Lactic acid bacteria a potential mold controlling and anti-Mycotoxigenic Agent: A Review

Belhadj Oussama Kamel*[1], Xu Li[1], Fuguo Xing[1], Sahnouni Fatima[2]

[1] *Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences/Key Laboratory of Agro-products Quality and Safety Control in Storage and Transport Process, Ministry of Agriculture, Beijing, 100193, P.R.China.*

[2] *Department of Biology, Faculty of Natural Sciences and Life, University of Mascara, Mascara 29000, Algeria*

Abstract

Contamination by molds and yeasts generally leads to the deterioration of the infected commodities, subsequently causing great economical loses and endangering humans and live stocks especially when mycotoxins are produced. Chemical and physical means utilized for controlling fungi and their occurrence are expressing limitations due to growing fungal resistance, mycotoxin stability, and public moral growing against chemical preservatives, resulting in high demand for bio-remediation methods capable of securing commodities. One potential candidate for that purpose is Lactic Acid Bacteria, having GRAS status and are recognized as promising antimicrobial agents. Despite that fact, their antifungal effect, mycotoxin binding, and detoxification potential are yet to be fully explored. This review aims at providing a summary of recent research and finding involving the antifungal effect of LAB and/ or their metabolites with a focus on bacteriocin involvement. Moreover, this review highlights LAB association with other antifungal agents to find greater inhibitory effects.

Keywords: *lactic acid Bacteria; Antifungal; Mycotoxin biodegradation; Biopreservation.*

* Corresponding author: *Belhadjoussamakamel@gmail.com*

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1. Introduction

Molds and yeasts are important food and feed spoilage organisms, they are microscopic plant-like organisms composed of long filaments called hyphae growing over the surface and inside nearly all substances from both plant and animal origin [1]. Genera: *Aspergillus*, *Fusarium*, *Penicillium*, *Mucor*, and *Saccharomyces* are common occurrences in food and produce highly toxicogenic metabolites known as mycotoxin: Aflatoxin, Ochratoxin are among the most potent ones [2], Certain environmental factors could stimulate the production of mycotoxins, therefore the extent of contamination will differ with geographic location, agricultural methods and the susceptibility of commodities to the penetration of fungi during storage and processing periods despite the said above they are detrimental for the food and render it unsafe for consumption [3]. The public moral progressively refused chemical preservative agent usage in food therefore the concept of biopreservation became an important approach to maintaining the hygienic quality of food products and controlling microbial and fungal deterioration without a negative impact on the sensory quality of the product [4], [5]. The use of microbiota and/or antimicrobial compounds in the process to prevent, control spoilage, and extend the shelf-life along with enhancing the safety of food [6], [7]. Lactic acid bacteria possess a major potential for use in biopreservation because they are generally recognized as safe (GRAS status), and they naturally dominate the microflora of many foods. Inhibitory properties of LAB are due to different factors such as the competition for nutrients and the production of antimicrobial, and antifungal metabolites [8]. Although the antibacterial effect of Lactic Acid Bacteria has been heavily studied, their antifungal effect was not researched with the same amplitude apart from some studies and reviews, According to Gerez *et al*. [9] Antifungal activities from the genera Lactobacillus inhibited both the growth and the Aflatoxin production of *Aspergillus parasiticus* and they produce an arsenal of metabolites that might have antifungal potency. In light of these facts, LAB should be researched more in their antifungal potency and their mycotoxin biodegradation ability. This review addresses LAB antifungal abilities and highlights their aspect as biocontrol agents for food product preservation against fungal contamination. Furthermore, it gives a summary of studies and research related to their implications as fungal control agents.

2. Fungal Spoilage of food and feed

Fungal contamination has an immense effect on agriculture products and noticeable economic losses are reported when processing agricultural products by the action of deleterious microorganisms. In Africa fermented foods are constituting a significant part of nutrition, the predictions on a global scale indicate that the market for fermented products and ingredients may reach up to $28.4 billion by 2022 [10]. However, the safety of these foods faces a significant threat by detrimental microorganisms and toxigenic fungal species including members belonging to the *Aspergillus*, *Penicillium*, and *Fusarium* genera known to add to the microbiota of fermented foods. when conditions are adequate These pervasive toxigenic fungal species produce toxic secondary metabolites called mycotoxins such as Aflatoxin [11], Ochratoxin [12], Fumonisins B1 [13], and Patulin [14]. When Theses toxin integrates into the commodity they prove difficult to eliminate and endanger both food, feed, and lives of stokes [15], [16]. Furthermore, losses range from loss of life to decreased production in animals, and increased costs of veterinary and human health care services. Severe contaminations lead to serious chemical reactions that induce offensive sensory changes in food and may cause total losses when the product is declared unfit for consumption, rejected by the market, and thus consequently destroyed [17]. Due to their ubiquity, various types of toxigenic mold

