Control of Aflatoxigenic Molds by Antagonistic Microorganisms: Inhibitory Behaviors, Bioactive Compounds, Related Mechanisms, and Influencing Factors

Xianfeng Ren 1,2, Qi Zhang 1,2,3,*, Wen Zhang 1,3, Jin Mao 1,4 and Peiwu Li 1,2,3,4,5,†,*

1 Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences, Wuhan 430062, China; renxianfenga@163.com (X.R.); zhangwen@oilcrops.cn (W.Z.); maojin106@whu.edu.cn (J.M.)
2 Key Laboratory of Biology and Genetic Improvement of Oil Crops, Ministry of Agriculture and Rural Affairs, Wuhan 430062, China
3 Key Laboratory of Detection for Mycotoxins, Ministry of Agriculture and Rural Affairs, Wuhan 430062, China
4 Laboratory of Risk Assessment for Oilseeds Products, Ministry of Agriculture and Rural Affairs, Wuhan 430062, China
5 Quality Inspection and Test Center for Oilseeds Products, Ministry of Agriculture and Rural Affairs, Wuhan 430062, China
† Corresponding authors addressed at: Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences, Wuhan 430062, China
* Correspondence: zhangqi01@caas.cn (Q.Z.); peiwuli@oilcrops.cn (P.L.);
Tel.: +86-27-8671-1839 (Q.Z.); +86-27-8681-2943 (P.L.); Fax: +86-27-8681-2862

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Abstract: Aflatoxin contamination has been causing great concern worldwide due to the major economic impact on crop production and their toxicological effects to human and animals. Contamination can occur in the field, during transportation, and also in storage. Post-harvest contamination usually derives from the pre-harvest infection of aflatoxigenic molds, especially aflatoxin-producing Aspergilli such as Aspergillus flavus and A. parasiticus. Many strategies preventing aflatoxigenic molds from entering food and feed chains have been reported, among which biological control is becoming one of the most praised strategies. The objective of this article is to review the biocontrol strategy for inhibiting the growth of and aflatoxin production by aflatoxigenic fungi. This review focuses on comparing inhibitory behaviors of different antagonistic microorganisms including various bacteria, fungi and yeasts. We also reviewed the bioactive compounds produced by microorganisms and the mechanisms leading to inhibition. The key factors influencing antifungal activities of antagonists are also discussed in this review.

Keywords: aflatoxin; biocontrol strategy; Aspergillus; prevention

Key Contribution: This review provides a comprehensive summary of biological agents with ability to inhibit aflatoxigenic molds and summarizes the knowledge and recent reports on the mechanisms leading to inhibition

1. Introduction

Aflatoxins are the most common contaminants occurring widely in oilseeds and grains. Aflatoxins B1, B2, G1, and G2 are a group of potent hepatotoxic and carcinogenic secondary metabolites produced mainly by Aspergillus section Flavi spp. like A. flavus and A. parasiticus [1–3]. Aflatoxigenic molds can cause a decrease in production, a loss of nutritional value, and a diminution of market
value of agricultural products, and also cause serious diseases like allergic reactions in humans and animals. Aflatoxin B1 (AFB1), the most toxic and commonly occurring one, has been classified as group I human carcinogen by the International Agency for Research on Cancer [4]. Aflatoxins M1 and M2, which are by-products of the above aflatoxins, may be found in dairy products from animals fed with contaminated feed and are closely related to the safety of dairy food.

Physical strategies such as field managements, physical separations, and moisture controls, and chemical strategies (e.g., using fungicides and chemical absorbents) have been applied to control aflatoxin and its producing molds. In most cases, the physical and chemical methods were inefficient, due to a nutritional loss of the processed foods, a difficulty in removing residues of the toxic compounds, or a development of resistant biotypes of pathogens. The biological control has been regarded as a more environmentally friendly and safer method [5,6], which was carried out generally at pre- and/or post-harvest. The post-harvest strategies focus mainly on the removal of aflatoxin [7–12]. However, once the agro-foods and feeds are contaminated, the contaminants such as aflatoxins can never be completely removed. Therefore, preventing aflatoxin production and fungal infection is the most efficient strategy.

In the past decades, many research studies have historically focused on the biocontrol of aflatoxigenic molds [13,14]. Three main modes of inhibitory actions are involved: antagonists grow rapidly to occupy ecological niche and compete for nutrients and/or living places, which leads to a displacement of pathogens; another involves inhibiting fungal growth, which leads to a reduction of fungal infection and colonization; the third is based on inhibiting aflatoxin biosynthesis. This review explores inhibitory behaviors, bioactive compounds, mechanisms of inhibitory actions, and factors influencing biological activities.

2. Antagonistic Microbes against Aflatoxigenic Strains

Various microorganisms including bacteria, fungi such as nontoxigenic Aspergillus, Trichoderma and penicillium spp., and yeast strains have been investigated as potential biocontrol agents against aflatoxigenic strains. As shown in Figure 1, the articles reporting bacterial antagonists were dominant (61%) compared with the articles reporting antagonistic fungi (27%) or yeasts (12%). Additionally, a comprehensive list of all microorganisms (approximately 50 different species) that have been well documented for their anti-aflatoxigenic potential is given in Table 1. Main characteristics and inhibitory behaviors of these antagonists are described as well.

![Figure 1](http://www.webofknowledge.com) Percentages of research articles related to different antagonists of aflatoxigenic fungi. We searched for research articles on the topic of “biocontrol of aflatoxigenic fungi” on Web of Science (http://www.webofknowledge.com). Related research articles account for approximately 150, and each slice of the pie represents a percentage of the articles reporting each sort of microorganisms.
Table 1. Species evaluated for their activities on aflatoxigenic molds.

| Microorganism | Genus                  | Specific Species | Activity                                      | References           |
|---------------|------------------------|------------------|-----------------------------------------------|----------------------|
| Bacteria      | Bacillus               | B. subtilis, B. amyloliquefaciens, B. megaterium, B. mojavensis, B. cereus, B. pumilus | Inhibit the growth of A. flavus and A. parasiticus | [15] [16] [17,18] |
|               |                        |                  | Inhibit aflatoxin production                  |                      |
|               | Pseudomonas            | P. fluorescens, P. chlororaphis, P. protegens | Inhibit A. flavus growth in grains            | [19–21] |
|               | Lactobacillus          | L. plantarum, L. rhamnosus, L. casei, L. fermentum, L. pentosus, L. parapantarum, L. delbrueckii subsp. Lactis | Bind aflatoxin M1, Inhibit aflatoxin production, Inhibit fungal growth | [22–24] [25,26] [27] |
|               | Streptomyces           | S. yanglinensis, S. anulatus, S. alboflavus, S. roseolus | Inhibit A. flavus growth                         | [28,29] [30,31] |
|               | Other bacteria         | Serratia marcescens, Stenotrophomonas sp., Ralstonia paucula, Burkholderia cepacia, Nannocystis exedens, Achromobacter xylosoxidans | Biocontrol A. flavus growth, Inhibit A. parasiticus growth, Inhibit aflatoxin production | [32,33] [34] [35,36] |
| Fungi         | Aspergillus            | A. flavus, A. parasiticus, A. niger, A. oryzae, A. clavatus | Inhibit A. flavus growth, Inhibit several plant pathogens | [37–40] [41,42] |
|               | Trichoderma            | T. harzianum, T. viride, T. longibrachiatum, | Biocontrol A. flavus growth                    | [43,44] |
|               | Penicillium            | P. chrysogenum, P. nalgiovense | Inhibit aflatoxin production                  | [45,46] |
|               | Yeast                  | xx               | Inhibit several common pathogenic fungi, Inhibit mycotoxins production | [47,48] [49,50] [51,52] |

2.1. Bacteria

2.1.1. Bacillus spp.

Bacillus spp. are a multifunctional group of bacteria. As shown in Figure 1, 21% of research articles reported Bacillus spp., which were most widely assessed in controlling aflatoxigenic strains. Aflatoxin accumulation in potato dextrose broth was almost totally inhibited by B. megaterium [16]. B. subtilis was also able to inhibit A. parasiticus growth and aflatoxin production by a percentage up to 92% and 100%, respectively [15]. Thus, B. megaterium and B. subtilis showed the highest biocontrol activity, inhibiting the growth of as well as aflatoxin production by aflatoxigenic strains, while B. amyloliquefaciens was also able to reduce A. parasiticus growth as well as degrade aflatoxins B1, B2, G1, and G2 after several days of co-cultivation [53,54]. González et al. [18] demonstrated that B. mojavensis,
B. cereus, and B. mycoides isolated from soil had ability to significantly inhibit A. parasiticus growth. Isolates of B. pumilus were also demonstrated with ability to inhibit aflatoxin production [17]. As reviewed by Schallmey et al. [55], Bacillus spp. were intensively assessed as biological agents, probably because they grew rapidly, produced a wide range of antimicrobial compounds, and generally were recognized as safe species.

2.1.2. Pseudomonas spp.

It was found that *P. fluorescens* could reduce AFB1 production by *A. flavus* in peanut medium at a rate of 99.4% [20], as well as inhibit conidial germination of *A. flavus* by up to nearly 20% [56]. A known fact is that *Pseudomonas* is one of the most prevalent genera isolated from soil (plants rhizosphere or nonrhizosphere). Palumbo et al. [19] demonstrated that the chitinolytic *P. chlororaphis* strains isolated from maize fields and maize rhizospheres could completely inhibit *A. flavus* growth. Mannaa et al. [21] found that *P. protegens* strain AS15 isolated from rice grains also significantly inhibited aflatoxin production by and mycelial growth of *A. flavus* at rates of 82.9% and 68.3%, respectively. Several other *Pseudomonas* strains were also demonstrated with an ability to completely inhibit the growth of *A. flavus* in different media [34].

2.1.3. Lactobacillus spp.

Lactic acid bacteria (LAB) are bacteria producing organic acids—mainly lactic acid—by carbohydrate fermentation. In food production, these bacteria are traditionally used to prevent spoilage and increase shelf life of foods. As shown in Table 1, *L. plantarum*, *L. rhamnosus*, *L. casei*, *L. fermentum*, *L. pentosus*, *L. paraplantarum*, and *L. delbrueckii subsp. Lactis* have been identified as biocontrol agents against aflatoxigenic fungi. Ahlberg et al. [57] demonstrated that LAB strains showed an ability to physically bind aflatoxins. In another study of Ahlberg et al. [26], 171 LAB strains were tested against *A. flavus*, and the species with the highest antifungal ability was identified as *L. plantarum*. The genus *Lactobacillus*, mainly the species *L. plantarum*, has been widely found to inhibit aflatoxigenic strains in various living environments [22,58–61]. LAB strains are mainly divided into four genera: *Lactobacillus*, *Lactococcus*, *Pediococcus*, and *Leuconostoc*. As was reported by Sangmanee and Hongpattarakere [22], the supernatant obtained from *L. plantarum* culturing broth could inhibit the mycelial growth and aflatoxin production of *A. flavus* by 100%. *L. casei*, *L. fermentum*, *L. reuteri*, and *L. acidophilus* were also proved to have an inhibitory effect higher than 80% on *Aspergillus niger*, *Penicillium* sp., and *Fusarium graminearum* [25]. Ahlberg et al. [26] demonstrated that *Lactobacillus* spp. with high or moderate anti-mycotoxigenic activities were identified as *L. pentosus*, *L. paraplantarum*, and *L. plantarum*. Species of *L. delbrueckii subsp. Lactis* were also found to completely inhibit aflatoxin G2 production and significantly control *A. parasiticus* growth [27].

