l-Cys (1 mg/l) or BSA (5 mg/l), were submitted to a gastrointestinal digestion in the presence of *L. casei* BL23 (final OD<sub>595</sub> 4), following the protocol described in Section 2.3. Then the concentrations of mercury in the soluble fraction and the cell pellet were determined.

#### 2.4.6. Effect of the components solubilized during the digestion of food

BL23 cells (final OD<sub>595</sub> 4) were incubated for 30 min with the bioaccessible fraction obtained from samples of tuna and mushrooms following the protocol described in Section 2.3, without LAB. Then the bacteria were centrifuged at 11000g for 5 min, the pellet was washed with PBS and it was centrifuged again. The pellet was placed in contact with a solution of Hg(II) or CH<sub>3</sub>Hg (1 mg/l), prepared in PBS, and it was incubated for 1 h at 37 °C. Then the samples were centrifuged (11000g, 5 min), and mercury concentrations in the cell pellet and the supernatant were analysed.

#### 2.5. Effect of the application of heat treatment to LAB on mercury uptake

BL23 cells were heat-treated at 80 °C for 30 min, which produced a loss of viability of more than seven logarithmic units, as assayed by plate counting. Then they were added to solutions of Hg(II) and CH<sub>3</sub>Hg (1 mg/l) prepared in PBS or to cooked samples of swordfish and mushroom. These mixtures were submitted to the gastrointestinal digestion (Section 2.3).

#### 2.6. Determination of mercury

The samples were digested using a microwave accelerated reaction system (MARS, CEM, Vertex, Spain). The samples of cooked food (0.5 g), soluble fractions (4 ml) or cell pellet were placed in Teflon reactors, followed by addition of 4 ml HNO<sub>3</sub> and 1 ml of H<sub>2</sub>O<sub>2</sub>. The reactors were irradiated (180 °C, 15 min) and the

digested solutions obtained were allowed to rest for 12 h to eliminate nitrous vapour. Then they were made up to volume with 0.6 M HCl and mercury was quantified by cold vapour atomic fluorescence spectrometry (CV-AFS) (Millennium Merlin PSA 10.025, PS Analytical, UK). The analytical conditions employed were: reducing agent, 2% (m/v) SnCl<sub>2</sub> in 1.8 M HCl, 4.5 ml/min; reagent blank, 0.6 M HCl, 9 ml/min; carrier gas, argon, 0.3 L/min; dryer gas, air, 2.5 L/min; delay time, 15 s; analysis time, 40 s; memory wash time, 60 s.

Quality control for quantification by CV-AFS was performed by analysing a liquid reference material (QCI-049-1 Trace Metals AA Sample 1, LGC Standards, Spain) with a certified mercury concentration of 40.8 ± 1.19 µg/l.

#### 2.7. Statistical analysis

Statistical analysis was performed by means of an one-factor analysis of variance (ANOVA) with multiple post-hoc comparisons using the Tukey HSD test or a Student's *t*-test (SigmaPlot, version 12). Differences were considered significant for *p* < 0.05.