can easily contaminate food and agricultural commodities Vegetables and fruits can be infested with a large number of spoilage microorganisms because of their contact with soil during growth and harvesting [15], and the fungus flora involved in the spoilage of fresh vegetables are *Botrytis cinerea, Rhizoctonia solani, Sclerotinia sclerotiorum,* various species of the genera *Alternaria, Aspergillus, Cladosporium, Colletotrichum, Phomopsis, Fusarium, Penicillium, Phoma, Phytophthora, Pythium, Rhizopus* and some mildews, The blue mold rot is a clear example of detrimental fungal contamination, it's caused by fungi including various species of *Penicillium*, *Botrytis cinerea*, and *Monilinia laxa*, as well as other fungi that produce mycotoxins [18–21]. Industries are struggling with this issue more frequently, that's why it's revered seriously to be dealt with by prevention measures and vigorous regulations to lower both financial risks and health hazards. As consequence, the general public required more efficient procedures to reduce fungal and mycotoxin harm while casting aside chemical preservatives and aiming towards a more natural way of preserving food.

3. Mycotoxin and their occurrence

Mycotoxins are fungal secondary metabolites, some of which have pharmacological activities and they are used in antibiotics production, growth promoters, or in other classes of drugs [22]. Diseases caused by molds are called mycoses while the diseases induced by their secondary metabolites are mycotoxicoses [23]. From thousands of species of fungus, only about 100 belonging to the genera Aspergillus, Penicillium, and Fusarium are known to produce mycotoxin [24]. Only a handful of mycotoxins are of serious implication in food and feed commodities and can cause hazardous risks affecting both human life and livestock, among which: Aflatoxin, Ochratoxin, deoxynivalenol (DON or vomitoxin), Zearalenone, Fumonisin, T-2 toxin, and T-2 like toxins (trichothecenes). Mycotoxin contamination is seen as an unavoidable naturally occurring problem, even where good agricultural practices are implemented thus representing a great challenge to food safety. Their destruction is very difficult due to their crystallization state and/or their high resistance under gamma radiation, high temperatures, and even physical and chemical influence [25], [26]. Consumption of a mycotoxin-contaminated diet may induce: acute and long-term chronic toxic effects in animals and humans, teratogenicity, potent carcinogenic effects, and/or estrogenic or immunosuppressive and can be especially hepatocarcinogens [27]. Besides, mycotoxin contamination of the animal feed supply chain can reduce animal productivity, The direct effects include reduced feed intake, feed refusal, poor feed conversion, diminished body weight gain, increased disease incidence, and reduced reproductive capacities that all lead to economic losses [28].

Exposure to mycotoxins is present worldwide, even though there are geographic and climatic differences in their production and occurrence. Fungi are a normal part of the microflora of field and stored crops, but the production of mycotoxins depends upon the type of fungi, agronomic practices, the composition of the commodity, the conditions of harvesting, handling, and storage [29]**.** Aflatoxin, Ochratoxin, and Fumonisin contamination occur in Cereal grains [30], [31] especially Rice (*Oryza sativa)* since it is cultivated in flooded irrigation conditions and high moisture levels, making it highly susceptible to getting infected by mold and to subsequent mycotoxin contamination [32–34], In fresh fruits as Strawberry fruits affected by mycotoxin express gray mold, rhizopus rot, Mucor fruit rot [35] even in their dried state: prunes [36], raisins[37], black olives [38] can be affected. Nuts, almonds, and peanuts were also reported to be affected by mycotoxin [39], A study conducted by Riba et al. [40] demonstrated that pistachio and unshelled walnuts were found to have Aflatoxins (AFs) trace when tested. Fermented

food is not exempt from fungal contamination and mycotoxin accumulation since they can be a favorable media for fungal growth consequently when the contamination occurs and the factor is favorable mycotoxin production is noticed in these products [41]. Therefore, developing, finding, and innovating ways to predict mycotoxin occurrence and/or detoxification process is important, furthered more to implement targeted prevention strategies. In 2018, Ksenija et al. [42] Highlighted the need to develop predictive models to detect mycotoxins in the food and feed industry, new detection measures are continually being updated while classic ones are being improved.

4. Current fungal contamination control measures and their limitation

The classic approach for controlling and preventing fungal contamination involved many physical, chemical, and biological methods of plant diseases and insect control. The use of compatible fungicides at the right time and in the appropriate way reduces contamination risks. A variety of agricultural practices are implemented, e.g. crop rotation, soil tillage, irrigation, weed control, growing crop varieties that have proved to be more resistant to fungi and insect injuries coupled with biological control using microbial antagonists or competitors can be integrated with contamination control strategies by spraying agents on plants to eradicate or limit the growth of toxin producers. As for Strategies to combat mycotoxins, they can be divided into preharvest treatments to reduce or inhibit the production of the toxins in the field, and postharvest remediation of contaminated commodities. Early harvest followed by drying of the grains can help avoid increased mycotoxin contamination. During harvest, it is of utmost importance to use adequate harvesting equipment that is correctly adjusted to avoid damage to the kernel, since damaged kernels are predisposed to infection during storage. Proper preharvest conditions are crucial to prevent fungal growth and mycotoxin accumulation in the harvested commodities [29], [43]. Therefore the priority remains preventing toxin accumulation directly on the field (preharvest) or thereafter (transport and storage) [44]. The chemical approach is based on washing procedures, radiation, ultrasound, and extraction with organic solvents, e.g. A chemical such as ammonia vapors are used to inactivate mycotoxin [45], calcium hydroxide mono-methylamine, and several others in Table 1. Their implementation needs special equipment and additional time leads to an increase in costs, while It is helpful to some extent but it contradicts the aim of developing “clean label” food products that do not contain any synthetic chemical preservatives [46]. The efficiency of these methods highly depends on the level of contamination and the distribution of mycotoxins throughout the grain [47]. Mycotoxin control measures have been implemented for agricultural commodities entering international trade or located in countries with centralized or large-scale buying and distribution systems. Despite this fact, in developing countries where local food consumption or subsistence agriculture is practiced by as much as 70% of the population, such measures would be difficult to implement [48], [49]. These countries are currently researching for a more optimal solution to avoid chemical fungicides since some of these are not authorized for postharvest treatment and several have been removed from the market due to possible toxicological risks. According to ‘Directive 91/414/CEE’ of the EU and the growing public concern about the use of pesticide approaches along with the development of resistance to fungicides by several fungal pathogens [50], [51].

