Use of lactic acid bacteria and yeasts to reduce exposure to chemical food contaminants and toxicity

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ABSTRACT

Chemical contaminants that are present in food pose a health problem and their levels are controlled by national and international food safety organizations. Despite increasing regulation, foods that exceed legal limits reach the market. In Europe, the number of notifications of chemical contamination due to pesticide residues, mycotoxins and metals is particularly high. Moreover, in many parts of the world, drinking water contains high levels of chemical contaminants owing to geogenic or anthropogenic causes.

Elimination of chemical contaminants from water and especially from food is quite complex. Drastic treatments are usually required, which can modify the food matrix or involve changes in the forms of cultivation and production of the food products. These modifications often make these treatments unfeasible. In recent years, efforts have been made to develop strategies based on the use of components of natural origin to reduce the quantity of contaminants in foods and drinking water, and to reduce the quantity that reaches the bloodstream after ingestion, and thus, their toxicity. This review provides a summary of the existing literature on strategies based on the use of lactic acid bacteria or yeasts belonging to the genus *Saccharomyces* that are employed in food industry or for dietary purposes.

Keywords: metals, mycotoxins, cyanotoxins, pesticides, lactic acid bacteria, *Saccharomyces*.

1. INTRODUCTION

Dietary exposure to chemical contaminants of natural or anthropogenic origin is a matter of concern for food safety organizations. Legislation on these food contaminants is increasingly restrictive and constantly revised. At the same time, vigilance has increased in recent decades. Nevertheless, food alerts related to chemical contamination keep being issued, indicating that the problem is not solved. The latest report of the European Union's Rapid Alert System for Food and Feed (RASFF, 2015) states that food safety events notified as a result of chemical contamination are especially high for mycotoxins (495 notifications), pesticide residues (405), and metals (209). Most common notifications of chemical contamination concern to the presence of aflatoxins (AF) and ochratoxin A (OTA) in nuts and derivatives as well as spices, chlorpyrifos (CP) in fruits, vegetables, cocoa and derivatives, coffee, and tea, and mercury (Hg) in seafood products.

Drinking water deserves separate mention. The case of arsenic (As) is especially well known; its presence in water in certain geographical areas is connected with its natural occurrence in the local bedrocks, and the levels of As in drinking water can be two orders of magnitude greater than the limit recommended by the World Health Organization (0.01 mg/L; WHO, 2012), causing situations classified as public health emergencies (Smith et al., 2000; Bundschuh et al., 2012). Water is also the main vehicle for oral exposure to cyanotoxins such as microcystin-LR (MC-LR) or cylindrospermopsin (CYN) (WHO, 1999; Dietrich and Hoeger, 2005). If water is obtained from a surface source during a cyanobacterial bloom, there is risk of contamination with toxins released after cell decomposition. Episodes of contamination with cyanotoxins in raw water, during drinking water treatment, and even in finished water, are numerous and problematic worldwide (Dietrich and Hoeger, 2005). Food contamination with these toxins has also been reported, especially in fish, edible crops, and supplements (Testai et al., 2016).

Reducing or eliminating these chemical contaminants of water and food is complex. To achieve this aim, changes have been made in the production systems (forms of cultivation, geographical localization of cultivation, area of capture or breeding), in the raw material storage processes and in the processing methods. However, the success of these actions is conditioned by the contaminant in question so that for some contaminants, such as Hg in large predatory fishes, the problem remains unsolved. In recent years' research has been conducted with the aim of reducing chemical contaminants in ingested food, or even the amount of contaminant that reaches the systemic circulation by adding components of natural origin to food and drinking water. This review provides a summary of the existing literature on strategies that employ lactic acid bacteria (LAB) or yeasts belonging to the *Saccharomyces* genus that are employed in food industry or for dietary purposes. LAB are a broad group of Gram-positive, non-spore forming, microaerophilic or anaerobic bacteria that characterizes for producing lactic acid as the major end product of sugar fermentation. The group includes various genera that are important for the food industry, such as *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, and *Weissella*, all of them belonging to the phylum *Firmicutes*. Bifidobacteria have been classically considered as LAB due to their phenotypical similarities to Firmicutes LAB, although they are phylogenetically unrelated (the belong to the phylum *Actinobacteria*), *Saccharomyces cerevisiae* is the most widely used yeast species, and some strains and their products are used for baking, alcoholic fermentation, or as nutritional supplements for humans and animals. The widespread application of many LAB species and *S. cerevisiae* in foods and their status as Generally Recognized as Safe (GRAS) organisms make them a suitable basis for strategies devised to diminish oral exposure to chemical contaminants.

2. BINDING CAPACITY OF LAB AND *SACCHAROMYCES*

Many LAB strains and yeasts of the *Saccharomyces* genus are safe microorganisms, easy to grow on a large scale and a common by-product of food industry. They also have a great capacity for adsorption and/or uptake of various kinds of chemical contaminants that are present in aqueous solution. These characteristics have driven a number of studies on their use in the areas of environment, health, and food safety as strategies to reduce exposure to chemical contaminants, and some applications of them have been patented. The mechanisms of interaction LAB or Saccharomyces/chemical food contaminants are varied (figure 1) and depend on the nature of the contaminant, the microbial strain and the physicochemical conditions.

2.1. Metals and metalloids. Most of the interactions of LAB with metals/metalloids involve "surface processes", i.e. adsorption of metal contaminants to functional groups on the cell surface. The surface of LAB is composed of a thick layer of peptidoglycan, teichoic acids, protein, and polysaccharides. Some strains also produce exopolysaccharides, which are released into the surrounding media but are also loosely attached to the bacteria, forming an amorphous layer outside the cell wall peptidoglycan (Lahtinen et al., 2004) (figure 1). These structures contain various negatively charged groups (carboxyl, hydroxyl, and phosphoryl) capable of binding cations.

