Review

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Sourdough lactic acid bacteria as antifungal and mycotoxin-controlling agents

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Abstract

Sourdough starter cultures are rich sources of endogenous lactic acid bacteria. The extended shelf lives of sourdough breads are attributed to a large array of organic acids and low-molecular-weight metabolites produced during the fermentation process. Different species belonging to the lactic acid bacteria group of microorganisms, mainly *Lactobacillus* and *Leuconostoc*, are increasingly gaining the attention as possible means for inhibiting mold growth in animal feed and human food chains. In addition, certain lactic acid bacteria strains isolated from sourdough starters were also shown to reduce mycotoxins concentrations in contaminated products either by binding or degradation. This short review will summarize the findings in this context that pertain to lactic acid bacteria isolated specifically from sourdough starters and acquaint the reader with the most recent advancements in this bio-preservation trend.

Keywords

Sourdough, lactic acid bacteria, antifungal, mycotoxins

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INTRODUCTION

Fungi are ubiquitous microorganisms that invade different food and feed commodities with diverse composition and water activities (a_w). Low a_w that usually form natural barriers for bacterial growth do not affect this group of microorganisms. In fact, fungi growth was reported at a_w as low as 0.61 (Mishra et al., 2009; Nevarez et al., 2008). The growth of fungi on food products has several detrimental effects including physical damage, consumer rejection, nutrient depletion, and finally and most importantly, the potential of mycotoxin presence (Dutton and Kinsey, 1995; Naicker et al., 2007). More than 400 different mycotoxins have been identified in the past 30 years (Hoogenboom et al., 2001; Kuiper-Goodman, 1995). Some of them are highly toxic, carcinogenic, and can cause acute and chronic effects in both humans and animals. The five most common agriculturally important mycotoxins are aflatoxins, fumonisins, ochratoxin A (OTA), zearalenone (ZEN), and deoxynivalenol (DON) (Coker et al., 2000; Smith et al., 1995; Wild and Gong, 2010). From the safety prospective, forborne bacteria are the only hazard that exceeds mycotoxin contamination in human food whereas mycotoxins pose the greatest threat within animal feed (Cardwell and Miller, 1996; Mashinini and Dutton, 2006). It is estimated that more than 4.5 billion people living in developing countries are chronically exposed to large uncontrolled amounts of mycotoxins (Vasanthi and Bhat, 1998; Williams et al., 2004).

Consumer concerns about the safety of chemical preservatives (such as benzoic, propionic, and sorbic acids) used to control fungi have stimulated the search for potential alternatives such as naturally occurring lactic acid bacteria (LAB) in a phenomenon that is widely known today as ''bio-preservation.''

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LAB is generally recognized as safe (GRAS) microorganisms, and more importantly, they found their way to human food chain since pre-historic times with centuries of empirical use in food fermentation (Galvez et al., 2007; Settanni and Corsetti, 2008). Microorganisms of the genera *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Streptococcus*, and *Pediococcus* are involved in numerous food fermentation processes. Both *Lactobacillus* and *Bifidobacterium* are considered as part of the normal human intestinal microflora that is believed to exert positive effects on human health (Daly et al., 1996).

There have been a plethora of published papers and reviews related to the antimicrobial and antifungal compounds/characteristics of LAB in the past few years and readers are referred to these in-depth reviews (Montalban-Lopez et al., 2011; O'Shea et al., 2013; Pawlowska et al., 2012; Rouse and van Sinderen, 2008; Zendo, 2013). To avoid redundancy, this review will focus on LAB isolated from sourdough starters and acquaint the reader with the most recent advancements in the bio-preservation field related particularly to this group of microorganisms and their interesting ability to inhibit fungi growth and potentially eliminate mycotoxins from food or feed.

MICROBIOLOGY OF SOURDOUGH STARTERS

Sourdough starters are symbiotic cultures of LAB and yeasts used traditionally to leaven bread. Historically, sourdough breads were most common in Europe and made of rye-flour due to the unfavorable climatic conditions in northern and eastern parts of Europe which make it difficult to grow wheat (De Vuyst and Vancanneyt, 2007; Moroni et al., 2009). Rye flour does not contain much gluten, so the structure of rye bread relies on gelatinized starch (Hammes and Tichaczek, 1994). Sourdough bread has a very distinctive taste, mainly due to its lactic acid content produced by the LAB during the fermentation process before baking (Poutanen et al., 2009).

