Lactic acid bacteria – Potential for control of mould growth and mycotoxins: A review

D.K.D. Daliéa,*, A.M. Deschampsb, F. Richard-Forgetaa

Unité MycSA, UPR 1264, INRA, 71 Avenue Edouard Bourlaux, BP 81, 33883 Villenave d’Ornon Cedex, France
bUMR INRA 1219 Oenologie, ISVV, 210 Chemin de Leysottes, 33882 Villenave d’Ornon Cedex, France

Abstract

Most data dealing with the biopreservative activity of lactic acid bacteria (LAB) are focused on their antibacterial effects. Food spoilage by mould and the occurrence of their mycotoxins constitute a potential health hazard. Development of biological control should help improve the safety of products by controlling mycotoxin contamination. Data have actually shown that many LAB can inhibit mould growth and that some of them have the potential to interact with mycotoxins.

This review summarizes these findings and demonstrates that LAB are promising biological agents for food safety.

© 2009 Elsevier Ltd. All rights reserved.

Keywords:
Lactic acid bacteria
Mould
Mycotoxin
1. Introduction

Mycoxinogenic moulds such as Aspergillus, Fusarium and Penicillium play an undeniable role in the deterioration of the marketable quality and hygiene of foodstuffs by synthesizing highly toxic metabolites known as mycotoxins. Several of these toxins have been identified but quite a few could be responsible for significant problems in foodstuffs. Six classes of mycotoxins are frequently encountered in different food systems: aflatoxins, fumonisins, ochratoxins, patulin, trichothecenes and zearalenone. Concerning the importance and diversity of their toxic effects - carcinogenic, immunotoxic, teratogenic, neurotoxic and hepatotoxic - the occurrence of mycotoxinogenic moulds in foods is potentially dangerous for public health and also constitutes a major economic problem. For example, in Western Europe, the economic losses related to the presence of moulds in bread are estimated to be more than 200 million euros per year (Legan, 1993). Physical and chemical methods have been developed to control the occurrence of these microorganisms and their toxins, but no efficient strategy has yet been proposed to reduce the presence of mycotoxins. Moreover, some moulds have acquired the ability to resist chemical treatments and some preservatives. For example, some Penicillium can grow in the presence of potassium sorbate (Davidson, 2001) and other moulds possess the ability to degrade sorbate (Nielsen & De Boer, 2000). The reduction of such moulds in food production is thus of primary importance and there is great interest in developing efficient and safe strategies for this purpose. Biopreservation, the control of one organism by another, has received much attention in the last ten years (Magnusson, Ström, Roos, Sjögren, & Schnürer, 2003).

Among natural biological antagonists, LAB have several potential applications. These microorganisms are widely used for the production of fermented foods and are also part of intestinal microflora. Research reports indicate that LAB have beneficial health effects in humans. These bacteria have a long history of use in foods. They produce some antagonistic compounds able to control pathogenic bacteria and undesirable spoilage microflora, in particular. Using LAB to control mould growth could be an interesting alternative to physical and chemical methods because these bacteria have been reported to have strong antimicrobial properties. However, the antifungal activity of lactic strains remains to be elucidated. A limited number of reports have shown that a good selection of LAB could allow the control of mould growth and improve the shelf life of many fermented products and, therefore, reduce health risks due to exposure to mycotoxins (Gourama & Bullerman, 1995).

In this review, the ability of LAB to control mycotoxinogenic mould growth, the antifungal substances that have been characterized as of this time and the interactions of these organisms with some mycotoxins are successively investigated.

2. Lactic acid bacteria are able to control mycotoxinogenic mould growth

Due to their nutritional requirements, LAB are generally cultivated in enriched media and are found in dairy products, meat, meat-derived products and cereal products (Carr, Chill, & Maida, 2002). These bacteria are mainly divided into four genera: Lactococcus, Lactobacillus, Leucanostoc and Pediococcus. They are traditionally used as preservative agents to prevent spoilage and to extend the shelf life of food and feed. According to Magnusson et al. (2003), three mechanisms may explain the antimicrobial efficiency of LAB: the yield of organic acid, competition for nutrients and production of antagonistic compounds. Several species or subspecies such as Lactococcus lactis subsp. lactis, Lc. lactis subsp. cremoris, Lc. lactis subsp. diacetylactis, Lactobacillus acidophillus, Lactobacillus plantarum and Lactobacillus curvatus are able to synthesize peptides or antimicrobial proteins known as bacteriocins, whose activity is only directed against closely taxonomically-related bacteria. Numerous studies have reported that these molecules are inactive against Gram-negative bacteria and eucaryotic microorganisms such as yeasts or moulds (Batish, Roy, Lal, & Grover, 1997). Moreover, the action of the antifungal properties of LAB on some mycotoxinogenic moulds have also been reported by a few authors. As of this time, the main LAB recognised for their ability to prevent or limit mycotoxinogenic mould growth belong to the genera Lactococcus and Lactobacillus and, to a lesser extent, to Pediococcus and Leuconostoc (Table 1).

Roy, Batish, Grover, and Neelakantan (1996) isolated 2100 colonies of LAB and screened them using several types of moulds and an agar well diffusion assay on potato dextrose agar containing 0.1% Triton X-100. Six colonies were identified for their antifungal activity against Aspergillus flavus IARI, and one of them showed a broad spectrum of antifungal activity against A. flavus IARI, A. flavus NCIM 555, Aspergillus parasiticus NCM 898 and Fusarium spp. This isolate was identified as Lc. lactis subsp. lactis CHD 28.3. Aspergillus IARI was the most sensitive indicator of the antifungal metabolite produced by this lactic strain.

