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Control of mould growth and mycotoxin production by lactic acid bacteria metabolites

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Abstract

A wide spectrum of filamentous fungi is often found in various food commodities, where they can cause extensive damage and lead to sizable economic losses. The occurrence of their toxic metabolites – mycotoxins - constitutes a high risk for human and animal health. Although prevention of fungal growth and mycotoxin production on plants and in feedstuffs is usually considered as the best approach to impede the harmful effects on animal and human health, decontamination/detoxification of contaminated products is also of prime importance. Since the general public requires high quality, preservative free, safe but mildly processed food with extended shelf life, biopreservation, the control of one organism by another, has received much attention lately. Among the different potential decontaminating microorganisms, the group of the lactic acid bacteria has been considered as the most promising natural biological antagonists. Data have shown that many lactic acid bacteria can inhibit mould growth and that some of them have the potential to interact with mycotoxins. This review summarizes recent data about potential control of mould growth and mycotoxin production by lactic acid bacteria and highlights that they are very promising biological agents for food safety.

Key words: moulds, mycotoxins, lactic acid bacteria, interaction, control

Introduction

A wide spectrum of filamentous fungi and yeasts is often found in various food commodities, where they can cause extensive damage and lead to sizable economic losses. Fungal infection leads to food spoilage such as off-flavors, discoloration, rotting and disintegration of the food structure. The very important aspect involved in spoilage of food by fungi is also the formation of toxic secondary metabolites - mycotoxins. Concerning the importance and diversity of their toxic effects – carcinogenic, teratogenic, mutagenic, immunotoxic, neurotoxic, nephrotoxic and hepatotoxic – the occurrence of mycotoxinogenic moulds in foods constitutes a high risk for human and animal health [1, 2, 3, 4].

Although prevention of fungal growth and mycotoxins production on plants and in feedstuffs is usually considered as the best approach to impede the harmful effects on animal and human health, decontamination/detoxification of contaminated products is also of prime importance [5, 6] Several physical and chemical techniques are used for the preservation of food and feed. Drying, freeze drying, cold storage, modified atmosphere storage and heat treatments are all means of physical methods of food preservation [7]. Several chemical additives also function as preservatives, and those are organic acids - acetic, lactic, sorbic, benzoic and propionic, as well as some antibiotic, such as natamycin, produced by *Streptomyces natalensis* [8].

However, some moulds and yeasts have acquired the ability to resist chemical treatments and some preservatives. For example, some *Penicillium*, *Saccharomyces* and *Zygosaccharomyces* species can grow in the presence of potassium sorbate, and other moulds possess the ability to degrade sorbate [8, 9]. *Penicillium roqueforti* isolates have been found to be resistant to benzoate and *P.discolor* to natamycin [10]. There is a great risk that the resistance phenomenon will increase in the future due to the frequent use of antibiotic and preservatives. The food industry has been put under pressure to find how to inhibit the growth of toxigenic moulds and the synthesis of mycotoxins in raw materials and end products, while the general public requires high quality, preservative free, safe but mildly processed food with extended shelf life. Biopreservation, the control of one organism by another, could be an interesting alternative to physical and chemical methods, and it has received much attention lately [11]. Among the different potential decontaminating microorganisms, the group of the lactic acid bacteria has been considered as the most promising natural biological antagonists.

The action of the antifungal properties of lactic acid bacteria (LAB) on some mycotoxinogenic moulds have been reported by few authors, but the number of published studies on antifungal LAB is still very low. A limited number of reports have shown that a good selection of LAB could allow the control of mould growth and therefore reduce health risks due to exposure to mycotoxins [3]. There is an open area for research possibilities for prevention of mould growth and elimination of mycotoxins from food or their transformation into less dangerous compounds, using the strains of lactic acid bacteria.

The present review outlines the ability of lactic acid bacteria to prevent mycotoxinogenic mould growth and synthesis of mycotoxins, as well as their potentials to decontaminate and detoxify already contaminated foods.

