Use of *Saccharomyces cerevisiae* to Reduce the Bioaccessibility of Mercury from Food

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Running header: Reduction of Hg bioaccessibility by *Saccharomyces cerevisiae*

ABSTRACT

Food is the main pathway of exposure to inorganic mercury [Hg(II)] and methylmercury (CH₃Hg). Intestinal absorption of these mercury species is influenced by their chemical form, the luminal pH or the diet composition. In this regard, strategies have been proposed for reducing mercury absorption using dietary components.

The present study evaluates the capacity of *Saccharomyces cerevisiae* to reduce the amount of mercury solubilized after gastrointestinal digestion which is available for intestinal absorption (bioaccessibility). The results show that *S. cerevisiae* strains reduce mercury bioaccessibility from aqueous solutions of Hg(II) ($89 \pm 6\%$) and CH₃Hg ($83 \pm 4\%$), and from mushrooms (19-77%), but not from seafood. The formation of mercury-cysteine or mercury-polypeptide complexes in the bioaccessible fraction may contribute to the reduced effect of yeasts on mercury bioaccessibility from seafood. Our study indicates that budding yeasts could be useful for reducing the intestinal absorption of mercury present in water and some food matrices.

Keywords: Mercury, bioaccessibility, *Saccharomyces cerevisiae*, seafood, mushrooms.

1. INTRODUCTION

Mercury is a metal found in the environment, where it is of natural or anthropogenic origin. Food is the main pathway of exposure to inorganic divalent mercury [Hg(II)] and methylmercury (CH₃Hg). Seafood products, especially large predators, are main dietary sources of CH₃Hg, the concentrations of which often exceed the maximum limits contemplated by current legislation (1 mg/kg).¹ The European Food Safety Authority (EFSA) reports a maximum CH₃Hg intake of 1.57 µg/kg body weight/week in the European adult population.² This value is close to the tolerable weekly intake (TWI) proposed by the Joint FAO/WHO Expert Committee on Food Additives (JEFCA) (1.6 µg/kg body weight).³ With regard to inorganic mercury, the food groups “fish and other seafood” and “non-alcoholic beverages” are among the greatest contributors to the intake of this mercury species in the European population.² However, mushrooms are the food matrices with the highest inorganic mercury concentrations, reaching levels in the order of 20 mg/kg dry weight.⁴ JEFCA reports a mean weekly intake of inorganic mercury from food of 2.16 µg/kg body weight for the adult population, this value being lower than the TWI proposed by the JEFCA (4 µg/kg body weight).³

Mercury toxicity is dependent upon the chemical form of the element, dose, age of the individual, duration of exposure, exposure route and dietary habits. Methylmercury affects mainly the nervous system, whereas the main targets of inorganic mercury are the kidneys.^{2,3} Furthermore, CH₃Hg is considered to be neurotoxic during development,⁵ and mainly because of this the international public health safety authorities have established recommendations referred to the consumption of certain fish species in vulnerable population groups.^{6,7}

EFSA states that in some population groups the intake of CH₃Hg is up to 6-fold higher than the recommended levels, and that this can result in health problems. On the other hand, EFSA also underscores that decreasing the intake of seafood is a strategy that cannot be applied

without also taking into account that these foods are an important source of nutrients.² It is therefore of interest to seek alternatives allowing a decrease in mercury exposure through foods. One possibility is to reduce the amount of ingested mercury that is actually absorbed and reaches the bloodstream, i.e., to act upon the oral bioavailability of mercury. It is known that the absorption of Hg(II) is variable and relatively low, with percentages of between 1-38% that moreover depend on the solubility of the mercury salt involved.³ In this respect, halides, sulfates and nitrates show high solubility, with moderate absorption levels, while mercury sulfide is scantily soluble and thus undergoes minimum absorption.³ In contrast, the reported absorption rates for CH₃Hg exceed 80%,² even when consumed through seafood.⁸