Some other Lactococcus strains identified as Lc. lactis (Coallier-Ascah & Idziak, 1985; Luchese & Harrigan, 1990; Wiseman & March, 1981), Lc. lactis subsp. diacetylactis DRCI (Batish, Lal, & Grover, 1989) and Lc. subsp. cremoris (Florianowicz, 2001) and vegetable products (Sathe, Nawani, Dhakephalkar, & Kapadnis, 2002) have been isolated from different environments such as sourdough (Corsetti, Gobetti, Rossi, & Damiani, 1998; Hassan & Bullerman, 2008), grass silage (Magnusson & Schnürer, 2001; Magnusson et al., 2003) and vegetable products (Sathe, Nawani, Dhakephalkar, & Kapadnis, 2002). A 10-fold concentrated culture filtrate of Lb. plantarum 21B isolated from sourdough and grown in wheat flour hydrolysate was shown to possess an efficient antifungal activity against Penicillium corylophilum, Penicillium roqueforti, Penicillium expansum, Aspergillus niger, A. flavus, and Fusarium graminearum (Lavermicocca et al., 2000).

These authors demonstrated that part of the antifungal activity of Lb. plantarum 21B was ascribed to the production of phenylactic and 4-hydroxy-phenylactic acids. Less than 7.5 mg/ml of phenylactic acid was required to obtain full inhibition of mould growth (Lavermicocca, Valerio, & Visconti, 2003). Earlier, Niki-Paavola, Laitila, Mattila-Sandholm, and Haikara (1999) described the ability of Lb. plantarum VTDE-78076 to suppress the growth of Fusarium VTD-80147. The antifungal activity of this strain was detected in low molecular fractions eluted from a chromatography column loaded with culture supernatant. Antifungal activity was ascribed...
to the occurrence of benzoic acid, an imidazolidinedione derivative and a piperazinedione derivative.

Lb. plantarum strains VTTE-78076 and VTTE-79098 have also been described as being active against different plant pathogenic, toxigenic and gushing-active Fusarium fungi (Laitila, Alakomi, Raaska, Mattila-Sandholm, & Haikara, 2002). Using automated turbidimetry as well as direct and indirect impedimetric methods, the previous authors showed that Lb. plantarum strains VTTE-78076 and VTTE-79098 were effective against Fusarium species such as Fusarium avenaceum, Fusarium culmorum, F. graminearum and Fusarium oxysporum with efficiency depending on the target organism.

Lactobacillus corynformis subsp. corynformis S13, isolated from grass silage, was able to inhibit the growth of a great number of mycotoxigenic moulds including Aspergillus fumigatus, Fusarium avenaceum, Fusarium culmorum, F. graminearum and Fusarium oxysporum (Flanagan et al., 2000). In liquid medium, the production of antifungal metabolites by Lb. corynformis subsp. corynformis S13 was shown to be a growth phase-dependent process. Ethanol has been reported to enhance the antifungal activity of this metabolite, which was irreversibly lost after treatment with proteolytic enzymes including proteinase K, trypsin and pepsin (Magnusson & Schnürer, 2001). After a partial purification, the molecular mass of the potent antifungal compound produced by Lb. corynformis subsp. corynformis S13 was estimated to be close to 3 KDa, to be heat stable, sensitive to proteolytic enzymes and active within a narrow pH range. These characteristics are in accordance with those of bacteriocins of subclass II (Klaenhammer, 1993).

Other species of Lactobacillus, including Lactobacillus casei (Florianowicz, 2001; Gourara, Nomura, & Morichi, 1991), Lactobacillus sanfrancisco CB1 (Corsetti et al., 1998) and Lactobacillus rhombo- sus (Stiles, Penkar, Packova, Chumchalova, & Bullerman, 2002), have also been described as being able to inhibit toxigenic mould growth. Moreover, several papers have reported the ability of the genus Pediococcus to control mycotoxigenic mould growth (Effat, Ibrahim, Tawfik, & Sharaf, 2001; Mandal, Sen, & Mandal, 2007; Rouse, Harnett, Vaughan, & van Sinderen, 2008).

Using vacuum-packed fermented meat, Mandal et al. (2007) isolated an antifungal lactic strain, identified as Pediococcus acidilactici LAB 5, that exhibited varying degrees of antifungal activity against A. fumigatus, A. parasiticus, Fusarium oxysporum and Penicillium sp. To our knowledge, only one paper to date has described the antifungal property of the genus Leuconostoc; the strain studied was identified as Leuconostoc mesenteroides and was effective against Penicillium and Aspergillus (Suzuki et al., 1991).

### 3. Factors that influence the antifungal activity of lactic acid bacteria

A well-designed selection of potential antifungal LAB could reduce the problem of toxigenic moulds. However, relevant use of antifungal LAB requires thorough knowledge of the parameters that modulate their antifungal properties. Numerous parameters have been considered, including temperature, time of incubation, growth medium, pH and nutritional factors (Batish et al., 1997).

### 3.1. Temperature and incubation period

Temperature and incubation period are essential factors that modulate LAB growth and significantly affect the amounts of antifungal metabolites produced (Batish et al., 1997). Reddy and Ranganathan (1985) have reported that the greatest production of antifungal metabolites by L. lactis subsp. diacetylactis occurred after three to four days of incubation. The maximal yield of antimi- crobial compounds produced by Lc. lactis subsp. diacetylactis S5-67-C was recorded at 25 °C.

### Table 1

Lactic acid bacteria isolated as of this time with the ability to inhibit toxinogenic mould growth.