2.1.4. Streptomyces spp.

*Streptomyces* spp. are gram (+) filamentous bacteria that widely grow in soils and on plants. A *Streptomyces* strain isolated from peanuts was found to completely inhibit, directly or via secondary metabolites, mycelial growth and conidial germination of *A. flavus* [62]. *Streptomyces* strain ASBV-1 was found to be able to reduce the viability of *A. parasiticus* spores and subsequently, inhibit aflatoxin accumulation in peanut grains [63]. Verheecke et al. [64] reported that several soil-born *Streptomyces* isolates had a strong bioactivity against aflatoxin B1 and B2 production by *A. flavus*. Several other *Streptomyces* species (Table 1) have been evaluated as bioactive agents providing an antagonistic activity against aflatoxigenic isolates. Shakeel et al. [28] demonstrated that culture filtrates and crude extracts of *S. yanglinensis* could completely inhibit mycelial growth of *A. flavus*. Studies demonstrated that *S. anulatus* [29], *S. alboflavus* [30], and *S. roseolus* [31] also exerted an effective antifungal activity toward aflatoxigenic strains and other common agricultural crops pathogens.

2.1.5. Other Bacteria Species
Serratia marcescens strain JPP1 isolated from peanut hulls is an endophytic bacterium which lives inside the plant tissue and does not cause visible morphological changes. Strain JPP1 exhibited remarkable inhibitory effects on aflatoxin production (rate >98%) and mycelial growth (rate >95%) of A. parasiticus [32]. Stenotrophomonas sp., a soil bacterium, could produce inhibitors against aflatoxin production, but without affecting fungal growth [33]. Nannocystis exedens, a myxobacterium commonly found in soil, had a potential to control the growth of A. flavus and A. parasiticus by lysing pathogens’ colony [35]. Palumbo et al. [34] isolated 171 bacteria from California almond orchard samples; apart from the familiar genera Bacillus and Pseudomonas, Burkholderia cepacia, B. pyrrocinia, Delftia acidovorans, D. acidovorans, andRalstonia paucula were also demonstrated with potential activity against A. flavus growth. Achromobacter xylosoxidans, a gram-negative and catalase-positive bacterium, is already known to have wide biological control abilities [65]. Yan et al. [36] demonstrated that A. xylosoxidans could produce inhibitory substances remarkably inhibiting A. flavus and A. parasiticus growth.

According to the current studies, antagonistic bacteria were actually highly effective on aflatoxigenic strains in vitro. However, their colonization in soil and on crops has not been evaluated under field conditions. Due to genetic and environmental differences, it is not easy to bring the bacterial cells to the Aspergilli infection sites. This may be the reason why most of the anti-aflatoxigenic studies are performed only in vitro, and no bacterial agents are already commercialized.

2.2. Fungi

2.2.1. Nontoxigenic Aspergillus spp.

A. flavus are variable in respect to aflatoxin-producing ability, and were described into S (small) and L (large) strains on the basis of sclerotial morphological types [66]. On average, S strains produce higher levels of AFB1 with less variation in aflatoxin production [67,68]; L strains are more variable in aflatoxin production and even include nonproducers entirely lacking the ability to produce aflatoxins [69]. Currently, introduction of nontoxigenic A. flavus into fields is the most promising strategy for preventing pre-harvest aflatoxin contamination. The use of nontoxigenic A. flavus to competitively exclude aflatoxigenic strains was first introduced by Cotty and Bayman [70]. As shown in Figure 1, there have been many studies subsequently focusing on nontoxigenic Aspergillus. Prevention of aflatoxin accumulation by inoculation with nontoxigenic A. flavus CT3 and K49 was assessed in a 4-year field study [37], with results indicating that the reduction percentages of aflatoxin on southern US corns were 65%–94%. Alaniz Zanon et al. [38] also found that the nontoxigenic A. flavus had a higher biocontrol efficacy against aflatoxin accumulation (inhibition rate = 78%–90%) in a two-year study in northern Argentina. In addition, nontoxigenic A. flavus isolates were demonstrated with an ability to reduce aflatoxin contamination of maize by a rate higher than 80% in Kenya [71]. Importantly, nontoxigenic A. flavus strains, AF36 (NRRL 18543) and Afla-Guard® (NRRL 21882), have been commercialized for use in groundnut and maize production, respectively, in USA. This biocontrol approach has also been proved to be effective on peanut [72,73], cottonseed [69], and corn [37,74] under field conditions. An application of nontoxigenic A. parasiticus in the field was also able to reduce aflatoxin contamination in storage [39]. Nontoxigenic A. niger strain FS10, isolated from fermented soybean, could not only significantly inhibit A. flavus growth, but also inhibit AFB1 production (rate = 94.5%) [40,75]. A. oryzae, the nontoxigenic domesticated ecotype of A. flavus, is used as a “Generally Recognized As Safe (GRAS)” microorganism for food fermentation [76,77]. Alshannaq et al. demonstrated that co-inoculation with A. oryzae and A. flavus on peanuts with a ratio of 1:100 could effectively inhibit AFB1 production [41]. The species of A. clavatus could secrete ribonuclease [78], while Skouri-Gargouri and Gargouri revealed that A. clavatus could inhibit the growth of several plant pathogens such as Fusarium oxysporum and Aspergillus niger due to the secretion of an antifungal peptide [42].

2.2.2. Trichoderma spp.
Trichoderma spp. comprise a large number of rhizocompetent filamentous strains in soils and root ecosystems. Their potential as fungal biocontrol agents against plant pathogenic fungi has been known for a long time [79]. The majority of Trichoderma isolates used industrially for biological control belong to the species *T. harzianum*, including strains T22 and T39 [80,81]. *Trichoderma* species can not only control crop diseases, but also exert beneficial effects on root growth and enhance crop productivity [82]. There have been several *Trichoderma* species reported showing varying degrees of control of aflatoxigenic strains since last century [79]. *T. harzianum* and *T. viride* were proved to be highly antagonistic and inhibit mycelial growth and aflatoxin production of *A. flavus* by a rate higher than 80% [43]. Evaluation of *Trichoderma* spp. for biocontrol of pre-harvest seed infection by *A. flavus* in groundnut was performed by Anjaiah et al. [44], with results indicating that in greenhouse and field experiments, the treatment of seeds with *Trichoderma* spp. including *T. harzianum*, *T. longibrachiatum*, *T. viride*, and *T. auroviride* reduced *A. flavus* populations (as cfu) by a percentage higher than 50%. A known fact is that *Trichoderma* species are historically a group of the most studied beneficial filamentous fungi. Sarrocco and Vannacci [14] gave a list of commercial bio-pesticides containing 14 different *Trichoderma* strains that belong to *T. harzianum*, *T. atroviride*, *T. viride*, *T. asperellum*, *T. gamsii*, and *T. polysporum*; however, these species have not been investigated as commercialized products to biocontrol aflatoxicogenic molds.

2.2.3. *Penicillium* spp.

Several species of *Penicillium* are able to grow rapidly in the presence of toxigenic strains [83]. The strain RP42C of *P. chrysogenum* was reported as antifungal-protein producer with a biological activity against the growth of aflatoxigenic strains on dry-cured ham [45,84]. *P. chrysogenum*, a fungal starter culture for mold-fermented foods production, is related to *P. nalgiovense*. Nielsen et al. [85] demonstrated that *P. nalgiovense* showed a higher inhibitory effect on the growth of the common fungal pathogens. Additionally, Geisen [46] demonstrated that *P. nalgiovense* had a greater inhibition on the secondary metabolites production of fungal strains. As fungal starter cultures, the antifungal activity of *Penicillium* species would play an important role in the safety of mold-fermented food.

2.3. Yeast Strains

Due to the ability to consume lactic acid in the presence of oxygen, yeast strains have been regarded as deteriorating agents for a long time. Yeast strains are also popular in household because of the ability of leavening dough. Marine yeast *Debaryomyces Hansenii BCS003* strain can decrease mycelial growth by almost 98% in a radial inhibition assay against *Aspergillus* strains [47], while native *D. Hansenii* strains were also demonstrated with a significantly antagonistic activity on the growth rate and aflatoxin production of *A. parasiticus* in meat products [48]. *Saccharomyces cerevisiae* RC008 and RC016 are strains demonstrated with the ability of inhibiting the growth of and AFB1 production by *A. parasiticus* under different regimes of water activities, pH values, temperatures, and oxygen availabilities [49]. As shown in Table 1, *Kluyveromyces*, *Pichia anomala*, and *Candida maltosa* isolates were also demonstrated to have an impact on mycelial growth, conidial germination, or aflatoxin production when interacting with aflatoxigenic *Aspergillus* strains [48,50,52,86–88].

2.4. A Conclusion of Antagonistic Microbes

Many bacteria agents have been demonstrated with an ability to inhibit aflatoxigenic molds; however, none of bacterial agents has been commercialized. At current research status, only nontoxigenic *A. flavus* NRRL 18543 and NRRL 21882 have been commercialized and applied in fields [72], and *Trichoderma* species are just showing a high potential to be commercialized for the future use [14]. For yeast strains, however, we need further studies to look for strains with high efficacy.

3. Inhibitory Compounds Produced by Different Antagonistic Microbes

Secondary metabolites produced by various microorganisms are high-value natural products, many of which exhibit significant pharmacological properties. The inhibitory compounds discussed
here are secondary metabolites with powerful bioactive properties in biological control of aflatoxin-producing fungi. Based on the results obtained in vitro experiments, inhibitory compounds produced by various antagonistic microorganisms and their bioactivities against aflatoxigenic molds are listed in Table 2. These compounds are divided into four different types of substances, including micromolecular organics, organic acids, antibiotics, and enzymes (Figure 2). The following paragraphs describe the producers, anti-aflatoxigenic activities, and main characteristics of these compounds in more detail.