### 3. Results

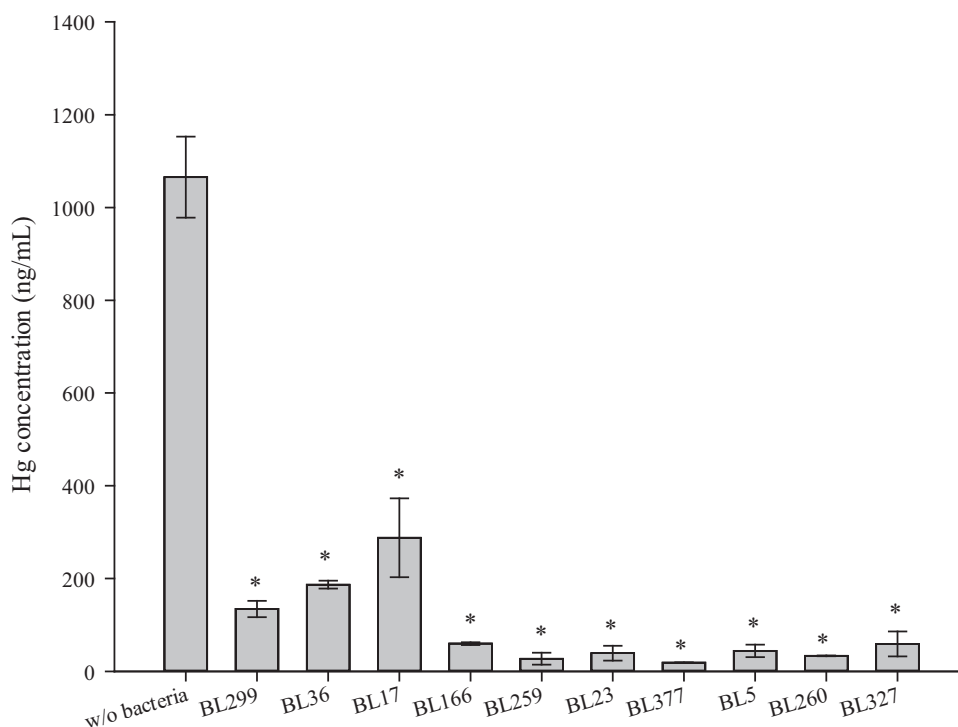
#### 3.1. Effect of LAB on bioaccessibility of mercury

Figs. 1 and 2 show the soluble mercury concentration after applying the gastrointestinal digestion to standard solutions of Hg(II) and CH<sub>3</sub>Hg, respectively, in the presence of bacteria. All the strains assayed reduced the bioaccessibility of mercury significantly, and the reduction was very similar in the two species [Hg(II): 72–98%; CH<sub>3</sub>Hg: 74–97%]. Of the quantity added initially (1 mg/l), the amount that was soluble after gastrointestinal digestion in the presence of bacteria and therefore that was available for absorption varied between 0.019 and 0.29 mg/l for Hg(II) and between 0.023 and 0.23 mg/l for CH<sub>3</sub>Hg. The reductions observed for mercury after gastrointestinal digestion were similar to those observed in saline solutions (Kinoshita et al., 2013), which indicates that the gastrointestinal conditions did not affect binding between LAB and mercury.

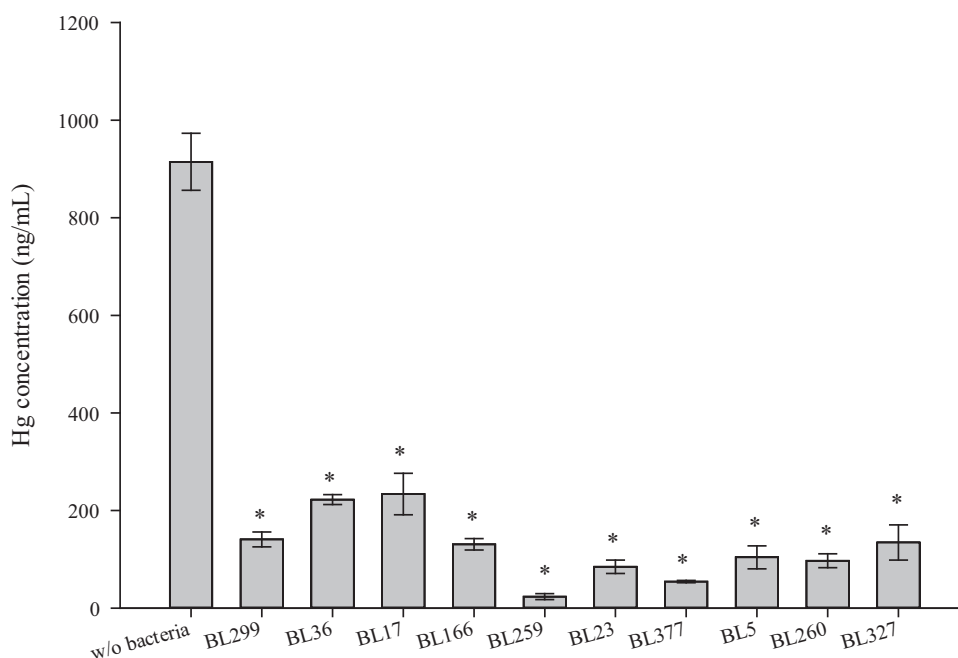
Application of the digestion to the swordfish samples produced bioaccessibilities ranging between 21 and 39%, with variable soluble concentrations (0.25–1.12 mg/kg). In contrast to what was observed in aqueous solutions, the presence of bacteria during the digestion process did not produce substantial changes in the soluble fraction of mercury obtained after digestion of swordfish (data not shown).