<table>
<thead>
<tr>
<th>Genus Lactobacillus</th>
<th>Activity spectrum</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lncorynformis subsp. corynformis S13</td>
<td>Broad spectrum</td>
<td>Magnusson and Schnürer (2001)</td>
</tr>
<tr>
<td>Lb. plantarum</td>
<td>Penicillium spp., Aspergillus spp.</td>
<td>Lachemmer (2001)</td>
</tr>
<tr>
<td>Lb. plantarum VTTE 78076</td>
<td>P. expansum</td>
<td>Suzuki et al. (1991)</td>
</tr>
<tr>
<td>Lb. plantarum</td>
<td>P. expansum</td>
<td>Batish et al. (2000)</td>
</tr>
<tr>
<td>Lb. plantarum</td>
<td>A. flavus, F. graminearum</td>
<td>Sjögren et al. (2003)</td>
</tr>
<tr>
<td>Lb. reuteri 1100</td>
<td>F. graminearum</td>
<td>Gere et al. (2009)</td>
</tr>
<tr>
<td>Lb. sanfranciscensic CB1</td>
<td>Fusarium spp., Penicillium spp., Aspergillus spp.,</td>
<td>Corsetti et al. (1998)</td>
</tr>
<tr>
<td>Lnc. mesenteroides</td>
<td>Penicillium spp., Aspergillus spp.</td>
<td></td>
</tr>
</tbody>
</table>
The studies carried out by Sathe et al. (2007) demonstrated that antifungal activity of \textit{Lb. plantarum} CUK501 was maximal (1280 AU/ml) at 30 °C, when the culture was at the end of its logarithmic phase.

Over 48 h of incubation, a decrease in antifungal activity was observed when the culture entered the stationary phase. These results were consistent with those previously obtained by Batish, Lal, and Grover (1990), who observed that the antifungal activity of a \textit{Lb. acidophilus} strain was maximal at 30 °C after 48 h incubation, whereas increasing the incubation period resulted in a lower antifungal activity. This decrease was imputed to a metabolism of active compounds or to their enzymatic degradation.

3.2. Effect of growth media

Little is known about the impact of the growth medium on the production of antifungal metabolites by LAB, even if some authors have claimed that the culture medium significantly modulates their metabolism (Batish et al., 1990; Roy et al., 1996). Elliker’s broth was shown to be the best medium for the production of antifungal compounds by \textit{L. lactis} subsp. \textit{lactis} CHD 28.3 against \textit{A. flavus} IARI, compared to M17 and MRS media (Roy et al., 1996). These results were in agreement with those previously obtained by Batish et al. (1990), who demonstrated that \textit{L. lactis} subsp. \textit{lactis} bio- var. \textit{diacetylactis} produced the greatest amounts of antifungal metabolites in Elliker’s broth. In other media such as MRS broth, Nutrient Broth, Yeast Extract, Dextrose Broth and Charmer’s Modified Medium, the production of antifungal metabolites was negligible. Moreover, in milk culture media, \textit{Lc. lactis} subsp. \textit{diacetylactis} produced a small amount of antifungal compound against \textit{A. fumigatus}, which could not be detected by the agar well diffusion assay (Batish et al., 1990).

3.3. Effect of nutritional factors

Effat et al. (2001) studied the influence of nutritional factors on the production of antifungal metabolites by \textit{Lb. rhamnosus} and \textit{Pc. acidilactici}. The authors indicated that yeast extract and glucose, as well as NaCl and CaCl$_2$ modulate the production of antifungal substances. Addition of up to 1% and 2% glucose led to an enhancement of the production of antifungal metabolites by \textit{Pc. acidilactici} and \textit{Lb. rhamnosus}, respectively. It was also demonstrated by Effat et al. (2001) that Yeast Extract increased the antifungal activity of \textit{Lb. rhamnosus} and \textit{Pc. acidilactici}; optimal inhibition was observed with a concentration of 1.5% for both bacteria. Some compounds such as xylose, casein-hydrolysate and proteose-peptone have been reported to delay the production of antifungal substances by \textit{Lc. lactis} subsp. \textit{diacetylactis} (Reddy & Ranganathan, 1983). An increase in the concentration of NaCl from 0.5% to 3% in \textit{Lb. rhamnosus} growth medium led to a higher antifungal efficiency (Effat et al., 2001). This enhancement was assumed to be related to a synergic effect between NaCl, organic acids and antifungal substance (Effat, 2000).

However, high NaCl concentrations were reported to reduce the production of antifungal metabolites by \textit{Lc. lactis} subsp. \textit{diacetylactis} (Batish et al., 1989). A supplementation of broth media with CaCl$_2$ increased the production of antifungal substance by \textit{Lb. rhamnosus} and \textit{Pc. acidilactici}; a maximum increase was observed when the concentration of CaCl$_2$ rose from 0.05% to 0.15% (Effat et al., 2001).

3.4. Effect of pH

The pH conditions greatly modulate the production of antifungal metabolites by LAB (Batish et al., 1997; Sathe et al., 2007). \textit{Lc. lactis} subsp. \textit{diacetylactis} was able to produce substantial amounts of antifungal substance in a narrow range of pH (5.5–7); although maximal production occurred at pH 6.8 (Batish et al., 1997; Reddy & Ranganathan, 1985). Similar data were obtained by Corsetti et al. (1998), who reported an optimal production of inhibitory substances by \textit{Lactobacillus sanfrancisco} CB1 at pH 6. The pH effect was shown to be linked to many factors such as substrate, incubation period, temperature, mould strains and the occurrence of competing microflora (Gourama & Bullerman, 1995).

4. Antifungal metabolites produced by lactic acid bacteria

Several compounds with a strong antifungal activity have been isolated from bacterial cultures. As of this time, the majority of identified antifungal substances are low-molecular-weight compounds composed of organic acids, reuterin, hydrogen peroxide, proteinaceous compounds, hydroxyl fatty acids and phenolic compounds (Table 2).