Table 2. Inhibitory compounds produced by antagonists against aflatoxigenic molds.

| Antagonists            | Inhibitory Compounds                                      | Main Characteristics of the Compounds                          | References |
|------------------------|-----------------------------------------------------------|-----------------------------------------------------------------|------------|
| Bacillus spp.          | Lipopeptides: surfactin, iturin A and fengycin            | Stable after autoclaving                                        | [18,89]    |
|                        | Bacillomycin D                                            | Completely inhibit *A. flavus* growth                           | [90]       |
|                        | Protease                                                  | Stable under high alkaline conditions                           | [15]       |
|                        | Oligopeptide (L-Asp-L-Orn)                                | Be able to enter into cells of *A. flavus*                      | [91]       |
| P. fluorescens         | Chitinolytic enzyme                                       | Extracellular enzyme                                           | [56]       |
|                        | Lactic acid                                               | With 60% antifungal activity at 0.02 mg/mL                      | [22,25]    |
|                        | Phenyllactic (PLA)                                        | Lose activity after neutralization treatment                    |            |
| Lactobacillus spp.     | Hydroxyphenyllactic acid (OH-PLA)                         | Show strong antifungal ability at the lowest concentration of 1 mg/mL | [92]       |
|                        | Indole lactic acid (ILA)                                  | About 1 mg/mL was sufficient to inhibit aflatoxins production by 90% | [22][24]  |
|                        | 2-butyl-4-hexyloctahydro-1H-indene, Oleic acid, palmitic acid, linoleic acid and 2,4-di-tetbutylphenol | In cell-free supernatant; resistant to sterilization and proteolytic enzymes | [22][24]  |
|                        | Peptides                                                  | Completely inhibit *A. flavus* growth on corn                   | [59]       |
|                        | 2-methylisoborneol                                         | A volatile organic compound with ability against storage fungi such as *F. moniliforme* and *A. flavus* in vitro | [30]       |
| Streptomyces spp.      | Alastatin A                                               | Completely inhibit *A. parasiticus* growth at a concentration of 0.5 μg/mL | [93]       |
|                        | Dioctatin A                                               | Strongly inhibit aflatoxin production                           | [94]       |
|                        | Dimethyl trisulfide                                       | Completely control *A. flavus* growth                           | [95]       |
|                        | Dimethyl disulfide                                        | Affect mycelial growth and sporulation                          | [30]       |
|                        | Benzenamine                                               | Completely inhibit *A. flavus* growth at 1 mL/L                 | [95]       |
Chitinase With thermal stability and broad pH stability \[29,96\]

| Yeast strains | Bioactive compounds against aflatoxigenic molds |
|---------------|------------------------------------------------|
| 2-phenylethanol | Inhibit conidial germination and aflatoxin production \[51\] |
| Isoamyl acetate | Inhibit the growth of several pathogenic fungi \[52\] |
| Isoamyl alcohol | Chitinase With ability to cause hyphal lysis and deterioration \[98\] |
| 4-Hydroxyphenethyl alcohol | Yeast strains In cell-free supernatant extract; stable at high temperatures \[97\] |
| 4,4-Dimethyloxazole | 1,2-Benzenedicarboxylic acid dioctyl ester |
| 4-Hydroxyphenethyl alcohol | Yeast strains In cell-free supernatant extract; stable at high temperatures \[97\] |
| 1,2-Benzenedicarboxylic acid dioctyl ester | Yeast strains In cell-free supernatant extract; stable at high temperatures \[97\] |

\[\beta\text{-1,3-gluconase}\]

T. harzianum Protease P6281 Stable in pH = 2.5–6.0; with ability to inhibit conidial germination and mycelial growth \[99\]

Serratia marcescens Chitinase With ability to degrade fungal cell walls \[32\]

Penicillium chrysogenum Antifungal protein PgAFP Molecular mass is 6494 Da; belong to small, cysteine-rich, and basic proteins \[84\]

Aspergillus clavatus Antifungal peptide Molecular mass = 5773 Da; with thermostability \[42\]

Achromobacter xylosoxidans Cyclo(L-Leucyl-L-Prolyl) Inhibit aflatoxin production by repressing transcription of aflatoxin-related genes \[36\]

Micro-molecular organics

| Micro-molecular organics | Bioactive compounds against aflatoxigenic molds |
|--------------------------|------------------------------------------------|
| Allylic compound | 2-butyll-4-hexyl-1-hydro-H-indene C\textsubscript{10}H\textsubscript{16}O (PubChem CID: 22215320) |
| 2-methylisoborneol C\textsubscript{12}H\textsubscript{18}O (PubChem CID: 167513) |
| Aflatoxin A C\textsubscript{2}H\textsubscript{12}N\textsubscript{2}O\textsubscript{8} (PubChem CID: 54607500) |
| 2,4-di-tetradecyloxyphenol C\textsubscript{36}H\textsubscript{74}O\textsubscript{2} (PubChem CID: 7331) |
| 2-phenylethanol C\textsubscript{8}H\textsubscript{10}O (PubChem CID: 6054) |
| Benzylamine C\textsubscript{7}H\textsubscript{11}N (PubChem CID: 6115) |
| 4-Hydroxyphenethyl alcohol C\textsubscript{8}H\textsubscript{10}O\textsubscript{2} (PubChem CID: 10393) |
| 1,2-Benzenedicarboxylic acid dioctyl ester C\textsubscript{16}H\textsubscript{14}O\textsubscript{4} (PubChem CID: 8346) |
| 4,4-Dimethyloxazole C\textsubscript{2}H\textsubscript{12}N\textsubscript{2}O\textsubscript{8} (PubChem CID: unknown) |

Aromatic compound

| Aromatic compound | Bioactive compounds against aflatoxigenic molds |
|------------------|------------------------------------------------|
| Leucine acid | 2-Hydroxyethyl-lactic acid C\textsubscript{5}H\textsubscript{10}O\textsubscript{3} (PubChem CID: 9378) |
| Indole-lactic acid C\textsubscript{6}H\textsubscript{7}NO\textsubscript{5} (PubChem CID: 53700591) |
| Phenyl-lactic acid C\textsubscript{6}H\textsubscript{9}NO\textsubscript{3} (PubChem CID: 1393) |
| Lactic acid C\textsubscript{3}H\textsubscript{6}O\textsubscript{2} (PubChem CID: 612) |
| oleic acid C\textsubscript{17}H\textsubscript{33}O\textsubscript{2} (PubChem CID: 445639) |
| Linoleic acid C\textsubscript{18}H\textsubscript{32}O\textsubscript{2} (PubChem CID: 5204050) |
| Palmitic acid C\textsubscript{16}H\textsubscript{34}O\textsubscript{2} (PubChem CID: 998) |
| Docosahexaenoic acid C\textsubscript{22}H\textsubscript{36}O\textsubscript{2} (PubChem CID: 102287023) |

Chitinase With ability to cause hyphal lysis and deterioration \[98\]

Figure 2. Bioactive compounds produced by microorganisms with antagonistic activities against aflatoxigenic molds. These compounds were divided into four different types of substances (micromolecular organics, organic acids, antibiotics, and enzymes). PubChem CID is listed at the end of each molecule. Details such as structures, molecular formula, and chemical and physical properties could be obtained in the following link: https://pubchem.ncbi.nlm.nih.gov/.
3.1. Antibiotics and Proteases Produced by Bacillus spp.

Bacillus species generally have characteristics to produce antimicrobial substances, mainly including lipopeptides, protease antibiotics, and bacteriocins [100]. These structurally diverse compounds exhibit a wide range of antimicrobial activity [101], especially the lipopeptides secreted by Bacillus presenting antifungal activity [102].

Bacillus strains isolated from aquatic environments were evaluated for their antifungal effect on A. flavus and A. carbonarius, producers of AFB1 and ochratoxin A, respectively [89]. Results showed that the lipopeptides (iturin A and surfactin isomers in extracts) produced by Bacillus sp. P1 strain exhibited high anti-Aspergillus activities on mycelial growth, conidial germination, and AFB1 and ochratoxin A production. Veras et al. [89] also analyzed the extracts from supernatants and cell pellets, and results indicated that lipopeptides were extracted mainly from cell-free supernatants. González Pereyra et al. [18] also demonstrated that lipopeptides, the extracellular compounds produced by soil Bacillus strains, were able to almost completely inhibit A. parasiticus growth and AFB1 production. In another report [103], mutants of B. subtilis obtained after varying doses of gamma irradiation could significantly inhibit A. flavus growth and aflatoxin production in pistachio nuts compared with the parental strain, because lipopeptides production of mutants increased. Additionally, Farzaneh et al. [104] reported that cell-free supernatants from B. subtilis had a significant effect on A. flavus spores viability, and the mass spectrometric analysis revealed that surfactin and fengycin were responsible for the biocontrol activity. These studies indicated that fengycin, surfactin, and iturin families of lipopeptides produced by Bacillus species were the dominant compounds potentially reducing Aspergillus spp. growth or aflatoxins production and, generally, these compounds were obtained from cell-free supernatants. Bacillomycin D, a lipopeptide substance produced by B. subtilis, was also demonstrated with abilities of significantly affecting mycelial growth, sporulation, and destabilizing the cell wall and cell membrane of A. flavus [90].

Proteases, especially alkaline proteases, are the well-known products of Bacillus strains. B. subtilis and B. amyloliquefaciens were able to inhibit A. parasiticus growth and showed a good proteolytic activity [15]. Additionally, three peptides of L-Asp-L-Orn (D1O), L-Asp-L-Asn (D1N), and L-Asp-L-Asp-L-Asn (D2N) produced by B. megaterium could significantly inhibit the growth of A. flavus [91]. Another study reported that unknown volatiles produced by B. megaterium could inhibit aflatoxin production, mycelial growth, and conidial germination of A. flavus in rice grains [105].

Overall, we can conclude from these studies that extracellular compounds of Bacillus species were able to inhibit aflatoxigenic molds. The compounds, especially lipopeptides and proteases may be the main effective antifungal factors inhibiting aflatoxin production, sporulation, and conidial germination and reducing mycelial growth.

3.2. Chitinolytic Enzyme Produced by Pseudomonas spp.

Akocak et al. [56] demonstrated that the chitinolytic enzyme produced by P. fluorescens could reduce the growth of A. flavus by inducing the morphological changes on conidial germination and mycelial growth. As reviewed by D’Aes et al. [106], biosurfactants such as cyclic lipopeptide and rhamnolipid produced by Pseudomonas spp. were involved in important functions of biocontrol. Phenazines produced by Pseudomonas strains were also major determinants controlling several plant pathogens [107]. However, biological activities of bio-surfactants and phenazines against aflatoxigenic strains have not been investigated. Therefore, speeding up the identification of bioactive compounds could potentially enhance application values of Pseudomonas species.
3.3. Organic Acids and Peptides Produced by Lactobacillus spp.

As revealed by Russo et al. [61], Lactobacillus spp. have broad antifungal activities because of the high production of lactic acid. Apart from lactic acid, phenyllactic acid (PLA), hydroxyphenyllactic acid (OH-PLA), and indole lactic acid (ILA) were also found to strongly inhibit aflatoxin-producing fungi [25,92]. Additionally, the antifungal compounds secreted by L. plantarum were investigated against the growth of and aflatoxin production by A. flavus and A. parasiticus, with results indicating that the antifungal compounds obtained from the cell-free supernatant, apart from lactic acid, majorly were 2-butyl-4-hexyloctahydro-1H-indene, oleic acid, palmitic acid, linoleic acid, and 2,4-di-tertbutylphenol [22].

Apart from organic acids, inhibitory peptides produced by L. plantarum were also demonstrated to be effective against A. flavus and A. parasiticus [59,60], while organic acids were dominant, probably associated with their low pH values [61].