Halttunen et al. (2007a) evaluated three *Lactobacillus* strains, three *Bifidobacterium* strains, and two commercial mixed LAB starter cultures, and demonstrated that up to 99% of cadmium (Cd) and 97% of lead (Pb) were removed from solutions with concentrations from 0.1 to 1 mg/L. They showed that binding of Cd and Pb to LAB is immediate, with maximum uptake after 5 minutes of contact, and pH-dependent, with higher accumulations being observed at pH values close to neutral. The authors suggested that the dependence on pH indicates that ion

exchange might be partly responsible for metal binding. They also showed that temperature (4–37 °C) does not affect binding, suggesting that the process is not energy-dependent. Teemu et al. (2008) showed that binding of Cd and Pb by *Lactobacillus fermentum* ME3 and *Bifidobacterium longum* 46 is reduced by chemical modifications when the negative charge of carboxyl and phosphoryl groups is neutralized, suggesting that both groups have a significant role in binding these metals. The relevance of the carboxylate groups of proteins of the S-layer was also shown by Fourier transform infrared spectroscopy analysis of the interaction of these proteins obtained from *Lactobacillus kefir* with a number of metal ions (Cd, Zn, Pb, and Ni) (Gerbino et al., 2011).

Another study investigated the binding capacity of inorganic Hg [Hg(II)] and the organic form methylmercury (CH_3Hg) to various strains of *Lactobacillus* (Alcántara et al., 2017). Binding of both forms was immediate and mainly occurred at the cell wall. Cell viability was not required for binding; in fact, an increase of binding capacity was observed for heat-killed cells (Alcántara et al., 2017). Furthermore, *Lactobacillus casei* mutants affected in genes involved in the modulation of the negative charge of the cell wall anionic polymer lipoteichoic acid showed increased Hg biosorption.

Kinoshita et al. (2013) determined that binding capacity of a number of LAB followed the order Hg(II) > Pb > Cd >>As(III), being As(III) binding very low in all cases. The difficulty of removing inorganic As from aqueous solutions by LAB has been shown in other studies (Halttunen et al., 2007b, Elsanhoty et al., 2016). These studies suggest that the negative charge of As species prevented their adsorption to the negatively charged surface of LAB. Halttunen et al. (2007b) carried out a chemical modification of the surface of the bacteria by means of methylation to neutralize the negative charge of carboxylic groups, or by amination to change the negative charge of carboxylic groups to the positive charge of amino groups. The results showed that native, methylated and aminated *L. casei* DSM20011 were all ineffective in As(III)

removal at all tested conditions, suggesting that electrostatic repulsion at the cell surface was not the main factor explaining poor As(III) binding. Removal of As(V) was also not achieved with native and methylated strains, and only aminated *L. casei* removed up to 38%, the maximum being obtained at low concentrations (0.1 mg/L) and a short incubation time (8 min). The authors concluded that, despite this removal, the use of aminated *L. casei* may not be practical because the biomass removal capacity is low and interactions between As(V) and the bacteria are weak.

Yeasts belonging to the *Saccharomyces* genus have also been evaluated as possible biosorbents of metals and metalloids (Wang and Chen, 2006). Infante et al. (2014) showed that *S. cerevisiae* in the form of a commercial dry preparation of baker's yeast had a high capacity for uptake of Pb (86%) and Hg(II) (70%) in aqueous solution. Volesky et al. (1993) showed that *S. cerevisiae* had greater potential than other yeasts such as *Saccharomyces uvarum* and *Candida utilis* in biosorption of Cd in aqueous solution. As occurred with LAB, binding capacities were high (up to 75%) at short times (5 min), with better retention being observed in yeast biomass from the exponential growth phase than in commercial wet pressed or dried biomass.

Seki et al. (2005) showed the poor ability of dry yeast to retain As(V). They demonstrated that methylation of the carboxyl groups on the surface of the yeast favored biosorption of the metalloid, with a maximum at pH 7, although they did not observe a high uptake in any case. It must be emphasized that the concentrations that they used were very high (0.5–2.5 mM; 156–780 mg/L). Shen et al. (2012) showed that *S. cerevisiae* was effective in removing As(V) from the medium at concentrations of 120 ng/L; however, at concentrations of 10 mg/L the reduction of soluble As(V) was less than 10%. The same result was found for As(III). Wu et al. (2012) demonstrated high retention of As(III) by *S. cerevisiae* at concentrations of 100 ng/L. The use of *Saccharomyces* for removal of another oxyanion, chromate [Cr(VI)], has also been shown to

be effective (Krauter et al., 1996). In that study the authors demonstrated that the effectiveness was greater for viable cells (61–100%) than for heat-treated cells (0–70%). Some studies have immobilized yeasts for biosorption of metal ions from water. *S. cerevisiae* immobilized in chitosan by forming beads of chitosan/lignosulphonate matrix has proved to be highly effective for removing Cr(VI), with complete removal of the metal at concentrations ≤ 100 mg/L (Saifuddin and Raziah, 2007).

Evidence available indicates that *S. cerevisiae* accumulates metals by means of two processes with clearly differentiated characteristics (figure 1). There is an initial stage of rapid accumulation that is not dependent on metabolism and that involves metal cation binding to the cell surface. This stage is followed by a slower accumulation process that depends on cell metabolism and involves internalization of the metal (Suh et al., 1998). As a result, viable cells accumulate a greater quantity of metal(loid) than non-viable cells. The yeast cell wall is composed of several layers bearing anionic groups. The inner layer consists of β-D-glucans [β(1,3)- and β(1,6)-D-glucans] and chitin, and represents 50–60% of the cell wall dry weight. The outer layer is made of heavily glycosylated mannoproteins (figure 1). The role of phosphomannans and carboxylic groups of cell wall proteins of the yeast *S. cerevisiae* in the binding of metal cations has been identified (Seki et al., 2005). Some of the transporters involved in internalization of metals/metalloids have also been described. Gomes et al. (2002) verified that Zrt1, a transporter responsible for high affinity Zn uptake in yeast, mediates Cd uptake, since control *S. cerevisiae* absorbed 23% of Cd from the medium after 24 h, while *zrt1* mutant cells did not remove Cd at all. Engineered strains overexpressing the Pho84 phosphate transporter internalized up to 750 μg of As(V) per gram of cells in solutions containing high As(V) concentrations (12–30 mg/L), a 50% improvement over control strains without Pho84 overexpression (Shen et al., 2012). Indeed, at high concentrations of As(V) in the medium, the presence of 50 μM phosphate reduces As uptake. Shah et al. (2010) also showed that *S.*

cerevisiae cells overexpressing the aquaglyceroporin Fps1p removed 40% more As(III) from the extracellular medium than control yeast.

