1 ***IN VITRO* EVALUATION OF THE EFFICACY OF LACTOBACILLI AND YEASTS IN**
2 **REDUCING BIOAVAILABILITY OF INORGANIC ARSENIC**

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10 **Abstract**

11 This study evaluates the potential of strains of *Lactobacillus* and *Saccharomyces cerevisiae* to
12 reduce inorganic arsenic [iAs, As(III)+As(V)] bioavailability. Removal of iAs from aqueous
13 solutions by these strains was evaluated and their effect on iAs bioaccessibility was analyzed by *in*
14 *vitro* gastrointestinal digestion. The effect of the strains on iAs absorption was also tested, using
15 intestinal cells. The results showed that iAs removal by the yeast strains was low (<10%). In
16 contrast, some lactobacilli substantially reduced As(V) concentration in solution (25–40%) but only
17 when the concentration of phosphate was ≤ 1 mM. Both microorganisms were able to reduce cellular
18 transport of iAs added as aqueous solution (11–58%). Moreover, a strain of *Lactobacillus*
19 *plantarum* reduced cellular transport of iAs from rice by up to 45%. We conclude that, *in vitro*,
20 some lactobacilli and yeasts could reduce intestinal absorption of inorganic As present in water and
21 food, and consequently reduce its bioavailability.

22

23 **Keywords:** inorganic arsenic, bioaccessibility, intestinal absorption, bioavailability, *Saccharomyces*
24 *cerevisiae*, *Lactobacillus*

25 **1. Introduction**

26 The toxicokinetics and toxicity of arsenic (As) largely depend on its chemical form. Inorganic As
27 species [As(III) + As(V)] are more toxic than organic As forms and can be found in food and water.
28 These species are considered by the International Agency for Research on Cancer as carcinogenic to
29 humans (Group 1; IARC, 2004). Chronic exposure to inorganic As is associated with greater
30 prevalence of cancer of lung, urinary tract, liver, kidney, and skin (Chen, Chen, Wu, & Kuo, 1992;
31 Smith et al., 1999).

32 Drinking water and food are the main sources of dietary exposure to inorganic As. According to
33 the World Health Organization (WHO), concentrations of As in water intended for human
34 consumption should not exceed 10 µg/L (WHO, 2012). However, in 2009 it was estimated that 140
35 million people in 50 countries were consuming water with As concentrations higher than the
36 recommended value. In many cases there is not an alternative source of water available to the
37 population, and it is used for drinking and cooking (Rahman et al., 2012; WHO, 2012). This occurs
38 in parts of Argentina, Chile, Mexico, Bangladesh, China, and India, among other places (Buchet &
39 Lison, 2000; Bundschuh et al., 2012). For the population that consumes drinking water with an As
40 concentration that complies with the WHO recommendation, food is the main source of the toxic
41 element. Health problems that associate with consumption of foods containing As are not as
42 evident, although it has been reported that some foods may represent a risk. For example, in its
43 latest report on inorganic As the European Food Safety Authority indicates that a risk cannot be
44 ruled out for frequent consumers of rice and products derived from algae, and it recommends trying
45 to reduce the intake of inorganic As (EFSA, 2009).

46 One way of acting to avoid high exposure to As through food and drinking water is to reduce the
47 quantity of the element that reaches the systemic circulation after its ingestion (bioavailability). A
48 reduction of bioavailability can be achieved by various mechanisms: reducing the concentration that
49 remains soluble for absorption by the intestinal mucosa after digestion (bioaccessibility), or
50 reducing transport of the element across the intestinal wall (intestinal absorption). Previous studies

51 have shown that the use of certain dietary components (glutathione, cysteine, tannic acid, iron salts,
52 cellulose) (Clemente, Devesa, & Vélez, 2016; Clemente, Devesa, & Vélez, 2017) leads to
53 substantial reductions of bioaccessibility and transport of the inorganic forms of As across the
54 intestinal mucosa.

55 Some strains of lactic acid bacteria and *Saccharomyces cerevisiae* have the ability to bind metal
56 cations, and even some oxyanions such as chromate (Monachese, Burton, & Reid, 2012;
57 Chiocchetti et al., 2018). Most of these studies were performed by placing the microorganisms in
58 contact with water spiked with the toxic elements and then determining cellular retention.
59 Furthermore, Jadán-Piedra et al. showed the effectiveness of various strains of lactic acid bacteria
60 and *Saccharomyces* in reducing the bioaccessibility and transport across intestinal cells of inorganic
61 mercury and methylmercury (Jadán-Piedra et al., 2017a; Jadán-Piedra, Baquedano, Puig, Vélez, &
62 Devesa, 2017b). These authors showed that this capability is due to the ability of these
63 microorganisms to adsorb/internalize forms of mercury. Some studies also show that administration
64 of *Lactobacillus* can reduce *in vivo* bioavailability of Cd and Pb (Tian et al., 2012; Zhai et al.,
65 2013). All these studies suggest that these microorganisms may be useful to develop strategies to
66 reduce exposure to toxic trace elements.