#### 3.2. Factors that influence the effect of LAB on bioaccessibility of mercury from food

In view of the lesser effectiveness of the LAB strains in reducing the bioaccessibility of mercury in samples of cooked swordfish, a series of factors were evaluated that might cause modification of the effect observed in aqueous solutions. For this and subsequent assays, strain *L. casei* BL23 was selected for its high capacity of mercury binding and easy handling. To determine whether the effect was dependent on the matrix, other food types were assayed (tuna and mushrooms). The results indicated that the addition of *L. casei* BL23 (final OD<sub>595</sub> 4) at the beginning of the digestion did not lead to reductions in soluble mercury concentrations in any of the food samples (data not shown). The cooking process also did not influence the effectiveness of *L. casei* BL23 in reducing bioaccessibility, as the bioaccessibility of mercury in raw swordfish (53 ± 8%) did not change in the presence of bacterial cells (52 ± 1%).



**Fig. 1.** Effect of lactic acid bacteria (LAB) on the bioaccessibility of Hg(II) from aqueous standard solution. Concentration of mercury (Hg) in the soluble fraction obtained after submitting a solution of Hg(II) (1 mg/l) to a gastrointestinal digestion, without bacteria, or with the addition of various bacterial strains (OD<sub>595</sub> 4). Values expressed as ng/ml (mean  $\pm$  SD, n = 4). Asterisks (\*) indicate statistically significant differences ( $p < 0.05$ ) with respect to the soluble fraction obtained without bacteria.

