Mycobiology

Mini-review

Microbe-Mediated Control of Mycotoxigenic Grain Fungi in Stored Rice with Focus on Aflatoxin Biodegradation and Biosynthesis Inhibition

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Abstract Rice contaminated with fungal species during storage is not only of poor quality and low economic value, but may also have harmful effects on human and animal health. The predominant fungal species isolated from rice grains during storage belong to the genera *Aspergillus* and *Penicillium*. Some of these fungal species produce mycotoxins; they are responsible for adverse health effects in humans and animals, particularly *Aspergillus flavus*, which produces the extremely carcinogenic aflatoxins. Not surprisingly, there have been numerous attempts to devise safety procedure for the control of such harmful fungi and production of mycotoxins, including aflatoxins. This review provides information about fungal and mycotoxin contamination of stored rice grains, and microbe-based (biological) strategies to control grain fungi and mycotoxins. The latter will include information regarding attempts undertaken for mycotoxin (especially aflatoxin) bio-detoxification and microbial interference with the aflatoxin-biosynthetic pathway in the toxin-producing fungi.

Keywords Aflatoxin, Biodegradation, Biological control, Grain mold, Mycotoxin, Rice grain

Rice (*Oryza sativa* L.) is one of the most important food crops in the world. It is an intrinsic part of the Asian cultures, in which some people of Indonesia believed that it had a soul like humans. In Japan, rice was considered the food of gods and samurais, and individuals who squandered even a handful of rice were punished. Although these beliefs have changed over time, people still highly value rice, particularly in Asia [1]. Rice represents the primary food source for almost half of the world's population, providing 20% of the total human dietary energy supply (Food and Agriculture Organization [FAO] of the United Nations, http://faostat.fao.org/). In Korea, rice is the major cereal; the total area devoted to rice was 799,344 ha and total rice production was 4,327 thousand tons in 2015. Moreover, the annual consumption of rice is 62.9 kg/capita, higher than that of any other food crop. While the amount of harvested rice is increasing annually, consumer intake has gradually declined, decreasing by half between 1985 and 2015 (Statistics Korea, http://kostat.go.kr/portal/eng/). As a result, harvested rice is stored for increasingly longer periods, which alters its physical, chemical, and biological properties [2]. During storage, rice is prone to contamination by various fungal species that can reduce its quality and market value. Therefore, this review will cover the following topics: (1) previous studies on fungal genera and species frequently detected in stored rice grains, highlighting the adverse effects on the grains as a source of mycotoxins; (2) previously reported microbe-based (biological) methods to control fungal contamination during storage, focusing on mycotoxigenic fungi, especially *Aspergillus flavus* producing aflatoxins as the most serious mycotoxin; (3) possible microbial degradation of mycotoxins, especially aflatoxins, and suppression of aflatoxin biosynthesis in fungi via microbial interference; and (4) concluding remarks and suggestions on future studies.

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FUNGAL SPECIES IN STORED RICE GRAINS

Fungal contamination of rice grains, a good substrate for storage fungi, starts in the field before harvest. The high

Table 1. A list of previously reported fungal species associated with rice grains