67 The aim of the present study was to search for strains of *Lactobacillus* and *S. cerevisiae* capable
68 of reducing the quantity of bioavailable As. In order to do so, we evaluated the effectiveness of the
69 strains in reducing the bioaccessibility of inorganic As, using an *in vitro* model of gastrointestinal
70 digestion, and their ability to reduce the quantity of inorganic As absorbed, using a cellular model
71 of intestinal epithelium.

72

73 **2. Materials and methods**

74

75 *2.1. Samples*

76 For the bioaccessibility and transport assays, standard solutions of As(III) and As(V) and a
77 sample of white rice were used. The As(V) standard (1000 mg/L, As₂O₅) was acquired from Merck.
78 The As(III) standard (1000 mg/L) was prepared by dissolving 1.320 g of As₂O₃ (Riedel de Haën) in
79 25 mL of 20% (m/v) KOH (Panreac), neutralizing with 20% (v/v) H₂SO₄ (Merck), and diluting to 1
80 L with 1% (v/v) H₂SO₄.

81 The rice was cooked to dryness using water spiked with 3 mg/L of As(V) (rice/water proportion:
82 1/3). No additional ingredients were used in the cooking process. The cooked sample was milled,
83 homogenized, and stored at -20 °C until analysis.

84

85 2.2. Strains of *Lactobacillus* and *S. cerevisiae*

86 The various strains of *Lactobacillus* (Table 1) were grown in MRS medium (Man, Rogosa, &
87 Sharpe; BD Difco) at 30 °C in static conditions. The strains of *S. cerevisiae* (Table 1) were
88 maintained in YPD medium (Yeast Peptone Dextrose) consisting of 0.5% (w/v) of yeast extract, 1%
89 (w/v) of bacteriological peptone, and 2% (w/v) of glucose (Sigma). For their use in the various
90 assays, the strains were washed with phosphate buffered saline (PBS, HyClone), and after
91 centrifugation (3000×g, 10 min) they were resuspended in the appropriate medium for the assays.

92

93 2.3. Evaluation of the removal from solution of inorganic As by *Lactobacillus* and *S. cerevisiae* 94 strains

95 Cell suspensions of the strains of *Lactobacillus* (optical density, OD 10) and *S. cerevisiae* (OD 4)
96 were incubated with As(III) and As(V) (both at 1 mg/L) at 37 °C for 4 h in PBS. Then the samples
97 were centrifuged at 3000×g for 2 min. The As concentrations in the supernatant and the cell pellet
98 were analyzed by dry ashing and subsequent quantification by flow injection-hydride generation-
99 atomic absorption spectrophotometry (FI-HG-AAS), following the protocol described by Clemente
100 et al. (2016).

101 The influence of various factors on the removal of inorganic As was also evaluated. These assays
102 were performed with *Lactobacillus plantarum* Lpp+ and *S. cerevisiae* strain 10 (Kyokai 6). The
103 parameters evaluated were:

104 A) Influence of concentrations of As(III) and As(V). The incubation was performed in PBS for 4
105 h, using two concentrations of the As species (0.1 and 1 mg/L).

106 B) Influence of cell viability. The bacteria and yeasts were treated thermally at 80 °C for 30 min.
107 They were then spiked in standards of As(III) and As(V) (1 mg/L) prepared in PBS, and the
108 mixtures were incubated at 37 °C for 4 h.

109 C) Influence of incubation medium. The incubation was performed with standards of As(III) and
110 As(V) (1 mg/L), replacing the PBS medium with 0.9% NaCl (m/v) with or without addition of
111 glucose (20 mM, Sigma) or increasing concentrations of sodium phosphate (0.01, 0.1, 1, and 10
112 mM; Merck).

113

114 2.4. Evaluation of the effect of *Lactobacillus* or *S. cerevisiae* on bioaccessibility of inorganic As

115 A static digestion method that simulates the gastric and intestinal steps of the human digestion
116 process (Jadán Piedra et al., 2017a) was used to evaluate the bioaccessibility of aqueous standards
117 of As(III) or As(V) (1 mg/L). The standards were subjected to the gastrointestinal digestion in the
118 presence or absence of cells of *Lactobacillus* strains BL36, BL10, and Lpp+ (OD 10; final volume
119 10 mL) or *S. cerevisiae* strains 2, 3, and 9 (OD 4; final volume 25 mL). The As concentration in the
120 bioaccessible fraction was quantified in accordance with the protocol indicated in section 2.3. The
121 bioaccessibility of As was determined as: (As in the soluble fraction/As in the aqueous standard) ×
122 100.