4.1. Organic acids

Organic acids occurring in foods are additives or end-products of carbohydrate metabolism of LAB. Lactic and acetic acids are the main products of the fermentation of carbohydrates by LAB. These acids, generally recognised as safe agents for the preservation of foods (El-Ziney, 1998), diffuse through the membrane of the target organisms (Axelsson, 1990; Piard & Desmazeaud, 1997). The pH effect was shown to be linked to many factors such as substrate, incubation period, temperature, mould strains and the occurrence of competing microflora (Gourama & Bullerman, 1995).
1990) in their hydrophobic undissociated form and then reduce cytoplasmic pH and stop metabolic activities. Mechanisms other than cytoplasmic pH reduction have been associated with organic acids (Karaba & Eklund, 1991).

It is hypothesized that organic acids act on the plasmic membrane by neutralising its electrochemical potential and increasing its permeability, leading to bacteriostasis and eventually to the death of susceptible organisms. The same hypothesis could also explain the susceptibility of some mould cultures to organic acids (Batish et al., 1997). This property was demonstrated for acetic and propionic acids (Eklund, 1989). With a high dissociation constant, acetic acid was described as being more effective than lactic acid and was by far the best inhibitor of mould growth (Batish et al., 1997).

Other organic acids that show antifungal activity such as phenyllactic acid have been identified in silos inoculated with LAB (Broberg, Jacobsson, Ström, & Schnürer, 2007). This acid has frequently been involved in the antifungal activity of LAB (Gerez, Torino, Rollan, & de Valdez, 2009; Lavermicocca et al., 2000; Magnusson, 2003; Magnusson et al., 2003; Ström, Sjögren, Broberg, & Schnürer, 2002). Phenyllactic acid was first isolated from Lb. plantarum 21 B cell-free extract after ethyl acetate extraction and preparative silica gel thin-layer chromatography, chromatographic and spectroscopic analysis (Lavermicocca et al., 2000). Phenyllactic acid was able to inhibit the growth of P. expansum IMD/F52, A. niger FTDC3227 and IMD1, A. flavus FTDC3226 and F. graminearum IMD623 at a concentration of about 50 mg/ml (Lavermicocca et al., 2000). Lb. plantarum strain MiLAB 393 isolated from grass silage was also been reported to produce phenyllactic acid as an antifungal metabolite (Ström, Schnürer, & Melin, 2005). It was hypothesized that phenyllactic acid acted in a synergy pathway with unidentified antifungal substances produced by Lb. plantarum MiLAB 393 strain (Ström et al., 2002).

4.2. Phenolic compounds

According to our knowledge, only one paper has dealt with the involvement of a phenolic compound in the antifungal activity of LAB (Mandal et al., 2007). This phenolic compound, which remains to be identified, was produced by Pc. acidilactici LAB 5 and showed varying degrees of antifungal activity against a number of foods and feedborne moulds and plant pathogenic fungi.

4.3. Hydroxy fatty acids

Some LAB can produce antimicrobial fatty acids that improve the sensory quality of fermented products (Earnshaw, 1992). Caprylic acid isolated from Lb. sanfrancisco CB1 was by far the main potent antifungal substance produced by this strain (Corsetti et al., 1998). This compound could act in synergy with other acids such as propionic, butyric and valeric acids. Recently, the occurrence of hydroxylated fatty acids (C12) with a great deal of antifungal activity has been described (Sjögren, Magnusson, Broberg, Schnürer, & kenne, 2003). Among these fatty acids, the most active was shown to possess a 12-carbon atom chain length. Hydroxylated fatty acid compounds present a very broad inhibition spectrum and are efficient against moulds and yeasts. The minimum inhibitory concentration (MIC) of hydroxylated fatty acids ranges between 10 and 100 μg/ml (Sjögren et al., 2003). Their production kinetics follow the growth of the producing strains, suggesting that these compounds do not result from cell lysis (Sjögren et al., 2003). However, their action mechanism remains to be elucidated.

4.4. Hydrogen peroxide

Hydrogen peroxide is produced by most LAB in the presence of oxygen (Kandler, 1983). LAB are unable to produce catalase. They can therefore not degrade hydrogen peroxide that, after accumulation, oxidises the lipid membrane and cellular proteins of the target organisms (Lindgren & Dobrogosz, 1990). In some foods such as milk, the antimicrobial activity of hydrogen peroxide at non-lethal concentrations is ascribed to its reaction with thiocyanate, catalysed by a lactoperoxidase. The products of this reaction, such as hypothiocyanite and other intermediary molecules, can reduce the growth of several undesirable microorganisms (Schnürer & Magnusson, 2005). In order to find an alternative to postharvest fungicides currently used in the prevention and control of blue mould caused by P. expansum, Venturini, Blanco, and Oria (2002) assessed the in vitro antimicrobial activity and the minimum inhibitory concentration (MIC) of hydrogen peroxide with different end point methods such as agar diffusion assays, volatility measurements and agar and broth dilution MIC assays. The growth of P. expansum was completely repressed by a 5% hydrogen peroxide solution when tested by an agar diffusion assay; the minimum inhibitory concentration (MIC) ascribed to hydrogen peroxide was less than 0.025%. Venturini et al. (2002) therefore suggested that the application of small quantities of hydrogen peroxide to apple skin might be an alternative to fungicides to inhibit P. expansum.