Some in vivo studies have described modifications in the toxicokinetics of mercury in the presence of food components or dietary supplements. An increase in CH₃Hg elimination has been reported in animals fed different types of dietary fiber, the authors attributing this to interruption of the enterohepatic recirculation.⁹ Orct et al.¹⁰ recorded a decrease in plasma mercury levels in rats co-administrated with HgCl₂ (6.45 μmol kg⁻¹ body weight) and Na₂SeO₃ (19.4 μmol kg⁻¹ body weight). In addition to food components, studies have also examined the possible role of probiotics in reducing mercury entry into the bloodstream. A recent study has reported a decrease in blood mercury levels in pregnant women treated with *Lactobacillus rhamnosus* GR-1.¹¹ Kinoshita et al.¹² described the capacity of lactobacilli to bind mercury in vitro, this possibly being responsible for the decrease in bioavailability observed in the population based study. Likewise, certain strains of *Saccharomyces cerevisiae* have been shown to be useful in reducing Hg(II) present in solution,¹³ and therefore might exert effects similar to those described for lactobacilli in human populations.

The present study seeks to identify strains of *S. cerevisiae* capable of reducing the amount of mercury contained in food that can be absorbed in the gastrointestinal tract. For this purpose an in vitro study has been made to evaluate the capacity of different strains of *S. cerevisiae* to

bind to Hg(II) and CH₃Hg and their effect upon the bioaccessibility (i.e., the soluble amount obtained after gastrointestinal digestion that is available for absorption) of mercury present in water and food.

2. MATERIALS AND METHODS

2.1. Culture conditions of *Saccharomyces cerevisiae*. The strains of *S. cerevisiae* used in the present study are described in Table 1. Yeasts were maintained on solid YPD medium [1% (w/v) of yeast extract, 2% (w/v) of bacteriological peptone, 2% (w/v) of glucose (Sigma, Spain), and 1.5% (w/v) of bacteriological agar]. For each assay, independent yeast colonies were incubated overnight in liquid synthetic complete medium [0.17% (w/v) of yeast nitrogen base without amino acids and without (NH₄)₂SO₄, 0.5% (w/v) of (NH₄)₂SO₄, 0.2% (w/v) synthetic complete drop-out Kaiser mixture (Formedium, Norfolk, United Kingdom), and 2% (w/v) of glucose] at 30°C and 190 rpm. Next, yeast cells were collected by centrifugation at 4,000 rpm for 2 min, washed twice with phosphate buffered saline (PBS, Hyclone, Fisher, Spain), and then used for the assayed described below.

2.2. Mercury binding assays. Laboratory yeast strain BY4741 (strain #1) was incubated under agitation with 10 mL of the standard solutions of Hg(II) and CH₃Hg (1 mg/L) prepared in PBS from commercial standards of Hg(NO₃)₂ (Merck, Spain) and CH₃HgCl (Alfa Aesar, Spain) respectively. Incubation was performed at an approximate concentration of 5×10^7 cells/mL [4 optical densities (OD)/mL], at different temperatures (30°C and 37°C), and at 37°C for different times (30, 60 and 120 min). After the incubation period, the samples were centrifuged at 4,000 rpm for 2 min. The mercury content in the supernatant and in the cell pellet was analyzed according to the protocol described in section 2.5.

2.3. Mercury bioaccessibility assays. Bioaccessibility was evaluated using the in vitro gastrointestinal digestion model described by Jadán-Piedra et al.,¹⁴ with minor modifications.

Two different assays were performed:

- a) Study of the mercury biosorption capacity of *S. cerevisiae* during the gastrointestinal digestion of standard solutions of Hg(II) and CH₃Hg.
- b) Study of the mercury biosorption capacity of *S. cerevisiae* during the digestion of swordfish (*Xiphias gladius*), yellow fin tuna (*Thunnus albacares*) and dehydrated mushrooms (*Boletus edulis* and *Amanita caesarea*).

For this study we chose *S. cerevisiae* strain BY4741, which was added at a concentration of 4 OD/mL to standard solutions of mercury (1 mg/L) or food (5 g of seafood or 0.5 g of mushrooms), and the combination was then subjected to digestion. In both cases, the volume of the gastrointestinal digestion process was 50 mL. The pH was adjusted to 2.0 with 6 M HCl (Merck), and a sufficient volume of pepsin solution (0.1 g of pepsin/mL prepared in 0.1 M HCl) was added to provide 0.2 mg of pepsin/mL of digestion solution. The mixture was incubated at 37°C during 2 h under constant agitation (120 rpm). After gastric digestion, the pH was increased to 6.5 with 1 M NH₃ (Panreac, Spain), and a solution of pancreatin and bile extract [0.004 g/mL of pancreatin and 0.025 g/mL of bile extract in 0.1 M NH₄HCO₃ (Merck)] was added to yield a final concentration of 0.025 mg of pancreatin/mL of solution and 0.3 mg of bile extract/mL of solution. The mixture was incubated at 37°C during 2 h under constant agitation (120 rpm).