123

124 2.5. *Effect of Lactobacillus and S. cerevisiae on intestinal permeability of inorganic As*

125

126 2.5.1. *Maintenance of cell cultures*

127 Two human cell cultures of the colon epithelium were used: NCM460 (non-transformed
128 absorptive cells) and HT29-MTX (mucosecretory cells from an adenocarcinoma). The NCM460
129 cell line was acquired from Incell Corporation and maintained in 75 cm² flasks with 10 mL of
130 modified Dulbecco medium (DMEM) with glutamine (0.87 g/L) and high glucose content (4.5 g/L)
131 at pH 7.2. The medium was supplemented with 10% (v/v) of fetal bovine serum (FBS), 1 mM of
132 sodium pyruvate, 10 mM of *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 100
133 U/mL of penicillin, 0.1 mg/mL of streptomycin, and 0.0025 mg/L of amphotericin B (NCM-
134 DMEMc). The assays were performed with cells between passages 11 and 19.

135 The HT29-MTX cell culture was provided by Dr. Thécla Lesuffleur (Institut National de la Santé
136 et de la Recherche Médicale, INSERM UMR, France). Cell maintenance was performed in DMEM
137 with glutamine and high glucose content supplemented with 10% of FBS, 1 mM of sodium
138 pyruvate, 10 mM of HEPES, 100 U/mL of penicillin, 0.1 mg/mL of streptomycin, and 0.0025 mg/L
139 of amphotericin B (HT-DMEMc). The assays were performed with cells between passages 17 and
140 25.

141 The cells were incubated at 37 °C in an atmosphere with 95% relative humidity and CO₂ at 5%,
142 with a change of medium every 3 days. When the cell monolayer reached 80–90% confluence, the
143 cells were detached with a solution of trypsin (0.5 g/L) and EDTA (ethylenediaminetetraacetic acid,
144 0.2 g/L) and reseeded at a density of 1.5×10^4 cells/cm². All the reagents used for culture
145 maintenance were obtained from HyClone.

146

147 2.5.2. *Evaluation of apparent permeability of inorganic As in aqueous solution*

148 The assays were performed in 12-well plates with polyester membrane inserts (diameter 12 mm,
149 pore size 0.4 µm, Transwell, Corning). In this system the cells are seeded on the porous membrane

150 of the insert that separates the well into two compartments: apical (upper) and basolateral (lower).
151 The cells were seeded at a density of 1.8×10^5 cells/cm² in supplemented DMEM, combining the
152 two cell types to obtain NCM460/HT29-MTX co-cultures (80/20). On the basolateral side, 1.5 mL
153 of supplemented DMEM was added. The cells were kept at 37 °C in an atmosphere with 5% CO₂
154 and 95% relative humidity, with a change of medium every 3 days until the time of the assay (9–10
155 days post-seeding).

156 The permeability assays were performed at 37 °C in the apical-basolateral direction (absorptive
157 direction), using various strains of *Lactobacillus* (OD 0.6) and *S. cerevisiae* (OD 1). Before
158 evaluating all the strains, a preliminary permeability assay was performed with the *Lactobacillus*
159 *rhamnosus* LGG strain and *S. cerevisiae* strain 2 (BY4743) to determine the most favorable form of
160 exposure. The conditions assayed were the following:

161 a) Preincubation: the monolayers were incubated for 2 h with the microorganisms in non-
162 supplemented DMEM added on the apical side. After incubation, the medium with the
163 microorganisms was removed and 0.5 mL of the As(III) and As(V) standards (0.75 mg/L) prepared
164 in Hank's balanced salt solution (HBSS) with 10 mM HEPES was added to the apical compartment.
165 In the basolateral compartment, 1.5 mL of HBSS medium with 10 mM HEPES was added.

166 b) Coexposure: 0.5 mL of the As(III) and As(V) standards (0.75 mg/L) prepared in HBSS with
167 10 mM HEPES and with the microorganisms was added to the apical compartment.

168 After exposure to each of the treatments, aliquots (1 mL) were taken from the basolateral
169 compartment at various timepoints and replaced with an equal volume of fresh medium (HBSS with
170 10 mM HEPES). Arsenic was quantified in the aliquots that were obtained as previously outlined
171 (section 2.3).

172 The coefficients of apparent permeability (P_{app}) were calculated using the equation: $P_{app} =$
173 $(dC/dt) \times [V_r/(A \times C_o)]$; where dC/dt is the flow (μg/s) determined by the linear slope of the
174 equation that governs the variation in the As concentrations, corrected for dilution, versus time; V_r
175 is the volume of the receptor compartment (1.5 mL); A is the surface occupied by the cell

176 monolayer (1.12 cm^2), and C_o is the initial concentration of As added to the donor compartment
177 ($0.75 \text{ }\mu\text{g/mL}$ in the assays with standards).