3.4. Micromolecular Organics, Organic Acids, and Enzymes Produced by Streptomyces spp.

Streptomyces spp., known to produce over 7500 bioactive compounds including anticancer agents, vitamins, and antibiotic compounds, have a better tolerance to water stress [28]. They usually do not secrete toxic residues that may contaminate environments because of their natural origin. 2-methylisoborneol, the volatile organic compound generated by S. alboflavus, was proved to have an ability of inhibiting A. flavus, Fusarium moniliforme, and Penicillium citrinum in vitro [30]. Aflastatin A, extracted from mycelial cake of Streptomyces sp., was a strong inhibitor of aflatoxin production [93]. Dimethyl trisulfide and Benzenamine, the small molecular organic compounds generated by S. alboflavus, played an important role in controlling aflatoxin production and A. flavus growth [95]. Dimethyl disulfide, the micromolecular volatile organic identified from the volatiles of S. alboflavus, was proved to act as an antagonistic substance against some plant pathogens in vitro [30]. Dioctatin A, an organic acid, produced by Streptomyces spp., was found to strongly inhibit aflatoxin production and conidiation of A. parasiticus [94]. The thermostable endochitinase purified from Streptomyces sp. [96] and the chitinase (Chi242) obtained from the culture supernatant of S. anulatus [29] have been found to inhibit the mycelial growth of A. parasiticus and A. niger, respectively. From these studies, we are able to see out that inhibitory compounds produced by Streptomyces spp. were highly species-specific. As Manivasagan et al. [108] reviewed, Actinomycetes, especially Streptomyces spp., have a tremendous potential to produce various secondary bioactive metabolites. In this case, Streptomyces species definitely have a great potential to be used for the biocontrol of aflatoxigenic fungi.

3.5. Micromolecular Organics and Enzymes Produced by Yeast Strains

Yeast strains are increasingly targeted for the production of bioactive substances, especially the budding yeast species Saccharomyces cerevisiae, which has been proven to be a powerful microorganism for heterologous expression of biosynthetic pathways [109]. The biocontrol activity of Pichia anomala WRL-076 was attributed to the production of 2-phenylethanol, which was the major volatile compound affecting the growth, aflatoxin production, and gene expression of A. flavus [51]. Studies also demonstrated that isoamyl acetate and isoamyl alcohol produced by Candida maltosa were able to inhibit the conidial germination of Aspergillus brasilienis [52]. 4-Hydroxyphenethyl alcohol, 4,4-Dimethylisoxazole, and 1,2-Benzenedicarboxylic acid diocetyl ester in the supernatant extracts of Saccharomyces cerevisiae provided the antifungal activity against aflatoxigenic growth and aflatoxins biosynthesis [97]. Tayel et al. [98] demonstrated that Pichia anomala was able to produce β-1,3-glucanase and exo-chitinase, which were suggested as a mode of antifungal action leading to cause hyphal lysis of A. flavus.

3.6. Protease and Extracellular Enzymes Produced by Trichoderma spp.

Regarding to Trichoderma species, only a few inhibitory compounds that play roles in their antagonistic interactions with aflatoxigenic fungi were reported. Deng et al. [99] demonstrated that
the aspartic protease P6281 secreted by *T. harzianum* could efficiently inhibit the conidial germination and the growth of *A. flavus*. Mostafa et al. [43] demonstrated that *T. harzianum* and *T. viride* showed a high antagonism and inhibited aflatoxins production of *A. flavus* by 90%, which were explained partially by the liberation of extracellular enzymes and the production of inhibitory volatile compounds.

3.7. Inhibitory Compounds Produced by the Other Microorganisms

Apart from the inhibitory compounds described in the above sections, chitinase produced by *Serratia marcescens* was able to efficiently degrade fungal cell walls [32]. The antifungal protein PgAFP produced by *Penicillium chrysogenum* could inhibit the growth of toxigenic molds [84]. Antifungal peptide produced by *Aspergillus clavatus* was thermostable and exhibited a strong inhibitory activity against mycelial growth of several plant pathogenic fungi [42]. Cyclo (L-Leucyl-L-Prolyl) produced by *Achromobacter xylosoxidans* was able to inhibit the growth of *A. parasiticus*, and it also remarkably repressed the transcription of the aflatoxin-biosynthesis related gene *aflR* [36].

3.8. A Conclusion of Inhibitory Compounds

Approximately 30 different compounds have been found to be bioactive against aflatoxigenic fungi. According to these studies, we identified three deficiencies in the research field that need improvement: (1) the variety of inhibitory compounds is still limited; and (2) all of the inhibitory compounds were tested only in vitro, in which case, it is difficult to relate with the real antagonistic efficacy in vivo because of the diversity of microbes in soils, differences of soil temperature, humidity, and pH, and the genetic and metabolic complexity of biocontrol antagonists; and (3) most, even all of the studies focused only on inhibitory efficiency, however, studies such as the resistance in *Aspergillus* and interactions among inhibitory compound, pathogen, antagonist, and environment were scarce. These deficiencies could be mirrored by the example of *Trichoderma* spp. The antagonistic *Trichoderma* strains have the ability to produce various compounds with antibiotic activity [81]. However, few antibiotic compounds have been identified from *Trichoderma* spp. for the biocontrol of aflatoxigenic molds. Although *Trichoderma* species play an important role in biocontrol of plant diseases, frequently enhance root growth, and induce systemic resistance responses of plants [82], the interaction among aflatoxigenic fungi, *Trichoderma*, soil, and plants has not been elucidated yet.

4. Mechanisms of Inhibitory Actions

4.1. Inhibitory Mechanisms by Antagonistic Bacteria

For antagonistic bacteria, their bioactive metabolites play a major role in controlling *Aspergillus* spp. growth and subsequent aflatoxin production. Inhibitory mechanisms by antagonistic bacteria mainly include (1) lysis of hyphae or spores by destabilizing structure and composition of cell wall; (2) probably affecting intracellular activities of mitochondria, cytoplasmic membrane, and nucleus; and (3) down-regulating expression of aflatoxin-synthesis related genes. Illustrations were made as follows: chitinolytic enzymes produced by *P. fluorescens* reduced the growth of *A. flavus* by altering the germination pattern of spores[56]; the cell-free supernatant of *L. plantarum* caused morphological changes in seven-day-old *A. flavus* and *A. parasiticus*, because of severe damage to the mitochondria and nucleus, formation of the membrane-bound vesicles, and degeneration of the cytoplasmic membrane [22]; and dicoctatin A produced by *Streptomyces* decreased expression of *aflR* and *brlA* (encoding a condition-specific transcription factor) and significantly inhibited the production of norsolorinic acid and sterigmatocystin that were precursors for aflatoxin synthesis [94].

4.2. Inhibitory Mechanisms by Nontoxigenic *Aspergillus* spp.

Fungal invasion, colonization, and competition between aflatoxigenic and atoxigenic strains of *A. flavus* have been studied [70,110]. Regarding nontoxigenic *Aspergillus* spp. as antagonist, two mechanisms are dominant: (1) toxigenic strains are physically excluded by the displacement of
nontoxigenic strains during infection; and (2) nontoxigenic strains competed for nutrients that were required for aflatoxin biosynthesis. However, as Ehrlich [111] reviewed, there were a lot of challenges to using nontoxigenic *Aspergillus* species. Primarily, due in part to inherent diversity of *Aspergillus* species and genetic complexity, genetic mutations may happen in nontoxigenic *Aspergillus* spp., which potentially leads atoxigenic strains to mutate to aflatoxigenic strains; therefore, from a long-term security, nontoxigenic *Aspergillus* strains were also suggested to be cautiously used [112–114].

4.3. Inhibitory Mechanisms by Antagonistic Yeasts

How did the antagonistic yeasts act as biological agents to control aflatoxigenic growth and aflatoxin production? That the yeast strain *Pichia anomala* could efficiently inhibit the growth of and aflatoxin production by *A. flavus* can be attributed to the production of 2-phenylethanol, which led to remarkable effects on conidial germination and expression of genes necessary for aflatoxin biosynthesis [51], and the production of chitinase and glucanase, which led to hyphal lysis and deterioration [98]. That *Debaryomyces Hansenii* was able to control *A. flavus* growth was attributed to the production of extracellular compounds and the competition for nutrients and spaces [47]. For *Saccharomyces cerevisiae*, the production of exochitinase and extracellular secondary metabolites could explain its mode of action for antifungal activity on the growth of *A. flavus* [97]. Therefore, for yeast strains, possible mechanisms of the inhibitory actions may involve two: (1) inhibiting aflatoxigenic growth by the production of extracellular enzymes and metabolites which lead to spores and hyphal deterioration, and (2) inhibiting aflatoxin production by down-regulating expression of aflatoxin biosynthesis genes.

4.4. Inhibitory Mechanisms by Antagonistic Trichoderma Strains

The antagonistic properties of *Trichoderma* strains are based on the activation of multiple physical and chemical mechanisms. The physical mechanisms included faster growth speed to compete for nutrients and living space, and mycoparasitism mediated by physical contact. Common interactions between antagonistic fungi and pathogens were divided into the following types [79]:

- 1 = antagonist overgrowing pathogen and pathogen stopped;
- 1/2 = antagonist overgrowing pathogen but pathogen still growing;
- 2/1 = pathogen overgrowing antagonist but antagonist still growing;
- 2 = pathogen overgrowing antagonist and antagonist stopped;
- 3 = mutual inhibition ≤2mm distance;
- 4 = extremely mutual inhibition >4mm distance.

Calistru et al. [115] discovered only three interaction types between *Trichoderma* and *A. flavus*, namely antagonist overgrowing pathogen with growth inhibition of pathogen, pathogen overgrowing antagonist with growth inhibition of antagonist, and mutual inhibition. By a scanning electron microscopical investigation, Calistru et al. [115] revealed that mycoparasitism is not the mechanism of the inhibitory interaction between *A. flavus* and *Trichoderma* spp. (*T. harzianum* and *T. viride*). Conversely, Mostafa et al. [43] drew a conclusion that the aggressive behavior towards *A. flavus* by *T. harzianum* was explained by mycoparasitism.

The chemical mechanisms were also involved in producing cell walllytic enzymes and inducing the plant’s defense system to resist pathogens [116]. The production of extracellular enzymes was responsible for the inhibitory effect of *T. viride* on toxigenic *A. flavus* [43]. *T. harzianum* actively attached to the toxigenic *Aspergillus* species followed by enzymatic lysis of the mycelial filaments [117]. Such, mechanisms of the inhibitory actions against the growth of *A. flavus* by *T. harzianum* are strains-specific and mainly include (1) faster growth speed to compete for nutrients and living space, (2) mycoparasitism, and (3) the production of extracellular enzymes, deteriorating aflatoxigenic mycelia. However, the research on the mechanism of inhibitory effects on aflatoxin production is still at initial stage.
4.5. A Conclusion of Mechanisms

According to all of the above studies, we listed four main mechanisms of inhibitory actions (Figure 3): (1) Physically competing for living spaces and nutrients, (2) destabilizing cell wall structure, (3) affecting intracellular activities of mitochondria, nucleus, and cytoplasmic membrane, and (4) down-regulating expression of aflatoxin-synthesis related genes. Importantly, inhibitory actions are most likely determined by a combination of different mechanisms, not by only one. We also listed some genes that have been analyzed under the treatment of different biocontrol agents (Figure 4).

![Figure 3](image-url)  
**Figure 3.** Mechanisms of inhibitory actions by antagonistic microorganisms against aflatoxigenic molds. For an inhibitory action, one of the four mechanisms may be dominant, but not the only one; inhibitory actions are most likely determined by a combination of different mechanisms.