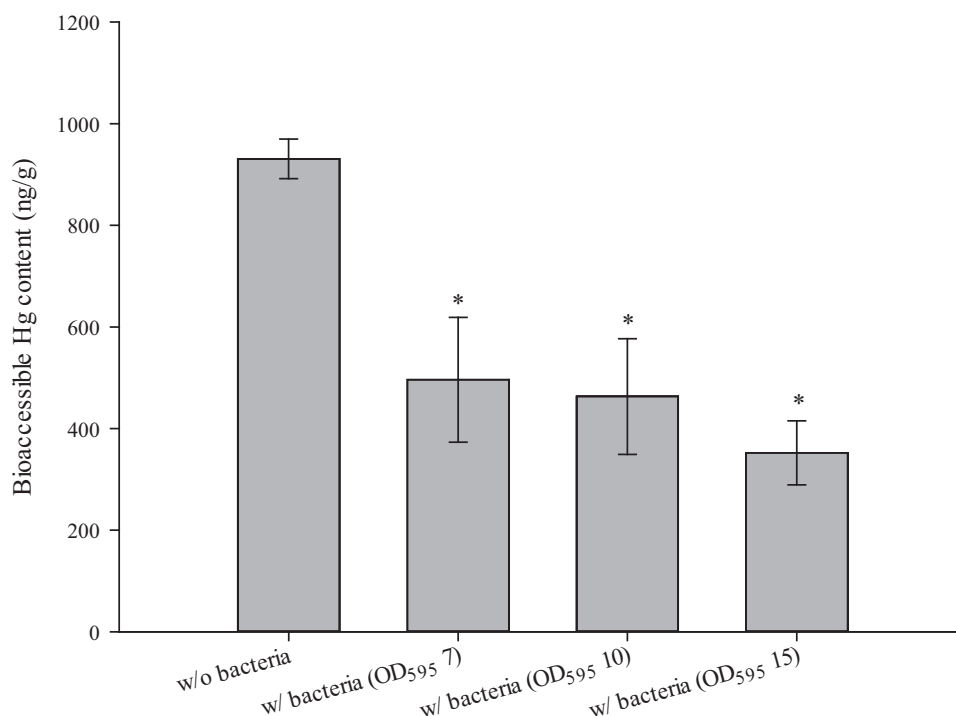


**Fig. 2.** Effect of lactic acid bacteria (LAB) on the bioaccessibility of CH<sub>3</sub>Hg from aqueous standard solutions. Concentration of mercury (Hg) in the soluble fraction obtained after submitting a standard solution of CH<sub>3</sub>Hg (1 mg/l) to a gastrointestinal digestion, without bacteria, or with the addition of various bacterial strains (OD<sub>595</sub> 4). Values expressed as ng/ml (mean  $\pm$  SD, n = 4). Asterisks (\*) indicate statistically significant differences ( $p < 0.05$ ) with respect to the soluble fraction obtained without bacteria.

The effect of the concentration of bacteria on the capture of mercury during digestion of food matrices was also evaluated. The use of a higher amount of cells (final OD<sub>595</sub> ranging from 7 to 15) did not affect bacterial retention of mercury during digestion of samples of fish (data not shown). The result for the mushroom samples was different. In this case, the increase in bacteria

amounts was accompanied by a reduction in bioaccessibility of mercury (Fig. 3). The reduction was high, and the differences between the three optical densities assayed were not significant (OD 7: 36–56%; OD 10: 42–59%; OD 15: 57–68%).

The differences observed between the assays with standard solutions and the assays with food matrices, especially the samples



**Fig. 3.** Effect of bacterial concentration on mercury (Hg) bioaccessibility from food. Concentration of Hg in the bioaccessible fraction obtained after submitting cooked mushrooms to a gastrointestinal digestion, without bacteria or with the addition of increasing concentrations of *Lactobacillus casei* BL23. Values expressed as ng/g of food (mean  $\pm$  SD,  $n = 4$ ). Asterisks (\*) indicate statistically significant differences ( $p < 0.05$ ) with respect to the bioaccessible fraction obtained without bacteria.

of seafood products, would suggest that soluble mercury from the matrix was in a chemical form that prevented it from interacting with the bacteria. Some studies have shown that mercury forms stable complexes with Cys or proteins (Watts, Howell, & Merl, 2014; Yasutake, Hirayama, & Inoue, 1990), and these complexes have been found in the bioaccessible fraction of seafood products (Cabañero, Madrid, & Cámara, 2007). These complexes might not interact with the bacteria. In order to test this hypothesis, the retention of mercury by *L. casei* BL23 in the presence of Cys or BSA in solutions was determined. Fig. 4a shows that the presence of Cys, irrespective of the concentration (1 and 5 mg/ml), prevented the retention of Hg(II) and CH<sub>3</sub>Hg by *L. casei* BL23. BSA produced a reduction in this capacity which was concentration-dependent, with the concentration of mercury in the bacteria being less than 10% at the highest concentration of BSA assayed (5 mg/ml). When it was investigated whether the presence of these components also affected the retention of mercury during the digestion process (Fig. 4b), once again Cys (1 mg/ml) abolished the retention capacity of the bacteria and all the mercury appeared in the soluble fraction. The effect of BSA (5 mg/ml) was different from the effect found previously (Fig. 4a); it reduced retention of Hg(II), although  $66 \pm 1\%$  still appeared in the bacteria, and it did not affect retention of CH<sub>3</sub>Hg. The data obtained in these assays suggest that the presence of complexes of mercury with Cys or proteins during digestion may affect the interaction of *L. casei* BL23 with the metal.