The pH was then adjusted to 7.2 with NH₃ and the samples were centrifuged at 10,000 rpm during 30 min at 4°C. The concentration of mercury in the soluble fraction obtained after centrifugation (bioaccessible fraction) was analyzed according to the protocol described in section 2.5. Bioaccessibility was calculated as a percentage using the following equation:

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

where A is the concentration of mercury in the bioaccessible fraction and B is the concentration of mercury in the standard solution or in the food before digestion.

The enzymes and bile salts used during digestion were acquired from Sigma-Aldrich, Spain: porcine pepsin (activity 944 U/mg of protein), porcine pancreatin (activity equivalent to the specifications of 4×US Pharmacopeia/mg of pancreatin) and bile extract (glycine, conjugates of taurine and other bile salts).

2.4. Study of the factors influencing the effect of *S. cerevisiae* upon the bioaccessibility of mercury in food. An evaluation was made of different factors that might influence the effect of the yeasts upon the bioaccessibility of mercury from food samples. BY4741 yeast strain was used in all assays, except for the study D. The factors analyzed were:

- A. Effect of yeast concentration. Three different yeast concentrations (4, 7 and 10 OD/mL) were added to samples of swordfish and mushrooms (*Amanita cesarea*). The resulting combination was subjected to the gastrointestinal digestion process described in section 2.3.
- B. Effect of the presence of cysteine (Cys) and bovine serum albumin (BSA). This assay was carried out to determine whether the binding of mercury to these compounds could affect its adsorption/uptake by yeast cells. The solutions of Hg(II) and CH₃Hg (1 mg/L) added with Cys (5 mg/L; Sigma) and BSA (5 mg/L; Biowest, Labclinic, Spain) were subjected to gastrointestinal digestion in the presence of *S. cerevisiae* (4 OD/mL), following the protocol described in section 2.3.
- C. Effect of the presence of Ca(II), Cu(II) and Fe(II). In this assay we determined whether the presence of divalent cations (ions with characteristics similar to those of mercury) could affect mercurial species retention by the yeasts through competitive mechanisms. Solutions of Hg(II) or CH₃Hg (1 mg/L) prepared in PBS were independently added with

CaCl₂ (100 mg/L; Panreac), CuSO₄ (40 mg/L; Panreac) or FeSO₄ (40 mg/L; Sigma), these being concentrations commonly found in food. The mixtures were incubated (2 h, 37°C) with *S. cerevisiae* (4 OD/mL) and then centrifuged at 4,000 rpm during 2 min. The cell pellet and supernatant were recovered to determine mercury according to the protocol described in section 2.5.

D. Effect of the *S. cerevisiae* strain. In addition to BY4741 strain, we studied the mercury biosorption capacity of 7 strains of *S. cerevisiae* from different sources (Table 1) by, incubating them at 4 OD/mL with solutions of Hg(II) and CH₃Hg (1 mg/L) prepared in PBS, as described in section 2.2.

Gastrointestinal digestions were then performed using three yeast strains (#3, #4 and #5) found to be most effective in reducing the solubility of mercury in standard solutions at a concentration of 4 OD/mL. These yeast strains were added to the samples of swordfish and mushrooms (*Tricholoma georgii*), and the combination was subjected to gastrointestinal digestion (section 2.3).

2.5. Determination of mercury. Microwave oven-assisted digestion (MARS, CEM, Vertex, Spain), with subsequent quantification by cold vapor atomic fluorescence spectrometry (CV-AFS; Millenium Merlin PSA 10.025, PS Analytical, Microbeam, Spain), was used for the determination of mercury in food samples, bioaccessible fractions, yeast cells and incubation media. For digestion, the samples were placed in a Teflon reactor, with the addition of 4 mL of 14 M HNO₃ (Merck) and 1 mL of H₂O₂ (30% v/v, Panreac). The reactor was irradiated in the microwave oven at a power setting of 800 W (180°C/15 min). After the digestion process, the digests were left to stand overnight to eliminate the nitrous vapors. Finally, the solution was made up to a final volume with HCl 0.6 M.