Fungi	Incidence[a]	Reference	Fungi	Incidence	Reference	Fungi	Incidence	Reference	Fungi	Incidence	Reference
Aspergillus amstelodami	*	[7]	*Curvularia cymbopogonis*	*	[4]	*Fusarium larvarum*	*	[4]	*Penicillium islandicum*	***	[9, 13]
Aspergillus candidus	***	[4, 7-9]	*Curvularia eragrostidis*	*	[4]	*Fusarium longipes*	*	[4]	*Penicillium* sp.	**	[4]
Aspergillus clavatus	**	[4]	*Curvularia geniculata*	**	[7]	*Fusarium moniliforme*	***	[4, 7]	*Penicillium verrucosum*	*	[9]
Aspergillus flavus	***	[4, 7-11]	*Curvularia inaequalis*	*	[4]	*Fusarium nivale*	*	[4]	*Periconia* sp.	*	[4]
Aspergillus fumigatus	***	[7, 8, 10, 11]	*Curvularia intermedia*	*	[4]	*Fusarium oxysporum*	*	[9-11]	*Pestalotia* sp.	**	[4, 7]
Aspergillus versicolor	**	[9, 10]	*Curvularia lunata*	***	[4, 7]	*Fusarium proliferatum*	*	[9, 10]	*Phaeotrichoconis crotolariae*	*	[4]
Aspergillus ochraceus	*	[9, 10]	*Curvularia oryzae*	***	[4]	*Fusarium semitectum*	***	[4, 7, 9, 10]	*Phoma* spp.	***	[4, 7]
Aspergillus parasiticus	*	[10, 11]	*Curvularia ovoidea*	*	[4]	*Fusarium solani*	**	[4, 7, 11]	*Phyllosticta* sp.	**	[4]
Aspergillus repens	*	[7]	*Curvularia pallescens*	**	[4]	*Fusarium tumidum*	*	[4]	*Phyllosticta glumarum*	*	[4, 7]
Aspergillus restrictus	*	[7]	*Curvularia stapeliae*	*	[4]	*Graphium* sp.	*	[4]	*Pinatubo oryzae*	**	[4]
Aspergillus terreus	**	[7, 10, 11]	*Cylindrocarpon* sp.	*	[4]	*Humicola* sp.	*	[4]	*Pithomyces maydicus*	**	[4]
Aspergillus niger	***	[4, 7, 9-11]	*Darluca* sp.	*	[4]	*Leptosphaeria sacchari*	*	[4]	*Pyrenochaeta* sp.	*	[4]
Absidia spp.	*	[11]	*Diarimella setulosa*	*	[4]	*Leptosphaeria* sp.	*	[4]	*Pyricularia grisea*	*	[4]
Acremoniella atra	*	[4]	*Diplodia* sp.	*	[4]	*Masonomyces claviformis*	*	[4]	*Rhizoctonia* sp.	**	[7]
Alternaria alternata	*	[4, 11]	*Drechslera cynodontis*	*	[4]	*Melanospora zamiae*	*	[4]	*Rhizopus* sp.	***	[4, 7, 9, 11]
Alternaria longissimi	**	[4]	*Drechslera dematioidea*	*	[4]	*Microascus cirrosus*	*	[4]	*Saccharomyces cerevisiae*	*	[11]
Alternaria padwickii	***	[4, 7]	*Drechslera halodes*	*	[4]	*Microdochium oryzae*	***	[4, 7]	*Sarocladium oryzae*	**	[4, 7]
Alternaria spp.	***	[9]	*Drechslera hawaiiensis*	*	[4]	*Monilia sitophila*	*	[7]	*Scopulariopsis brevicaulis*	*	[11]
Annellophragmia sp.	*	[4]	*Drechslera longirostrata*	*	[4]	*Monodictys levis*	*	[4]	*Septoria* sp.	*	[4]
Bipolaris oryzae	***	[4, 7]	*Drechslera maydis*	*	[4]	*Mucor racemosus*	*	[7]	*Spegazzinia deightonii*	*	[4]
Botrytis cinerea	*	[4]	*Drechslera rostrata*	*	[4]	*Mucor* spp.	*	[9, 11]	*Stachybotrys* sp.	*	[4]
Cephalosporium sp.	*	[4]	*Drechslera sachari*	*	[4]	*Nakataea sigmoidea*	**	[4, 7]	*Stemphylium* sp.	*	[4]
Cercospora janseana	*	[4]	*Drechslera sorokiniana*	*	[4]	*Nectria haematococca*	*	[4]	*Sterigmatobotris macrocarpa*	*	[4]
Chaetomium cochliodes	*	[7]	*Drechslera tetramera*	*	[4]	*Nigrospora oryzae*	**	[4, 7]	*Tetraploa aristata*	*	[4]
Chaetomium cuneatum	*	[7]	*Drechslera turcica*	*	[4]	*Nigrospora sphaerica*	*	[4]	*Tilletia barclayana*	**	[4, 7]
Chaetomium funicola	*	[7]	*Epicoccum purpurascens*	**	[4]	*Papularia* sp.	*	[4]	*Trichoderma* sp.	*	[4]
Chaetomium globosum	*	[4, 7]	*Eurotium* spp.	**	[9]	*Penicillifer fulcer*	*	[4]	*Trichosporiella* sp.	*	[4]
Chramyphora sp.	*	[4]	*Fusarium avenaceum*	*	[4]	*Penicillium chrysogenum*	*	[10]	*Trichothecium* sp.	*	[4]
Cladosporium sp.	**	[4, 7, 11]	*Fusarium decemcellulare*	*	[4]	*Penicillium frequentans*	*	[10]	*Tritirachium* sp.	*	[4]
Colletotrichum sp.	*	[4]	*Fusarium equiseti*	*	[4, 11]	*Penicillium oxalicum*	*	[10]	*Ulocladium* sp.	*	[4]
Corynespora sp.	*	[4]	*Fusarium expansum*	*	[9]	*Penicillium purpurogenum*	*	[10]	*Ustilaginoidea virens*	**	[4]
Cunninghamella sp.	*	[4]	*Fusarium fusarioides*	*	[4]	*Penicillium citrinum*	***	[7, 9, 10]	*Verticilium albo-atrum*	**	[4, 7]
Curvularia affinis	**	[4]	*Fusarium graminearum*	*	[4, 7, 9]	*Penicillium fellutanum*	***	[12]	*Verticillium* spp.	**	[7]