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Table 1. The classic chemical fungicide and their limitations

Agent	Chemical Formula	Mechanism	Spectrum	limitations	References
Calcium hydroxide nanoparticles	$Ca(OH)_2$	Inhibition of fungal growth through De-acidification	*Penicillium sp* *Corylophilum spp* *A. niger*	Low toxicity	[52]
Sodium bisulfite	$NaHSO_3$	Destruction of Aflatoxin	*A. flavus* *Aflatoxin degradation*	Limited efficacy against other microorganisms	[53]
Chlorine dioxide gas	ClO_2	Spores germination inactivation	*B. cinerea* *A. alternate* *S. vesicarium* *M. piriformis*	Potential health risks at high concentrations, vegetal tissue damage	[54] [55] [56]
Hydrogen peroxide	H_2O_2	Disruption of yeast cell	*Candida spp*	Limited efficacy against other microorganisms	[57]
Ascorbic acid	$C_6H_8O_6$	Antioxidant activity	*F. ananatum*	Limited spectrum	[58]
Sulfur dioxide	SO_2	inhibit fungal mycelia and spores	*B. cinerea* *Rhizopus sp*	Limited efficacy	[59]
formaldehyde	CH_2O	disorders permeability of cell wall	*P. chrysogenum* *T. harzianum* *A. niger*	Potential health risks at high concentrations.	[60]
ammonia	NH_3	Toxicity by modifying the wound environment	*A. parasiticus* *P. digitatum*	Potential damage to fruits at high concentrations	[61] [62]
Ammonium hydroxide	NH_4OH	ND	*A. flavus*	ND	[63]
Azole	$C_{22}H_{17}ClN_2$	Block synthesis of ergosterol	*Candidas spp* *P. corylophilum* *A. niger* *C.cladosporioides*	Can have limited efficacy against some strains of fungi; can have side effects and interactions with other drugs	[52]

[1] Not well defined

5. Lactic Acid Bacteria and Antifungal metabolites

Lactic Acid Bacteria (LAB) have several potential applications; they are widely used for the production of fermented foods, a part of intestinal microflora, and exerts beneficial

health effects on human. LAB protection of food from spoilage and pathogenic microorganisms could be an interesting alternative since researchers have left the classic platforms of chemical detoxifying methods towards a new strategy using lactic acid bacteria [64]. They represent ideal candidates for commercial exploitation due to their GRAS status (Generally Regarded As Safe) and their Qualified Presumption of Safety (QPS) status in the EU. Consequently, the scientific exploration of their potential as biocontrol agents have and still growing in interest [65]. According to Magnusson et al. [66] three mechanisms may explain the antimicrobial efficiency of LAB: the yield of organic acid, competition for nutrients, and production of antagonistic compounds counting: organic acids, hydrogen peroxide, diacetyl, and bacteriocins. However, the antifungal activity of lactic strains remains to be elucidated [67], [68]. In accordance, the inhibition of mold growth in the presence of lactic acid bacteria has been investigated by some authors Strom et al. [69] chose *Lactobacillus plantarum* to investigate one of the first antifungal (AF) interactions as A co-cultivation assay was devised using *Lb. plantarum MiLAB 393* and its target *Aspergillus nidulans,* its results come as promising potential. Pawlowska et al. [70] affirm that Careful selection of specific strains of LAB with antifungal properties can allow the reduction of molds and yeast genera and can therefore improve the shelf-life of many fermented products and reduce the presence of a mycotoxin.