178

179 *2.5.3. Evaluation of permeability of As present in foods*

180 The permeability assays were also performed with bioaccessible fractions of cooked white rice
181 obtained by applying the protocol described in section 2.4, using 5 g of rice for a final digestion
182 volume of 50 mL. The bioaccessible fraction was subjected to a thermal treatment at $90 \text{ }^\circ\text{C}$ for 5
183 min to inactivate the enzymes. For facilitating the maintenance of cell viability and activity during
184 the assays, glucose (final concentration 5 mM, Sigma) was added to the inactivated soluble fraction
185 and the osmolarity was adjusted with NaCl 3 M (Panreac) to values between 270 and 310
186 mOsmol/kg, using a cryoscopic Automatic Micro-Osmometer (Löser).

187 The inactivated bioaccessible fraction (0.5 mL) was added to the apical side of the cell cultures
188 seeded in two-chamber systems, with or without the strains of bacteria and yeasts, and the
189 permeability was evaluated in accordance with the protocol described in section 2.5.2.

190

191 *2.5.4. Evaluation of cell monolayer integrity for the transport assays*

192 To determine the validity of the transport assays we evaluated the P_{app} of Lucifer Yellow (LY,
193 Sigma), a fluorescent compound that is mainly transported paracellularly and whose transport is an
194 indicator of cell monolayer integrity. LY was added to the apical compartment at a concentration of
195 $100 \text{ }\mu\text{M}$ at the same time as the treatments. The fluorescence of LY in the aliquots collected from
196 the basolateral compartment was measured with a microplate reader (PolarSTAR OPTIMA reader,
197 BMG-Labtech) at excitation/emission wavelengths of 485/520 nm. The assays were considered
198 valid when the P_{app} values were $\leq 1 \times 10^{-5} \text{ cm/s}$.

199 The transepithelial electrical resistance (TEER) was determined at the beginning and end of the
200 assay, using a Millicell-ERS voltmeter (Millipore Corporation). The transport assays were

201 considered valid when the TEER values did not vary by more than 25% at the end of the assay with
202 respect to the initial measurement.

203

204 2.6. Statistical analysis

205 The statistical analysis was performed by applying Student's *t*-test or a one-way analysis of
206 variance (ANOVA) with multiple post hoc comparisons by means of the Tukey HSD test
207 (SigmaPlot, version 13.5). Differences were considered significant for $p < 0.05$.

208

209

210 3. Results

211

212 3.1. Capacity of strains of *Lactobacillus* and *S. cerevisiae* to bind inorganic As

213 The results obtained in the removal assays with lactobacilli and yeasts after contact with
214 solutions of As(III) and As(V) in PBS (1 mg/L) for 4 h showed that none of the strains evaluated
215 possessed a high capacity to retain inorganic As (<6% of the As added to the medium initially).

216 In order to characterize the type of interaction between the metalloid and the bacteria/yeasts
217 some factors that might influence the capacity of removing the metalloid by these microorganisms
218 were evaluated: concentration of As, viability of the microorganisms, and compounds present in
219 the incubation medium.

220

221 3.1.1. Concentration of As

222 Some studies on removal of metals by bacteria or yeasts indicate that as the concentration
223 increases their capacity of removing the element decreases (Chiocchetti et al., 2018). To determine
224 whether this was the reason for the low accumulation we worked with a lower concentration of the
225 As species (0.1 mg/L instead of 1 mg/L). The results obtained (Table 2) showed an increase in the

226 retention percentage in comparison with those obtained with a concentration of 1 mg/L; however,
227 the retentions were not high (lactobacilli: 2–12%; yeasts: 1–6%). These data indicate that the low
228 cellular retention observed at the higher concentration was not due to a process of saturation.

229

230 3.1.2. Viability of microorganisms

231 Figure 1 shows the removal of As by *L. plantarum* Lpp+ and *S. cerevisiae* (Kyokai 6; strain 10)
232 thermally treated (80 °C, 30 min) and untreated. Removal was low in both conditions, although
233 viability significantly favored cellular accumulation ($p < 0.05$). This was similar for As(III) and
234 As(V) in the *S. cerevisiae* strain, whereas in *L. plantarum* Lpp+ thermal treatment reduced the
235 incorporation of As(V) to a greater extent than what was observed for As(III). These results suggest
236 that removal is favored by a process that needs metabolism/cell transport. This mechanism of
237 uptake coexists with a surface interaction with cell components, especially notable in the uptake of
238 As(III) by the bacteria.