The quantification of mercury was carried out against a Hg(II) calibration curve in the concentration range of 0.05-2 ng/mL. Quality control was based on the analysis of the sample of RTC QCI1014 mercury certified water (certified value: 40.8 ± 1.19 µg/L, LGC Standards, Spain).

2.6. Statistical analysis. The statistical analysis of the results was based on the Student t-test for paired data or single-factor analysis of variance (ANOVA) with *post hoc* multiple comparisons using the Tukey HSD test (SigmaPlot, version 13.5). Statistical significance was considered for $p \leq 0.05$.

3. RESULTS

3.1. Studies on the mercury biosorption capacity of *S. cerevisiae*

3.1.1. Influence of temperature. The effect of *S. cerevisiae* upon uptake/adsorption of the different mercury species was initially evaluated at 30°C, this being the optimum temperature for growth of the yeast. The results showed laboratory yeast strain BY4741 (#1 in Table 1) to be able to accumulate a large percentage of added Hg(II) ($59 \pm 2\%$) and CH₃Hg ($76 \pm 4\%$) (Figure 1). However, since the purpose of our study was to evaluate the effect of *S. cerevisiae* during gastrointestinal digestion, and that the latter takes place at 37°C, we considered advisable to determine whether this difference in temperature affected the mercury biosorption capacity. The results obtained indicate that the temperature increment did not reduce the efficiency of the mercury uptake/adsorption process [Figure 1, Hg(II): $73 \pm 3\%$; CH₃Hg: $82 \pm 2\%$].

3.1.2. Influence of time. The kinetics of mercury binding by the laboratory yeast strain at 37°C was analyzed using incubation times of 30, 60 and 120 min (Figure 2). The biosorption of Hg(II) increased significantly with the duration of exposure, from $35 \pm 1\%$ after 30 min to 73

$\pm 3\%$ after 120 min. In contrast, the retention of CH_3Hg did not show significant variations over time ($88 \pm 3\%$ after 30 min and $82 \pm 2\%$ after 120 min), and was already found to be high after 30 min of exposure.

3.2. Studies on the capacity of *S. cerevisiae* to reduce bioaccessibility during gastrointestinal digestion

3.2.1. Assays in standard solutions. Figure 3 shows the amount of mercury retained by *S. cerevisiae* strain BY4741 after the gastrointestinal digestion of standard solutions of Hg(II) and CH_3Hg . Mercury retention by yeast was very high for both mercurial species, with percentages corresponding to Hg(II) ($89 \pm 6\%$) and CH_3Hg ($83 \pm 4\%$) similar to those obtained in the assays made without digestion (section 3.1). These results indicate that the digestion conditions used (pH, enzymes and salt concentrations) did not reduce the mercurial species uptake/adsorption capacity of *S. cerevisiae*.

3.2.2. Assays in food. Figure 4 shows the bioaccessible mercury contents following gastrointestinal digestion of food samples in the absence and presence of yeast strain BY4741 (4 OD/mL). The results indicate that yeast addition does not result in important changes in the amount of mercury present in the soluble fraction of swordfish and tuna. Importantly, a statistically significant decrease in bioaccessible content in mushrooms was recorded (19-33%). Therefore, these data indicate that budding yeast reduces mercury bioaccessibility from mushrooms but not from swordfish or tuna.

3.3. Factors influencing the reduction of mercury bioaccessibility from food

3.3.1. Yeast concentration. In samples of swordfish, the addition of yeast at higher optical densities (7-10 OD/mL) was not associated to increased mercury retention during digestion (data not shown) compared with that recorded at an optical density of 4 OD/mL (section 3.2.2). In the case of mushrooms, an increase in optical density from 4 to 7 OD/mL was associated to a nonsignificant further decrease in mercury bioaccessibility (4 OD/mL: 22%; 7 OD/mL: 28%). We concluded that, within the studied range, optical densities higher than 4 OD/mL were therefore not found to be a determining factor in mercury binding in food samples.