It was recently suggested that the F. graminearum spore germination rate might be affected by hydrogen peroxide (Pons, Pinson-Gadais, Verdel-Bonnin, Barreau, & Richard-Forget, 2006). Moreover, inoculation of spores that have been incubated with 0.5 mM hydrogen peroxide led to a significant reduction of mycotoxin accumulation, as in the case of trichothecene. Moreover, Gild-Ad and Mayer (1999) proved that fungi can rapidly break down hydrogen peroxide. In fact, non-lethal levels of exogenous hydrogen peroxide modulate fungal catalase and superoxide dismutase, two major enzymes involved in detoxification (Angelova, Pashova, Spasova, Vassilev, & Slokoska, 2005).

4.5. Reuterin

Reuterin is a product of glycerol fermentation produced by Lactobacillus reuteri (Chung, Axelsson, Lindgren, & Dobrogosz, 1989), Lactobacillus brevis, Lactobacillus buchneri (Schütz & Radlter, 1984), Lactobacillus collinoides (Claise & Lonvand-Funel, 2000) and Lb. coryniformis (Magnusson, 2003) under anaerobic conditions. It has been shown that in the target organisms, this compound was able to suppress ribonuclease activity, the main enzyme involved in the biosynthesis of DNA (Dobrogosz, Casas, & Karlsson, 1989). Reuterin has been reported to be able to inhibit the growth of Asparagus and Fusarium (Chung et al., 1989). The addition of glycerol to some LAB cultures that produce reuterin increases their antifungal activity (Magnusson, 2003).

4.6. Proteinaceous compounds

There is no clear evidence at this time of the role of protein compounds in the inhibition of mould growth by LAB. However, several authors have clearly reported that some lactic strains such as Lc. lactis subsp. lactis, (Roy et al., 1996), Lb. casei subsp. pseudo-plantarum (Gourama & Bullerman, 1997) and Pediococcus pentosaceus (Rouse et al., 2008) produced antifungal metabolites that were sensitive to proteolytic enzymes.

For example, the antifungal activity of the Lb. casei subsp. pseudo-plantarum strain was shown to be suppressed by a trypsin or α-chymotrypsin treatment (Gourama & Bullerman, 1997). After a partial characterisation, the previous authors suggested that the main molecule involved in this antifungal activity was a peptide with a molecular weight of less than 1 kDa. This result was consistent with further experiments conducted by Magnusson and Schnürer (2001), who showed that the antifungal metabolite pro-
duced by the *Lb. coryniformis* subsp. *coryniformis* S13 strain was a highly heat stable, small peptide (approximately 3 KDa), whose activity was fully inhibited by proteolytic enzymes. In addition, the antifungal peptide, characterised by a great hydrophobicity, rapidly adsorbed to the producing cells or alternatively formed spontaneous aggregates (Magnusson & Schnürer, 2001). Moreover, when a liquid culture of *Lb. coryniformis* subsp. *coryniformis* S13 strain was supplemented with either ethanol or formic or acetic acids, the antifungal activity increased (Magnusson & Schnürer, 2001).

Other types of antifungal peptides were identified to be cyclic dipeptides, including cyclo (Phe-Pro), cyclo (Phe-OH-Pro) and cyclo (Gly-L-Leu) (Magnusson, 2003; Niku-Paavola et al., 1999; Ström et al., 2002). Cyclo (Phe-Pro) and cyclo (Phe-OH-Pro) were produced by *Lb. plantarum* MiLAB 393 (Ström et al., 2002), *Pedicoccus pentosaceus*, *Lb. sakei* and *Lb. coryniformis* (Magnusson, 2003), while cyclo (Gly-L-Leu) was isolated from *Lb. plantarum* VTTE-78076 (Niku-Paavola et al., 1999).

We previously reported the wide diversity of antifungal metabolites that LAB are able to produce to control mould growth. However, with their efficient antifungal activities, would LAB be able to interact with the mycotoxin production? If so, how could these promising biological preservation agents modulate the accumulation of mycotoxins?

### 5. Lactic acid bacteria–mycotoxin interactions

Most data dealing with the effects of LAB on the accumulation of mycotoxins are related to aflatoxin-producing moulds. Wiseman and Marth (1981) revealed the existence of an amensalism relationship between *Lc. lactis* and *A. parasiticus*. When these authors added the spores of *A. parasiticus* to a 13-day-old culture of *Lc. lactis*, they observed the entire repression of aflatoxin production. When the fungal spore suspension and the lactic strain were inoculated simultaneously, an increase in aflatoxin production was observed. In contrast, Coallier-Asch and Idziak (1985) showed an inhibition of aflatoxin accumulation when both microorganisms were simultaneously cultivated in Lab-Lemco tryptone broth (LTB). Addition of glucose to the cultivation medium during the conidiation phase of the mould did not restore the production of aflatoxin.

According to Coallier-Asch and Idziak (1985), the inhibition of aflatoxin accumulation was not related to a pH decrease but rather to the occurrence of a low-molecular-weight metabolite produced by the LAB at the beginning of its exponential phase of growth. Inhibition of aflatoxin production by other LAB belonging to the genus *Lactobacillus* was also reported (Karunaratne, Wezenberg, & Bullerman, 1990). It was assumed that this inhibition resulted from the production of a metabolite different from hydrogen peroxide or organic acid (Gourama, 1991). Haskard, El-Nezami, Kankaanpää, Salminen, and Ahokas (2001) demonstrated that *Lb. rhamnosus* GG (ATCC 53103) and *Lb. rhamnosus* LC-705 (DSM 7061) were able to eliminate aflatoxin B1 from the culture medium by a physical process.