239

240 3.1.3. Incubation medium

241 Studies conducted in other cell systems have shown that part of the transport of As(V) takes
242 place by means of phosphate transporters, owing to the structural similarity of these two oxyanions
243 (Villa Bellosta & Sorribas, 2008; Calatayud, Barrios, Vélez, & Devesa, 2012). For As(III) other
244 kinds of transporters have been reported: glucose permeases, aquaporins, and organic anion
245 transporters (Liu et al., 2002; Liu et al., 2006; Calatayud et al., 2012). Therefore, transport of
246 inorganic As may be affected by the presence of substrates of these transporters as a result of
247 competition or by reduction of cell metabolism. To evaluate this, we performed incubations of *L.*
248 *plantarum* Lpp+ and *S. cerevisiae* strain 10 (Kyokai 6) with As in media with various compositions.

249 The results obtained showed that removal of As(III) was not affected by changes in the
250 composition of the medium (data not shown). However, there were differences in removal of As(V)
251 (Figure 2). Incubation in saline medium without phosphate (0.9% NaCl) significantly increased ($p <$

0.05) the retention of As(V) in both microorganisms with respect to the data obtained with PBS. The addition of inorganic phosphate to the saline medium at the same concentration as in the PBS (10 mM of inorganic phosphate) significantly reduced ($p < 0.05$) the quantity of As(V) accumulated by the bacteria and yeasts. The presence of glucose (20 mM) in the medium significantly increased ($p < 0.05$) the retention of As(V) in yeast with respect to that observed in 0.9% NaCl.

We also evaluated the concentration of inorganic phosphate required to reduce cellular accumulation of As(V) in bacteria and yeasts. Figure 3 shows the accumulation of As(V) in the two microorganisms without inorganic phosphate in the medium and with increasing concentrations of it (0.01–10 mM). The results show that with inorganic phosphate concentrations greater than 0.1 mM the retention of As(V) began to decrease significantly ($p < 0.05$). In lactobacilli, a 27% reduction in the accumulation was observed with 1 mM of inorganic phosphate, and an 88% reduction with 10 mM. The retention of As(V) at the phosphate concentrations ≤ 1 mM is high (38–40%). In *S. cerevisiae*, the reduction was 28% with an inorganic phosphate concentration of 0.1 mM and 60% with concentrations of 1 and 10 mM. Even without inorganic phosphate, the retention percentages in yeasts were low ($\leq 10\%$).

To determine whether the concentration of phosphate in the medium affected removal capacity of other strains of *Lactobacillus*, strains BL36, LGG, and BL10 were incubated with As(V) in saline medium with a phosphate concentration of 1 mM. In these conditions the retention of As was also considerable for strains BL36 (25%) and BL10 (28%). However, the LGG strain did not present high accumulation (9%).

3.2. Effect of lactobacilli and yeasts on bioaccessibility of inorganic As

The data obtained in the removal assays in aqueous medium demonstrated that the microorganisms employed have a limited capacity to reduce the quantity of soluble As. However, as the conditions of the digestion process are different from those employed in the removal assays and the digestive process involves the addition of various enzymes with activities that might modify the

278 surface of these microorganisms, we considered it useful to determine the retention of inorganic As
279 by these microorganisms during the gastrointestinal digestion.

280 Cellular accumulation of As(III) and As(V) (1 mg/L) in bacteria and yeasts after applying a
281 simulated gastrointestinal digestion was not substantial for any of the strains assayed ($\leq 9\%$, data not
282 shown).

283

284 3.3. *Effect of lactobacilli and S. cerevisiae on intestinal permeability of inorganic As in vitro*

285 Initial assays with *L. rhamnosus* LGG and *S. cerevisiae* BY4743 strains showed that for the
286 bacterium only preincubation favored a reduction in As permeability ($33 \pm 18\%$) using an intestinal
287 epithelial cell model, whereas for the yeast similar reductions were obtained (preincubation: $25 \pm$
288 7% ; coexposure: $22 \pm 4\%$). In view of these results, the remaining assays were performed with
289 preincubation for the *Lactobacillus* strains [BL17, BL36, LGG, and Lpp+ (Table 1)] and with
290 coexposure for the yeasts [strains 2, 3, 9, and 10 (Table 3)].

291

292 3.3.1. *Effect on permeability of aqueous solutions of As(III) and As(V)*

293 After preincubation for 2 h (OD 0.6) the assays with *Lactobacillus* strains showed significant
294 reductions ($p < 0.05$) in transport across the intestinal monolayer (Table 3). The reductions were
295 greater for As(III) (33–58%) than for As(V) (15–32%). Noteworthy reductions in permeability of
296 As(III) were observed with the BL36, LGG, and Lpp+ strains. With yeasts (Table 3), most of the
297 strains assayed (OD 1) also reduced the cellular permeability of As(III) and As(V) significantly ($p <$
298 0.05), with greater reductions in the transport of As(V). The reductions produced by strains 2
299 (BY4743) and 9 (UWOPS03-461.4) were especially noteworthy.