2.2. Mycotoxins. Hernández-Mendoza et al. (2009a) studied the ability of several strains of *L. casei* obtained from various ecological niches (cheese, corn silage, human feces, fermented beverage) to bind aflatoxin B1 (AFB1) during 4 h at 37 °C, showing a maximum biosorption of 49%. Haskard et al. (2000) showed greater retentions by *Lactobacillus rhamnosus* strain GG after 1 h of incubation with AFB1 (76–84%), without observing important differences between viable and non-viable cells, or between different pHs (2.5–8.5). Peltonen et al. (2001) evaluated the ability of 12 *Lactobacillus*, 5 *Bifidobacterium*, and 3 *Lactococcus* strains to bind AFB1 at 37 °C during 24 h. Between 6 and 60% AFB1 was removed from the solution by these strains. Binding is reversible, and AFB1 is released by repeated aqueous washes. As occurs with metals, binding takes place in the first moments of contact (El-Nezami et al., 1998; Peltonen et al., 2001).

Fuchs et al. (2008) studied the ability of 30 LAB strains to reduce the amount of OTA and patulin (PAT) present in aqueous solution after incubation at 37 °C for 4 h. Most strains displayed poor binding capacity with the noticeable exceptions of *Lactobacillus acidophilus* VM20 (97% OTA decrease), *B. longum* VM14 and LA02 (58% and 54% OTA decrease), *Lactobacillus plantarum* VM37 (44% OTA decrease), and *Bifidobacterium animalis* VM12 (82% PAT decrease). This study showed that the binding capacity of these compounds was strain-dependent. Furthermore, highest binding was achieved with viable bacteria at low concentration of the toxins (especially for PAT), high cell density ($\geq 10^8$ colony forming units (CFU) per mL), and a pH of 5. Niderkorn et al. (2006) demonstrated the effect of LAB on other mycotoxins present in foods, deoxynivalenol (DON), fumonisins B1 (FB1) and B2 (FB2), and

zearalenone (ZEA). LAB displayed higher removal capacity of FB2 and ZEA (57–100%), followed by FB1 (14–82%); while DON had the lowest removal percentage (10–55%).

Studies on the mechanisms of mycotoxin uptake by LAB show that binding appears to be predominantly extracellular (figure 1). After various chemical and enzymatic treatments, Lahtinen et al. (2004) concluded that exopolysaccharide can be ruled out as a possible AFB1 binding component, and that cell wall peptidoglycan or components bound covalently to peptidoglycan are the components most likely involved. Hernández-Mendoza et al. (2009b) showed that LAB with a deficiency in teichoic acids accumulate less AFB1, and they indicated that these components of the cell wall associated with peptidoglycan may be important in binding to this mycotoxin. Other studies have demonstrated the role of peptidoglycan in binding to OTA, PAT, and FB (Niderkorn et al., 2009; Wang et al., 2015). Niderkorn et al. (2009) suggested that the differences in FB binding capacity of different LAB species can be rationally explained by the variation in peptidoglycan structure, governed mainly by the amino acid sequence of peptide bridges that crosslink the conserved linear glycan chains. It has also been suggested that some mycotoxins are bound to bacteria by weak, non-covalent interactions, such as association with hydrophobic pockets on the bacterial surface (Haskard et al., 2001).

The use of LAB to reduce the quantity of mycotoxins is not confined only to their ability to bind these contaminants; in some cases, there have also been reports of their ability to inhibit growth of the fungi that produce them. Franco et al. (2011) showed that LAB isolated from wheat derivatives and kefir grain were capable of inhibiting the fungus *Fusarium graminearum*. Gomah and Zohri (2014) demonstrated that *L. rhamnosus* presented activity against growth of *F. graminearum*, *Fusarium culmorum*, and *Fusarium proliferatum*, which are the main producers of DON, ZEA, and FB1 mycotoxins, respectively. The presence of the bacteria completely suppressed mycelium growth of all the *Fusarium* species studied and consequently inhibited production of toxins. The antimicrobial activity of LAB may be related to competition

for nutrients in the medium, to production of organic acids, such as lactic acid, and to production of antagonistic compounds, such as phenyllactic acid and cyclic dipeptides, among others (Magnusson et al., 2003).

Binding of AF by yeast strains is also a fast and reversible process. However, their binding ability is generally lower than that of bacterial strains (Guan et al., 2011). Pizzolitto et al. (2011) showed variable reductions in the quantity of AFB1 (17–82%) related to the strain and the concentration of the mycotoxin but independent of the contact time (1–360 min). Armando et al. (2012) demonstrated the ability of various strains of *S. cerevisiae* to retain ZEA and OTA in aqueous solution after 1 h of incubation. The uptake was slightly influenced by the concentration of the mycotoxin. The retention ranged from 46 to 74% at 1 μg/mL of OTA, and from 14 to 58% at 10 μg/mL. The same tendency, but less marked, was observed for ZEA, for which the retentions at low concentrations (48–87%) were greater than those observed at higher concentrations (41–68%). Joannis-Cassan et al. (2011) evaluated the capacity of yeast-based products (yeast cell wall or inactivated yeast) for biosorption of ZEA, AFB1, and OTA, observing that adsorption improved when yeast cell wall was used. Several studies have shown the importance of the cell wall of *S. cerevisiae* in the removal of mycotoxins. Jouany et al. (2005) showed that β-D-glucans are the yeast component responsible for complexation of ZEA, and that the reticular organization of β-D-glucans and the distribution between β-(1,3)-D-glucans and β-(1,6)-D-glucans play a major role in the efficacy. Luo et al. (2015) demonstrated that the PAT adsorption capability of *S. cerevisiae* is mainly affected by cell wall thickness and 1,3-β-glucan contents.

2.3. Cyanotoxins. There are no reports of binding of these toxins to yeasts and there are few studies that show their biosorption by LAB. Three probiotics, *L. rhamnosus* strains GG and LC-705, and *Bifidobacterium lactis* strain Bb12, were found to bind MC-LR from aqueous solutions

(Meriluoto et al., 2005). The highest removal percentage was 35%, observed with 10^{10} CFU/mL and a MC-LR concentration of 0.5 mg/mL during an incubation of 24 h at 35 °C. Nybom et al. (2007, 2008a) showed higher retentions of this cyanotoxin (up to 60%) when working at lower concentrations (0.1 mg/mL). Unlike what is observed for metals and mycotoxins, binding was not immediate and it increased with exposure time. On the other hand, removal was much greater when working with viable cells and at optimum temperature (37 °C) (Nybom et al., 2007). The findings of Nybom et al. (2012) suggest that the disappearance of MC-LR is partly due to a process of degradation by the cell wall-associated proteinases of LAB (figure 1). This would explain why viable cells at optimum activity temperatures generate the greatest removals. In an earlier work (Nybom et al., 2008b), the same authors showed an improvement in MC-LR removal after addition of glucose, which suggests that an energy-consuming process is responsible for part of its removal by LAB. Removal of the cyanobacterial toxin CYN is not as effective as that of MC-LR; Nybom et al. (2008a) achieved reductions of less than 32% using incubations of 0.1 mg/L of toxin with 10^{10} CFU/mL for 24 h.