[a]*, **, and *** indicate low, moderate, and frequent as concluded based on frequency of isolation in at least one of the mentioned report, respectively.

relative humidity drops as the seeds enter the storage period, causing changes in the diversity and population of the fungi and the predominant fungal species [3]. In accordance with the rules of International Seed Testing Association, more than 500,000 seed lots have been tested by the International Rice Research Institute (IRRI) seed health unit and the fungal species detected were reported [4]. In general, rice is susceptible to a wide range of contaminating fungi, the growth of which is associated with grain spoilage, off-flavors, toxins, discoloration, and the production of harmful propagules [5]. The main reason for grain spoilage and tissue decomposition is the production of enzymes, such as lipases, proteases, carbohydrases, and volatile compounds (e.g., dimethyl disulfide, geosmin, and 2-methylisoborneol) by storage fungi [6]. As summarized from previous studies, fungal contamination and occurrence in rice grains [4, 7-13] are listed in Table 1.

Predominant *Aspergillus* and *Penicillium* species. As mentioned above, low relative humidity during rice storage influences the structure of fungal communities, favoring dry condition-tolerant fungi. The most frequently detected fungal genera in stored rice are *Aspergillus* and *Penicillium*, both of which are relatively tolerant to storage conditions.

Previously, Oh *et al.* [2] evaluated the occurrence of fungi and bacteria on rice grains stored in the rice-processing complexes of the National Agricultural Cooperative Federation in Korea. They reported that *Aspergillus* and *Penicillium* were the predominant fungal genera, in spite of the high fungal diversity between regions. In a later assessment of microbial populations and aflatoxin contamination, they identified three predominant *Aspergillus* species, which were *A. flavus*, *Aspergillus candidus*, and *Aspergillus fumigatus* [8]. These findings were consistent with other studies, which identified *Aspergillus*, *Penicillium*, and *Fusarium* as the predominant genera [14], and *A. candidus* and *A. flavus* as the predominant species [9].

Aspergillus spp. are ubiquitous in stored food, particularly in cereals. Among the most frequent and important species of rice-infecting fungi is *A. flavus*, which is widespread across the world. This species normally exists as a saprophyte in the soil, where it associates with a variety of decaying organic matter [15]. *A. flavus* can tolerate a wide range of temperatures and a low level of relative humidity; this specific feature enables the fungus to efficiently colonize the surface of stored rice grains. Along with rice, it can infect several other crops, such as peanuts, maize, and tree nuts. Contamination with *A. flavus* is very serious because of the presence of aflatoxin, a secondary metabolite that was first isolated from the fungus [16]. Aflatoxin is the most harmful mycotoxin and causes various adverse health effects that will be discussed in the next section. *A. candidus*, belonging to the subgenus *Circumdati*, is characterized by slow growth and white conidia. This fungus is a common contaminant of stored grains and can cause respiratory problems in individuals exposed to dust from the contaminated grains [17]. In contrast, *A. fumigatus* belongs to the subgenus *Fumigate* and is known for its fast growing, turquoise or dark-green colonies [18]. Abad *et al.* [19] stated that though *A. fumigatus* does not infect humans, inhaled conidia can give rise to aspergillosis in immune-compromised individuals with altered or weakened immune responses. In the review [19], they also mentioned factors that made *A. fumigatus* a successful pathogen, as well as the genes and molecules involved in causing the disease.