Lactic acid bacteria as biopreservatives

The group of lactic acid bacteria (LAB) occupies a central role in food fermentation processes and has a long and safe history of application and consumption of fermented food and beverages [12]. LAB occur naturally in foods or are added as pure culture to various food products. Their production of lactic and acetic acids, ethanol, aroma compounds, bacteriocins, exopolysaccharides and several enzymes is of importance for enhancing shelf life and microbial safety, improving texture and contributing to the pleasant sensory profile of the end product [12]. A general preservation effect is obtained by most fermentations due to the accumulation of organic acids and alcohols concomitantly with the reduction of the level of free sugars, depletion of oxygen and lowering pH [13, 14]. Various mechanisms have been suggested to be responsible for the inhibitory effects of the bacteria on fungal growth, such as nutritional competition, secondary metabolites, pH or combinations of these mechanisms [11, 15]. Even the precise mechanism of antimicrobial action is difficult to elucidate due to complex and commonly synergistic interactions between different compounds, several compounds with a strong antifungal activity have been isolated from LAB cultures [16]. The majority of them are low-molecular-weight compounds composed of organic acids, reuterin, hydrogen-peroxide, proteinaceous compounds, fatty acids and phenolic compounds [3].

Antifungal metabolites produced by lactic acid bacteria

Organic acids

Organic acids, such as lactic, acetic, propionic and phenyllactic, have frequently been involved in the antifungal activity of LAB. Lactic and acetic acids, the main products of the fermentation of carbohydrates by LAB, are generally recognized as safe agents for preservation of foods. It is hypothesized that they act on the plasmatic membrane by neutralizing its electrochemical potential and increasing its permeability. The undissociated hydrophobic form of the acid diffuses over the cell membrane and dissociates inside the cell,

releasing H^+-ions that acidify the cytoplasm and stop metabolic activities in susceptible mould cells [17, 18]. With a high dissociation constant, acetic acid was by far the best inhibitor of mould growth. Phenyllactic acid, isolated from *Lb.plantarum* 21 B, was able to inhibit the growth of *P.expansum* IDM/FS2, *A.niger* FTDC3227 and IDM1, *A.flavus* FTDC3226 and *F.graminearum* IDM623 at a concentration of about 50mg/ml [19].

Reuterin

Reuterin, a broad-spectrum antimicrobial substance originally described from *Lactobacillus reuteri*, is a product of glycerol fermentation produced by starving cells under anaerobic conditions. It is able to suppress the activity of ribonuclease, the main enzyme involved in the biosynthesis of DNA in the target organism [20]. The production of reuterin has also been reported from *Lb.brevis, Lb.buchneri, Lb.collinoides* and *Lb.coryniformis* [11, 21, 22]. Antifungal activity was shown against species of *Candida*, *Torulopsis*, *Saccharomyces*, *Aspergillus* and *Fusarium* [23]. The addition of glycerol to some LAB cultures that produce reuterin increases their antifungal activity [11].

Hydrogen peroxide

The most strains of LAB produce hydrogen peroxide in the presence of oxygen. Since LAB are unable to produce catalase, they cannot degrade the hydrogen-peroxide, which, therefore, accumulates in the environment. The antifungal activity of hydrogen peroxide is attributed to a strong oxidizing effect on the lipid membrane and cellular proteins of the target organisms [24].

Hydroxylated fatty acids

Lipolytic LAB can produce significant amounts of antimicrobial fatty acids that also contribute to the sensory quality of fermented foods [25]. Caproic acid isolated from *Lb.sanfrancisco* CB1 was by far the main potent antifungal substance, and it could act in synergy with other acids, such as propionic, butyric and valeric acids. J. SJÖRGEN & al. [26] observed that hydroxylated fatty acid compounds with 12 carbons had the strongest antifungal activity against a broad spectrum of moulds and yeasts. The minimum inhibitory concentrations (MIC) of these compounds against moulds and yeasts ranged between 10 and 100 µg/ml, which could be compared with standard antifungal drugs. However, their action mechanism remains to be elucidated.