300 3.3.2. Effect on permeability of As present in foods

301 Figure 4 shows the effect of the strains of bacteria and yeasts selected from the assays with aqueous
302 As standards (section 3.3.1) on the P_{app} of the As present in bioaccessible fractions of rice obtained
303 after an emulated digestion process. The data showed a significant reduction ($p < 0.05$) in the
304 permeability of As derived from rice for the Lpp+ bacterial strain (preincubation; $45 \pm 13\%$) and for
305 all the yeast strains assayed (coexposure; 19–27%).

306

307

308 4. Discussion

309 The use of microorganisms based on their ability to take up toxic trace elements (e.g., As, Cd,
310 Cr, Cu, Hg, Ni, or Pb) has spread in recent years, and their use as a bioremediation strategy has
311 given satisfactory results (Dixit et al., 2015; de Alencar, Navoni, & Do Amaral, 2017; Niloofar,
312 Morteza, & Seyyed, 2017). Retention of metals/metalloids by bacteria and yeasts takes place mainly
313 by means of two mechanisms, adsorption of the element to the surface and/or internalization.

314 The surface of lactic acid bacteria consists of a thick layer of peptidoglycans, (lipo)teichoic
315 acids, proteins, and polysaccharides. These structures contain various negatively charged groups
316 that can favor surface binding of metal cations (Teemu, Seppo, Jussi, Raija, & Kalle, 2008;
317 Gerbino, Mobili, Tymczyszyn, Fausto, & Gomez-Zavaglia, 2011). The cell wall of yeasts consists
318 of various layers that contain anionic groups. The role played by phosphomannans and carboxyl
319 groups in *S. cerevisiae* cell wall proteins in the binding of metal cations has been identified
320 previously (Seki, Suzuki, & Maruyama, 2005). The composition of the surface of these
321 microorganisms facilitates the adsorption of metal cations, as has been shown for Hg, Pb, and Cd
322 (Chiocchetti et al., 2018), but not of anions such as inorganic As. Results reported in this study are
323 in agreement with this notion as retention of inorganic As was not high (<6%). Halttunen, Finell
324 and Salminen (2007) showed that amination of the *Lactobacillus casei* DSM20011 cell wall, which

increased the positive charge, can increased the removal of As(V) from water, but not of As(III). However, other studies have reported efficient removal of As(III) by a strain of *L. acidophilus* (Singh & Sarma, 2010) or *S. cerevisiae* (Wu, Wen, Zhou, Dai, & Wu, 2012).

The fact that retention of As(III) and especially of As(V) decreases considerably when non-viable cells are used indicates that the incorporation of As takes place by means of a metabolic process, such as internalization in the cell mediated by a membrane transporter. Various membrane transporters involved in the transport of inorganic As in these microorganisms have been identified. Shen, Shah, Chen, & Da Silva (2012) showed that overexpression of the phosphate transporter Pho84 in *Saccharomyces* strains produced a 50% increase in transport of As(V), which is a structural analogue of phosphate (HAsO_4^{-2} vs. HPO_4^{-2}). Shah, Shen, Chen, & Da Silva (2010) also showed that cells of *S. cerevisiae* that overexpress the aquaglyceroporin Fps1p take up 40% more of As(III) from the extracellular medium. In the bacterium *Escherichia coli*, As(V) can be internalized by means of two phosphate transporters, a high-affinity system (Pst) and a low-affinity system (Pit) (Yang, Fu, Lin, & Rosen, 2012). These data are compatible with the fact that transport of As(V) is inhibited competitively owing to the presence of phosphate in the medium. The low capacity of uptake of As(III) and the presence of appreciable concentrations of phosphate in the intestinal lumen would reduce the possibilities of using these microorganisms to decrease the soluble fraction of inorganic As obtained during the gastrointestinal digestion.

Given that the possibility of reducing exposure to inorganic As by decreasing bioaccessibility is low, we studied the possibility of reducing exposure by modifying intestinal absorption of the toxic element. *In vitro* studies of transport across intestinal epithelial cells using the two types of microorganisms showed considerable reductions with the Lpp+ bacterial strain and some *S. cerevisiae* strains (absorption reduced by 11 to 49%). These reductions were obtained both with aqueous standards of inorganic As (Table 3) and with As conveyed by means of food (Figure 4). The reduction in absorption may be a consequence of the formation of an As/microorganism complex that is not absorbed or else a modulation of cell monolayer permeability by

351 microorganisms that reduces As transport. This study has shown that inorganic As has a low
352 interaction with the bacteria and yeasts assayed; therefore, the reduction of transport must be a
353 consequence of the effect of these microorganisms on intestinal cells permeability.