3.3.2. Presence of cysteine and albumin. A possible explanation for the inefficacy of yeast in reducing the bioaccessibility of mercury in the seafood samples could be that the mercury solubilized from the food matrix is present in a chemical form that cannot interact with yeast cells. In this regard, some studies have described the formation of complexes of mercury with Cys or with polypeptides or proteins,^{15,16} which might be unable to interact with yeast. Figure 5 shows mercury uptake/adsorption by yeast BY4741 in the presence of Cys or BSA during the digestive process. Both compounds were associated to significant reductions in the amount of mercury retained by yeast cells compared with those assays in which Cys or BSA was not added to the medium. The effect of Cys was particularly notorious, with reductions of over 95%. These data suggest that the formation of complexes of Hg(II) and CH₃Hg with sulfated amino acids or proteins could prevent the interaction of mercury with *S. cerevisiae*, thereby resulting in no reduction in mercury bioaccessibility.

3.3.3. Presence of divalent cations. Another possible explanation for the lesser effect of yeast upon the bioaccessibility of mercury in food would be the interaction of yeast with other matrix components for which they exhibit greater affinity, thereby preventing interaction with

mercury. We explored the above possibility using divalent cations with characteristics similar to those of the mercurial species studied, and which could compete for the same binding sites or for the same transport mechanisms. Therefore, we studied mercury binding to yeast in the absence of such cations and following the individual addition of Ca(II), Cu(II) and Fe(II). As shown in Figure 6, the presence of these divalent cations exerted no effect upon CH₃Hg, though significant reductions were observed in the uptake/adsorption of Hg(II) with Ca(II) ($18 \pm 3\%$) and particularly with Cu(II) ($36 \pm 2\%$).

3.3.4. Saccharomyces cerevisiae strain. To distinguish whether the effect in mercury retention exerted by the laboratory yeast BY4741 was strain specific or a feature of *S. cerevisiae* cells, we determined the amount of soluble mercury after incubating Hg(II) and CH₃Hg standards during two hours at 37°C with seven additional *S. cerevisiae* strains from different sources including laboratory, wine, dietetic, wild and sake yeast strains (Table 1). As shown in Figure 7, all the tested strains significantly reduced the mercury contents in solution. Following incubation, the initial amount of added Hg(II) (9200 ng) was found to have decreased to between 404 and 2241 ng (Figure 7a). In the case of CH₃Hg, with an initially added amount of 8432 ng, the soluble content following exposure to the yeasts ranged between 324 and 3028 ng (Figure 7b). These results demonstrate that mercury retention is an intrinsic characteristic of *S. cerevisiae*, but they also suggest that there are differences among yeast strains that significantly affect mercury bioaccessibility.

From these studies standard solutions, we selected wine strain VRB (strain #4), wine strain T73 (strain #3) and dietetic strain Ultralevura (strain #5) that exhibited a strong effect in decreasing Hg(II) and CH₃Hg solubility, for assessing their effects upon the bioaccessibility of mercury present in swordfish and mushrooms. As previously shown for laboratory strain BY4741 (section 3.2.2), none of these strains modified the bioaccessibility of mercury in

swordfish (data not shown). The effect in mushrooms was different (Figure 8), with reduction in the bioaccessible mercury contents superior to 50% for the 3 strains (strain #3: $55 \pm 3\%$; strain #4: $74 \pm 4\%$; strain #5: $61 \pm 8\%$). These results strongly suggest that the reduction in mercury bioaccessibility obtained from mushroom samples is a general characteristic of *S. cerevisiae* species.

4. DISCUSSION

Reducing mercury exposure through the diet is not easy. Extraction of the metal from food before marketing^{17,18} results in important changes in the physical and nutritional properties of the food due to the application of relatively long treatment processes or the need for food matrix transformation. Another option for reducing exposure is to lower the amount of mercury that reaches the systemic circulation after intake. This can be done in two complementary ways: reducing the amount of mercury rendered soluble after digestion and/or reducing absorption through direct action upon transport across the intestinal barrier. Jadán-Piedra et al.¹⁴ suggest the use of food components (tannins, lignin, pectin and some celluloses) in order to lower the bioaccessibility of mercury from seafood. The addition of these components to food significantly reduces ($\leq 98\%$) the amount of toxic element available for absorption after digestion. The present study has continued the search for dietary strategies aimed at lowering the oral bioavailability of mercury, focusing on the use of yeast strains belonging to the species *S. cerevisiae*.