Interactions between lactic strains and fusariotoxins such as zearalenone (ZEN) and its derivative, α-zearalenol, were also investigated. A significant proportion (38–48%) of both toxins was trapped in the bacterial pellet and no degradation product of zearalenone or α-zearalenol was detected (El-Nezami, Polychrónaki, Salminen, & Mykkänen, 2002a), leading to the conclusion that binding and not metabolism was the mechanism by which the toxins were removed from the media. Similar results were obtained with other mycotoxins including ochratoxin A (Del Prete et al., 2007; Fuchs et al., 2008) and fumonisin B1 and B2 (Niderkorn, Boudra, & Morgavi, 2006). Therefore, two specific processes such as binding and inhibition of biosynthesis may be involved in the interaction between LAB and the accumulation of some mycotoxins.

### 6. Inhibition of mycotoxin biosynthesis by lactic acid bacteria

Several papers dealing with the inhibition of mycotoxin biosynthesis by LAB have focused on aflatoxins (Thyagaraja & Hosono, 1994). During cell lysis, it is possible that LAB releases molecules that potentially inhibit mould growth and therefore lead to a lower accumulation of their mycotoxins (Gourama & Bullerman, 1995). These “anti-mycotoxinogenic” metabolites could also be produced during LAB growth. Gourama (1991), using a dialysis assay, demonstrated the occurrence of a metabolite that inhibits aflatoxin accumulation in *Lactobacillus* cell-free extracts. It was suggested that this inhibition of aflatoxin biosynthesis was not the result of a hydrogen peroxide production or a pH decrease (Karunaratne et al., 1990). These findings were consistent with those of Gourama (1991), who suggested that inhibition of aflatoxin biosynthesis by *Lactobacillus*-cell free supernatants was probably due to specific bacterial metabolites. Coallier-Asch and Idziak (1985) reported a significant reduction of aflatoxin biosynthesis by *Lactobacillus*-cell free supernatants and suggested that this inhibition was related to a heat stable, low-molecular-weight inhibitory compound. Although *Lactobacillus* spp. were found to delay aflatoxin biosynthesis, other lactic strains such as *Lc. lactis* were found to stimulate aflatoxin accumulation (Luchese & Harrigan, 1990).

### 7. Binding of mycotoxins by lactic acid bacteria

The cell walls of some LAB such as *Leuconostoc* and *Streptococcus* have been reported to be able to bind some mutagenic compounds such as amino acid pyrolysates and heterocyclic amino acids produced during cooking. Similar results were obtained with other LAB isolated from fermented products (Rajendran & Ohta, 1998; Thyagaraja & Hosono, 1994). Further investigations have been conducted to evaluate the ability of LAB to remove other food-contaminating substances including mycotoxins, known for their mutagenic effects.

#### 7.1. Binding of aflatoxins by lactic acid bacteria

Aflatoxin B1 is a well-known carcinogen and is classified by the International Agency for Research in Cancer as a class 1 human carcinogen. Therefore, reducing its bioavailability is of great interest for human health. Several LAB have been found to be able to bind aflatoxin B1 in vitro and in vivo (Kankaanpää, Tuomola, El-Nezami, Ahokas, & Salminen, 2000; Gratz et al., 2004), with an efficiency depending on the bacterial strain (Shah & Wu, 1999). El-Nezami, Kankaanpää, Salminen, and Ahokas (1998) have evaluated the ability of five *Lactobacillus* to bind aflatoxins in vitro and have shown that probiotic strains such as *Lb. rhamnosus* GG and *Lb. rhamnosus* LC-705 were very effective for removing aflatoxin B1, with more than 80% of the toxin trapped in a 20 μg/ml solution (Haskard, El-Nezami, Peltonen, Salminen, & Ahokas, 1998). It was assumed that aflatoxin B2, G1, and G2 were less sensitive to this binding process (El-Nezami et al., 2002b).

In vitro binding of aflatoxin B1 by LAB was described as a fast (no more than 1 min) and reversible process (Bueno, Casale, Pizzolitto, Salano, & Olivier, 2006), strain- and dose-dependent (Kankaanpää et al., 2000). Data found in the literature indicate that other organisms such as *Saccharomyces cerevisiae* have the potential to bind aflatoxin B1 (Santin et al., 2003; Baptista et al., 2004). In order to clarify the in vitro aflatoxin B1 removal by LAB and *S. cerevisiae*, a mathematical model (Fig. 1) has been proposed (Bueno et al.,...
This model suggests the attachment of aflatoxin B1 molecules to the surface of the organism and takes two processes into consideration: binding (adsorption) and release (desorption) of aflatoxin to and from the binding site on the surface of the microorganisms (Bueno et al., 2006; Lee et al., 2003). This model allows us to estimate the number of aflatoxin B1 binding sites \( M \), the system equilibrium constant \( K_{eq} \) and the effectiveness of cells to remove aflatoxin B1 from a liquid medium \( \frac{M}{[AFLB]} \times K_{eq} \). Using this model, it has been demonstrated that the different abilities of strains to trap aflatoxin B1 were directly linked to the number of binding sites \( M \) presented by each microorganism. The yeast \( S. \) cerevisiae was reported to be the most efficient microorganism for aflatoxin B1 quenching (Bueno et al., 2006).

7.2. Binding of trichothecenes by lactic acid bacteria

The ability of \( Lb. \) rhamnosus GG and \( Lb. \) rhamnosus LC-705 to remove zearealenone and its derivative \( \alpha \)-zearealenol from a liquid medium was investigated by El-Nezami et al. (2002a). A significant proportion of both toxins (38% and 46%) was trapped by the bacterial pellet. No degradation products of either zearealenone or \( \alpha \)-zearealenol were observed after three days of incubation. Both heat-treated and acid-treated bacteria were able to remove zearealenone and \( \alpha \)-zearealenol, suggesting that binding, not metabolism, was the mechanism by which both toxins were removed from the media. The process was also fast and depended on the bacterial cells and toxin concentrations. Coincubation of zearealenone and \( \alpha \)-zearealenol significantly decreased the percentage of bound toxins, indicating that both toxins might have the same binding site on the bacterial surface.