These bread varieties are about twice as acidic as conventional bread because of the presence of lactic and acetic acids. A moderate decrease in acidity (increase of pH) usually occurs during baking but the final pH of a ripe sourdough will be still between 3.6 and 4.0. The low pH aids not only in extending the shelf life of the final product but in reality influences the formation of aroma compounds during the baking process that gives the distinct taste of sourdough breads. Guerzoni et al. (2007) showed how the decreased acidity in these breads lead to increased concentrations of particular aroma compounds such as medium-chain fatty acids and isovaleric acid, a key odorant in various fermented foods. The same principle that was used in the past to prepare rye breads (low in gluten) is being used successfully nowadays to produce gluten-free products that are commercially available for celiac disease patients (Di Cagno et al., 2008; Moroni et al., 2009; Poutanen et al., 2009).

From the microbiology point of view, sourdough fermentation process is a complicated yet fascinating one at the same time. A very recent paper studied the dynamics of such process using DNA and 16S rRNA next-generation sequencing techniques and tracked the different species that prevailed during the fermentation process (5–7 days of propagation) correlating these species with sourdough maturity. During wheat and rye sourdough preparation, the number of viable LAB, ratio between LAB and yeasts, and rate of acidification keep changing. More importantly, the microbial ecology during the preparation process keeps evolving. In their study, Ercolini et al. (2013) demonstrated that the starter flours were initially contaminated with *Acinetobacter*, *Pantoea*, *Pseudomonas*, *Comamonas*, *Enterobacter*, *Erwinia*, and *Sphingomonas* species, and the abundance of these species varied with flour source. However, soon and within one day of the fermentation process, the populations of most of those bacterial species were eliminated but the *Enterobacteriaceae* group, and were replaced with gram-positive bacteria represented by LAB, which was present initially at very low/intermediate abundance. It was suggested that flour-type contributes also to the bacterial biodiversity. For example in rye flour, members of the *Leuconostocaceae* (such as *Weissella* sp.) were the dominating species during the entire propagation process to be flanked later by *Lactobacillus sakei* group. Differently, species that succeeded during the 10 days of propagation in wheat sourdoughs fluctuated between dominating and sub-dominating populations of *L. sakei*, *Leuconostoc* sp., *Weissella* sp., and *Lactococcus lactis* even though that other sub-dominant species such as *Lactobacillus plantarum* were detected throughout the propagation. On the yeast side, *Saccharomyces cerevisiae* was the most dominating species and occurred in all sourdoughs with a LAB and yeasts ratio stable near ca. 100:1, at maturity.

In essence, only one fermentation cycle is needed to completely turn the microbial diversity in sourdough from mainly *Proteobacteria* to *Firmicutes* and to simplify the initial microbial diversity to a more adaptable species that remain constant thereafter.

ANTIFUNGAL ACTIVITY OF SOURDOUGH BREAD LAB

LAB, as mentioned earlier, have the potential to be used in bio-preservation methods because (a) they are

safe to consume; (b) they naturally dominate the microflora of many foods during storage/fermentation; and (c) they produce metabolites that are able to inhibit fungi growth under the native growth conditions. LAB usually competes with other microorganisms by secreting antagonistic compounds and modifying the surrounding microenvironment by such metabolites. Several active compounds and their modes of action have been characterized in recent years (Ahmad Rather et al., 2013; Andersson et al., 1988; Bizani et al., 2005; Crowley et al., 2013; Fhoula et al., 2013; Motta et al., 2008; Niku-Paavola et al., 1999; Pangsomboon et al., 2006). These antimicrobial and antifungal metabolites produced by sourdough LAB can generally be divided into two groups: (1) low-molecular-mass compounds (below 1000) and (2) bacteriocins (30–60 amino acid residues). Bacteriocins have an inhibitory effect only on closely related species and a lot of them are highly characterized and some already found their way to industrial scale use. On the other hand, the biologically active low-molecular-mass compounds produced by LAB are poorly characterized although the existence of such compounds has frequently been reported (Ahmad Rather et al., 2013; Ray et al., 2000; Van Belkum and Stiles, 2000). These substances differ from bacteriocins in their wide spectrum of activity against both gram-positive and gram-negative bacteria and fungi (including some yeast).

A large numbers of published papers attribute the antifungal activity of LAB to organic acids (such as lactic, acetic, caproic, formic, propionic, and butyric) produced during fermentation leading to decreased pH values/increased acidity (Cabo et al., 2002; Lind et al., 2005) which later inhibit mold growth.

The role of such organic acids is usually neglected in favor for other compounds during commercial applications. In an elaborate approach, Zhang et al. (2010) explored the possibility of incorporating two efficient propionate producers, *Lactobacillus buchneri* and *Lactobacillus diolivorans*, in bread preservation. Propionic acid formation ranged from 9 to 48 mM correlating with the ash content of the prepared sourdoughs. The increased concentrations of propionate translated into inhibited mold growth for more than 12 days in comparisons to control samples inoculated with *Aspergillus clavatus*, *Cladosporium* spp., *Mortierella* spp., or *Penicillium roquefortii*.