Since ancient times, yeasts belonging to the genus *Saccharomyces*, particularly *S. cerevisiae*, have been used in many food-related fermentation processes. Recently, certain strains of *S. cerevisiae* have been adopted as food supplements due to their high contents in vitamins, minerals, fiber and proteins,¹⁹ and have been used as probiotics in the treatment of chronic, recurrent or acute diarrhea.^{20,21} Interest in the use of this yeast as bioabsorbing agents for

eliminating metals and metalloids has increased in recent years, since they are safe and easy to produce on a large scale, they are a subproduct of the food industry, and they are easy to study due to their genetic characteristics.²² The capacity of this yeast to retain or bind metals such as lead, cadmium, copper, aluminum, chromium, nickel, zinc and mercury has been demonstrated in aqueous solutions.^{13,23-25} They are also effective in eliminating metals from waste waters, acting as chelating and flocculating agents.^{26,27} In this regard, although the application of such yeasts has been limited to environmental samples, their chelating capacity could also be applicable to other areas, including health. Probiotics of the genus *Lactobacillus* have recently been used in a population-based intervention designed to reduce the absorption of metals ingested via the oral route.¹¹ These authors demonstrated a slight decrease in blood mercury levels in pregnant women. Some strains of *S. cerevisiae* could also be used in interventions of this kind. Their application moreover would afford other benefits related to their antioxidant and, in some cases, anti-inflammatory effects,²⁸ thereby countering two of the toxic effects associated to continued mercury exposure.

The above considerations led us to use *S. cerevisiae* in the present study. The most relevant results are summarized in table S1 and S2 of supplementary data. *Saccharomyces cerevisiae* is able to retain mercury under the conditions of gastrointestinal digestion, with reductions in aqueous solutions of the soluble fraction of Hg(II) (89%) and CH₃Hg (83%). This yeast was also effective in lowering the bioaccessibility of mercury present in mushrooms (up to 77%), though no such decrease was noted in seafood. This lesser efficacy is possibly attributable to the way in which mercury is released from seafood during digestion, or to the formation of complexes of mercury with amino acids, polypeptides or proteins solubilized during the digestive process. Likewise, certain components of the food matrix itself, such as divalent cations, might exert a negative effect in this regard.

The application of simulated digestion of mercury standards in the presence of Cys showed this amino acid to significantly reduce the capacity of the yeast to retain Hg(II) and CH₃Hg during digestion. Cabañero et al.²⁹ found Hg/Cys complexes to be present in the bioaccessible fraction obtained after the digestion of samples of swordfish, which would explain the lack of efficiency of yeast in reducing the bioaccessibility of mercury from this type of fish. The fact that yeast is more effective reducing mercury solubilized from mushrooms may be due to the fact that these food matrices are deficient in sulfated amino acids,^{30,31} and therefore the mercury released from the matrix might not form complexes with Cys.

The presence of copper also significantly reduced the retention of Hg(II) by *Saccharomyces* (38%). This decrease could be a result of competition with mercury for the same binding sites and/or same internalization mechanisms. In fact, studies on the biosorption of Cu(II) by *S. cerevisiae* have revealed important retention of this element.³² An X-ray absorption spectroscopic study with intact *S. cerevisiae* cells, found accumulated copper to be exclusively linked to sulfide groups.³³ The binding of Hg(II) and CH₃Hg to thiol groups has also been widely documented, and is even considered to constitute one of the main ways in which mercury accumulates and exerts its toxic activity in other eukaryotic cells.³⁴ Furthermore, synchrotron-based X-ray spectroscopic studies of bacteria exposed to Hg(II) have identified sulfhydryl groups as the dominant mercury binding groups in the micromolar and submicromolar range.³⁵ Further studies are necessary to decipher how copper interferes with this type of interaction.