7.3. Binding of zearalenone by lactic acid bacteria

The ability of \( Lb. \) rhamnosus GG and \( Lb. \) rhamnosus LC-705 to remove zearalenone A by lactic acid bacteria

The ability of \( Lb. \) rhamnosus GG and \( Lb. \) rhamnosus LC-705 to remove zearalenone and its derivative \( \alpha \)-zearealenol from a liquid medium was investigated by El-Nezami et al. (2002a). A significant proportion of both toxins (38% and 46%) was trapped by the bacterial pellet. No degradation products of either zearealenone or \( \alpha \)-zearealenol were observed after three days of incubation. Both heat-treated and acid-treated bacteria were able to remove zearealenone and \( \alpha \)-zearealenol, suggesting that binding, not metabolism, was the mechanism by which both toxins were removed from the media. The process was also fast and depended on the bacterial cells and toxin concentrations. Coincubation of zearealenone and \( \alpha \)-zearealenol significantly decreased the percentage of bound toxins, indicating that both toxins might have the same binding site on the bacterial surface.

7.4. Binding of ochratoxin A by lactic acid bacteria

Piotrowska and Zakowska (2005) screened 29 strains of LAB belonging to \( Lactobacillus \) and \( Lactococcus \) genera for their sensitivity to ochratoxin A and their ability to remove this toxin from liquid media. All the strains tested were able to reduce the concentration of ochratoxin A and most of them were insensitive to the toxin. The greatest adsorptions, more than 50% of the initial ochratoxin A content, were obtained with \( Lb. \) acidophilus CH-5, \( Lb. \) rhamnosus GG, \( Lb. \) plantarum BS, \( Lb. \) brevis and \( Lb. \) sanfranciscensis. Similar results were reported for the ability of wine LAB to bind ochratoxin A. The amount of ochratoxin A removed during bacterial growth was shown to vary between 8% and 28% and no degradation product was detected, suggesting that ochratoxin A removal by wine LAB was a binding process (Del Prete et al., 2007).
7.5. Binding of fumonisins by lactic acid bacteria

Niderkorn et al. (2006) were the first to examine in vitro interactions between LAB and fumonisins. The ability of 29 LAB and three strains of Propionibacterium to remove fumonisin B₁ and B₂ from acidified MRS broth samples (pH 4.0) was evaluated. Niderkorn et al. (2006) demonstrated that fumonisin B₁ was not as effectively removed as fumonisin B₂.

Most of the strains were able to remove both toxins, but considerable differences were observed among these strains. The Propionibacterium strains were less efficient than the LAB. Binding efficiency was affected by the pH since at pH 7, LAB were unable to trap fumonisins B₁ and B₂ (Niderkorn et al., 2006).

8. Mechanism of mycotoxin binding by lactic acid bacteria

Few investigations have been conducted to elucidate the mechanism by which some mycotoxins such as aflatoxins, zearalenone and fumonisins are trapped by LAB pellets. It has been demonstrated that when heat or acid treatments were applied to LAB, their ability to remove aflatoxin B₁ increased (El-Nezami et al., 1998). According to this result, supplementation of some basic compounds (NaOH and Na₂CO₃) and isopropanol was shown to negatively influence this binding. Viability of LAB strains was not essential, suggesting that binding probably took place on the cell wall. Accessibility of bound aflatoxin B₁ to specific monoclonal antibody confirmed the surface nature of binding (Haskard et al., 2001). It was suggested that carbohydrates and/or protein components of LAB played a major role in aflatoxin B₁ binding since the effect of pronase E and periodate (periodate causes oxidation of cis OH groups to aldehydes and carbon acid groups) on heat-killed, acid-killed and viable LAB strains resulted in a considerable decrease in aflatoxin B₁ binding (Haskard, Binnion, & Ahokas, 2000).

It was assumed that no fatty acids were involved in this interaction since treatment of LAB strain with lipases did not induce a significant decrease in aflatoxin B₁ binding. The study conducted by Lahtinen, Haskard, Ouwehand, Salminen, and Ahokas (2004) corroborates the previous data and leads to the conclusion that cell wall peptidoglycans are responsible for aflatoxin B₁ removal by LAB strains. Regarding the chemical interactions involved in this process, hydrophobic ones were the most plausible since aflatoxin B₁ removal was significantly decreased when heat-and acid-killed LAB were treated with an anti-hydrophobic agent such as urea. Heat and acid treatments are responsible for protein denaturation, leading to the exposure of more hydrophobic surfaces. Moreover, when the cells were treated with organic solvents, bound toxin was rapidly extracted, confirming a potential role of hydrophobic interaction in aflatoxin B₁ binding (Haskard et al., 2000). Haskard et al. (2000) also reported that hydrogen bonding and electrostatic interaction were not involved in aflatoxin B₁ binding by LAB since this process was not significantly affected by mono and divalent ions or by variations in pH (2.5–8.5).

On the basis of the chemical moieties and interactions involved in zearalenone and α-zearalenol binding by LAB, it is likely that carbohydrates and proteins were the bacterial cell components involved in the process (El-Nezami et al., 2004). Enzymatic treatments of viable LAB with pronase E did not decrease the capacity of lactic strains to bind zearalenone and its derivative. However, the effect of the same enzyme on heat- and acid-killed LAB significantly affected the binding capacity, suggesting that the new binding sites exposed after a heat or acidic treatment were proteins. Lipase did not affect this interaction while urea decreased it.