Lactic acid bacteria are used as bio-protective for a range of foods including; bread, fresh fruits, vegetables, animal feed, and dairy products. The use of LAB is one of the oldest methods for preserving food and it is estimated to date back more than 3000 years [71]. While a plethora of studies have assessed LAB antibacterial effects [72] their antifungal activity has not been the object of similar excessive studies, Although it has been proven in some cases [73]. Various screenings have been undertaken with aim of identifying LAB with antifungal properties as Table 2. Gives a summery of the different isolated sources including Cereals [74], [75], Vegetables [76], [77], Sourdough [9], [78], [79], and Dairy food [80], [81]. While the precise antifungal factor(s) from lactic acid bacteria is yet vague due to the variety of metabolites secreted by LAB, various hypothesizes and experiments were done to collect data related to this subject. Magnusson J [77] in his research identified Lactic acid bacteria with antifungal abilities, **He** draws attention to the fact that upon the addition of proteinase K disappearance of antifungal activity was noted. Furthermore, Lavermicocca and Strom [79], [82] **In their** analysis have attributed the antifungal effect to the secretion of phenyl lactic acid (PLA), an organic acid that is produced as a by-product from the metabolism of phenylalanine. Afterward, A study by Cortés-Zavaleta et al. [83] listed a correlation between PLA production with antifungal strength. PLA has the potential to retard the growth of many fungal species including species belonging to *Aspergillus, Fusarium, and Penicillium* [84]. Other metabolites such as carbon dioxide, ethanol, reuterin, diacetyl, proteinaceous compounds or low-molecular-weight peptides, and even phenolic compounds, or a combination of these factors, have been also hypothesized as responsible for their antifungal strength [85], [86].

Table 2. Publication reporting the antifungal potency of Lactic Acid bacteria

Lactic isolate	Source	Antifungal spectrum	Compound/mechanism	References
L. plantarum BCH-1 L. coryniformis BCH-4	rice rinsed water	*A. fumigatus* *A. flavus*	organic acids	[87]

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L. paracasei LUHS244 L. uvarum LUHS245 L. farraginis LUHS206	from a spontaneously fermented cereal	*Penicillium sp* *Aspergillus sp*	organic acids	[88]
W. paramesenteroides LC11	fermented cassava	*A. niger* *A. tubingensis* *P. crustosum*	Acetic acid hydroxy fatty acids	[74] [89]
L. fermentum	Forastero cocoa bean	*S. cerevisiae* *T. delbrueckii* *C. ethanolica* *H. uvarum*	Organic acid and/ or proteinaceous metabolites	[90]
L. acidophilus	Isolated culture	*C. albicans* *C. krusei* *C. kefyr* *C. glabrata.*	ND[1]	[91]
L. plantarum *L. delbrueckii* *La. fermentum*	'Kunu' is traditionally fermented Millet and sorghum beverage	*A. flavus*	ND[1]	[92]
P. pentosaceus	Whole Barley Sourdough	*A. flavus* *A. niger*	Organic acids Low molecular mass compounds	[93]
L. hammesii DSM 16381	Sourdough	*A. niger* *P. roqueforti*	fatty acid	[94]
L. rhamnosus BIOIII28	Czech Republic dairy product (Yogurt)	*Y. lipolytica* *R. mucilaginosa*	ND[1]	[95]
Leu. mesenteroides DU15	Malaysian fermented foods.	*A. niger*	low molecular peptides	[96]
Lb. curvatus A61	Azerbaijan's traditional homemade cheese	*Cladosporium sp* *Fusarium ssp.*	organic acids other compounds.	[97]
L. plantarum FST 1.7	Sourdough	*F. graminearum* *F. culmorum*	Cyclic dipeptides	[98]
.plantarum CRL 778	Sourdough	*A. niger* *Penicillium* *F. graminearum*	Phenyllactic PLA	[9]
L. sakei subsp. ALI033	kimchi	*P. brevicompactum*	organic acid	[99]
L. citreum *L. rossiae*	Italian durum wheat semolina	*A. niger*	Lactic and acetic acids.	[100]

[1]: Not Determined

5.1. Organic acids

Lactic acid bacteria produce multiple metabolites including organic acids (Lactic, Acetic, propionic, and several others. They are considered major secreted metabolites that dramatically affect the growth of fungi through inhibition of mycelial growth, they cause rapid acidification of the raw material through their production [101], [102]. Batish et al. [103] underlined that LAB activity against fungal contamination has been attributed to organic acids among other factors**,** The reports regarding the role of organic acids from microbial sources in suppressing fungal growth have remained quite insufficient. Presser

et al. [104] highlighted that at acidic pH the ratio of dissociated and undissociated acids is shifted toward the undissociated form and was reported to have a higher inhibitory activity than the dissociated forms. Furthermore, Sadiq et al. [105] state that in their protonated or undissociated form organic acids are lipophilic and thus readily diffuse across the fungal cell membrane and get accumulated in the cytoplasm.

Among organic acids produced by LAB strains, lactic acid is usually produced in the highest quantity and is thus considered the major metabolite of LAB. However, the authors underlined that it exhibits less inhibitory activity against fungal growth as compared to other organic acids such as acetic acid and propionic acid. In the study done by Trias et al. [106], the antimicrobial substances produced by the LAB strains described were organic acids, which exhibited anti-fungi activity. Just a few reports describe the influence of these acids in the production of mycotoxins [107–109]. However, LAB antifungal compounds including organic acids, fatty acids, and carboxylic acids that affect fungal growth and mycotoxin production present interesting potential [110].