Other recent studies demonstrated the presence of novel acids that form during the fermentation process and were responsible for the observed antifungal activity regardless of their contribution to the overall pH value of the sourdough (Table 1). Lavermicocca et al. (2000) isolated LAB strains from sourdough cultures that showed strong antifungal activity when tested using conidial germination assay. One of the tested strains completely inhibited *Penicillium roqueforti*, *P. expansum*, *Aspergillus niger*, *A. flavus*, and *Fusarium graminearum*. Two novel antifungal compounds, phenyllactic acid (PLA) and 4-hydroxy-phenyllactic acid (OH-PLA), were identified in culture filtrates. These studies were later expanded, and PLA was reported in the supernatant of *Lactobacillus plantarum* cultures that showed the most inhibitory activity toward the tested fungi. Less than 7.5 mg of PLA per ml was required to obtain 90% growth inhibition of all

Table 1. Some of the natural compounds produced during the fermentation process initiated by sourdough starter cultures which were identified to exert an inhibitory effect on fungi growth

Compound	Chemical structure	Molecular weight ($g\,mol^{-1}$)	Reported mode of action
Acetic acid	$C_2H_4O_2$	60.05	Intracellular acidification/ inhibition of glycolysis
Lactic acid	$C_3H_6O_3$	90.08	Intracellular acidification
Caproic/hexanoic acid	$C_6H_{12}O_2$	116.16	Alters fungal membrane permeability
Formic acid	CH_2O_2	46.03	Inhibition of electron transport chain/ uncoupling of phosphorylation
Propionic acid	$C_3H_6O_2$	74.08	Intracellular acidification
Butyric acid	$C_4H_8O_2$	88.11	Reduces colonization
Phenyllactic acid (PLA)	$C_9H_{10}O_3$	166.174	Inhibitor of phenylalanine dehydrogenase/ inhibit spore synthesis
4-Hydroxy-phenyllactic acids (OH-PLA)	$C_9H_{11}O_4$	183.2	Similar to PLA
Monohydroxy octadecenoic acid	$C_{18}H_{35}O_3$	299.461	Increase membrane permeability
Peptide mixtures (known/unknown sequences)	Various	Various	Unknown

tested fungi. Similar conclusions about the effects of PLA on filamentous fungi sporulation and radial growth inhibition were confirmed recently (Svanstrom et al., 2013).

Valerio et al. (2004) investigated the possibility of increasing the antifungal efficacy of LAB cultures by phenylalanine supplementation coupled with tyrosine limitation. This usually results in increased amounts of PLA and OH-PLA. The authors suggested that selecting LAB strains which show high ability to convert such precursors to active compounds might be the base for future applications of such strains on the industrial level. Another evidence for the involvement of LAB metabolites is the recent study that pointed out the role of a hydroxy fatty acids (resulting from linoleic acid conversion) in controlling mold growth (Black et al., 2013). The formation of monohydroxy octadecenoic acid, for example, by one *L. hammesii* strain, exerted an antifungal effect in the resulting sourdough breads.

In a similar fashion, Ryan et al. (2011) reported the isolation of several antifungal compounds produced in media inoculated with *Lactobacillus amylovorus* DSM19280. The reported list contained carboxylic and fatty acids in addition to various cyclic-dipeptides. While some of the identified chemicals look promising, the feasible usage of some of the inhibitory compounds reported on the list as a ''use alone'' fungi inhibitors is questionable (with a minimal inhibitory concentrations (MICs) >50–$200\,mg\,mL^{-1}$). The high MIC of some of these inhibitors should not undermine their significance in future synergistic applications. For example, Ryan et al. (2008) investigated a synergistic approach of utilizing antifungal sourdough starters in combination with calcium propionate to reduce the amounts of chemical additives during bread preservation. The synergistic effect was strong enough to inhibit the outgrowth of some resistant *Penicillium roqueforti* spores that were not affected earlier either by antifungal sourdoughs or calcium propionate when each was incorporated separately.