Phenolic compounds

V. MANDAL & al. [27] isolated a phenolic compound, which remain to be identified, from *Pediococcus acidilactici* LAB 5, that showed varying degrees of antifungal activity against a number of foods and feedborne moulds and plant pathogenic fungi.

Control of mould growth by lactic acid bacteria

For many years now it has been clear that the most effective means to prevent contamination of food with mycotoxins is to avoid growth of mycotoxinogenic fungi [18]. The interaction between foodborne fungi, especially mycotoxin-producing ones, and other microorganisms is a common phenomenon in nature that can affect fungal growth and/or production of mycotoxins.

R. MUÑOZ & al. [28] investigated the effect of different fermenting organisms – two LAB, *Lactobacillus fermentum* and *Lactobacillus rhamnosus*, and *Saccharomyces cerevisiae*, on growth of mycotoxin-producing *Aspergillus nomius*. They carried out the assays by simultaneous inoculation of one of the possible inhibiting microorganisms and the fungus, or subsequent inoculation of one of the microorganisms followed by the fungus. All three microorganisms assayed showed growth inhibition of the mycotoxin-producing *Aspergillus* strain, but the *Lb.rhamnosus* O236 isolated from sheep milk showed the highest fungal inhibition of the microorganisms assayed.

U. SCHILLINGER & J.V. VILLARREAL [29] investigated the ability of a numerous of LAB strains to inhibit the growth of an ochratoxin-producing *Penicillium nordicum* (BFE

487). They used a 69 different LAB strains isolated from various food sources, including some probiotic and bioprotective bacteriocin-producing strains. For the detection of antifungal activity, a modified agar spot assay was used. Briefly, the test strains were spot inoculated onto MRS agar plates and allowed to grow at 30°C for up to 3 days in anaerobic jars. The plates then were overlaid with malt extract agar (0.7%, 9 ml) containing about 10^5 spores of *P.nordicum* BFE 487 per ml. After incubation for up to 5 days at 25°C, the plates were examined for the formation of inhibition zones around the bacterial colonies. Of the 69 LAB strains that were screened, 37 of the strains were able to produce zones of inhibition on MRS agar plates. These 37 strains included the well known commercial probiotic cultures *Lb.rhamnosus* LGG and *Lb. plantarum* 299v. The size of the inhibition zones increased when the incubation period before overlaying the plates with the mould was extended from 24 to 48 or 72 h.

Apart from the well-known milk and meat fermented products, in some southern regions of Italy traditional breads are produced by subjecting the durum wheat semolina to LAB fermentation. The LAB involved in cereal fermentation originate from natural contamination of the flour and include various species of *Lactobacillus*, *Lactococcus*, *Enterococcus, Pediococcus, Leuconostoc* and *Weissella* genera. F. VALERIO & al. [30] subjected thirty samples of Italian durum wheat semolina to microbiological analyses in order to explore their LAB diversity and to find strains with antifungal activity. Seventeen of isolated strains, each characterized by a different REP-PCR pattern, were screened for their antifungal properties. They were grown in a flour-based medium, comparable to a real food system and the resulting fermentation products were tested against fungal species generally contaminating bakery products, *Aspergillus niger, Penicillium roqueforti* and *Endomyces fibuliger*. Eight out of seventeen tested LAB strains, including *Weissella cibaria* (3 strains), *W.confusa* (1), *Leuconostoc citreum, L. mesenteroides, Lactobacillus plantarum* and *Lb.rossiae,* almost completely inhibited (more than 90%) the growth of *E.fibuliger*, with respect to growth in control medium. *P.roqueforti* was inhibited by almost all strains at a percentage higher than 65.52%. The inhibitory effect of these strains was comparable to that obtained with the common preservative calcium propionate (0.3% v/v). The most effective strain was *Lb. plantarum* C21-41. This research also highlighted the unexplored antifungal activity of *L.citreum, Lb.rossiae* and *W.cibaria*, which inhibited all fungal strains to the same or a higher extent compared with calcium propionate, for example, *A.niger* was completely inhibited (>98%) by these strains.