Concerning the mechanisms of action involved in the removal of fumonisins by LAB, Niderkorn (2007) suggested that peptidoglycans were the most plausible fumonisin binding sites. The quenching ability of LAB was increased when bacteria were killed using different physical and chemical treatments, while lysozyme and mutanolysin enzymes that target peptidoglycans partially inhibited it. It was also reported that tricarballylic acid chains found in fumonisins molecules played an important role in the binding process since hydrolysed fumonisin had less affinity for LAB, and free amine group inactivation had no effect on the binding process (Niderkorn, 2007). The same article attempted to explain the low affinity of fumonisin B₁ using a molecular modelling approach. In fact, an additional hydroxyl group in fumonisin B₁ could form a hydrogen bond with one of the tricarballylic acid chains, resulting in a spatial configuration where the tricarballylic acid chain is less available to interact with bacterial peptidoglycans.

Removal of fumonisins by LAB was ascribed to adhesion to cell wall components rather than covalent binding or metabolism, since the dead cells fully retained their binding ability. Peptidoglycans probably play a key rule in this binding process. Therefore, elucidating the differences between bacterial cell wall components of LAB strains might make it possible to select LAB species with the potential to act as biopreservative agents capable of reducing exposure from fumonisins that occur in food and feed.

9. Stability of the lactic bacteria–mycotoxin complex

Potential future applications of LAB to reduce mycotoxin availability rely on the relative stability of the complexes formed. In the case of weak binding interactions, mycotoxins may be released by the continual washing of the bacterial surface in the gastrointestinal tract. Several studies have attempted to assess the stability of the complexes formed between mycotoxins and LAB and have concluded that the binding strength significantly depends on the strain and on environmental conditions. Thus, among a panel of autochthonous LAB isolated from Iranian sourdough and dairy products, Lb. casei was reported to be the strongest binder of aflatoxin compared to other Lb. plantarum and fermentum strains (Fazeli et al., 2009). A similar study was performed concerning the ability of lactic and propionic acid bacteria to scavenge fumonisins (Niderkorn et al., 2006). According to the former authors, the strength of the mycotoxin–LAB interaction was influenced by the peptidoglycan structure and, more precisely, by its amino acid composition (Niderkorn, Morgavi, Aboab, Lemaire, & Boudra, 2009). The fact that non-viable LAB conserve their high binding capacity is an essential point that has to be considered. The survival of viable bacteria is in fact significantly reduced by the low pH conditions occurring in the stomach. Moreover, certain LAB that show considerable adhesion to intestinal cells lose this property when they bind to mycotoxins such as aflatoxin B₁ (Gratz et al., 2004; Kankaanpää et al., 2000). Consequently, in the gastrointestinal tract, the bacteria–mycotoxin complex is rapidly excreted. Use of these LAB could be beneficial for humans and animals constantly exposed to mycotoxins by reducing the absorption of these toxins and increasing the excretion of mycotoxin bound to bacterial cells. The above discussion clearly illustrates the fact that any in vitro results must be supported by in vivo experiments concerning the species in question in order to assess the real effect of LAB on mycotoxin bioavailability and toxicity.

10. Conclusion

The analysis of data available in the literature dealing with antifungal activity of LAB has highlighted the ability of some strains to repress mycotoxinogenic mould growth through the production of several low-molecular-weight antifungal metabolites. Even if some of these antifungal metabolites, including cyclic dipeptides, phenylactic acids and 3-hydroxylated fatty acids, have been success-
fully purified, most of these low-molecular-mass compounds remain to be identified due to the lack of suitable assay procedures to isolate very small amounts of active metabolites. Therefore, more research should be devoted to the development of efficient methods for detecting antifungal compounds in complex biological systems. Controlling optimal conditions responsible for better antifungal metabolite production in vitro and in the food matrix could enhance the potential offered by LAB as natural food-grade agents. Some selected LAB were also shown to be able to trap mycotoxins. Mycotoxin quenching was described as a reversible phenomenon, strain- and dose-dependent, and did not affect the viability of LAB. The ability of LAB to trap toxins was even more effective when bacteria were treated with different physical and chemical treatments aimed at killing bacteria. The sequestration of mycotoxin from food and feed prior to their consumption could be one of the most effective mycotoxicosis prevention strategies. The binding property displayed by some selected LAB, resulting in a decrease of mycotoxin bioavailability, could therefore be used in novel approaches to decontaminate food and feed. However, little is known at this time about the stability and toxicity of LAB–mycotoxin complexes. Many questions must still be answered before they can be practically used at the industrial level. Among these, the potential release of toxin after ingestion requires further study, as does the toxicity of the bound toxin compared to its unbound form. Thus, the use of lactic acid bacteria and their metabolites to control mould development and mycotoxin accumulation appears to be a promising biocontrol strategy in perishable foods or feed frequently contaminated by toxigenic fungal strains, including vegetable products and particularly cereals, fruits and by-products.

The use of lactic acid bacteria has been mainly limited to fermentation processes. Lastly, due to their ability to trap mycotoxins, some lactic acid bacteria might reduce the availability and toxicity of toxins in the gastrointestinal tract of humans and animals and might therefore be used as a probiotic agent.

With the increasing interest in food safety throughout the world, LAB cultures with high antifungal, antimycotoxigenic and mycotoxin binding potential could be of immense value in limiting mycotoxin exposure. However, the introduction of large scale bio-preservation of food requires careful safety assessment and risk analysis.

Acknowledgements

This work is part of Doguieal Dalé PhD project financially supported by the “Ministère de l’Enseignement Supérieur et de la Recherche Scientifique” of Côte d’Ivoire, as part of the Integrated Research Project “Qualité Sanitaire des Aliments en Aquitaine 2006–2008”.

References