![Figure 4](image-url)  
**Figure 4.** The genes down-regulated by different biocontrol agents. Different biocontrol agents acted on different aflatoxin synthesis genes which were demonstrated to be down-regulated. For example, *Bacillus subtilis* and *Pseudomonas fluorescens* could down-regulate the expressions of *Nor-1* and *aflR*. The clustered genes in aflatoxin biosynthetic pathway were plotted according to reports of Yu et al. [118].
5. Factors Influencing Antifungal Activities

It is well-known fact that the growth rate of and aflatoxin production by aflatoxigenic strains were strongly influenced by environments, cultural conditions, and nutritional factors. The combined effects of incubation time, temperature, water activity (aw), and CO2 on the growth and aflatoxin production by *A. flavus* were studied [119]. Nutritional sources were also demonstrated to have a significant influence on fungal growth and mycotoxin production [120,121]. Additionally, the expression of aflatoxin-synthesis related genes were demonstrated to be highly in relation to changes in water activity and temperature levels [122–124]. Similarly, antifungal activities of various biocontrol microbes were also related to these biotic and abiotic factors. Examples are described here below.

5.1. pH Value

Studies showed that the bioactivity of *L. plantarum* was pH-dependent. The low pH was responsible for the highlighted bioactivity of *L. plantarum* against aflatoxin-producing strains [22,61]. Gerez et al. [25] demonstrated that the antifungal activity of some *Lactobacillus* strains was lost after the neutralization treatment because the acidic nature of the antifungal metabolites was destroyed. In addition, *Saccharomyces cerevisiae* RC008 and RC016 showed a great antagonistic activity at pH 4, where strains can highly decrease the growth rate of *A. parasiticus* [49]. Conversely, the bacterium *Bacillus pumilus* grew very slightly at pH 4, where it showed the lowest anti-aflatoxigenic activity only with 38% inhibition of aflatoxin production [17]. These studies indicated that the best pH value for different antagonists against aflatoxigenic molds is remarkably species-dependent.

5.2. Temperature and Water Activity

Culturing temperature and water activity (aw) are also key factors. The maximum activity of protease P6281 produced by *T. harzianum* was observed at 40 °C [99]. The appropriate conditions for the growth of *Kluyveromyces* spp. were 60 min of incubation at 45 °C and 0.95 aw [125], while Penna and Etcheverry [86] demonstrated that *Kluyveromyces* isolates could impact both *A. flavus* growth and AFB1 accumulation at a wide range of water activities (0.93–0.99). La Penna et al. [50] found that several *Kluyveromyces* isolates showed anti-aflatoxigenic activity and inhibitory activity on aflatoxin production at all water activities tested. A notable finding was that the yeast strains of *Debaryomyces hansenii* could stimulate aflatoxins production by *A. parasiticus* at water activity of 0.99, whereas significantly reduce aflatoxins production at 0.92 aw [48]. Therefore, temperature and water activity are also important factors influencing antifungal efficiency of antagonists.

5.3. Other Factors such as Incubation Time, Culturing Medium, and Mutagenesis

Furthermore, incubation time is also a key factor affecting the production of anti-aflatoxigenic metabolites. Munimbazi and Bullerman [17] gave an evident proving that the greatest inhibitory activity arose up after 3 and 4 days incubation of *Bacillus pumilus*, and aflatoxin production was completely inhibited in supernatant obtained only from 3 and 4 day old bacterium. Whipps [79] demonstrated that different media appeared to be related to antifungal behaviors. Afsharmanesh et al. [103] found that a random mutagenesis of *Bacillus subtilis* could significantly inhibit *A. flavus* growth and aflatoxin production compared with the parental strain. This shows that mutant study can potentially improve biocontrol activity in inhibiting aflatoxigenic strains.

As shown in Figure 5, incubation conditions such as growing period, temperature, water activity, pH values, and nutritional sources could not only influence pathogens’, but also antagonists’ growth and/or metabolism. Therefore, dynamic growing conditions should be taken into account in performing strategies to biocontrol aflatoxigenic molds and eliminate aflatoxin risk by aflatoxigenic fungi.
Figure 5. Key factors influencing antifungal activities against aflatoxigenic fungi. These key factors have influences on both aflatoxigenic and antagonists’ growth and metabolisms. As a result, the combination of these factors is playing an important role in biocontrol efficacy.

6. Perspective and Conclusion

The biocontrol strategy for preventing aflatoxigenic fungi has been discussed in this review. It is clear that some microbes, including various bacteria, nontoxicogenic Aspergillus, Trichoderma, and yeasts have shown potentials to biocontrol aflatoxigenic molds. The inhibitory compounds that have potential biocontrol effects on aflatoxigenic strains, together with mechanisms and influencing factors of the bioactive actions are also reviewed. The current research status is still not very optimistic, because there are still many aspects needing urgent improvements. The above reviewed research works do, however, suggest that deeper practical works must be conducted to identify effective and environmental biocontrol agents, substantially to reach an advanced stage of application and commercialization. Additionally, a comprehensive and systematic study, covering inhibitory behaviors, mechanisms, factors, and pathogen–antagonist–plant interactions, is also urgently needed.

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References

1. Giorni, P.; Magan, N.; Pietri, A.; Bertuzzi, T.; Battilani, P. Studies on Aspergillus section Flavi isolated from maize in northern Italy. *Int. J. Food Microbiol.* 2007, 113, 330–338, doi:10.1016/j.ijfoodmicro.2006.09.007.
2. Varga, J.; Frisvad, J.C.; Samson, R.A. Two new aflatoxin producing species, and an overview of Aspergillus section Flavi. *Stud. Mycol.* 2011, 69, 57–80, doi:10.3114/sim.2011.69.05.
3. Bennett, J.W.; Klich, M. Mycotoxins. *Clin. Microbiol. Rev.* 2003, 16, 497.
4. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Some Traditional Herbal Medicines, S.M., Naphthalene; Styrene. Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene. *IARC Monogr. Eval. Carcinog. Risks Hum.* 2002, 82, 1–556.
5. Ji, C.; Fan, Y.; Zhao, L. Review on biological degradation of mycotoxins. *Anim. Nutr.* 2016, 2, 127–133.
6. Zhu, Y.; Hassan, Y.I.; Watts, C.; Zhou, T. Innovative technologies for the mitigation of mycotoxins in animal feed and ingredients-A review of recent patents. *Anim. Feed Sci. Technol.* 2016, 216, 19–29, doi:10.1016/j.anifeedsci.2016.03.030.
7. El-Nezami, H.; Kankaanpaa, P.; Salminen, S.; Ahokas, J. Ability of dairy strains of lactic acid bacteria to bind a common food carcinogen, aflatoxin B-1. *Food Chem. Toxicol.* 1998, 36, 321–326, doi:10.1016/s0278-6915(97)00160-9.
8. Haskard, C.A.; El-Nezami, H.S.; Kankaanpaa, P.E.; Salminen, S.; Ahokas, J.T. Surface binding of aflatoxin B(1) by lactic acid bacteria. Appl. Environ. Microbiol. 2001, 67, 3086–3091, doi:10.1128/AEM.67.7.3086-3091.2001.

9. Gonçalves, B.L.; Rosim, R.E.; de Oliveira, C.A.F.; Corassin, C.H. The in vitro ability of different Saccharomyces cerevisiae—Based products to bind aflatoxin B1. Food Control 2015, 47, 298–300, doi:10.1016/j.foodcont.2014.07.024.

10. Wu, Q.; Jezkova, A.; Yuan, Z.; Pavlikova, L.; Dohnal, V.; Kuca, K. Biological degradation of aflatoxins. Drug Metab. Rev. 2009, 41, 1–7, doi:10.1080/03622530802563850.

11. Das, A.; Bhattacharya, S.; Palaniswamy, M.; Angayarkanni, J. Aflatoxin B1 degradation during co-cultivation of Aspergillus flavus and Pleurotus ostreatus strains on rice straw. 3 Biotech. 2015, 5, 279–284, doi:10.1007/s13205-014-0228-7.

12. Teniola, O.D.; Addo, P.A.; Brost, I.M.; Farber, P.; Jany, K.D.; Alberts, J.F.; van Zyl, W.H.; Steyn, P.S.; Holzapfel, W.H. Degradation of aflatoxin B(1) by cell-free extracts of Rhodococcus erythropolis and Mycobacterium fluoranthenivorans sp. nov. DSM44556(T). Int. J. Food Microbiol. 2005, 105, 111–117, doi:10.1016/j.ifoodmicro.2005.05.004.

13. Torres, A.M.; Barros, G.G.; Palacios, S.A.; Chulze, S.N.; Battilani, P. Review on pre- and post-harvest management of peanuts to minimize aflatoxin contamination. Food Res. Int. 2014, 62, 11–19, doi:10.1016/j.foodres.2014.02.023.

14. Sarrocco, S.; Vannacci, G. Preharvest application of beneficial fungi as a strategy to prevent postharvest mycotoxin contamination: A review. Crop. Prot. 2018, 110, 160–170, doi:10.1016/j.cropro.2017.11.013.

15. Shahmoshteh, F.; Hamidi-Esfahani, Z.; Spadaro, D.; Shams-Ghahfarokhi, M.; Razzaghi-Abyaneh, M. Unraveling the mode of antifungal action of Bacillus subtilis and Bacillus amyloliquefaciens as potential biocontrol agents against aflatoxigenic Aspergillus parasiticus. Food Control 2018, 89, 300–307, doi:10.1016/j.foodcont.2017.11.010.

16. Kong, Q.; Chi, C.; Yu, J.; Shan, S.; Li, Q.; Li, Q.; Guan, B.; Nierman, W.C.; Bennett, J.W. The inhibitory effect of Bacillus megaterium on aflatoxin and cyclopiazonic acid biosynthetic pathway gene expression in Aspergillus flavus. Appl. Microbiol. Biotechnol. 2014, 98, 5161–5172, doi:10.1007/s00253-014-6563-2.

17. Munimbazi, C.; Bullerman, L.B. Inhibition of aflatoxin production of Aspergillus parasiticus NRRL 2999 by Bacillus pumilus. Mycopathologia 1998, 140, 163–169.

18. González Pereyra, M.L.; Martínez, M.P.; Petroselli, G.; Erra Balsells, R.; Cavagliera, L.R. Antifungal and aflatoxin-reducing activity of extracellular compounds produced by soil Bacillus strains with potential application in agriculture. Food Control 2018, 85, 392–399, doi:10.1016/j.foodcont.2017.10.020.

19. Palumbo, J.D.; O’Keefe, T.L.; Abbas, H.K. Isolation of maize soil and rhizosphere bacteria with antagonistic activity against Aspergillus flavus and Fusarium verticillioides. J. Food Prot. 2007, 70, 1615–1621, doi:10.4315/0362-028x-70.7.1615.

20. Yang, X.; Zhang, Q.; Chen, Z.Y.; Liu, H.; Li, P. Investigation of Pseudomonas fluorescens strain 3JW1 on preventing and reducing aflatoxin contaminations in peanuts. PLoS ONE 2017, 12, e0178810, doi:10.1371/journal.pone.0178810.

21. Manna, M.; Oh, J.Y.; Kim, K.D. Microbe-mediated control of Aspergillus flavus in stored rice grains with a focus on aflatoxin inhibition and biodegradation. Ann. Appl. Biol. 2017, 171, 376–392, doi:10.1111/aab.12381.

22. Sangmanee, P.; Hongpattarakere, T. Inhibitory of multiple antifungal components produced by Lactobacillus plantarum K35 on growth, aflatoxin production and ultrastructure alterations of Aspergillus flavus and Aspergillus parasiticus. Food Control 2014, 40, 224–233, doi:10.1016/j.foodcont.2013.12.005.