5.2. Phenyl lactic acid (PLA)

Phenyl lactic (PLA) and p-OH-phenyl lactic acids (OH-PLA) play a role in inhibiting fungal growth; LAB antifungal aspect has become particularly important with the identification of PLA as an antifungal compound. the inhibitory properties of PLA have been demonstrated against several fungal species isolated from bakery products, Cereals, including some mycotoxigenic species such as *Aspergillus ochraceus*, *Penicillium verrucosum*, and *Penicillium citrinum* in addition to certain contaminating bacteria, namely *Listeria sp*, *Staphylococcus aureus*, and *Enterococcus faecalis*. [83], [100], [111–114]. Lavermicocca et al.[115] conclude that phenyl-lactic acid and 4-hydroxy-phenyl-lactic acid from a sourdough isolate of *L. plantarum* had broad-spectrum fungicidal activity. Followed by Gerez et al. [9] who outlined the presence of antifungal metabolite produced by *Lb. reuteri 1100* in sourdough, revealed as Acetic acid, phenyl lactic acid, and exhibited antifungal ability against braid mold spoilage. Dallagnol et al. [116] described the production of PLA by an *L. Plantarum* strain is affected by the influence of biosynthetic precursors, intermediates, and electron acceptors. The fact that poses a particular challenge is that this metabolite exhibits an effect at high concentrations.

5.3. Reuterin and Reutericyclin

One of the most intensively studied low molecular weight inhibitory compounds, Reuterin is a broad-spectrum antimicrobial substance originally described from *Lactobacillus reuteri*. Chemically identified as a mixture of monomeric hydrated monomeric and cyclic dimeric forms of a b-hydroxypropionaldehyde compound which is in equilibrium with its hydrated monomeric and cyclic dimeric forms, it is highly soluble in neutral pH [117–121]. Reuterin has been reported to have inhibitory activity against different microorganisms including gram-positive and gram-negative bacteria, yeast, and fungi. the antifungal activity of these molecules was studied against species of *Candida*, *Torulopsis*, *Saccharomyces*, *Aspergillus*, and *Fusarium*. However, there is still considerable ambiguity in explaining this claim [119], [122].

Lactobacillus reuteri isolates produce two compounds; reuterin, and reutericyclin, both active toward Gram-positive bacteria. Reutericyclin is a tetrameric acid derivative with a broader spectrum of inhibitory activity, including Gram-negative bacteria, fungi, and protozoa [123–125], It was Dobrogosz et al. [126] who provided the hypothesis that

under anaerobic conditions this compound was able to suppress ribonuclease activity, the main enzyme involved in the biosynthesis of DNA. Afterward, Schaefer et al. [127] gave a similar hypothesis for reuterin's antimicrobial activity relating it to the dimeric form. The structure of the HPA dimer is similar to that of a ribose sugar, and it works as a competitive inhibitor of the enzyme ribonucleotide reductase, thus blocking DNA synthesis. This mechanism is difficult to be determined due to the enzyme's active site containing a thiol group.

Oliveira et al. [108] demonstrated that the cell-free supernatant (CFS) of medium fermented for 48 h with this strain showed antifungal activity against *F. culmorum*, As it belongs to the species L. reuteri, the strain is likely to be a reuterin producer. Some results indicated that *L. reuteri* isolates reduced aflatoxins B1, B2, G1, and G2, respectively. The effect of the isolate on aflatoxin B1 was significantly higher than the other toxins [128]. Despite all the cited above, Returin and Reutericyclin production has not yet been authorized for commercialization.

5.4. Fatty acids

The antimicrobial activity of fatty acids has been recognized for many years, Hartling et al. [129] reported that unsaturated fatty acids are active against Gram-positive bacteria and studied the relative antifungal activity of volatile fatty acids (VFAs) on grain. They found propionic acid to be an effective antifungal substance at a level of 0.8% on grains having 20% moisture. The VFAs were also found to be fungicidal in experiments where grains were inoculated with mycotoxigenic molds; This indicates that VFAs seem to have the potential for use as food biopreservatives against molds.

The main limitation of VFAs is that they could adversely affect the flavor of food and thus be of limited utility. Gould et al. [130] expressed that the antifungal activity of fatty acids is dependent on chain length, concentration, and pH of the medium. Sjogren et al. [131] performed one of the first studies that discovered several 3-hydroxylated fatty acids with antifungal activity from *Lactobacillus plantarum MiLAB 14* as there were no previous reports available on the antifungal activity of hydroxylated fatty acids produced. They pointed out that a hydroxylated fatty acid with 12 carbons had the strongest antifungal activity in comparison to other carbon chain length fatty acids. Besides, Their results expressed a strong antifungal activity against a broad spectrum of yeasts and molds where yeasts were generally more sensitive to the fatty acids than molds. The minimum inhibitory concentrations (MIC) of the hydroxylated fatty acids against molds and yeasts ranged between 10 and 100 μg ml^{-1}. Other recent studies confirmed the cited above as Unsaturated fatty acids (UFAs) and hydroxy unsaturated fatty acids (HUFA) include compounds with antifungal activity [132], [133].