As occurs with mycotoxins, there are reports of LAB activity against the cyanobacteria that produce the toxins. Kang et al. (2016) showed that *Lactobacillus paraplantarum* (10^8 cells/mL) strongly inhibited *Anabaena flos-aquae* and *Anabaena crassa* (1.1×10^5 cells/mL), species of a genus linked with production of the majority of the known hepatotoxins (microcystins) and neurotoxins (anatoxins and saxitoxins) (Becker et al., 2010). However, the same treatment with LAB was not able to inhibit the three cyanobacteria of the *Microcystis* genus assayed. Kang et al. (2016) demonstrated that cyanobactericidal bacteria could lyse cells of *A. flos-aquae* indirectly without cell-to-cell contact during the whole experimental period.

The studies described in this section indicate that various strains of LAB are effective in uptaking cyanotoxins. However, there are no studies that verify whether the use of LAB can

reduce the levels of these contaminants in contaminated water or food (section 3) or reduce their ability to reach the systemic circulation and prevent their toxicity (sections 4 and 5).

2.4. Pesticides. The pesticides that have been most studied are organophosphorus (OP) pesticides. The role of LAB in removing them is mostly associated with a process of bacterial degradation (figure 1). The metabolism of CP, an OP pesticide, by two lactic acid bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) was reported by Shaker et al. (1988). The culture medium in which the bacteria were grown initially contained 7.4 mg/L of CP, but after 96 h displayed a 72–83% loss of pesticide. *Leuconostoc mesenteroides* WCP907, *Lactobacillus brevis* WCP902, *L. plantarum* WCP931, and *Lactobacillus sakei* WCP904, isolated from kimchi (a fermented cabbage), are also capable of biodegrading OP pesticides such as CP, coumaphos, diazinon, parathion, and methylparathion (Cho et al., 2009). These four LAB strains exhibited a more rapid degradation of CP (from 100 mg/L to 32–39 mg/L) until day 3, decreasing over time. These results support the hypothesis that these strains are able to utilize CP to support their growth.

Harishankar et al. (2013) showed that the end product degradation profiles are different in various LAB strains under the same conditions, suggesting that different pathways may be operating in these strains. Three classes of bacterial enzymes are known to degrade OP, phosphotriesterases, methyl parathion hydrolases, and organophosphorus acid anhydrolases, and all operate via hydrolysis of the R3 phosphoester bond (Russell et al., 2011). Some of them have been reported in LAB. Islam et al. (2010) isolated a gene (*opdB*) encoding an organophosphorus hydrolase enzyme (OpdB) from *L. brevis* WCP902.

It has also been shown that there is bacterial degradation of organochlorates, pyrethroids, and carbamates (Kataoka and Takagi, 2013; Arya and Sharma, 2014; Cycoń and Piotrowska-Seget, 2016), but there have been no studies on whether LAB are capable of performing it. Only

one study has shown degradation of lindane (an organochlorate pesticide) in wastewater in the presence of a broad community of microorganisms among which there were LAB and *S. cerevisiae*, although the degradation could not be attributed to one microbial group in particular (Ismail et al., 2015).

The effect of yeast has also been evaluated. Lal and Lal (1987) observed some degree of degradation of CP by *S. cerevisiae*. Only half the initial CP was recovered 12 h after the cultures were inoculated with 1–10 mg/L of yeast biomass. Bizaj et al. (2011) showed that in *S. cerevisiae* the removal of pesticides is not only due to their degradation ability. After 7 days of incubation with viable and non-viable yeast cells (8.8 mg/mL), they observed a substantial removal of pyrimethanil (up to 69%) and fenhexamid (up to 46%). The fact that non-viable yeasts remove pesticides from the medium suggests that there is adsorption on the yeast biomass and that the removal is not only a consequence of metabolic degradation.

3. APPLICATION FOR DECONTAMINATION IN FOOD PRODUCTS

Most of the studies on the effectiveness of LAB and *Saccharomyces* in removing these contaminants have used artificially contaminated water. However, the natural samples (drinking water or food) in which these microorganisms might be used as bioremediation techniques are much more complex and there may be components in them that interfere with binding of contaminants to LAB or *Saccharomyces*. The existing data on their application in decontamination of foods are limited to a restricted number of studies, mainly in liquid samples. Most of the studies show that the treatments are highly effective.

3.1. Metal and metalloid removal from food. The application of LAB to reduce levels of metals/metalloids in food is limited to two studies showing that the effect varies depending on the food matrix. Schut et al. (2011) demonstrated that the high copper (Cu) binding capacity of

Lactobacillus buchneri DSM 20057 decreased in must, wine, and grape juice samples. The observed decreased effectiveness was attributed not only to the low pH values of the food samples but also to the presence of metal cations, organic acids, and phenolic compounds. Zhai et al. (2016), however, obtained high reductions (46–62%) of the Cd present in vegetable and fruit juices with *L. plantarum* CCFM8610 (10^7 CFU/mL), with the magnitude of the reduction being dependent on the nature of the juice. These authors also tested the LAB strain as a starter culture, fermenting the juices for 36 h, a treatment that also produced significant decreases in Cd concentration (56–81%).

S. cerevisiae has also been studied as a possible biosorbent of Cu during fermentation of wine. Sun et al. (2015) showed that *S. cerevisiae* was capable of retaining Cu during fermentation of must containing 0.5 mM of the element (58–67%). This ability decreased as the concentration of Cu in the must increased, which seems to be associated with an effect of the metal on yeasts, since Cu stress delays and even stagnates growth of *S. cerevisiae*, and reduces sugar uptake and ethanol production. Da Silva et al. (1999) also demonstrated uptake of Cd by *S. cerevisiae* (10% w/w) during the process of sugarcane must fermentation. These authors observed a linear relation between Cd uptake and Cd concentration in the must, revealing that at the concentrations tested (0.5–5 mg/kg) the metal did not have a toxic effect on the yeast.