Generally speaking, isolating and identifying proteinaceous inhibitory compounds for sourdough starters that fall within the circle of interest is technically complicated due to the fact that fungi are sensitive to normal LAB by-products of metabolism, most notably acetic and lactic acids. Different media have been used to study antifungal activities of LAB. Some of these media are quite suitable for maximizing the antifungal compounds production/secretion in the supernatant by mimicking the conditions in which sourdough LAB can originally grow. An example of such media is wheat flour hydrolysate (WFH) (Lavermicocca et al., 2000). Other media that were used in earlier studies to enumerate LAB such as de Man, Rogosa, and Sharpe (MRS) were found to interfere with a reliable antifungal activity assessment as it increases the chances of selecting false positives (Stiles et al., 2002). In order to decrease such risks, the MRS media was suggested to be reformulated by eliminating sodium acetate, an antifungal agent by itself, before screening for LAB (Stiles et al., 2002). Thus, test media should be carefully considered and selected in order to enhance the possibility of selecting active inhibitors and to minimize aberrant interpretations.

Despite the difficulties connected with such investigations, Garofalo et al. (2012) identified a mixture of peptides in the active antifungal fraction of one LAB isolate, namely *Lactobacillus rossiae* LD108, after screening 216 LAB cultures obtained from bread-/panettone-doughs and sourdoughs against *Eurotium repens*, *Aspergillus japonicus*, and *Penicillium roseopurpureum*. The peptides, analyzed by MALDI-TOF Mass Spectroscopy, corresponded to wheat α-gliadin proteolysis fragments and were able to present strong antifungal properties when explored against *A. japonicas* in inhibitory bioassays.

A better understanding for the role of different mixtures of defined and undefined peptides that possess antifungal properties is emerging. In general, these peptides are produced during the fermentation phase established by certain isolates of LAB such as the four different peptides reported by Rizzello et al. (2011). The reported peptides were the results of sourdough fermentation by two *Lactobacillus* strains (*L. plantarum* LB1 and *L. rossiae* LB5) and led to the noticeable inhibition of various fungi isolated from Italian bakeries. The inhibitory effect of these peptides was complemented by the presence of formic acid in the mixture as reported by the authors.

In a further study carried out by the same team (Coda et al., 2011), they evaluated the antifungal properties of *Lactobacillus plantarum* 1A7- or *Wickerhamomyces anomalus* LCF1695-fermented sourdoughs. The water-/salt-soluble extracts, with large inhibitory spectrum, were found to contain several novel antifungal peptides in addition to ethanol and ethyl acetate. Bread making by the incorporation of both of these microorganisms in the fermentation step, controlled fungi contamination later up to 28 days of storage.

Within the same aim, the antifungal effect of some of proteinaceous compounds/peptide mixtures was reported also earlier by Coda et al. (2008). In this case, the inhibitory water-soluble extracts were obtained from *Lactobacillus brevis* AM7-fermented sourdoughs with a MIC close to 40 mg of peptide/ml.

Overall, the aforementioned studies indicate the possibility of isolating LAB strains that have either a wide or narrow spectrum of antifungal activities.

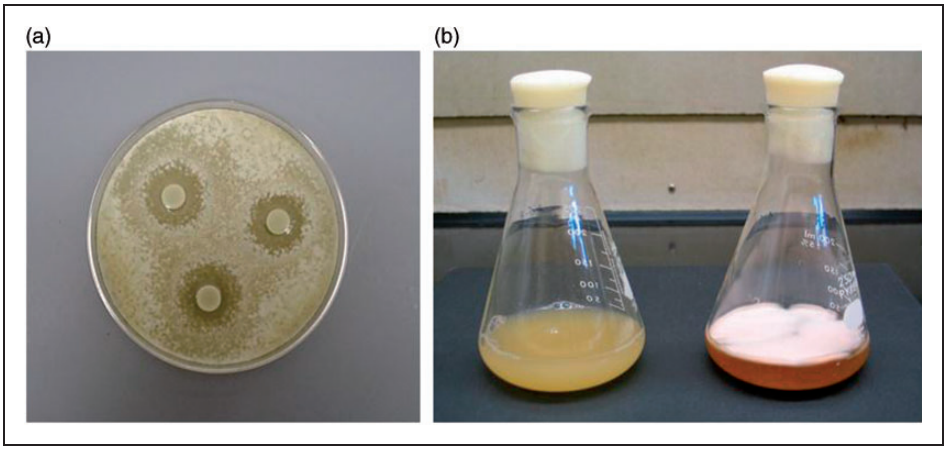

Figure 1. The reported inhibitions of fungal growth are swords of two edges. While visually detected inhibitions of fungi growth (*Penicillium expansum* on the solid media in (a) and *Fusarium graminearum* in the liquid broth in (b)) by certain microorganisms (*Lactobacillus paracasei* subsp. *tolerans* L17 in both (a) and (b)) looks promising, the actual concentrations of secreted mycotoxins should be investigated as a number of studies started to report an increased levels of secreted mycotoxins despite the visually limited growth patterns of the fungi possibly as part of a defense-mechanism induced by encountering unfavorable environmental conditions. [In (b), the left flask with no visible signs of fungal growth was found to contain higher levels of deoxynivalenol (>200 ng/ml) compared to the right side flask (<25 ng/ml)].