5.5. Cyclic dipeptides

Also known as 2,5 dioxopiperazines, they are among the most common peptide derivatives found in nature. Various bioactive properties are associated with these dipeptides including antimicrobial and antitumoral activities, while they may also be involved in quorum-sensing processes [134]. There are few insights on the antifungal activity of certain LAB where the active compounds have been characterized as cyclic dipeptides, hydroxy fatty acids, 3-phenyl lactic acid, and other low molecular weight compounds [135]. In some cases the antifungal peptides were identified to be cyclic dipeptides such as the work of Ström et al. [136] The cyclic dipeptides identified in their work have antifungal effect namely cyclo(L-Phe–trans-4-OH-L-Pro). The cyclic

dipeptides have antifungal activity at higher concentrations and hence are much less effective than the hydroxylated fatty acids. Li et al. [137] successfully isolated *Lact. Casei AST18* from Cheese that inhibited *Penicillium. sp* and the antifungal metabolite revealed to be Cyclo-(Leu-Pro), 2,6-diphenyl–piperidine, and 5,10-diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2- a;10,20-d] pyrazine. This, confirms that cyclic dipeptide has antifungal potential however, the limitation remains in that they exhibited this effect at higher concentrations.

5.6. Phenolic compounds

Lactic Acid Bacteria also secrets phenolic compounds as one of their metabolites which have antifungal promising properties yet to be fully understood. Mandal et al. [86] performed a study that revealed the presence of a phenolic compound produced by *Pc. acidilactici* in fermented meat and illustrated varying degrees of antifungal activity against many foods and feeds borne molds and plants pathogenic fungi including *A. fumigatus, A. parasiticus, F. oxysporum, and Penicillium sp. A. fumigates* and *R. mucilaginosa,* but it remains to be identified and characterized. Phenolic compounds (4-hydroxy benzoic acid, vanillic acid) and other volatile compounds can act as chaotrophic stressors and according to Cray et al. are considered antimicrobial substances operating in a manner that is not target-specific thus impacting the structure and metabolic interactions of macromolecular systems by reducing (water: macromolecule) interactions. Chaotrophic and hydrophobic stressors of biotic origin have potent activity against plant-pathogenic fungi including *Rhizoctonia solani* and *Fusarium spp* at μM to mM concentrations depending on the substance [138–142].

Luz et al. [143] conducted a recent study to evaluate a water-soluble extract from sourdoughs fermented with the lactic acid bacteria (LAB) for antifungal reflection on loaf bread, they cited that several antimicrobial phenolic acids were found. The strain of *L. bulgaricus* in the wheat dough produced phenyl-lactic and sinapic acids. the bread with sourdough addition with *L. plantarum and L. bulgaricus* expressed fungal growth at 4 and 5 days of incubation then the control one expressed it in 3 days of incubation, thus evidence of the shelf life is improved.

5.7. Bacteriocin

Klaenhammer was the first to expose the presence of a metabolite secreted by some Lactic acid bacteria equipped with antimicrobial activity namely bacteriocin. Since then, it has become an interest for several studies as a potential antibiotic substitution. Bacteriocins from the LAB with GRAS status (generally recognized as safe) have received significant attention as a novel approach to the control of pathogens in foods [144], [145]. Their antimicrobial potential has become well-known against several foodborne pathogens. Despite the amount of research that was made in the last decade, studies on the antifungal properties of LAB are yet to be more detailed and especially their bacteriocins. Several authors have reported that the antifungal activity of LAB is lost after treatment with proteolytic enzymes, thereby significance of the relation between the secreted compound protein nature and the antifungal potency [146–148]. Since then the increasing interest in these molecules has stimulated new LAB isolation and characterization of their metabolites.

Magnusson et al. [77] isolated and studied the broad-spectrum proteinaceous antifungal compound produced by *Lactobacillus coryniformis* subsp. *coryniformis* strain Si3 and stated that the observed decrease in activity could be caused by proteolytic

degradation. The claim was confirmed in Magnusson, J's next work [66] taking that last hypothesis in mind, He found that a proteinaceous compound produced by *Lactobacillus coryniformis subsp. coryniformis* strain Si3 had an antifungal effect against several molds and the yeasts *Debaromyces hansenii* and *Kluyveromyces marxianus*. The peptide was small (approx 3kDa), heat-stable, active in the pH range of 3-6, and inactivated by proteinase K or trypsin.

In a similar case, The antifungal activity of CFS from *L. sucicola* JT03, *W. paramesenteroides* JT13, and *P. acidilactici* JY03 was lost after being treated by trypsin, which suggested that the main antifungal component of the CFS might be proteinaceous compounds [149]. Another more recent study performed by Hammami et al. [150] cited the purification and characterization of a novel bacteriocin named BAC IH7 that demonstrated antifungal and antibacterial properties toward Gram-positive, Gram-negative bacteria as well as against fungi (*Fusarium sp., A. tumefaciens, C. tropicalis*). Similar findings were reported by Cizeikiene et al. [151] where bacteriocin-like inhibitory substances (BLIS) producing LAB showed fungistatic activities against *P. expansum, A. Versicolor, A. fumigatus, P. chrysogenum,* and *F. culmorum,* whereas fungicidal activities were observed against *A. niger*., Table 3. resume recent identified bacteriocin and research of their antifungal spectrum.