We can conclude that *S. cerevisiae* is able to retain in vitro the main mercurial species in water and foods. The binding capacity of this yeast is maintained under the conditions of the gastrointestinal digestion process employed in our study, resulting in a decrease in the bioaccessibility of the mercury present in aqueous solutions or in mushrooms. However, effective biosorption was not observed in swordfish or tuna. This may be attributable to the formation of mercury-cysteine or mercury-polypeptide complexes in the soluble fraction, which

are not retained by yeast, or to the presence of matrix components that interact with yeast cells in the same way as mercury. These effects of *S. cerevisiae* could be modified in vivo due to the composition and interactions found in the lumen, which are more complex than those simulated by the in vitro digestion process used in our study. In vivo studies are therefore needed to confirm the results obtained here. It also would be interesting to characterize the mechanisms involved in mercury retention, with a view to designing adequate strategies for reducing oral exposure to mercury.

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FIGURE CAPTIONS

Figure 1. Effect of temperature upon the retention of Hg(II) and CH₃Hg (1 mg/L) by *S. cerevisiae* laboratory strain BY4741 (4 OD/mL) following an incubation period of 120 min. Values expressed as ng of mercury (mean $\pm$ SD, n=3). Asterisks indicate statistically significant differences in yeast retention at 37°C with respect to 30°C ($p \leq 0.05$).

Figure 2. Effect of incubation time upon the retention of Hg(II) and CH₃Hg (1 mg/L) by *S. cerevisiae* strain BY4741 (4 OD/mL) at 37°C. Values expressed as ng of mercury (mean $\pm$ SD, n=3).

Figure 3. Yeast mercury retention after subjecting the aqueous standard solutions of Hg(II) and CH₃Hg (1 mg/L) with *S. cerevisiae* strain BY4741 (4 OD/mL) to gastrointestinal digestion. Values expressed as ng of mercury (mean $\pm$ SD, n=3).

Figure 4. Bioaccessible mercury content after subjecting samples of swordfish (*Xiphias gladius*), mushrooms (*Amanita cesarea* and *Boletus edulis*) and tuna (*Thunnus albacares*) to gastrointestinal digestion in the absence or presence of *S. cerevisiae* strain BY4741 (4 OD/mL). Values expressed as ng of mercury (mean $\pm$ SD, n=3). Asterisks indicate statistically significant differences with respect to digestion without yeast ($p \leq 0.05$).

Figure 5. Mercury retention by *S. cerevisiae* strain BY4741 (4 OD/mL) during the digestion of standard solutions of Hg(II) and CH₃Hg (1 mg/L) in the absence or presence of cysteine (Cys, 5 mg/L) or albumin (BSA, 5 mg/L). Values expressed as ng of mercury (mean $\pm$ SD, n=3). Asterisks indicate statistically significant differences with respect to digestion without BSA and Cys ($p \leq 0.05$).

Figure 6. Mercury retention by *S. cerevisiae* strain BY4741 (4 OD/mL) during the incubation of standard solutions of Hg(II) and CH₃Hg (1 mg/L) in the absence or presence of Ca(II) (100 mg/L), Fe(II) (40 mg/L) and Cu(II) (40 mg/L). Values expressed as ng of mercury (mean $\pm$ SD,

n=3). Asterisks statistically significant differences with respect to incubation without cations ($p \leq 0.05$).

Figure 7. Soluble mercury contents after two hours of incubation at 37°C of different strains of *S. cerevisiae* (4 OD/mL) with a 1 mg/L solution of Hg(II) (Figure 7a) or CH₃Hg (Figure 7b). Values expressed as ng of mercury (mean $\pm$ SD, n=3). Asterisks indicate statistically significant differences with respect to incubation without yeast ($p \leq 0.05$). See Table 1 for a description of the yeast strains used.

Figure 8. Bioaccessible mercury content after subjecting the mushroom samples (*Tricholoma georgii*) to gastrointestinal digestion in the absence or presence of different strains of *S. cerevisiae* (4 OD/mL). Values expressed as ng of mercury/g of sample (mean $\pm$ SD, n=3). Asterisks indicate statistically significant differences with respect to digestion without yeast ($p \leq 0.05$). See Table 1 for a description of the yeast strains used.

Table 1. *Saccharomyces cerevisiae* strains used in the study.

<i>Strain number</i>	<i>Name</i>	<i>Characteristics</i>
1	BY4741	Haploid laboratory
2	BY4743	Diploid laboratory
3	T73	Wine
4	VRB	Wine
5	Ultralevura	Dietetic
6	YPS128	American wild
7	UWOPS03-461.4	Malaysian wild
8	Kyokai 6	Sake

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.

Figure 7.

Figure 8.