These isolates will have the potential later to be used in food preservation. Testing and evaluation methods should be scrutinized carefully to select rigorously for strains with high potential and reduce the number of false positives. The under-laying mechanism of inhibition for most of the reported antifungal compounds is still under investigation and should be taken in our opinion to the molecular level in order to cautiously understand the mode of action for the best utilization in food processing. This is especially true as some isolates can in fact inhibit the visual fungal growth (Figure 1(b)) but at the same time cause an elevated levels of mycotoxin secretion as part of the natural defense of the stressed fungi due to the limiting growth conditions (Hassan and Bullerman, 2008b).

SOURDOUGH LAB AND MYCOTOXIN ELIMINATION

Another interesting property of LAB that was noticed in the last two decades is their ability to detoxify mycotoxins already present in the surrounding environment. This activity came to the center of attention after the noticeable advancement of chemical and immunological analysis techniques (especially high-performance liquid chromatography, HPLC, and enzyme-linked immunosorbent assays, ELISA) enabling for a reliable measure of mycotoxins eliminated by such isolates (He et al., 2007, 2009; Tangni et al., 2011).

Different removal and detoxification methods of mycotoxins including physical and chemical approaches have been explored in the past but not with much success either because of the high costs associated with these techniques or the production of environmentally unfriendly by-products or simply because the resulting products had some issues related to safety or marketability. With the increased growth of global trade and hence the amount of imported/exported cereals between countries (Wu, 2006; Wu and Guclu, 2012), the horizon of mycotoxin-detoxification studies started to reappear once again with large number of practical applications pending the development and adaptation of reliable and reproducible methods of detoxification. While some physical and chemical detoxification methods look promising, the recent focus has shifted to the biological methods mainly due to (a) the mild reaction conditions without the need for extreme pH/temperatures, (b) the possibility of incorporating such detoxification steps at any point of feed/food processing (pre- or post-harvesting), (c) product specificity as most of these reactions are enzymatic, and finally (d) the high acceptance rates among consumers for the end-products compared to other detoxification methods (He and Zhou, 2010).

The field of biological detoxification of mycotoxins has already explored different routes including but not limited to transformation by symbiotic microflora of invertebrates, use of LAB in fermentation and ensiling, transformation/binding in the digestive tract of mammals, and transformation of secreted mycotoxins by the same species of fungi that produce them. While the focus of this short review is mainly on LAB obtained from sourdough starters, but we believe it is worthy to introduce the reader to some key studies that reported

the use of other sources of living microorganisms, yet rich at the same time in endogenous LAB similar to sourdough flora, to eliminate mycotoxins (Fuchs et al., 2002; Takahashi-Ando et al., 2002).

Generally speaking, the mycotoxins elimination activities of sourdough LAB can be categorized mainly under two mechanisms: (a) mycotoxin biodegradation/bio-conversion and (b) mycotoxin binding.

THE BIO-DEGRADATION OF SECRETED MYCOTOXINS BY SOURDOUGH LAB

Kiessling et al. (1984) studied the effect of rumen microorganisms on six different mycotoxins including aflatoxin B_1 (AFB_1), OTA, ZEN, T-2 toxin, diacetoxyscirpenol, and DON. Of the mycotoxins that were incubated with intact rumen fluids, four were completely metabolized: OTA was cleaved to ochratoxin alpha and phenylalanine; ZEN was reduced to alpha-zearalenol and to a less degree to beta-zearalenol; and diacetoxyscirpenol and T-2 toxin were deacetylated to monoacetoxyscirpenol and HT-2 toxin, respectively. In a similar study, Swanson et al. (1988) demonstrated the role of fecal and intestinal microflora on the metabolism of trichothecenes. Suspensions of microflora obtained from the feces of horses, cattle, dogs, rats, swine, and chickens were incubated anaerobically with diacetoxyscirpenol (DAS). The microflora obtained from rats, cattle, and swine completely transformed DAS primarily to deepoxy monoacetoxyscirpenol (DE-MAS) and deepoxy scirpentriol (DE-SCP). This experiment suggested clearly that the role of intestinal microflora was more than just binding DAS. A similar finding was reported for the ability of chicken intestinal microbes to degrade trichothecene mycotoxins (Young et al., 2007). Two pathways were observed with this microflora, the deacylation (predominant) and deepoxidation of the tested mycotoxins.