3.2. Mycotoxin removal from food. Hatab et al. (2012) showed that inactivated cells of various LAB strains were capable of removing up to 80% of PAT added to apple juice, and that this treatment did not alter the quality of the juice. During the steeping/fermentation steps of production of ogi, a porridge obtained through fermentation of cereals in West Africa, Okeke et al. (2015) showed substantial reductions of AFB1, FB1, ZEA, aflatoxin M1 (AFM1), citrinin (CIT), and cyclopiazonic acid (CPA) by a bacterial community in which various strains of LAB

predominated. Elsanhoty et al. (2014) showed that yogurt produced from milk spiked with AFM1 (50 μg/L) and fermented by 50% yoghurt culture (*S. thermophilus* and *L. bulgaricus*) and 50% *L. bulgaricus*, or *L. acidophilus* presented a significant reduction (55–70%) in the level of AFM1 at the end of the storage period (7 days, 5 °C).

It has also been shown that LAB can have an inhibitory effect on fungi that produce mycotoxins during the manufacture of foods. Liske et al. (2000) found that 12 isolates of LAB out of 25 tested were capable of reducing the amount of *F. culmorum* during malting of wheat and barley. Laitila et al. (2002) also demonstrated a reduction in the population of *Fusarium avenaceum*, *F. culmorum*, *F. graminearum*, and *Fusarium oxysporum* with *L. plantarum* during barley malting, achieving greater reductions (up to 50%) when the degree of contamination of the cereal was lower. These authors indicated that, owing to the intensive growth of *Fusarium* at the beginning of the steeping step, the addition of *L. plantarum* in the early stage of malting is essential.

Saccharomyces strains have also been used successfully to remove mycotoxins from foods. Bejaoui et al. (2004) found that oenological *S. cerevisiae* and *Saccharomyces bayanus* were able to remove OTA from synthetic and natural grape juices, but no degradation of the toxins was observed. The removal was more efficient when non-viable yeasts were used rather than commercial yeast walls. Yue et al. (2011) showed that *S. cerevisiae* inactivated strains could remove over 50% of PAT in apple juice within 24 h, with the highest reduction rate being 72%. Furthermore, juice quality parameters of the treated apple juice were similar to those of the untreated patulin-free juice (Yue et al., 2011).

After addition of *S. cerevisiae*, AFB1 contamination in peanuts inoculated with *Aspergillus parasiticus* was reduced by 74 and 56% after 7 and 15 days, respectively (Prado et al., 2011). The treatment was more effective when the yeast was added 3 h before inoculation of the toxigenic fungus than when the two microorganisms were added simultaneously. Da Silva et

al. (2015) showed a reduction in generation of AF in peanuts contaminated with *A. parasiticus* when *Saccharomyces boulardii* (66%) was added, and the reduction increased when this yeast was combined with *Lactobacillus delbrueckii* (96%), a bacterium that on its own does not produce significant reductions in the level of toxins. The effectiveness of *S. cerevisiae* in removing mycotoxins during the making of bread has also been demonstrated. The effect of making bread with various kinds of yeasts on aflatoxins AFB1, AFB2, and AFG1 indicated significant depletion during dough fermentation (190 min, 35 °C), whereas the baking stage (30 min, 200 °C) had the least effect on aflatoxin contents (Milani et al., 2014). The authors suggested that the greater loss during the first fermentation was mainly due to the fact that it was a long step and it was performed at a temperature closer to optimum for *S. cerevisiae*.

3.3. Pesticide removal from food. All the strategies for reducing levels of pesticides in food using LAB or *Saccharomyces* have been applied on OP contaminated products and are based on their ability to degrade this type of pesticide. Reductions in the levels of OP residues (CP, CP-methyl, diazinon, dichlorvos, fenthion, malathion, phorate, pirimiphos-methyl, and trichlorphon) during production of yogurt have been shown after addition of LAB (7–65%) or yogurt starters (7–19%) to samples of skim milk (Zhou and Zhao, 2015). Higher phosphatase production of the strains was observed to bring about greater pesticide degradation in the milk. Cho et al. (2009) demonstrated degradation of OP pesticides by *Lc. mesenteroides*, *L. brevis*, *L. plantarum*, and *L. sakei* during fermentation of kimchi.

The application of LAB during silage of cereals has proved to be an effective technique for removing pesticides. The addition of a combination of strains of *L. plantarum* to whole corn contaminated with CP and phorate permitted reduction of the levels of the pesticides (25–33%) during silage at room temperature for 10 weeks (Zhang et al., 2016). Wang et al. (2016) demonstrated that rice straw inoculated with *L. casei* WYS3 presented increased removal of CP

after ensiling. These authors showed that organophosphorus hydrolase gene expression in LAB was tripled in the groups that were grown in the presence of CP, compared with controls without CP.

The incorporation of *S. cerevisiae* during fermentation of food is also a practice that reduces the levels of some pesticides. Low et al. (2005) investigated the ability of *S. cerevisiae* to degrade the herbicide glyphosate during the fermentation cycle of the breadmaking process, showing a 21% mean decline after 1 h of incubation. The authors indicated that this reduction was due to degradation of the herbicide, but they did not perform assays to confirm it. Đorđević and Đurović-Pejčev (2015) fortified wheat grains with various concentrations of CP-methyl (3, 15, and 45 mg/kg), and after sterilization *S. cerevisiae* was inoculated (8% v/w) and the mixture was submitted to a fermentation process. The main loss of contaminant took place during autoclaving (up to 80%), whereas fermentation with *S. cerevisiae* produced a reduction of 14–19%, independently of the concentration of CP-methyl added.