23. Quattini, M.; Bernardi, C.; Stuknyte, M.; Masotti, F.; Passera, A.; Ricci, G.; Vallone, L.; De Noni, I.; Brasca, M.; Fortina, M.G. Functional characterization of Lactobacillus plantarum ITEM 17215: A potential biocontrol agent of fungi with plant growth promoting traits, able to enhance the nutritional value of cereal products. Food Res. Int. 2018, 106, 936–944, doi:10.1016/j.foodres.2018.01.074.

24. Elsanhoty, R.M.; Salam, S.A.; Ramadan, M.F.; Badr, F.H. Detoxification of aflatoxin M1 in yoghurt using probiotics and lactic acid bacteria. Food Control 2014, 43, 129–134, doi:10.1016/j.foodcont.2014.03.002.

25. Gerez, C.L.; Torres, M.J.; de Valdez, G.F.; Rollan, G. Control of spoilage fungi by lactic acid bacteria. Biol. Control 2013, 64, 231–237, doi:10.1016/j.biocontrol.2012.10.009.

26. Ahlberg, S.; Joutsjoki, V.; Laurikkala, S.; Varmanen, P.; Korhonen, H. Aspergillus flavus growth inhibition by Lactobacillus strains isolated from traditional fermented Kenyan milk and maize products. Arch. Microbiol. 2017, 199, 457–464, doi:10.1007/s00203-016-1316-3.
Toxins 2020, 12, 24

27. Ghanbari, R.; Molaei Aghaei, E.; Rezaie, S.; Jahed Khaniki, G.; Alimohammadi, M.; Soleimani, M.; Noorbakhsh, F. The inhibitory effect of lactic acid bacteria on aflatoxin production and expression of aflR gene in Aspergillus parasiticus. J. Food Saf. 2018, 38, doi:10.1111/jfs.12413.

28. Shakedel, Q.; Luu, A.; Zhang, J.; Wu, M.; Li, G.; Hsiang, T.; Yang, L. Biocontrol of Aspergillus flavus on Peanut Kernels Using Streptomyces yanglinensis 3-10. Front. Microbiol. 2018, 9, 1049, doi:10.3389/fmicb.2018.01049.

29. Mander, P.; Cho, S.S.; Choi, Y.H.; Panthi, S.; Choi, Y.S.; Kim, H.M.; Yoo, J.C. Purification and characterization of chitinase showing antifungal and biodegradation properties obtained from Streptomyces anulatus CS242. Arch. Pharmacal Res. 2016, 39, 878–886, doi:10.1007/s12272-016-0747-3.

30. Wang, C.; Wang, Z.; Qiao, X.; Li, Z.; Li, F.; Chen, M.; Wang, Y.; Huang, Y.; Cui, H. Antifungal activity of volatile organic compounds from Streptomyces aboiflavus TD-1. FEMS Microbiol. Lett. 2013, 341, 45–51, doi:10.1111/1574-6968.12088.

31. Caceres, I.; Snini, S.P.; Puel, O.; Mathieu, J. Streptomyces roseolus, A Promising Biocontrol Agent Against Aspergillus flavus, the Main Aflatoxin B1 Producer. Toxins 2018, 10, 442, doi:10.3390/toxins10110442.

32. Wang, K.; Yan, P.S.; Cao, L.X.; Ding, Q.L.; Shao, C.; Zhao, T.F. Potential of chitinolytic Serratia marcescens strain JPP1 for biological control of Aspergillus parasiticus and aflatoxin. Biores. Int. 2013, 397142, doi:10.1155/2013/397142.

33. Jermnak, U.; Chinaphuti, A.; Poapolathep, A.; Kawai, R.; Nagasawa, H.; Sakuda, S. Prevention of aflatoxin contamination by a soil bacterium of Stenotrophomonas sp. that produces aflatoxin production inhibitors. Microbiology 2013, 159, 902–912, doi:10.1099/mic.0.065813-0.

34. Palumbo, J.D.; Baker, J.L.; Mahoney, N.E. Isolation of bacterial antagonists of Aspergillus flavus from almonds. Microb. Ecol. 2006, 52, 45–52, doi:10.1007/s00248-006-9096-y.

35. Taylor, W.J.; Draughon, F.A. Nannocystis excedens: A potential biocompetitive agent against Aspergillus flavus and Aspergillus parasiticus. J. Food Prot. 2001, 64, 1030–1034, doi:10.4315/0362-028x-64.7.1030.

36. Yan, P.S.; Song, Y.; Sakuno, E.; Nakajima, H.; Nakagawa, H.; Yabe, K. Cyclo(Leucyl-L-prolyl) produced by Achromobacter xylosidans inhibits aflatoxin production by Aspergillus parasiticus. Appl. Environ. Microbiol. 2007, 70, 7466–7473, doi:10.1128/AEM.70.12.7466-7473.2004.

37. Abbas, H.K.; Zabloutwicz, R.M.; Bruns, H.A.; Abel, C.A. Biocontrol of aflatoxin in corn by inoculation with non-aflatoxicogenic Aspergillus flavus isolates. Biocontrol Sci. Technol. 2007, 16, 437–449, doi:10.1080/09583150500532477.

38. Alaniz Zanon, M.S.; Barros, G.G.; Chulze, S.N. Non-aflatoxicogenic Aspergillus flavus as potential biocontrol agents to reduce aflatoxin contamination in peanuts harvested in Northern Argentina. Int. J. Food Microbiol. 2016, 231, 63–68, doi:10.1016/j.ijfoodmicro.2016.05.016.

39. Dorner, J.W.; Cole, R.J. Effect of application of nontoxicogenic strains of Aspergillus flavus and A-parasiticus on subsequent aflatoxin contamination of peanuts in storage. J. Stored Prod. Res. 2002, 38, 329–339, doi:10.1016/s0022-474x(01)00035-2.

40. Xu, D.; Wang, H.; Zhang, Y.; Yang, Z.; Sun, X. Inhibition of non-toxicogenic Aspergillus niger FS10 isolated from Chinese fermented soybean on growth and aflatoxin B1 production by Aspergillus flavus. Food Control 2013, 32, 359–365, doi:10.1016/j.foodcont.2012.12.013.

41. Alshannaq, A.F.; Gibbons, J.G.; Lee, M.K.; Han, K.H.; Hong, S.B.; Yu, J.H. Controlling aflatoxin contamination and propagation of Aspergillus flavus by a soy-fermenting Aspergillus oryzae strain. Sci. Rep. 2018, 8, 16871, doi:10.1038/s41598-018-35246-1.

42. Skouri-Gargouri, H.; Gargouri, A. First isolation of a novel thermostable antifungal peptide secreted by Aspergillus clavatus. Peptides 2008, 29, 1871–1877, doi:10.1016/j.peptides.2008.07.005.

43. Mostafa, A.A.; Al-Rahmah, A.N.; Abdel-Megeed, A.; Sayed, S.R.; Hatamleh, A.A. Antagonistic Activities of Some Fungal Strains against the Toxigenic Aspergillus flavus Isolate and its Aflatoxins Productivity. J. Pure Appl. Microbiol. 2013, 7, 169–178.

44. Anjiaiah, V.; Thakur, R.P.; Koedam, N. Evaluation of bacteria and Trichoderma for biocontrol of pre-harvest seed infection by Aspergillus flavus in groundnut. Biocontrol Sci. Technol. 2007, 16, 431–436, doi:10.1080/09583150500532337.

45. Bernález, V.; Rodríguez, A.; Martín, A.; Lozano, D.; Córdoba, J.J. Development of a multiplex qPCR method for simultaneous quantification in dry-cured ham of an antifungal-peptide Penicillium chrysogenum strain used as protective culture and aflatoxin-producing moulds. Food Control 2014, 36, 257–265, doi:10.1016/j.foodcont.2013.08.020.
46. Geisen, R. *P-nalgiovense* carries a gene which is homologous to the paf gene of *P-chrysogenum* which codes for an antifungal peptide. *Int. J. Food Microbiol.* 2000, 62, 95–101, doi:10.1016/s0168-1605(00)00367-6.

47. Medina-Côrdoval, N.; López-Aguilar, R.; Ascencio, F.; Castellanos, T.; Campa-Côrdoval, A.I.; Angulo, C. Biocontrol activity of the marine yeast *Debaryomyces hansenii* against phytopathogenic fungi and its ability to inhibit mycotoxins production in maize grain (*Zea mays L.*). *Biol. Control* 2016, 97, 70–79, doi:10.1016/j.biocontrol.2016.03.006.

48. Peromingo, B.; Andrade, M.J.; Delgado, J.; Sanchez-Montero, L.; Nunez, F. Biocontrol of aflatoxicogenic *Aspergillus parasiticus* by native *Debaryomyces hansenii* in dry-cured meat products. *Microbiol. 2016, 82, 269–276, doi:10.1016/j.jfoodmicro.2019.01.024.

49. Armando, M.R.; Dogi, C.A.; Rosa, C.A.; Dalcero, A.M.; Cavagliari, L.R. *Saccharomyces cerevisiae* strains and the reduction of *Aspergillus parasiticus* growth and aflatoxin B1 production at different interacting environmental conditions, in *vitro*. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.* 2012, 29, 1443–1449, doi:10.1080/19440049.2012.698655.

50. La Penna, M.; Neschi, A.; Etcheverry, M. In vitro studies on the potential for biological control on *Aspergillus* section *Flavi* by *Kluyveromyces* spp. *Lett. Appl. Microbiol.* 2004, 38, 257–264, doi:10.1111/j.1472-765X.2003.01467.x.

51. Hua, S.S.; Beck, J.J.; Sarreal, S.B.; Gee, W. The major volatile compound 2-phenylethanol from the biocontrol yeast, *Pichia anomala*, inhibits growth and expression of aflatoxin biosynthetic genes of *Aspergillus flavus*. *Mycores Res.* 2014, 30, 71–78, doi:10.1111/s1255-014-0189-z.

52. Ando, H.; Hatanaka, K.; Ohata, I.; Yamashita-Kitaguchi, Y.; Kurata, A.; Kishimoto, N. Antifungal activities of volatile substances generated by yeast isolated from Iranian commercial cheese. *Food Control* 2012, 26, 472–478, doi:10.1016/j.foodcont.2012.02.017.

53. Siahmoshteh, F.; Siciliano, I.; Banani, H.; Hamidi-Esfahani, Z.; Razzaghi-Abyaneh, M.; Gullino, M.L.; Spadaro, D. Efficacy of *Bacillus subtilis* and *Bacillus amyloliquefaciens* in the control of *Aspergillus parasiticus* growth and aflatoxins production on pistachio. *Int. J. Food Microbiol.* 2017, 254, 47–53, doi:10.1016/j.ifoodmicro.2017.05.011.

54. Etcheverry, M.G.; Scandolara, A.; Neschi, A.; Vilas Boas Ribeiro, M.S.; Pereira, P.; Battiliani, P. Biological interactions to select biocontrol agents against toxigenic strains of *Aspergillus flavus* and *Fusarium verticillioides* from maize. *Mycopathologia* 2009, 167, 287–295, doi:10.1007/s11046-008-9177-1.

55. Schallmey, M.; Singh, A.; Ward, O.P. Developments in the use of *Bacillus* species for industrial production. *Can. J. Microbiol.* 2004, 50, 1–17, doi:10.1139/w03-076.