354 Earlier studies showed that the transport of inorganic As species has a predominantly
355 paracellular component (Calatayud, Gimeno, Vélez, Devesa, & Montoro, 2010; Calatayud, Devesa,
356 Montoro, & Vélez, 2011), a component which is largely governed by proteins that form tight
357 junctions (Anderson & Van Itallie, 2009). It has been shown in *in vitro* and *in vivo* assays that some
358 strains of lactic acid bacteria can increase expression of tight junction proteins and modulate
359 intestinal permeability (Ulluwishewa et al., 2011; Bron et al., 2017). Also, some strains of *S.*
360 *cerevisiae* favor the restoration of tight junctions and the permeability of the intestinal barrier after
361 bacterial infections (Moslehi-Jenabian, Pedersen, & Jespersen, 2010). Therefore, the modulation of
362 the permeability by lactobacilli and yeast can be responsible for the considerable reductions in As
363 transport across the intestinal monolayers reported here.

364

365 **5. Conclusions**

366 The *in vitro* results obtained in the present study suggest that strains of lactic acid bacteria and
367 yeasts could reduce exposure to inorganic As by decreasing intestinal transport possibly through
368 modulation of tight junctions. However, studies in animal models are needed to confirm our results.
369 This would make it possible to determine whether microbial strains could be used as a future
370 intervention strategy in populations with high exposure to inorganic As.

371

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503

504 **Table 1. Strains of *Lactobacillus* and *Saccharomyces cerevisiae* assayed.** ^aDeutsche Sammlung
505 von Mikroorganismen und Zellkulturen; ^bAmerican Type Strain Culture Collection; ^cColección
506 Española de Cultivos Tipo (CECT); ^dCentre National de Recherches Zootechniques (CNRZ);
507 ^eKleerebezem et al. (2003); ^fCollections in other laboratories; ^gCollection of the Lactic Bacteria and
508 Probiotics Laboratory [Instituto de Agroquímica y Tecnología de Alimentos (IATA)-Consejo
509 Superior de Investigaciones Científicas (CSIC)]; ^hYeast strains obtained from Dr. Amparo Querol
510 collection (IATA-CSIC).

511

Code	Strain	Origin
<i>Lactobacillus</i>		
BL7	<i>Lactobacillus brevis</i> DSMZ ^a 1268	Beer
BL10	<i>Lactobacillus acidophilus</i> ATCC ^b 9224	Sour milk
BL17	<i>Lactobacillus acidophilus</i> ATCC ^b 4356	Human intestine
BL23	<i>Lactobacillus casei</i> CECT ^c 5275	Dairy products
BL36	<i>Lactobacillus brevis</i> ATCC ^b 14869	Human intestine
BL73	<i>Lactobacillus acidophilus</i> CNRZ ^d 55	Rat feces
BL75	<i>Lactobacillus acidophilus</i> CNRZ ^d 217	Rat digestive microbiota
BL166	<i>Lactobacillus plantarum</i> WCFS1 ^e	Human intestine
BL221	<i>Lactobacillus crispatus</i> M247 ^f	Feces from weaning baby
BL278	<i>Lactobacillus crispatus</i> DSMZ ^a 20584	Isolated from tears
BL279	<i>Lactobacillus acidophilus</i> CECT ^c 4529	Chicken intestine
BL280	<i>Lactobacillus acidophilus</i> CECT ^c 4179	Rat feces
LGG	<i>Lactobacillus rhamnosus</i> ATCC ^b 53103	Human intestine
Lpp+	<i>Lactobacillus plantarum</i> ^g	Laboratory strain
<i>Saccharomyces cerevisiae</i>^h		
2	BY4743	Diploid laboratory strain
3	T73	Wine strain
4	VRB	Mannoprotein-producing strain
7	Ultralevura	Probiotic strain (UPSA medica S.L.)
8	YPS128	Wild strain of American origin
9	UWOPS03-461.4	Wild strain of Malaysian origin
10	Kyokai 6	Sake strain

512

513 **Table 2. Removal of inorganic As (0.1 mg/L) by strains of *Lactobacillus* and *Saccharomyces***
514 ***cerevisiae*.** Cellular retention of As after exposure of the strains of *Lactobacillus* (OD 10) and *S.*
515 *cerevisiae* (OD 4) to As(III) and As(V) (0.1 mg/L, 5 mL) in PBS for 4 h. Values expressed as ng As
516 (mean $\pm$ SD, n = 3) and as percentage of retention with respect to the amount of As(III) or As(V)
517 added.