4. EFFECTS ON INTESTINAL ABSORPTION AND TISSUE DISTRIBUTION OF FOOD CONTAMINANTS

The ability of LAB and *Saccharomyces* to interact with food contaminants and the results derived from assays in foods suggest that they could be used to reduce the amount of these contaminants that reaches the blood stream, i.e. to reduce their bioavailability. In addition to a mechanism of interaction between the contaminants and LAB or *Saccharomyces*, the reduction in absorption may also be due to direct action of the microorganism on the intestinal mucosa, by altering its permeability or its absorption mechanisms. This dietary strategy of reducing exposure to the contaminant has even been assayed in population interventions through consumption of dairy products with LAB. The use of this kind of strategy is of great interest because in addition to reducing the bioavailability of the contaminant it could be a supplement

of high biological value to the diet, which may even counteract the effects of the contaminant, as commented below.

4.1. Metals and metalloids. *In vitro* studies have shown that the ability of certain strains of LAB and *S. cerevisiae* to bind Hg(II), CH_3Hg, and Cd is maintained in conditions of gastrointestinal digestion (Jadán Piedra et al., 2017a, 2017b). In fact, these authors have demonstrated that there is a substantial reduction in bioaccessibility of Hg (the quantity of the element that is soluble after digestion and that can therefore be absorbed) in samples of mushrooms, which indicates that this type of reduction strategy is valid not only for aqueous standards but also for foods. These studies also showed that the reduction in bioaccessibility by LAB and *Saccharomyces* was matrix-dependent, because it did not take place when applied to seafood products. The authors suggested that this might be due to the chemical form in which Hg is released from fish during digestion (complexes with cysteine or other thiol groups), with the result that uptake/adsorption by the microorganisms is not possible.

Studies to determine the effect of LAB on bioavailability in experimental animals have concentrated on Pb, Cd, and Hg, using drinking water as the dosing vehicle. We are not aware of any studies using strains of *S. cerevisiae*. Zhai et al. (2013) exposed rats to a single dose of Cd [60 mg/kg of body weight (bw)] and studied the effect of administering *L. plantarum* CCFM8610 (1×10^9 CFU with 0.5 mL skim milk). This probiotic was administered before (7 days, once daily) or after (1 h) exposure to Cd, and in both cases viable and non-viable bacteria were used. Elimination in feces increased significantly in all the treatments with *Lactobacillus*, fecal excretion being especially noteworthy in the group treated with *L. plantarum* CCFM8610 after exposure to Cd. Tissue accumulation (kidney and liver) was reduced similarly with viable and non-viable LAB, although the accumulation was less when the mice were dosed after the treatment with Cd. In a later study in which mice were subjected to chronic exposure (8 weeks)

through drinking water spiked with Cd (100 mg/L) with daily administration by gavage of *L. plantarum* CCFM8610 (1×10^9 CFU), once again significant reductions in Cd accumulation in kidney and liver and an increase in fecal elimination were observed (Zhai et al., 2014). In this study the authors demonstrated that these effects were due to Cd sequestration during its passage through the gastrointestinal tract, because when Cd was introduced intraperitoneally oral administration of CCFM8610 did not have an impact on tissue Cd accumulation.

A smaller quantity of Pb was also observed in blood and organs (liver, kidney, and stomach) of rats treated with Pb (1 g/L) in drinking water for 11 weeks, co-administered with *L. plantarum* from the beginning or from week 5 onwards (Tian et al., 2012). The best protection was obtained with co-administration of Pb/LAB from the beginning. These authors showed that the results with *Lactobacillus* were similar to those obtained using DMSA (meso-2, 3-dimercaptosucccinic acid), a known metal chelating agent employed in cases of acute intoxication by metals. Akhmetsadykova et al. (2013) demonstrated the effectiveness of fermented camel's milk in decreasing absorption and organ accumulation of Pb in cavies administered for 28 days with fermented milk with a low concentration of Pb (0.5 mg/L). Recently, the reducing effect of *L. plantarum* and spores of *Bacillus coagulans* on tissue accumulation and increase in fecal elimination in rodents treated with Hg(II) for 48 days has also been shown (Majlesi et al., 2017).

Finally, it is important to highlight the first population intervention aimed at reducing plasma levels of various metals. Bisanz et al. (2014) conducted a study on pregnant women and children in Tanzania. The children were followed over 25 days, and the pregnant women over their last two trimesters until birth. During this period, they were given yogurt containing 10^{10} CFU *L. rhamnosus* GR-1 per 250 g. The treatment did not have a statistically significant effect on the children. In the women the blood levels of Hg and As increased in the control group but remained stable in the one that received *Lactobacillus* during the follow-up period.

4.2. Mycotoxins. Kabak et al. (2009) showed *in vitro* the effect of *Lactobacillus* and *Bifidobacterium* strains on the bioaccessibility of AFB1 and OTA from samples of pistachio nuts, buckwheat, and two artificially contaminated infant formulae. The average bioaccessibility without LAB was about 30% for OTA and 90% for AFB1. After digestion in the presence of LAB (10^8 CFU/mL), the percentage of mycotoxin in the bioaccessible fraction presented a moderate reduction, especially for AFB1-spiked infant food. However, when the standard deviation was taken into account non-significant reductions were observed. Serrano-Niño et al. (2013) showed a reduction in bioaccessibility of the mycotoxin AFM1 after *in vitro* digestion of artificially contaminated milk (10 ng/mL) in the presence of LAB, with *Lactobacillus johnsonii* NRRL B-2178 (32%) and *Bifidobacterium bifidum* NRRL B-41410 (45%) being the most effective strains. It has also been demonstrated *in vitro* that the presence of *L. rhamnosus* strain GG reduces AFB1 transport across an intestinal cell monolayer generated by Caco-2 cells (Gratz et al., 2007). The apparent permeability coefficient of AFB1 (P_{app} 25 ± 3 × 10^6 cm/s) was reduced in the presence of 5 × 10^{10} CFU/mL (P_{app} 7 ± 5 × 10^6 cm/s), indicative of a possible reducing effect of LAB on intestinal absorption of this mycotoxin.