Interaction between the bacteria and other components of the matrix for which they have greater affinity might also explain why the addition of bacterial cells had little effect on the bioaccessibility of mercury in food. This possibility was evaluated by incubating *L. casei* BL23 with the soluble fractions of tuna and mushroom samples obtained after digestion, so that the soluble components of the digestion could interact with the bacteria and potentially block the mercury binding sites. The soluble fraction was then removed and the bacteria were incubated with saline solutions of mercury. Preincubation of *L. casei* BL23 with the bioac-

cessible fraction of the food matrices did not affect bacterial retention of mercury (Fig. 1, supplementary data), therefore, the components of the matrix that were solubilized during the digestion did not interfere with the binding of mercury to the bacteria.

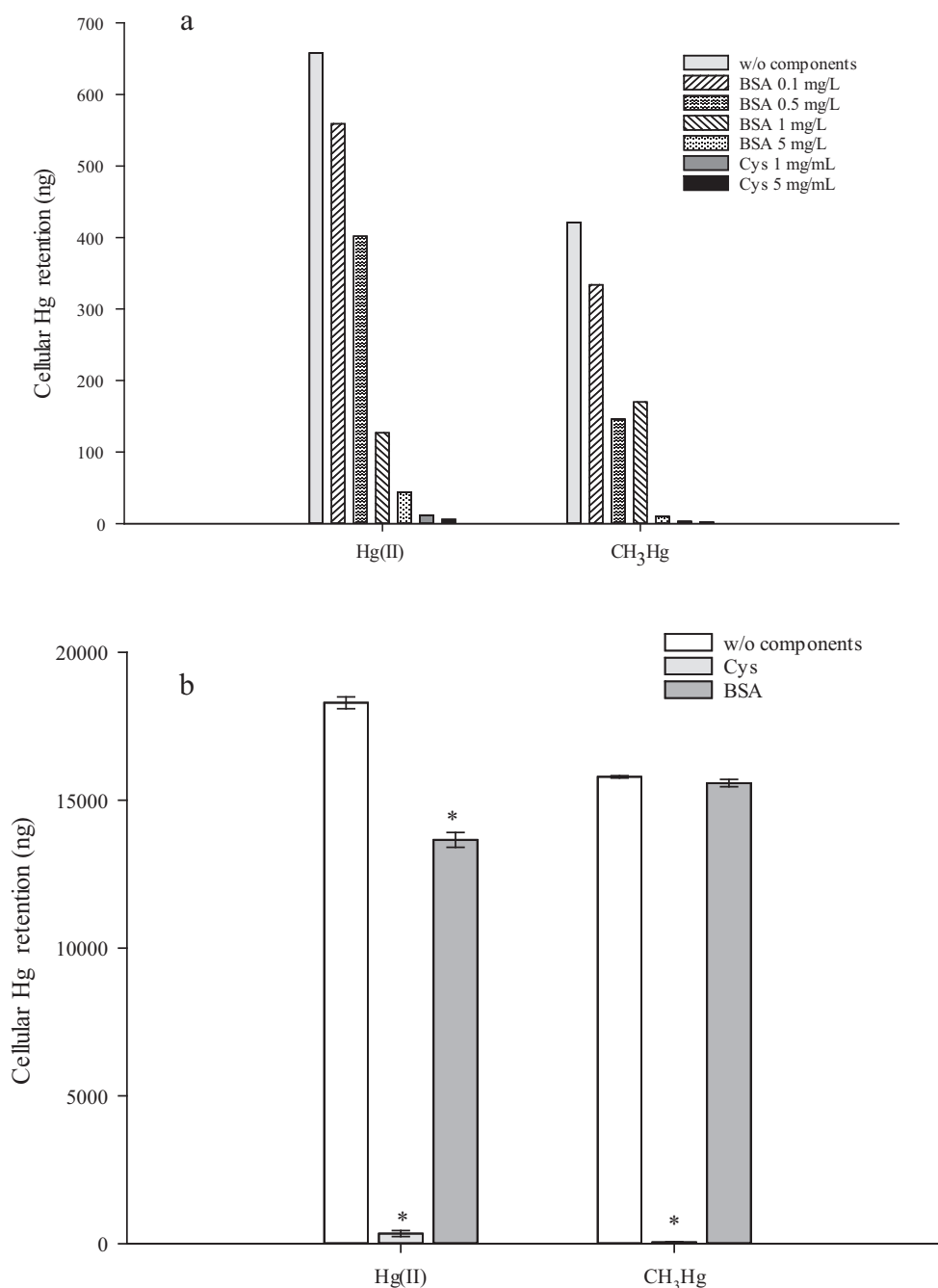
### 3.3. Effect of heat treatment of LAB on mercury uptake