518

	Strain	As(III)	As(V)
<i>Lactobacillus</i>	BL17	14.0 $\pm$ 1.3 (4.7%)	7.5 $\pm$ 0.2 (1.9%)
	BL36	21.4 $\pm$ 2.1 (7.1%)	19.4 $\pm$ 2.7 (5.4%)
	Lpp+	33.9 $\pm$ 2.3 (11.5%)	12.8 $\pm$ 1.5 (4.4%)
	BL75	9.1 $\pm$ 1.7 (3.1%)	7.7 $\pm$ 0.6 (2.2%)
<i>Saccharomyces cerevisiae</i>	2	27.3 $\pm$ 0.9 (5.5%)	22.6 $\pm$ 0.1 (4.5%)
	3	9.3 $\pm$ 0.1 (1.9%)	17.7 $\pm$ 3.2 (3.6%)
	8	23.4 $\pm$ 1.7 (4.9%)	6.6 $\pm$ 1.1 (1.1%)
	9	27.8 $\pm$ 3.2 (5.8%)	7.4 $\pm$ 1.1 (1.2%)
	10	16.0 $\pm$ 5.2 (3.3%)	6.2 $\pm$ 1.3 (1.0%)

519

520 **Table 3. Effect of lactobacilli and yeasts on transport of As(III) and As(V).** Reductions in the
 521 coefficients of apparent permeability (P_{app}) of NCM460/HT29MTX co-cultures (80/20)
 522 preincubated with bacteria (OD 0.6) for 2 h and subsequently exposed to As(III) or As(V) (0.75
 523 mg/L) for 2 h or coexposed to As(III) or As(V) (0.75 mg/L) and yeasts (OD 1) for 2 h. Values
 524 expressed as percentages of reduction with respect to the assays performed without microorganisms
 525 (mean $\pm$ SD; n = 4). Asterisks indicate statistically significant differences with respect to the assays
 526 without microorganisms ($p < 0.05$).

527

Code	Reduction of P_{app} (%)	
	As(III)	As(V)
<i>Lactobacillus</i>		
BL17	32.9 $\pm$ 5.2*	16.3 $\pm$ 2.3*
BL36	43.8 $\pm$ 10.0*	32.3 $\pm$ 12.1*
LGG	48.1 $\pm$ 15.6*	24.8 $\pm$ 4.8*
Lpp+	57.9 $\pm$ 12.2*	15.4 $\pm$ 3.7*
<i>Saccharomyces cerevisiae</i>		
2	32 $\pm$ 2.6*	49 $\pm$ 1.1*
3	11 $\pm$ 2.2*	34 $\pm$ 7.0*
9	21 $\pm$ 3.5*	45 $\pm$ 5.6*
10	21 $\pm$ 4.0*	22 $\pm$ 4.1*

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529

530 **FIGURE CAPTIONS**

531 **Figure 1.** Effect of strain viability on removal of inorganic As. Amounts of As(III) and As(V) (1
532 mg/L, 5 mL) retained after exposure of bacterial strain Lpp+ (OD 10) and yeast strain 10 (OD 4)
533 with or without prior heat treatment. Values expressed as ng As (mean $\pm$ SD, n = 3). Asterisks
534 indicate statistically significant differences in the retention of As between microorganisms
535 thermally treated or untreated ($p < 0.05$).

536 **Figure 2.** Effect of incubation medium on removal of inorganic As by lactobacilli and yeasts.
537 Cellular retention of As after exposure of bacterial strain Lpp+ (OD 10) and yeast strain 10 (OD 4)
538 to As(V) (1 mg/L, 5 mL) in various incubation media for 4 h. Values expressed as ng As (mean $\pm$
539 SD, n = 3). Asterisks indicate statistically significant differences with respect to the retention
540 observed in PBS ($p < 0.05$).

541 **Figure 3.** Effect of concentration of inorganic phosphate in the medium on removal of As(V) by
542 lactobacilli and *S. cerevisiae*. Cellular retention of As after exposure of Lpp+ strain (OD 10, Figure
543 3A) and *S. cerevisiae* strain 10 (OD 4, Figure 3B) to As(V) (1 mg/L, 5 mL) in saline medium (0.9%
544 NaCl) with various concentrations of inorganic phosphate for 4 h. Values expressed as ng As (mean
545 $\pm$ SD, n = 3). Asterisks indicate statistically significant differences with respect to the retention
546 observed without inorganic phosphate ($p < 0.05$).

547 **Figure 4. Effect of strains of *Lactobacillus* and yeasts on transport of As from rice.** Coefficients
548 of apparent permeability (P_{app}) of NCM460/HT29-MTX co-cultures (80/20) exposed for 75 min to
549 the bioaccessible fractions with or without strains of *Lactobacillus* and yeasts (mean $\pm$ SD; n = 6).
550 Asterisks indicate statistically significant differences in the values of P_{app} with respect to the
551 permeability assays performed without microorganisms (w/o microorganisms; $p < 0.05$).

552

553 **Figure 1.**

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556 **Figure 2.**
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560 **Figure 3.**
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565 **Figure 4.**

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