The reduction by LAB of the quantity of mycotoxin available for intestinal absorption has been verified *in vivo*. Rats dosed with a probiotic suspension (*L. rhamnosus* GG: 5 × 10^{10} CFU/0.5 mL PBS) by oral gavage 3 days before and 3 days after a single oral dose of AFB1 (1.5 mg/kg bw) showed increased fecal excretion of AFB1 (122%) and of the metabolite AFM1 (152%) within 24 h after AFB1 dosing (Gratz et al., 2006). Hernández-Mendoza et al. (2011) demonstrated that the effect of *L. reuteri* (0.68 × 10^9 CFU/mL, oral gavage, 7 days before AFB1 dosing) on absorption of AFB1 (16 mg/kg bw) in rats was due to its ability to bind the mycotoxin in the intestinal tract, mostly in the duodenum and ileum. El-Nezami et al. (2000)

carried out a pilot clinical trial to investigate the effect of a probiotic preparation containing *L. rhamnosus* LC-705 and *P. freudenreichii* spp. *shermanii* JS on the levels of AFB1 in fecal samples from healthy volunteers. The study involved 3 distinct periods: 1-week baseline, 2-weeks supplementation, and 1-week follow-up period. The fecal samples from 11 of the 20 recruited volunteers were positive for AFB1, with levels ranging between 1.8 and 6 μg AFB1/kg feces. For volunteers who were administered the probiotic preparation, there was a significant reduction in the level of AFB1 after the second week of the trial, a reduction that continued during the follow-up period.

Research with *S. cerevisiae* has been limited to *in vitro* studies in which gastrointestinal digestion was simulated and it was shown that in these conditions the yeast is able to capture mycotoxins. Dogi et al. (2011) demonstrated that various strains of *S. cerevisiae* bound AFB1 during a simulated ruminant digestion, with adsorption percentages ranging from 41 to 94%. Furthermore, the yeast-AFB1 complex was stable under physicochemical conditions comparable to the conditions in the gastrointestinal tract. Armando et al. (2012) showed that human gastrointestinal conditions produced an increase in the ability of *S. cerevisiae* to bind to ZEA and OTA, possibly because of an increase in superficial binding sites owing to enzyme activity. The data obtained from these studies suggest that *S. cerevisiae* could reduce mycotoxin bioavailability; however, there are no *in vivo* studies that confirm this ability.

4.3. Pesticides. Trinder et al. (2015) indicated that probiotic lactobacilli could be a potential prophylactic treatment for reducing pesticide absorption in humans and wildlife; however, there are no *in vivo* studies that provide data about this protective effect. Using the Caco-2 cell line as a model of the intestinal epithelium, the same authors evaluated transport of CP and parathion (100 μM) in the presence and absence of *L. rhamnosus* GG and GR-1 (10^9 CFU/mL). The

results showed a slight decrease in transport across the intestinal monolayer, although the data were not conclusive (Trinder et al., 2016).

5. EFFECTS ON TOXICITY GENERATED BY FOOD CONTAMINANTS

The effects of food contaminants are very diverse and affect various target organs. Among the mechanisms of action of these contaminants, one of the most studied is the generation of oxidative stress. Numerous studies have tried to reduce the oxidative stress generated by these contaminants by using various strategies, including treatment with strains of LAB and *Saccharomyces*. This protective effect has been demonstrated in *in vitro* studies and in *in vivo* assays employing laboratory animals.

5.1. Metals and metalloids. Tian et al. (2012) evaluated the effectiveness of *L. plantarum* CCFM8661 against Pb-induced toxicity, by treating mice with the probiotic during or after exposure to 1 g/L of Pb acetate in drinking water. The results showed that *L. plantarum* offered a significant protective effect against Pb toxicity by recovering blood γ-aminolevulinic acid dehydratase activity, and preventing alterations in the levels of glutathione (GSH), glutathione peroxidase, malondialdehyde (MDA), superoxide dismutase, and reactive oxygen species (ROS). The more effective treatment was the one administered consistently during the entire Pb exposure. Ozcan et al. (2009) revealed the beneficial effects of kefir given to rats treated with Pb (2 g/L). After 6 weeks of treatment, blood levels of GSH in the Pb group (45 mg/ dL) were significantly lower and MDA levels (5 μmol/ L) were higher than those observed in the group supplemented with kefir (GSH: 57 mg/dL, MDA: 4.2 μmol/L).

The protective effect of LAB against the toxicity of Cd has also been demonstrated. Strains of *L. acidophilus* and *B. longum* (5×10^8 CFU/g of food) significantly reduced Cd-induced genotoxicity (around 20%) in mice treated for 5 weeks with $CdCl_2$ (70 mg/L in drinking water)

(Jama et al., 2012). Zhai et al. (2014) also showed that *L. plantarum* CCFM8610 (10^9 CFU in 0.5 mL of skim milk once daily via gavage) reduced oxidative stress caused by exposure to Cd (10 mg/mL in drinking water during 8 weeks). This protection was not only a consequence of the ability of the bacteria in the intestinal lumen to bind to Cd, because *L. plantarum* also reduced oxidative stress when the exposure was intraperitoneal. The authors suggested that this reduction was due to induction of metallothioneins and gene expression changes in several Cd-toxicity-related pathways.

Al-Damegh et al. (2014) evaluated the effects of milk enriched with probiotics (2% of *S. thermophilus*, *L. acidophilus*, and *B. bifidum*) to ameliorate the toxic effects of As(V) (30 mg/L) in rats treated for 56 days through drinking water. The results showed that levels of creatinine, aspartate aminotransferase, and triglycerides were lower in the probiotic group compared with the rats treated with As(V) alone. Moreover, this study also showed a protection against testicular toxicity caused by As(V). Protection was possibly not a consequence of chelation of the metalloid, because, as indicated in section 4.1, the ability of LAB to bind arsenic species is very low. An effect on transport across the intestinal wall might account for this effect as it has been shown that LAB can increase expression of cell junction proteins, thus affecting intestinal permeability (Ulluwishewa et al., 2011).

5.2. Mycotoxins. Gratz et al. (2006) evaluated the effect of *L. rhamnosus* strain GG (5×10^{10} CFU) on hepatotoxicity caused by a single oral dose of AFB1 in rats (1.5 mg/kg bw), using plasma alanine transaminase (ALT) activity as an indicator of liver damage. The probiotic was administered daily by gavage, 3 days before and 3 days after the AFB1 treatment. ALT activity increased in the group receiving AFB1 alone (103.7 ± 84.9 U/L) compared to the controls (41.6 ± 18.7 U/L); however, LAB treatment did not reduce the AFB1-induced increase in ALT activity (56.4 ± 34.2 U/L). Khalil et al. (2015) analyzed the serum biochemical profile of

growing rats fed a diet with FB1-contaminated corn (50–200 mg/kg diet) for 4 weeks, and the role of *L. delbrueckii* subsp. *lactis* DSM 20076 and *Pediococcus acidilactici* NNRL B-5627 supplementation (10^{10} CFU/mL). Significant increases in serum liver and renal function markers [ALT, alanine aminotransferase, alkaline phosphatase, total bilirubin, urea, creatinine, and uric acid] were recorded in rats exposed to 100 and 200 mg of FB1/kg diet. Co-administration of LAB at doses of 1 mL/day ameliorated FB1 intoxication, reducing the changes in most of the serum parameters studied. The data suggest that those *Lactobacillus* strains effectively reduce FB1-induced hepatorenal toxicity.