Pertaining to baked products processing, Valle-Algarra et al. (2009) tracked the levels of OTA, DON, 3-acetyldeoxynivalenol (3-ADON), and nivalenol (NIV) during traditional bread making. The spiked wheat flours showed a significant reduction in OTA levels ranging from 29.8% to 33.5% during the fermentation process. The baking process added another layer of significant decrease of OTA, NIV, 3-ADON, and DON levels by 32.9%, 76.9%, 65.6%, and 47.9%, respectively. Another pilot scale study (Samar et al., 2001) evaluated the stability of naturally occurring DON during the fermentation stage of the bread-making processes. Two different products, French bread and Vienna bread, were prepared with naturally contaminated wheat flour (150 mg/kg) under controlled experimental conditions. Dough was fermented at 50 °C according to the standard procedures employed in Argentinean low-technology bakeries. Reductions of 56% and 41% were observed for the Vienna and French bread, respectively. The study concluded that part of DON detoxification takes place during the fermentation step while the rest of the reduction might be attributed to the thermal decomposition during the baking step.

The fate of DON and its conjugate deoxynivalenol-3-glucoside (DON-3-Glc) within infected wheat samples was tracked recently and the effect of milling and baking technologies were examined thoroughly (Kostelanska et al., 2011). The fractionation of DON-3-Glc and DON during milling was similar and the tested flours contained approximately 60% of the levels of unprocessed wheat grains. The fermentation effect in this study on these two mycotoxins levels was minimal compared to baking. Only a slight decrease in both DON-3-Glc and DON levels was noticed (10% and 13%, respectively). Contrary to Kostelanska findings (Kostelanska et al., 2011), a very recent study (Vidal et al., 2014) raised concerns about the fate of DON-3-G during sourdough fermentation/baking phases as the results, although preliminary, suggested that the levels of this masked mycotoxin were increased indeed. Furthermore, the same study showed that OTA levels were quite stable with no significant reduction throughout sourdough-making processes.

In essence, microorganisms that can modify mycotoxins and metabolize them do exist but its proving to be difficult to pinpoint the responsible enzymes or the involved metabolic pathways. With all the recent advances in the field of genomics, molecular biology, and the availability of chimeric microorganisms; the identification of genes responsible for mycotoxin biodetoxification is more possible nowadays. In a recent study, Poppenberger et al. (2003) reported the detoxification of DON by UDP-glucosyltransferase isolated from *Arabidopsis thaliana*. The enzyme catalyzes the transfer of glucose from UDP-glucose to the hydroxyl group at carbon 3 (C3) of DON. This enzyme, designated as deoxynivalenol-glucosyltransferase or DOGT1, was also able to detoxify the acetylated derivative of DON, 15-acetyldeoxynivalenol (15-ADON). The *TRI101* gene was also reported to act on the same carbon (C3) leading to the acetylation of the attached group and substantially decreasing the toxicity of the parent compound (100-fold decrease in toxicity) (Garvey et al., 2008). Takahashi-Ando et al. (2002) reported the purification of an enzyme responsible for ZEN detoxification. The enzyme is naturally produced by *Clonostachys rosea* IFO 7063. The team purified the enzyme from the reported fungus isolate to homogeneity and sequenced its internal polypeptide chains. Based on the retrieved sequence, the coding

gene was cloned into *Schizosaccharomyces pombe* and *Escherichia coli* which both showed later the ability to degrade ZEN. Such methods of cloning and introducing genes into chimeric microorganisms could possibly open the door for a new era of genetically modified LAB that can be incorporated into the fermentation and acidification of food products and at the same time have the ability to degrade mycotoxins possibly present in the growth medium.

CONTROLLING AND LIMITING MYCOTOXINS ABSORPTION THROUGH BINDING TO SOURDOUGH LAB

Within the context of mycotoxin elimination, Oatley et al. (2000) suggested that another potential method of reducing the adverse effects of mycotoxins on human health is by blocking their uptake via binding to bacteria that either make up the normal gut flora or abundantly present in fermented foods. Since various strains of *Bifidobacteria* bind AFB_1 in vitro, they investigated the binding affinities of large number of *Bifidobacteria* strains utilizing ELISA. Their isolates were found to bind significant quantities of AFB_1 ranging from 25% to nearly 60% of the added toxin.