56. Akocak, P.B.; Churey, J.J.; Worobo, R.W. Antagonistic effect of chitinolytic *Pseudomonas* and *Bacillus* on growth of fungal hyphae and spores of aflatoxicogenic *Aspergillus flavus*. *Food Biosci.* 2015, 10, 48–58, doi:10.1016/j.fbio.2015.01.005.

57. Ahlberg, S.H.; Joutsjoki, V.; Korhonen, H.J. Potential of lactic acid bacteria in aflatoxin risk mitigation. *Int. J. Food Microbiol.* 2015, 207, 87–102, doi:10.1016/j.ifoodmicro.2015.04.042.

58. Gupta, R.; Srivastava, S. Antifungal effect of antimicrobial peptides (AMPs’ LR14) derived from *Lactobacillus plantarum* strain LR14/14 and their applications in prevention of grain spoilage. *Food Microbiol.* 2014, 42, 1–7, doi:10.1016/j.fm.2014.02.005.

59. Muhialdin, B.J.; Hassan, Z.; Abu Bakar, F.; Saari, N. Identification of antifungal peptides produced by *Lactobacillus plantarum* IS10 grown in the MRS broth. *Food Control* 2016, 59, 27–30, doi:10.1016/j.foodcont.2015.05.022.

60. Luz, C.; Saladino, R.; Luciano, F.B.; Mañes, J.; Meca, G. In vitro antifungal activity of bioactive peptides produced by *Lactobacillus plantarum* against *Aspergillus parasiticus* and *Penicillium expansum*. *LWT Food Sci. Technol.* 2017, 81, 128–135, doi:10.1016/j.lwt.2017.03.053.

61. Russo, P.; Arena, M.P.; Fiocco, D.; Capozzi, V.; Drider, D.; Spano, G. *Lactobacillus plantarum* with broad antifungal activity: A promising approach to increase safety and shelf-life of cereal-based products. *Int. J. Food Microbiol.* 2017, 247, 48–54, doi:10.1016/j.ifoodmicro.2016.04.027.

62. Sultan, Y.; Magan, N. Impact of a *Streptomyces*(AS1) strain and its metabolites on control of *Aspergillus flavus* and aflatoxin B1 contamination in vitro and in stored peanuts. *Biocontrol Sci. Technol.* 2011, 21, 1437–1455, doi:10.1080/09583157.2011.632078.

63. Zucchi, T.D.; de Moraes, L.A.; de Melo, I.S. *Streptomyces* sp. ASBV-1 reduces aflatoxin accumulation by *Aspergillus parasiticus* in peanut grains. *J. Appl. Microbiol.* 2008, 105, 2153–2160, doi:10.1111/j.1365-2672.2008.03940.x.
64. Verheecke, C.; Liboz, T.; Darriet, M.; Sabaou, N.; Mathieu, F. In vitro interaction of actinomycetes isolates with Aspergillus flavus: Impact on aflatoxins B1 and B2 production. Lett. Appl. Microbiol. 2014, 58, 597–603, doi:10.1111/lam.12233.

65. Yuen, G.Y.; Schroth, M.N. Inhibition of Fusarium oxysporum f.sp. dianthi by iron competition with an Alcaligenes sp. Phytopathology 1986, 76, 171–176, doi:10.1094/Phyto-76-171.

66. Cotty, P.J. Virulence and Cultural Characteristics of Two Aspergillus flavus Strains Pathogenic on Cotton. Phytopathology 1989, 79, 808–814.

67. Cotty, P.J. Aflatoxin-producing potential of communities of Aspergillus section Flavi from cotton producing areas in the United States. Mycol. Res. 1997, 101, 698–704.

68. Horn, B.W.; Dorner, J.W. Regional Differences in Production of Aflatoxin B1 and Cyclopiazonic Acid by Soil Isolates of Aspergillus flavus along a Transect within the United States. Appl. Environ. Microbiol. 1999, 65, 1444–1449.

69. Cotty, P.J. Influence of field application of an atoxigenic strain of Aspergillus flavus on the populations of A. flavus infecting cotton bolls and on the aflatoxin content of cottonseed. Phytopathology 1994, 84, 1270–1277.

70. Cotty, P.J.; Bayman, P. Competitive exclusion of a toxigenic strain of Aspergillus flavus by an atoxigenic strain. Phytopathology 1993, 83, 1283–1287.

71. Probst, C.; Bandypadhyay, R.; Price, L.E.; Cotty, P.J. Identification of Atoxigenic Aspergillus flavus Isolates to Reduce Aflatoxin Contamination of Maize in Kenya. Plant Dis. 2011, 95, 212–218, doi:10.1094/PDIS-06-10-0438.

72. Dorner, J.W.; Cole, R.J.; Connick, W.J.; Daigle, D.J.; McGuire, M.R.; Shasha, B.S. Evaluation of biological control formulations to reduce aflatoxin contamination in peanuts. Biol. Control 2003, 26, 318–324, doi:10.1016/s1049-9644(02)00139-1.

73. Kachapulula, P.W.; Akello, J.; Bandypadhyay, R.; Cotty, P.J. Aspergillus section Flavi community structure in Zambia influences aflatoxin contamination of maize and groundnut. Int. J. Food Microbiol. 2017, 261, 49–56, doi:10.1016/j.ijfoodmicro.2017.08.014.

74. Alaniz Zanon, M.S.; Paz Clemente, M.; Noemi Chulze, S. Characterization and competitive ability of non-aflatoxigenic Aspergillus flavus isolated from the maize agro-ecosystem in Argentina as potential aflatoxin biocontrol agents. Int. J. Food Microbiol. 2018, 277, 58–63, doi:10.1016/j.ijfoodmicro.2018.04.020.

75. Xing, F.; Wang, L.; Liu, X.; Selvaraj, J.N.; Wang, Y.; Zhao, Y.; Liu, Y. Aflatoxin B1 inhibition in Aspergillus flavus by Aspergillus niger through down-regulating expression of major biosynthetic genes and AFB1 degradation by atoxigenic A. flavus. Int. J. Food Microbiol. 2017, 256, 1–10, doi:10.1016/j.ijfoodmicro.2017.05.013.

76. Abe, K.; Gomi, K.; Hasegawa, F.; Machida, M. Impact of Aspergillus oryzae genomics on industrial production of metabolites. Mycopathologia 2006, 162, 143–153, doi:10.1007/s11046-006-0049-2.

77. Machida, M.; Asai, K.; Sano, M.; Tanaka, T.; Kumagai, T.; Terai, G.; Kusumoto, K.I.; Arima, T.; Akita, O.; Kashiwagi, Y.; et al. Genome sequencing and analysis of Aspergillus oryzae. Nature 2005, 438, 1157–1161, doi:10.1038/nature04300.

78. Parente, D.; Raucci, G.; Celano, B.; Pacilli, A.; Zanoni, L.; Canevari, S.; Adobati, E.; Colnaghi, M.L.; Dosio, F.; Arpico, S.; et al. Clavin a type-1 ribosome-inactivating protein from Aspergillus clavatus IFO 8605-cDNA isolation, heterologous expression, biochemical and biological characterization of the recombinant protein. Eur. J. Biochem. 1996, 239, 272–280, doi:10.1111/j.1432-1033.1996.0272ux.x.

79. Whippis, J.M. Effect of media on growth and interactions between a range of soil-borne glasshouse pathogens and antagonistic fungi. New Phytol. 1987, 107, 127–142, doi:10.1111/j.1469-8137.1987.tb04887.x.

80. Harman, G.E. Myths and dogmas of biocontrol—Changes in perceptions derived from research on Trichoderma harzianum T-22. Plant Dis. 2000, 84, 377–393, doi:10.1094/pdis.2000.84.4.377.

81. Vinale, F.; Marra, R.; Scala, F.; Ghisalberti, E.L.; Lorito, M.; Sivasithamparam, K. Major secondary metabolites produced by two commercial Trichoderma strains active against different phytopathogens. Lett. Appl. Microbiol. 2006, 43, 143–148, doi:10.1111/j.1472-765X.2006.01939.x.

82. Vinale, F.; Sivasithamparam, K.; Ghisalberti, E.L.; Marra, R.; Woo, S.L.; Lorito, M. Trichoderma—plant-pathogen interactions. Soil Biol. Biochem. 2008, 40, 1–10, doi:10.1016/j.soilbio.2007.07.002.