C.L. GEREZ & al. [31] have also investigated the ability of some LAB strains to inhibit *Aspergillus, Fusarium* and *Penicillium*, the main contaminants in bread. They assayed 95 strains of LAB and indicated that only four strains – *Lb.plantarum* CRL 778, *Lb.reuteri* CRL 1100, *Lb. brevis* CRL 772 and *Lb.brevis* CRL 796 – displayed antifungal activity against tested moulds. They observed that the inclusion of antifungal LAB strains in the starter culture allowed a reduction in the concentration of calcium propionate by 50% while still attaining the shelf life similar to that of traditional bread containing 0.4% calcium propionate.

Interactions between mycotoxins and lactic acid bacteria

As it was highlighted above, the most efficient way to prevent contamination of food with mycotoxins is to avoid growth of mycotoxigenic fungi. Unfortunately, the contamination of various commodities with toxigenic moulds is unavoidable under certain environmental conditions (6). Therefore, several techniques to destroy mycotoxins in food have been developed [32]. They include heating, treatment with ammonia, screening and radiation, but they have not become popular because they are too expensive, impractical for commercial application or destroy vital nutrients of foods. Biological strategies for the management of mycotoxins in food and feed, which gain much attention lately, is promising method for inhibition of mycotoxin biosynthesis and detoxification of already contaminated food.

Biological decontamination of mycotoxins by different microorganisms, such as *Flavobacterium aurantiacum, Corynebacterium rubrum, Aspergillus niger, Rhizopus* spp., *Mucor* spp. and others, has been reviewed by several authors, especially in the last two decades [33, 34]. These organisms have been shown to enzymatically degrade mycotoxins, but question remains on the toxicity of products of enzymatic degradation and undesired effects of fermentation with non-native microorganisms on quality of food [32]. However, the number of reviews on decontamination of mycotoxins by microorganisms involved in food fermentation, such as yeasts and lactic acid bacteria, is very limited.

Inhibition of mycotoxin biosynthesis by LAB

One of the processes that may be involved in the interaction between LAB and the accumulation of some mycotoxins is inhibition of their biosynthesis. H. GOURAMA [35] 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 probably due to specific bacterial metabolite.

Binding of mycotoxins by LAB

Some LAB strains have been reported to be able to bind some mycotoxins [36, 37]. EL-NEZAMI & al. [38] 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. M. PIERIDES & al. [39] investigated the ability of dairy strains of lactic acid bacteria to remove aflatoxin M1 from contaminated phosphate buffer saline, skim and full cream milk. All tested strains, whether viable or heat-killed, could reduce the aflatoxin M1 content of a liquid medium, which indicates that bacterial viability is not prerequisite to toxin removal and suggests involvement of a cell wall-related physical phenomenon instead of a metabolic degradation reaction. H. EL-NEZAMI & al. [15] investigated the ability of the same strains to remove zearalenone and its derivative α-zearalenol from a liquid medium. A significant proportion of both toxins (38% and 46%) was trapped by the bacterial pellet, with no degradation products of these toxins after three days of incubation. According to M. PIOTROWSKA & Z. ZAKOWSKA [40], all 29 tested strains of LAB belonging to *Lactobacillus* and *Lactococcus* genera were able to reduce the concentration of OTA. The greatest adsorptions, more than 50%, of the initial ochratoxin A content, were obtained with *Lb. acidophilus* CH-5, *Lb. brevis* and *Lb. sanfranciscensis.* Potential future applications of surface binding in order to reduce mycotoxin bioavailability in animals or humans rely on the relative stability of the complex formed. If mycotoxin is bound noncovalently and extracellularly, it may be released by the continual washing of the bacterial surface in the gastrointestinal tract if the binding is insufficiently strong [41]. An understanding of the nature of the binding – if binding is intracellular or extracellular, or reversible or irreversible - is important in understanding the fate of bound mycotoxin.