A similar study conducted by Peltonen et al. (2001) explored AFB_1 binding affinity of 20 different commercial dairy strains of LAB belonging to *Lactobacillus*, *Lactococcus*, and *Bifidobacterium*. All the studied strains were able to bind part of the AFB_1 present in the solution but the percentage differed from one strain to another ranging from 5.6% to 59.7%. The top three isolates (removed at least 50% of the toxin) were found to belong to *Lactobacillus* genera (*Lactobacillus amylovorus* and *Lactobacillus rhamnosus*). Two important findings are to note in this study. First, AFB_1 binding is rapid with 52.6% to 76.9% of the toxin binding to the bacteria within 10 to 15 min. Second, the bound mycotoxin can be released back into washing solutions with multiple vigorous washes. This study attributed mycotoxin removal to cell-wall binding of these isolates rather than enzymatic degradation.

The interaction between ZEN and its derivative alpha-zearalenol with two food-grade strains of *Lactobacillus* was investigated too (El-Nezami et al., 2002). These toxins were incubated with either *Lactobacillus rhamnosus* strain GG or *L. rhamnosus* strain LC-705. Considerable proportions of both mycotoxins (38–46%) were recovered from the bacterial pellet, and no degradation products of ZEN or alpha-Zearalenol were detected using HPLC either in the supernatant of culturing media or methanol extracts of the bacterial pellets. Both heat-treated and acid-treated bacteria were capable of removing toxins, indicating that binding, not metabolism was the mechanism by which both mycotoxins were removed. El-Nezami et al. (1998) further subjected these two strains (GG and LC-705) to various chemical and physical treatments to see how that affects the binding affinity. In short, isolates were subjected to ethanol, UV, sonication, pH, hydrochloric acid and heat treatments, and promising results were obtained when bacterial pellets of both strains were treated with hydrochloric acid, which significantly enhanced their binding ability. This suggested that LAB are likely to bind more mycotoxins when they pass through the stomach of mono-gastric animals and humans where they undergo low pH and high acid conditions present in the stomach (with pH value around 1). Further mechanistic studies by Haskard et al. (2000) between the *L. rhamnosus* GG strain and AFB_1 showed that viable, heat-killed, and acid-killed bacteria responded similarly. The effect of pronase E, lipase and m-periodate on mycotoxin binding ability and release suggested that the binding predominantly occurs at the carbohydrate component of the bacterial cell walls while the effect of urea hinted that hydrophobic interactions might play a major role in explaining this binding mechanism.

Despite being a very rich source of LAB with diversified species and strains that possess unique genetic and physiological characteristics (Table 2), sourdough starters (in general) and the bacterial isolates (in particular) obtained from such starters are certainly understudied for their capacity to bio-transform/bind mycotoxins. Only few studies explored the ability of such rich microflora to eliminate mycotoxins.

Fazeli et al. (2009) reported the reduction of AFB_1 from the liquid media by the autochthonous LAB (*Lactobacillus casei*, *L. plantarum*, and *L. fermentum*) isolated from traditional Iranian bread. The identified strains were all capable of removing AFB_1 with a range of 25% to 61% of the original concentration in a strain dependent fashion with *L. casei* being a strong binder in comparison to the other two strains. The removal process was rapid with approximately 61% and 56% of the toxin taken instantly by *L. fermentum* and *L. plantarum*, respectively. The binding was of a reversible nature, and some of the bound AFB_1 was released back into the media by the repeated centrifugation and re-suspension of the bacterial pellets.

In a similar fashion, we recently reported the ability of one strain of LAB, *Lactobacillus paracasei* subsp. *tolerans*, isolated earlier (Hassan and Bullerman, 2008a, 2008b) from a sourdough starter culture to detoxify DON (but not ZEN) from liquid cultures. Our mechanistic studies indicated that surface binding rather than enzymatic biotransformation is the driving wheel behind the observed significant

Table 2. Sourdough starters are very rich and diverse sources of lactic acid bacteria

Species	Reference
L. paracasei	Meroth et al., 2003
Lactobacillus paracasei subsp. *tolerans*	Hassan and Bullerman, 2008a
L. plantarum	Gobbetti et al., 1994; Lavermicocca et al., 2000, 2003; and Meroth et al., 2003
L. aralimentarius sp. nov.	Cai et al., 1999
L. mindensis sp. nov.	Ehrmann et al., 2003 and Meroth et al., 2003
L. fermentum	Gobbetti et al., 1994; Hamad et al., 1997; and Meroth et al., 2003
L. reuteri	Hamad et al., 1997; Muller et al., 2000a, 2000b; and Meroth et al., 2003
Lactococcus lactis	Hamad et al., 1997 and Meroth et al., 2003
L. Sanfranciscensis	Ganzle et al., 1998 and Meroth et al., 2003
L. sanfrancisco	Corsetti et al., 1998
L. amylovorus	Muller et al., 2000a; Muller et al., 2000b
L. pontis	Muller et al., 2000a, 2000b and Meroth et al., 2003
L. brevis ssp. *lindneri*	Gobbetti et al., 1994
L. acidophilus	Gobbetti et al., 1994
L. farciminis	Gobbetti et al., 1994
L. alimentarius	Gobbetti et al., 1994
L. fructivorans	Gobbetti et al., 1994
L. crispatus	Meroth et al., 2003
L. frumenti	Meroth et al., 2003
L. nantensis sp. nov.	Valcheva et al., 2006
L. hammesii sp. nov.	Valcheva et al., 2005
L. panis	Meroth et al., 2003
L. johnsonii	Meroth et al., 2003
L. brevis	Meroth et al., 2003
L. curvatus	Meroth et al., 2003