Finally, studies were conducted to determine whether heat-killed bacteria had a different effect on the bioaccessibility of mercury from that observed with live bacteria. Some studies have shown that the effectiveness of non-viable bacteria in metal sequestration is greater than that obtained with viable cells (Topcu and Bulat, 2010). The results showed that the effect of the non-viable bacteria was similar to that of viable bacteria in solutions and in samples of seafood products (Fig. 5). However, at OD<sub>595</sub> 4 the non-viable bacteria reduced the bioaccessibility of the mercury present in mushrooms significantly in contrast to live bacteria. This reduction (32–45%) is similar to that observed when working with viable bacteria at greater densities (OD<sub>595</sub> 7; Fig. 3).

## 4. Discussion

The presence of mercury in some food matrices, especially in seafood products, is a public health problem. The annual reports of the European Rapid Alert System for Food and Feed (RASFF & Feed, European Food Safety Authority, 2014) have shown that metals are the group of abiotic contaminants that has generated the greatest number of notifications of contamination in food products, and mercury and chromium are the ones that present most problems. EFSA reports that the medians of 95th percentile dietary exposures to mercury are close to or above the tolerable weekly intake (TWI) for all age groups in the European population, and that high consumers of fish meat may exceed the TWI by up to six-fold (EFSA, 2012). Some international agencies have issued recommendations indicating the need to reduce mercury in the diet.