Detoxification of mycotoxins by LAB

FUCHS & al. [42] investigated the detoxification of two abundant mycotoxins – ochratoxin A (OTA) and patulin (PAT) by lactic acid bacteria. They analyzed the ability of thirty different LAB strains to remove the two toxins from liquid medium, in a screening trial by the use of HPLC coupled with UV detection (for PAT) or fluorescence detection (for OTA), and identified two highly effective strains: *Lactobacillus acidophilus* VM 20, which caused a decrease of OTA by ≥ 95%, and *Bifidobacterium animalis* VM 12, which reduced PAT levels by 80%. Subsequently experiments showed that the effectiveness of these strains is influenced by different factors, such as concentration of toxins, the cell density, the pH-value and the viability of the bacteria. Since chemical analytical studies do not provide a firm proof that the decrease of the mycotoxins results in a reduction of their toxic properties, the authors carried out additional experiments, in which the influence of the incubation of

bacteria with toxins was studied in human derived liver cells (HepG2). It was investigated if the treatment of the toxins with the bacteria has an effect on the inhibition of cell division and on the formation of micronuclei, which are formed as a consequence of chromosome breakage and aneuploidy [43]. A substantial decrease (39-59%) of OTA and PAT induced micronuclei formation was observed with the most effective strains detected in the chemical analyses. Also, the inhibition of the cell division rates by the toxins was significantly reduced.

J. RAŠIĆ & al. [44] have shown that the content of aflatoxin B_1 (AB_1), the most potent aflatoxin, may be decreased by yoghurt and milks acidified with citric, lactic and acetic acids. They found that AB_1 added to milk before fermentation was reduced considerably in yoghurts. A decrease of 97% was observed in samples with 600 μg/kg AB_1 and 90% in those with 1400 μg/kg. The greatest decrease in AB_1 added at a concentration of 1000 μg/kg was in milk acidified with citric acid (90%), followed by lactic acid (84%) and acetic acid (73%). Decrease of AB_1 content is probably related with its conversion to aflatoxin B_{2a}, the much less toxic compound, as shown by duckling tests and no bile duct hyperlapsia [45].

ŠKRINJAR & al. [46] investigated the possibility to reduce OTA levels by some yoghurt bacteria and bifidobacteria. Due to the growth of *Streptococcus salivarius* subsp. *Thermophilus* T4, *Lactobacillus delbruecki* subsp. *bulgaricus* LB-51 and *Bifidobacterium bifidum*, the OTA level in milk samples with 0.05 and 0.1 mg/l OTA was completely reduced, while a significant decrease of the toxin content was observed in milk sample with higher OTA concentrations. The decrease in the OTA content was the most conspicuous during the milk fermentation by yoghurt bacteria. The toxin completely disappeared in samples with 0.05, 0.1 and 0.5 mg/l of OTA, while a 36 and 26% drop, respectively, was observed in samples with 1.0 and 1.5 mg/l of OTA. The mechanism of removing OTA from the substrate is its conversion to isocumarin, known as ochratoxin α (OTα) and L-β phenylalanine by breaking amide bond that links those two moieties of OTA. Even not nontoxic, OTα is, at least, 1000 times less toxic than OTA, and its elimination half-time in the body (9.6h) is shorter than that of OTA (103h) [47].

Conclusion

For many years consumers have requested food preserved without chemical additives. The use of antifungal LAB and their metabolites instead of chemical preservatives would enable the food industry to grant this request, since it appears to be promising biocontrol strategy in perishable foods or feed frequently contaminated by toxigenic fungal strains.

Data available in the literature highlighted the ability of some LAB strains to repress mycotoxigenic mould growth through the production of several low-molecular weight antifungal metabolites, most of which remain to be identified. Investigation of optimal conditions responsible for better antifungal metabolite production *in vitro* and in the food matrix could enhance the potential use of LAB as natural food-grade agents. Some selected LAB strains have been reported 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 binding property displayed by LAB results in a decrease of mycotoxin bioavailability, which could be used in novel approaches to decontaminate food and feed. However, the introduction of large-scale biopreservation of food requires careful safety assessment and risk analysis. Many questions must still be answered before they can be practically used at the industrial level.

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