Examples of *Lactobacillus* strains isolated and identified from traditional sourdoughs.

reduction of DON concentrations (Hassan and Bullerman, 2013).

Seven trichothecenes (DON, NIV, HT-2 toxin, T-2 toxin, 15- and 3-ADON, and fusarenon-X) were tracked recently in bread production chains (wheat grains, intermediate products collected during milling and baking process, breads) (Lancova et al., 2008). The highest levels of DON were found in the bran fraction while the lowest were in the reduction flours. A significant decrease of DON occurred during the fermentation process; approximately 38% to 46% of the original content, but as expected, baking at 210 °C for 14 min had no effect on DON overall levels.

The presence of mycotoxin not only poses health risks to consumers, but it also affects the bread-making properties of wheat flours. Sub-optimally stored wheat samples with DON content ranging between 820 and 12,000 μg/kg were investigated for their dough- and bread-making properties (Antes et al., 2001). Even though only extremely high DON concentrations of some wheat samples led to a slight decrease of gluten proteins but lower levels affected the rheological properties of dough made of such flours decreasing their maximum resistance (MR). In comparison, the contamination of wheat with *Aspergillus* and *Penicillium* led to a higher decrease of gluten proteins resulting in and extremely decreased MR of the dough and very low bread volumes. Similarly Lancova et al. (2008) found that the rheological properties, such as proofing time and dough stability, were worse in samples that contained higher levels of DON confirming the above earlier observations.

As different parts of the world have different practices for the preparation of starchy products, the traditional nixtamalization method of making the dough (masa) for corn tortillas was evaluated for the detoxification of aflatoxins. The process reduced levels of AFB_1 by 94%, aflatoxin M_1 (AFM_1) by 90%, and

aflatoxin B(1)-8,9-dihydrodiol by 93%, respectively (Elias-Orozco et al., 2002). The reduction of the above mycotoxin was attributed to the use of both lime and hydrogen peroxide in the nixtamalization process (0.3% lime and 1.5% hydrogen peroxide). In spite the premising results of detoxifying aflatoxins in corn tortillas, the high levels of lime and hydrogen peroxide negatively affected the taste and aroma of the final products. In a similar study, Palencia et al. (2003) tracked the levels of Fumonisin B_1 (FB_1) in maize during tortilla preparation. The study reported a total decrease of fumonisins by 50% compared to original concentrations. They associated the decrease with the nixtamalization step utilized by Mayan communities which makes use of lime leading to the loss of tricarballylic acid side chains and yielding hydrolyzed FB_1 (HFB_1) by-products.

Collectively, it appears that binding is one of the major forces of LAB ability to detoxify mycotoxins but that does not negate the existence of certain strains that can bio-transform mycotoxins enzymatically. The possible use of such microorganisms should be scrutinized in depth and further studied to elucidate/confirm the related mechanisms.

CONCLUSIONS

LAB isolated from different sources, especially sourdough bread starters, show promising antifungal and mycotoxin binding/detoxification characteristics that deserve further investigation. Isolating LAB strains that have the ability to inhibit mycotoxigenic fungi growth, optimizing the processing conditions to attend the maximum inhibitory effect, and understanding the molecular mechanisms behind such inhibition will certainly lead to numerous applications in fungi control enhancing our ability to produce a safer food/feed and to minimize the devastating annual loss in agriculture commodities due to fungi infestation, contamination, and mycotoxin presence. Furthermore, such isolates will open the door for multi-hurdle approaches to decrease fungi-related problems in our food chain. No one isolate/strain will be able to address all fungi/food related issues but the use of some of these LAB isolates, already considered to be part of the natural micro-flora of some products, might increase the shelf life of the final products, enhance their stability, and yield in value-added products that possess high consumer acceptance rates.

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DECLARATION OF CONFLICTING INTERESTS

The authors declare that there is no conflict of interest.

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