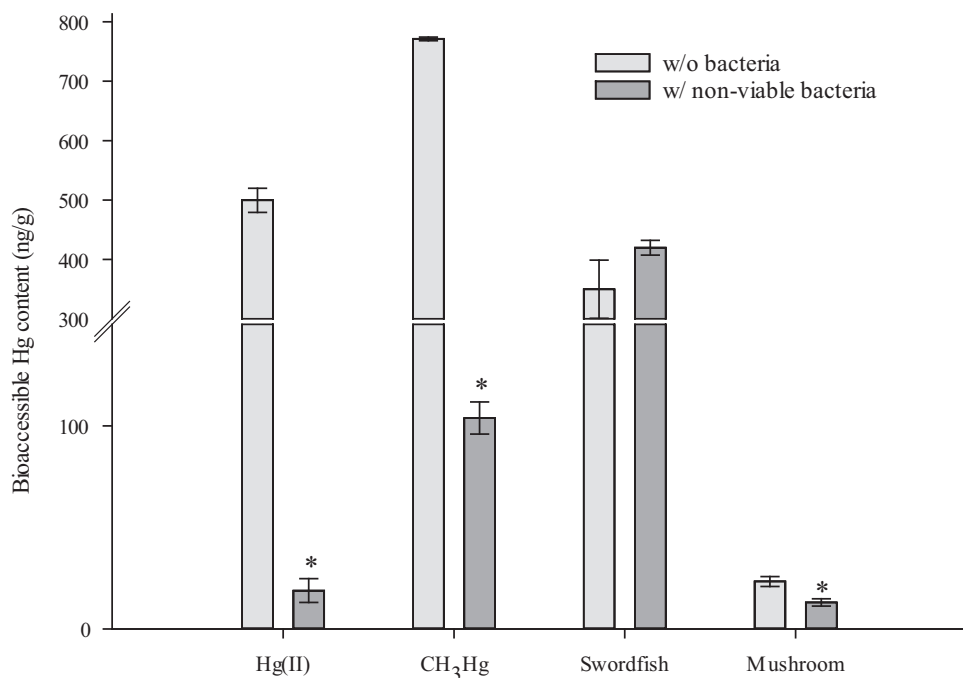


**Fig. 4.** Effect of L-Cysteine (Cys) and bovine serum albumin (BSA) on retention of mercury (Hg) by *L. casei* BL23. (4a) Cellular retention of Hg(II) and CH<sub>3</sub>Hg (1 mg/l) after incubation for 1 h in saline solution with *L. casei* BL23 (OD<sub>595</sub> 4) in the absence or presence of BSA (0.1, 0.5, 1 and 5 mg/ml) or Cys (1 and 5 mg/ml). Values expressed as mean (n = 2). (4b) Cellular retention of Hg after submitting standards of Hg(II) and CH<sub>3</sub>Hg (1 mg/l) supplemented with BSA (5 mg/ml) or Cys (1 mg/ml) to gastrointestinal digestion in the presence of *L. casei* BL23. Values expressed as mean ± SD (n = 3). In Fig. 4b, asterisks (\*) indicate statistically significant differences (p < 0.05) with respect to the retention obtained without Cys or BSA.

However, these recommendations clash with the fact that seafood products are important sources of nutrients and components with a high biological value, such as unsaturated fatty acids, and this makes it difficult to issue general recommendations to eliminate them or to reduce their presence in the diet. In fact, EFSA (2012) has said that “if measures to reduce methylmercury exposure are considered, the potential beneficial effects of fish consumption should also be taken into account”.

Studies conducted with a view to reducing dietary exposure to mercury have concentrated on seafood products because they are the ones that contribute most mercury to the diet. A study on cul-

tured bluefin tuna showed that it is possible to reduce the levels of mercury in this seafood product by controlling the feed given to the fish (Nakao et al., 2009). However, this strategy is not viable for large predators because they are not usually raised in captivity. A number of studies have aimed to remove mercury from fish after they have been caught. Cysteine was identified as the complexing agent with the greatest potential for use. Different assays have shown that high effectiveness (40–79%) was obtained by using long incubation times (24 h), acidic pHs (<3) and precooked or minced samples (Aizpurua, Tenuta-Filho, Sakuma, & Zenebon, 1997; Schab, Sachs, & Yannai, 1978; Yannai & Saltzman, 1973),



**Fig. 5.** Effect of non-viable bacteria on mercury (Hg) bioaccessibility. Concentration of Hg in the bioaccessible fraction of standard solutions of Hg(II) and CH<sub>3</sub>Hg (1 mg/l) or samples of swordfish and mushroom submitted to gastrointestinal digestion in the presence or absence of thermally treated *L. casei* BL23 (OD<sub>595</sub> 4). Values expressed as ng/g of food or ng/ml of standard solution (mean  $\pm$  SD; n = 4). Asterisks (\*) indicate statistically significant differences ( $p < 0.05$ ) with respect to the bioaccessible fraction obtained without bacteria.

but not with whole fillets, short times and neutral pHs. Other strategies have made use of chelators such as EDTA (Hajeb & Jinap, 2012), which is allowed to be used in foods as a sequestrant (E385) by the European Union (EU, 2011), but consumption is not recommended for children and pregnant women and in countries such as Australia its use as a food additive is not allowed. Unlike what happens with other toxic trace elements, cooking does not produce a reduction of the mercury present in seafood products, and it may even lead to an increase in the concentration owing to loss of water and other soluble components (Morgan, Berry, & Graves, 1997).

In view of the difficulty of reducing the mercury concentration in a seafood product before it is consumed, an alternative that might be considered as a way of reducing exposure could be to modify its arrival in the systemic circulation after ingestion. In an earlier study, Jadán-Piedra et al. (2016) suggested the use of food components (tannins, lignin, pectin and some celluloses) to reduce intestinal absorption of mercury from seafood products. The addition of these components to food significantly reduces the quantity of toxic element that is available for absorption after digestion (up to 98%); however for some treatments a reduction in absorption of minerals is also observed. This investigation has continued in the present study with the search for dietary strategies to reduce the oral bioavailability of mercury by using LAB strains. Most LAB have probiotic qualities, they are currently marketed, and they have good public acceptance. Furthermore, they are adapted to develop in the gastrointestinal tract, making them an attractive strategy for reducing heavy metals bioavailability through their delivery to this location (Monachese et al., 2012).