LAB are also successful agents for counteracting the oxidative stress and protecting against the genotoxicity induced by mycotoxins (Deabes et al., 2012). *L. rhamnosus* GG (10^{10} CFU) reduced MDA contents and increased GSH levels and superoxide dismutase activity in kidney and liver of mice exposed to a mixture of aflatoxins (AFB1, AFB2, AFG1, AFG2, 0.7 mg/kg bw) for 7 days. Moreover, probiotic administration before aflatoxin gavage significantly reduced frequencies of chromosomal aberrations in bone marrow and spermatocytes, and recovered mitotic and meiotic activities. Abbès et al. (2013) showed the protection of LAB against the immunotoxic effect generated *in vivo* by AFM1. A daily oral dose of AFM1 (100 μg/kg bw, 2 weeks) induced immune damage, as evidenced by significant decreases in $CD4^+$, $CD8^+$, $CD54^+$, and $CD56^+$ levels. However, co-treatment of mice with *L. rhamnosus* GAF01 enhanced blood T-cells to values comparable to those observed in control animals.

The protection against the toxicity generated by mycotoxins is not due solely to a direct effect on the reduction of its intestinal absorption. This was shown in a study conducted in rats exposed intraperitoneally to AFB1 (450 μg/kg bw, twice a week for 6 weeks) together with oral administration for 24 weeks of milk fermented by *L. rhamnosus* GG and *L. casei* Shirota (Kumar et al., 2011). By week 25, a reduction of tumor incidence was observed in animals that received the fermented milk, as well as a reduction in DNA damage and oncogene expression.

Madrigal-Santillán et al. (2006) exposed mice with AFB1-contaminated corn at two levels (0.4 and 0.8 mg/kg), with or without co-administration of *S. cerevisiae* (10^8 CFU/g of diet) and determined the frequency of micronucleated normochromatic erythrocytes (MNNE), an indicator of genotoxicity. Control and *S. cerevisiae*-fed mice had a low MNNE rate, whereas mice fed only with AFB1 showed a significant MNNE increase. The same authors conducted a similar study in which they treated mice with AFB1-contaminated corn (0.25 mg/kg of corn) and mannan (50–500 mg/kg) instead of dosing with *S. cerevisiae* (Madrigal-Santillán et al., 2007). The results demonstrated an antigenotoxic effect of this component of the yeast wall, with the best response found in mice fed with AFB1 plus 500 mg/kg of mannan.

5.3. Pesticides. The protective effect of LAB on the toxicity associated with exposure to pesticides has not been extensively studied. Using *Drosophila melanogaster* as a model and co-exposure of CP and *L. rhamnosus* strain GG showed positive results (Trinder et al., 2016). Newly eclosed *D. melanogaster* flies were exposed acutely to food containing 10 μM CP with or without supplementation with live *L. rhamnosus* GG (10^9 CFU). Treatment with lactobacilli mitigated CP-induced mortality. Kamaladevi et al. (2016) showed that *L. casei* exhibited a protective effect on reproduction, feeding, locomotion, and survival of the nematode *Caenorhabditis elegans* against toxicity of malathion (300 mM). Pretreatment with *L. casei* 4 hours before CP exposure increased the level of acetylcholinesterase and upregulated the phase-II detoxification enzymes encoding metallothionein and glutathione-S-transferase genes in this model organism.

6. CONCLUSIONS AND FUTURE RESEARCH

The data compiled in this review show the ability of food grade microorganisms (LAB and Saccharomyces strains) to reduce exposure and toxicity of chemical contaminants. Some of the

underlying processes for these beneficial effects are being studied but there are still many unexplored mechanisms and it is necessary to achieve a better characterization of contaminant/microorganism interaction, especially in food samples. Most of the *in vitro* and *in vivo* applications have been developed using aqueous solutions of standards of the toxic compounds. However, the food/feed matrix has received little attention when trying to confirm the efficacy of these microorganisms-based strategies. Therefore, to confirm the validity of these strategies in chelation, absorption, and toxicity of contaminants, studies are required, in which the contaminant is not only dosed by using aqueous solutions but also delivered through contaminated food/feed.

The use of artificial systems emulating the gastrointestinal passage provide a valuable tool for strain testing and selection before *in vivo* experiments in animals and final trials in exposed populations are undertaken. Future research needs to establish how these microorganisms can be delivered to the exposed populations; in the form of supplements, after their incorporation in food formulations, by using them in food/feed fermentations, or whether they can be used in decontamination processes of the main dietary sources of these contaminants. Furthermore, it is necessary to ensure that these strategies do not entail associated risks, especially in chronic exposures, such as reduction in the bioavailability of other important nutrients (e.g. minerals or oligoelements). These studies will complement the evidence base available so far and allow the selection of appropriate strategies for each combination of food and contaminant.

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FIGURE CAPTIONS

Figure 1. Schematic representation of the cell wall structure in LAB and yeast and proposed processes involved in the interaction with chemical contaminants. Adsorption to surface components of different nature have been reported as the major mechanism for binding heavy metals/metalloids, microbial toxins and pesticides. In yeast, specific transporters such as Zrt1, Pho84 or Fps1 have been demonstrated to be involved in Cd, As(V) and As(III) uptake, respectively. In LAB, no specific transporters involved in toxics accumulation have been determined, although internalization by specific or unspecific mechanisms is not discarded. In LAB, enzymatic activities such as proteinases, phosphotriesterases, methyl parathion hydrolases and organophosphorus acid anhydrolases were shown to be involved in degradation of cyanobacterial toxins and organophosphorous pesticides (see text for details). EPS, exopolysaccharides; SLP, S-layer proteins; PG, peptidoglycan; TA, teichoic acids; LTA, lipoteichoic acids; M, cytoplasmic membrane; MP, mannoproteins; GLN, glucan; CH, chitin.