Another example is Bacteriocin F1 which was produced by *L. paracasei* subsp. *tolerans* FX-6 had a strong antifungal effect on A. flavus, Aspergillus niger, Rhizopus nigricans, and Penicillium glaucum and was proven to be thermal-stable even in boiled water for 60 min [152]. Despite these cited facts of antifungal effect from LAB bacteriocins, the only one authorized for commercialized purposes remains Nisin, The successful development of Nisin from an initial biological observation through regulatory approval for commercial applications [153] and a further implication of bacteriocin remain to be clarified and utilized.

Table 3. Compilation of publication reporting Bacteriocin and bacteriocin-like inhibitory substances antifungal potency

Lactic Strain	Antifungal spectrum	agent	references
Lactobacillus Y153	*B. cinerea*	Bacteriocin	[154]
P. pentosaceus *P. acidilactici*	*F. culmorum* *A. niger* *A. Versicolor*	BLIS[1]	[155]
L. mesenteroides DU15 *L. plantarum TE10,*	*A. niger*	Low molecular peptides	[96]
L. plantarum strain LR/14	*A. niger* *R. stolonifer* *M.racemosus*	AMPs[2] / LR14	[156]
L. plantarum MTCC 9503	*Aspergillus* *Penicillium* *Fusarium* *Alternaria*	Bacteriocin	[157]
P. pentosaceus LBM 18	*Aspergillus spp*	BLIS[1]	[158]

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L. curvatus A61	*Cladosporium ssp* *Fusarium ssp*	Bacteriocin	[97]
L. plantarum IS10	*A. flavus MD3,* *P. roqueforti MD4* *E. rubrum MD5*	Bacteriocin	[159]
L. bulgaricus BB18 *E. faecium MH3*	*Candida sp*	Bulgaricin BB18 Enterocin MH3	[160]
W. cibaria	*Fusarium oxysporum*	proteinaceous substances	[75]
L. plantarum	*A. Parasiticus* *Penicillium expansum*	SGADTTFLTK peptide	[161]
L. paracasei FX-6	*A. flavus* *A niger,* *R. nigricans* *P. glaucum*	Bacteriocin F1	[152]
L. fermentum CRL 251	*A. niger* *Penicillium sp.* *F. graminearum*	fungus-inhibitory peptide	[111]
L. coryniformis Si3	*A.fumigatus* *A. nidulans* *P. commune* *F. poae* *F. graminearum*	proteinaceous antifungal compound	[162]

[1:] Bacteriocin Like Inhibitory Substances, [2:] Anti-microbial peptides

6. LAB mycotoxin binding and biodegradation

Controlling pathogenic fungi in food and feed is an urgent matter since when cases of contamination occur mycotoxin is produced and accumulates in the food commodities, and removing them is mandatory to guarantee the security criteria of the food. Chemical detoxification is one approach to handle this issue, early study performed by McKenzie et al. [163] investigated the chemical degradation and detoxification of common mycotoxins in the presence of high concentrations of ozone (O_3). they stated that Zearalenone (ZEA) was degraded at 15 seconds, with no by-products detectable by HPLC, and the toxicity of these compounds (measured by a mycotoxin-sensitive bioassay) was significantly decreased following treatment by O_3 for 15 s.

Enzymatic biodegradation is another possibility, Takahashi et al. [164] identified and characterized a lactonohydrolase enzyme in *Clonostachys rosea* capable of converting ZEA to a less oestrogenic compound. similar findings were revealed afterward by Varga et al. [165] affirming ZEA complete degradation by several *Rhizopus* isolates including *R. stolonifer*, *R. oryzae*, and *R. microsporus* strains, but further studies are needed for the identification of ZEA-degrading enzymes in Rhizopus isolates.

Bio-controlling pathogenic fungi can be ensured by involving lactic acid bacteria (LAB) to produce a variety of fermented food [6]. Searching for alternatives to reduce the risks associated with fungal spoilage, LAB are currently being researched as a potential mycotoxin reduction agent. as Piotrowska et al. [166] indicated ochratoxin removal from

culture and the mechanism was said to be adsorption to the cell wall of *L. Plantarum*. however, several mechanisms can be involved in this degradation.

LAB strains hold promising effective degradation of dietary mycotoxins because of their unique mycotoxin-degrading characteristics. The biological degradation of mycotoxins after adsorption is the most widely known mechanism of mycotoxins reduction by LAB [167]. El-Nezami et al. [168] established one of the first investigations aiming to reveal the reduction, binding, and detoxification potential of mycotoxin by Lactic acid bacteria as five isolates of lactic acid bacteria including *Lactobacillus rhamnosus* strains GG and LC 705, *L. acidophilus, L. gasseri,* and *L. casei Shirota* were screened for Aflatoxin binding. both the probiotic *L rhamnosus* strains effectively removed aflatoxin B1 from contaminated culture media. The removal was by a rapid process removing as much as 80% (w/w) of toxin immediately. They continued their observation further and published that *L. rhamnosus* strains GG and LC 705 have most effectively bound aflatoxin B1 than aflatoxin B2, G1, and G1 [169].