Studies conducted to determine mercury complexation by bacteria have shown that mercury-tolerant environmental strains isolated from soils, effluents and river sediments, sequestered Hg(II) extracellularly as spherical or amorphous deposits. Moreover, killed bacterial biomass also generated spherical extracellular mercury deposits, with a sequestration capacity (40–120 mg mercury

per g of biomass) superior to that of live bacteria (François et al., 2012). Subsequently, Kinoshita et al. (2013) showed that this chelating capacity extends to LAB, which are able to reduce the solubility of Hg(II) in aqueous solution. The present study evaluated whether LAB are also capable of chelating mercury under gastrointestinal digestion conditions, and whether it is possible to use LAB to reduce the bioaccessibility of mercury ingested with food matrices.

The results obtained show that LAB decreased the bioaccessibility of mercury in gastrointestinal conditions, with remarkable reductions of the soluble fraction in solutions of Hg(II) and CH<sub>3</sub>Hg (72–98%). However, this reduction was not observed when seafood matrices were employed and only occurred with heat-killed bacteria or high concentrations of viable bacterial cells in mushrooms. After evaluating various factors that might affect the reduction, it is suggested that the main cause of this reduced effectiveness in seafood products is the way in which mercury is released from the food matrix during digestion or the complexes that this element forms with other components solubilized during the digestive process.

The application of the simulated digestion of mercury solutions supplemented with Cys clearly showed that this amino acid significantly reduces and almost abolishes the mercury uptake capacity of *L. casei* BL23, possibly as a result of formation of mercury–Cys complexes which probably prevent interaction of mercury with the bacteria. Cabañero et al. (2007) demonstrated that, after application of an *in vitro* gastrointestinal digestion to swordfish, the solubilized mercury, mainly CH<sub>3</sub>Hg, appeared to be mostly bound to Cys. The reason why the bacteria were only effective in reducing the mercury solubilized from mushrooms might be that, in these samples, the mercury released during the digestion forms complexes with Cys in a lower proportion. It must be noted that most mushrooms are deficient in sulphur-containing amino acids (Al-Enazi, El-Bahrawy, & El-Khateeb, 2012).

## 5. Conclusions

This is the first time that the potential of LAB for reducing mercury exposure from foods is evaluated under realistic and controlled conditions (presence of food and emulated gastrointestinal digestion). In this respect, only one study in humans exists in which blood levels of several heavy metals were determined in a population that regularly consumes fish after intake of yoghurt containing an *L. rhamnosus* strain (Bisanz et al., 2014). In this study, it was reported that a low (3.2 nM) but significant reduction in mercury levels was obtained in pregnant women. The data obtained in this study showed that LAB are capable of taking up inorganic mercury and methylmercury in the conditions existing in gastrointestinal digestion and that, in principle, these bacteria could be useful to avoid absorption of this metal when it is ingested. However, there is a lack of effectiveness with seafood products. This situation, which could be due to the appearance of mercury–Cys or mercury–polypeptide complexes in the digest that cannot easily be taken up by the bacteria, might be modified *in vivo* by the composition and interactions in the lumen, which are more complex than those emulated with the *in vitro* digestion.

Furthermore, this study showed once again that evaluation of bioavailability using trace element solutions may give a general idea but the presence of a food matrix could cause considerable variations in what happens. The findings derived from previous studies about the beneficial effect of LAB on the absorption and toxicity of other toxic trace elements conducted with oral dosing of aqueous standards cannot necessarily be extrapolated for the design of strategies to reduce the risk of exposure to contaminants conveyed mainly by foods.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2017.01.157>.

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