83. Acosta, R.; Rodriguez-Martin, A.; Martin, A.; Nunez, F.; Asensio, M.A. Selection of antifungal protein-producing molds from dry-cured meat products. Int. J. Food Microbiol. 2009, 135, 39–46, doi:10.1016/j.ijfoodmicro.2009.07.020.
84. Rodriguez-Martin, A.; Acosta, R.; Liddell, S.; Nunez, F.; Benito, M.J.; Asensio, M.A. Characterization of the novel antifungal protein PgAFP and the encoding gene of Penicillium chrysogenum. Peptides 2010, 31, 541–547, doi:10.1016/j.peptides.2009.11.002.
85. Nielsen, M.S.; Frisvad, J.C.; Nielsen, P.V. Protection by fungal starters against growth and secondary metabolite production of fungal spoilers of cheese. Int. J. Food Microbiol. 1998, 42, 91–99, doi:10.1016/s0168-1605(98)00700-1.
86.Penn, M.L.; Etcheverry, M. Impact on growth and aflatoxin B1 accumulation by Klyuyveromyces isolates at different water activity conditions. Mycopathologia 2006, 162, 347–353, doi:10.1007/s11046-006-0071-4.
87. Hua, S.S.T. Progress in Prevention of Aflatoxin Contamination in Food by Preharvest Application of a Yeast Strain, Pichia Anomala WRL6. Mod. Multidiscip. Appl. Microbiol. 2008, 322–326.
88. Hua, S.S.T.; Baker, J.L.; Flores-Espiritu, M. Interactions of saprophytic yeasts with a nor mutant of Aspergillus flavus. Appl. Environ. Microbiol. 1999, 65, 2738–2740.
89. Veras, F.F.; Correa, A.P.F.; Welke, J.E.; Brandelli, A. Inhibition of mycotoxin-producing fungi by Bacillus strains isolated from fish intestines. Int. J. Food Microbiol. 2016, 238, 23–32, doi:10.1016/j.ijfoodmicro.2016.08.035.
90. Gong, Q.; Zhang, C.; Lu, F.; Zhao, H.; Bie, X.; Lu, Z. Identification of bacillomycin D from Bacillus subtilis fmbJ and its inhibition effects against Aspergillus flavus. Food Control 2014, 36, 8–14, doi:10.1016/j.foodcont.2013.07.034.
91. Chen, Y.; Kong, Q.; Liang, Y. Three newly identified peptides from Bacillus megaterium strongly inhibit the growth and aflatoxin B1 production of Aspergillus flavus. Food Control 2019, 95, 41–49, doi:10.1016/j.foodcont.2018.07.040.
92. Guimaraes, A.; Santiago, A.; Teixeira, J.A.; Venancio, A.; Abrunhosa, L. Anti-aflatoxicogenic effect of organic acids produced by Lactobacillus plantarum. Int. J. Food Microbiol. 2018, 264, 31–38, doi:10.1016/j.ijfoodmicro.2017.10.025.
93. Ono, M.; Sakuda, S.; Suzuki, A.; Isogai, A. Aflastatin A, a novel inhibitor of aflatoxin production by aflatoxigenic fungi. J. Antibiot. 1997, 50, 111–118, doi:10.7164/antibiotics.50.111.
94. Yoshinari, T.; Akiyama, T.; Nakamura, K.; Kondo, T.; Takahashi, Y.; Muraoka, Y.; Nonomura, Y.; Nagasawa, H.; Sakuda, S. Dioctatin A is a strong inhibitor of aflatoxin production by Aspergillus parasiticus. Microbiology 2007, 153, 2774–2780, doi:10.1099/mic.0.2006/005629-0.
95. Yang, M.; Lu, L.; Pang, J.; Hu, Y.; Guo, Q.; Li, Z.; Wu, S.; Liu, H.; Wang, C. Biocontrol activity of volatile organic compounds from Streptomyces albiflavus TD-1 against Aspergillus flavus growth and aflatoxin production. J. Microbiol. 2019, 57, 396–404, doi:10.1007/s12275-019-8517-9.
96. Gomes, R.C.; Semedo, L.; Soares, R.M.A.; Linhares, L.F.; Ulhoa, C.J.; Alviano, C.S.; Coelho, R.R.R. Purification of a thermostable endochitinase from Streptomyces RC1071 isolated from a cerrado soil and its antagonism against phytopathogenic fungi. J. Appl. Microbiol. 2001, 90, 653–661, doi:10.1046/j.1365-2672.2001.01294.x.
97. Abdel-Kareem, M.M.; Rasmey, A.M.; Zohri, A.A. The action mechanism and biocontrol potentiality of novel isolates of Saccharomyces cerevisiae against the aflatoxigenic Aspergillus flavus. Lett. Appl. Microbiol. 2019, 68, 104–111, doi:10.1111/lam.13105.
98. Tayel, A.A.; El-Tras, W.F.; Moussa, S.H.; El-Agamy, M.A. Antifungal action of Picha anomala against aflatoxigenic Aspergillus flavus and its application as a feed supplement. J. Sci. Food Agric. 2013, 93, 3259–3263, doi:10.1002/jsfa.6169.
99. Deng, J.J.; Huang, W.Q.; Li, Z.W.; Lu, D.L.; Zhang, Y.; Luo, X.C. Biocontrol activity of recombinant aspartic protease from Trichoderma harzianum against pathogenic fungi. Enzym. Microb. Technol. 2018, 112, 35–42, doi:10.1016/j.enzmictec.2018.02.002.
100. Abriouel, H.; Franz, C.M.; Ben Omar, N.; Galvez, A. Diversity and applications of Bacillus bacteriocins. FEMS Microbiol. Rev. 2011, 35, 201–232, doi:10.1111/j.1574-6976.2010.00244.x.
101. Mondol, M.A.; Shin, H.J.; Islam, M.T. Diversity of secondary metabolites from marine Bacillus species: Chemistry and biological activity. Mar. Drugs 2013, 11, 2846–2872, doi:10.3390/md11082846.
102. Zhao, H.; Shao, D.; Jiang, C.; Shi, J.; Li, Q.; Huang, Q.; Rajoka, M.S.R.; Yang, H.; Jin, M. Biological activity of lipopeptides from Bacillus. Appl. Microbiol. Biotechnol. 2017, 101, 5951–5960, doi:10.1007/s00253-017-8396-0.
103. Afsharmanesh, H.; Ahmadzadeh, M.; Javan-Nikkhah, M.; Behboudi, K. Improvement in biocontrol activity of *Bacillus subtilis* UTB1 against *Aspergillus flavus* using gamma-irradiation. *Crop Prot.* 2014, 60, 83–92, doi:10.1016/j.cropro.2014.02.013.

104. Farzaneh, M.; Shi, Z.Q.; Ahmadzadeh, M.; Hu, L.B.; Ghasempour, A. Inhibition of the *Aspergillus flavus* Growth and Aflatoxin B1 Contamination on Pistachio Nut by Fengycin and Surfactin-Producing *Bacillus subtilis* UTBSP1. *Plant Pathol.* J. 2016, 32, 209–215, doi:10.5423/PPJ.OA.11.2015.0250.

105. Mannaa, M.; Oh, J.Y.; Kim, K.D. Biocontrol Activity of Volatile-Producing *Bacillus megaterium* and *Pseudomonas protegens* against *Aspergillus flavus* and Aflatoxin Production on Stored Rice Grains. *Mycobiology* 2017, 45, 213–219, doi:10.5941/MYCO.2017.45.3.213.

106. D’Aes, J.; De Maeyer, K.; Pauwelyn, E.; Hofte, M. Biosurfactants in plant-*Pseudomonas* interactions and their importance to biocontrol. *Environ. Microbiol. Rep.* 2010, 2, 359–372, doi:10.1111/j.1758-2229.2009.00104.x.

107. Chin-A-Woeng, T.F.C.; Bloemberg, G.V.; Lugtenberg, B.J.J. Phenazines and their role in biocontrol by *Pseudomonas* bacteria. *New Phytol.* 2003, 157, 503–523, doi:10.1046/j.1469-8137.2003.00686.x.

108. Manivasagan, P.; Kang, K.H.; Sivakumar, K.; Li-Chan, E.C.; Oh, H.M.; Kim, S.K. Marine actinobacteria: An important source of active bioactive natural products. *Environ. Toxicol. Pharmacol.* 2014, 38, 172–188, doi:10.1016/j.etap.2014.05.014.

109. Siddiqui, M.S.; Thodey, K.; Trenchard, I.; Smolke, C.D. Advancing secondary metabolite biosynthesis in yeast with synthetic biology tools. *FEMS Yeast Res.* 2012, 12, 144–170, doi:10.1111/j.1567-1364.2011.00774.x.

110. Hruska, Z.; Rajasekaran, K.; Yao, H.; Kincaid, R.; Darlington, D.; Brown, R.L.; Bhatnagar, D.; Cleveland, T.E. Co-inoculation of aflatoxigenic and non-aflatoxigenic strains of *Aspergillus flavus* to study fungal invasion, colonization, and competition in maize kernels. *Front. Microbiol.* 2014, 5, doi:10.3389/fmicb.2014.00122.

111. Ehrlich, K.C. Non-aflatoxigenic *Aspergillus flavus* to prevent aflatoxin contamination in crops: Advantages and limitations. *Front. Microbiol.* 2014, 5, 50, doi:10.3389/fmicb.2014.00050.

112. Ehrlich, K.C.; Cotty, P.J. An isolate of *Aspergillus flavus* used to reduce aflatoxin contamination in cottonseed has a defective polyketide synthase gene. *Appl. Microbiol. Biotechnol.* 2004, 65, 473–478, doi:10.1007/s00253-004-1670-y.

113. Chang, P.K.; Horn, B.W.; Dorner, J.W. Sequence breakpoints in the aflatoxin biosynthesis gene cluster and flanking regions in nonaflatoxigenic *Aspergillus flavus* isolates. *Fungal Genet. Biol.* 2005, 42, 914–923, doi:10.1016/j.fgb.2005.07.004.

114. Hulikunte Mallikarjuniah, N.; Jayapala, N.; Puttaswamy, H.; Siddapura Ramachandrappa, N. Characterization of non-aflatoxigenic strains of *Aspergillus flavus* as potential biocontrol agent for the management of aflatoxin contamination in groundnut. *Microb. Pathog.* 2017, 102, 21–28, doi:10.1016/j.micpath.2016.11.007.

115. Calistru, C.; McLean, M.; Berjak, P. In vitro studies on the potential for biological control of *Aspergillus flavus* and Fusarium moniliforme by *Trichoderma* species—A study of the production of extracellular metabolites by *Trichoderma* species. *Mycopathologia* 1997, 137, 115–124, doi:10.1023/a:1006802423729.

116. Benitez, T.; Rincon, A.M.; Limon, M.C.; Codon, A.C. Biocontrol mechanisms of *Trichoderma* strains. *Int. Microbiol.* 2004, 7, 249–260.

117. Braun, H.; Wotitsch, L.; Hetzer, B.; Geisen, R.; Zange, H.; Schmidt-Heydt, M. *Trichoderma harzianum*: Inhibition of mycotoxin producing fungi and toxin biosynthesis. *Int. J. Food Microbiol.* 2018, 280, 10–16, doi:10.1016/j.ijfoodmicro.2018.04.021.

118. Yu, J.; Chang, P.K.; Ehrlich, K.C.; Cary, J.W.; Bhatnagar, D.; Cleveland, T.E.; Payne, G.A.; Linz, J.E.; Woloshuk, C.P.; Bennett, J.W. Clustered Pathway Genes in Aflatoxin Biosynthesis. *Appl. Environ. Microbiol.* 2004, 70, 1253–1262, doi:10.1128/aem.70.3.1253–1262.2004.

119. Mousa, W.; Ghazali, F.M.; Jinap, S.; Ghazali, H.M.; Radu, S.; Salama, A.E.-R. Temperature, water activity and gas composition effects on the growth and aflatoxin production by *Aspergillus flavus* on paddy. *J. Stored Prod. Res.* 2016, 67, 49–55, doi:10.1016/j.jspr.2016.01.003.

120. Ma, H.; Zhang, N.; Sun, L.; Qi, D. Effects of different substrates and oils on aflatoxin B1 production by *Aspergillus parasiticus*. *Eur. Food Res. Technol.* 2014, 240, 627–634, doi:10.1007/s00217-014-2364-x.

121. Al-Saad, L.A.; Al-Badran, A.I.; Al-Jumayli, S.A.; Magan, N.; Rodriguez, A. Impact of bacterial biocontrol agents on aflatoxin biosynthetic genes, *afID* and *afQR* expression, and phenotypic aflatoxin B1 production by *Aspergillus flavus* under different environmental and nutritional regimes. *Int. J. Food Microbiol.* 2016, 217, 123–129, doi:10.1016/j.ijfoodmicro.2015.10.016.
122. Gallo, A.; Solfrizzo, M.; Epifani, F.; Panzarini, G.; Perrone, G. Effect of temperature and water activity on gene expression and aflatoxin biosynthesis in *Aspergillus flavus* on almond medium. *Int. J. Food Microbiol.* 2016, 217, 162–169, doi:10.1016/j.ijfoodmicro.2015.10.026.

123. Schmidt-Heydt, M.; Abdel-Hadi, A.; Magan, N.; Geisen, R. Complex regulation of the aflatoxin biosynthesis gene cluster of *Aspergillus flavus* in relation to various combinations of water activity and temperature. *Int. J. Food Microbiol.* 2009, 135, 231–237, doi:10.1016/j.ijfoodmicro.2009.07.026.

124. Abdel-Hadi, A.; Carter, D.; Magan, N. Temporal monitoring of the nor-1 (*aflD*) gene of *Aspergillus flavus* in relation to aflatoxin B(1) production during storage of peanuts under different water activity levels. *J. Appl. Microbiol.* 2010, 109, 1914–1922, doi:10.1111/j.1365-2672.2010.04820.x.

125. Montemarani, A.; Nesci, A.; Etcheverry, M. Production of *Kluyveromyces* spp. and environmental tolerance induction against *Aspergillus flavus*. *Ann. Microbiol.* 2013, 64, 935–944, doi:10.1007/s13213-013-0726-6.

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