As stated by Ghazvini et al, [170] Both *Bifidobacterium* and *Lactobacillus* significantly reduced the mycelial growth rate of *A. parasiticus* along with a reduction of AFB1, B2, and G1 production while this reduction was not significant in the case of AFG2. The exhibited inhibitory effects on the standard solution of AFTs were from 88.8% to 99.8% in comparison with the control. further examples are to be cited, *L. sakei subsp. ALI033* was isolated and identified from kimchi in Imsil and used in the experiment of Huh et al. [99] the strain was found to exhibit antifungal effects on *Penicillium sp*. Owing forward the strain *Lactobacillus reuteri R29* is reported to have a broad spectrum of antifungal activity and suitability for application in food systems. [171]. LABS are among the future potential bio-preserving agents heavily researched in the field of food and feed science. Studies of their antifungal effect were performed in various models counting; bakery [96], Sourdough [172], Soft Wheat [173], Citrus [149], yogurt production [174], and cereal-based beverages [175] among several others.

7. Association of LAB with other antifungal molecules

The combination of antimicrobial molecules for the search for greater effect and spreading of the synergic inhibition to a wider spectrum is not a new concept to bio-preservation. It has been the focus of several researchers who keep testing new combinations having the potential as food biopreservatives against food spoilage microorganisms. For instance, the combination of Essential Oils (EOs) and bacteriocin bacLP17 as seafood biopreservatives to control against *L. monocytogenes* proposed in the investigation done by Iseppi et al. [176] has produced encouraging results, consistent with the previous studies by Turgis et al. [177]. According to Singh et al. [178] The combined use of EOs and bacteriocins could also help to overcome the problem of bacteriocin resistance in Gram-positive bacteria, thus the combination of nisin (0-200 IU/ml) and garlic extract (0-6 mg/ml) presented promising inhibition effect.

The same concept can be utilized for antifungal purposes, it's becoming more needed and research is being conducted to find means of limiting fungal growth and/or preventing mycotoxin formation. Since the influence of such a combination on fungus is worthy of being tested and the mechanism of inhibition yet to be well characterized. Furthermore, Early studies from ADAMS and HALL [179] uncovered that a combination of different organic acids such as lactic and propionic has a synergistic fungistatic effect. Effat et al, [180], [181] stated that the association between chemical treatment and newly found antifungal metabolites from LAB illustrates the enhancement of the antifungal effect. *Lb. rhamnosus* produced more antifungal metabolites in a higher concentration of NaCl. It

was assumed to be related to a synergic effect between NaCl, organic acids, and antifungal substances.

Similar approaches were realized by Zhang et al. [182] explored the combination of acetate with other antifungal compounds to reduce or prevent the adverse impact of individual organic acids on bread flavor. prior studies using sourdough containing propionic and acetic acids, the synergic effect of sourdough and antifungal organic acids was proven to delay the fungal spoilage of bread [183]. According to Leyva et al. [184] Combination between *L. plantarum L244* and *L. harbinensis L172* in a yogurt model revealed inhibition of *P. commune, G. geotrichum, and Y. lypolitica.* However, the fact that some combination between Lactic acid bacteria may lead to mutual inhibition of the antifungal effect is to be considered like the case of the expected Synergistic effects between *L. plantarum* CCDM 583 and *L. plantarum* MP2 in the investigation of Horackova et al. [172], contrary to the expectancy the efficiency of *L. plantarum* CCDM 583 against *F. culmorum* DMF301 was reduced when grown together with strains 361 and MP2. Further combinations are yet to be tested and characterized for potential usage in the technical process for fungal control in food and feed.

8. Conclusion and Future Perspective

After reviewing the literature concerning lactic acid bacteria involvement as potential antifungal agents, several points can be drawn. There has been a notable shift from chemical and physical process preservatives toward bio-preservation approaches since they expressed limitations in controlling fungal contamination along with the development of fungicide toleration, resistance, and mycotoxin heat stability. The antifungal activity of Lactic acid bacteria is a promising candidate yet to be fully understood.

Research for new metabolites and new combinations that can restrain fungal contamination is at its prime, *Lactobacillus Plantarum* strains are among the most referred to as possessing the antifungal potential to be used as bio-preservatives. However, their interactions and limitation have yet to be unraveled fully. Although biodegradation of toxins is a permanent solution, cell wall binding is still considered as advantageous over metabolic degradation of certain toxins because of the following reasons: biodegradation is not only a time-consuming process, but it may also convert some mycotoxins into more harmful metabolites. For instance, AFB1 has been reported to be bio-transformed in some cases into a harmful metabolite namely Aflatoxin [185].

Different lactobacilli strains may individually exhibit antifungal potential however when used in the association they might inhibit each other in mixed cultures, which may lead to a subsequent reduction of their antifungal effect. Concerning the knowledge gained so far, little information exists on control measures that would mitigate mycotoxigenic fungi using lactic acid bacteria. Nevertheless, with the growing scholarly output by researchers on LAB potential revilement, Research findings in the future could favor their use as commercial biopreservatives agents.

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Conflicts of Interest:

The authors declare no conflict of interest.

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