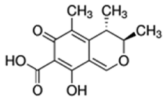
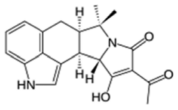
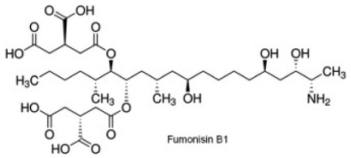
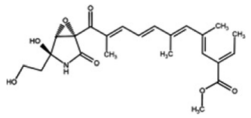
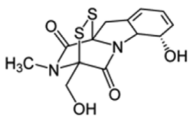
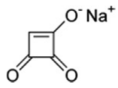
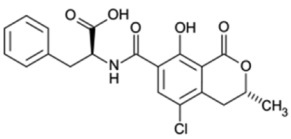
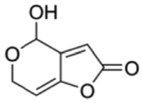
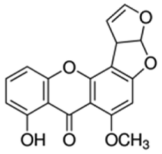
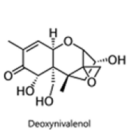
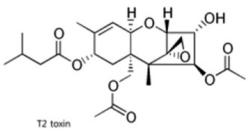
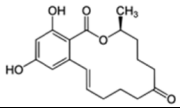
Compared with the genus *Aspergillus*, *Penicillium* (the second most important fungal group in stored rice) is a more diverse genus in terms of both species number and habitat range [20]. *Penicillium* spp. usually have bluish or greenish gray-colored colonies and a closed texture [21]. Members of the genus *Penicillium* are known as serious grain contaminants owing to their production of mycotoxins (e.g., ochratoxin, patulin, and citrinin) and related fungal metabolites (e.g., alkaloids, amino acids, anthraquinone, and azaphilone) [22, 23]. It is difficult to identify a specific *Penicillium* species due to their morphological similarities, which might have led to several misidentifications, particularly, of the ochratoxin-producing species. Recently, *Penicillium verrucosum* (the main source of ochratoxin contamination of cereals) and *Penicillium nordicum* have been accepted as ochratoxin-producing species of the genus *Penicillium* [24]. In Korea, two *Penicillium* species, *Penicillium islandicum* and *Penicillium fellutanum*, were found to be abundant in stored rice [12, 13]. *P. islandicum* has been detected in imported and polished rice (15/45 samples) in Korea, harvested rice in Japan (3/100 samples), and paddy and milled rice in Argentina and Paraguay [9, 13, 25]. Besides, *P. fellutanum* is known to produce ergot alkaloids (agroclavine 1 and epoxyagroclavine 1) and diketopiperazine alkaloids (fellutanine A, B, C, and D) [22, 26, 27].

MYCOTOXINS ASSOCIATED WITH STORED RICE GRAINS

Mycotoxins are chemically diverse, toxic secondary metabolites produced by certain fungi, mostly those from the genera *Aspergillus, Fusarium*, and *Penicillium*. They contaminate food sources and have several toxicological effects on humans and animals when consumed directly or indirectly [28]. The earliest report on the impact of mycotoxins on human health was related to ergot-poisoning in Europe, which resulted in thousands of people suffering from severe symptoms, and also leading to death. The disease was named "Holy fire" or "St. Anthony's fire" but is known today as ergotism and is caused by the consumption of ergot-infected cereals [16]. The toxicity of the ergot-infected products is related to ergot alkaloids. These ergot alkaloids were originally named after their first known source, the sclerotia (ergot) of the fungus *Claviceps purpurea*. The ergot alkaloids have various toxic effects, such as painful spasms, diarrhea, nausea, headache, or gangrenous symptoms in the fingers and toes. However, the alkaloids have potential, biological functions,

such as anti-herbivory defense and pharmaceutical applications, due to their structural similarities with three neurotransmitters, which can bind serotonin, dopamine, and adrenergic receptors [29, 30]. Another famous early outbreak of mycotoxicosis was associated with the consumption of contaminated, discolored yellow rice in Japan in the early 19th century, which was later attributed to a toxigenic entity [31].

Table 2. Mycotoxins and their related fungi associated with rice grains

Mycotoxins	Producing fungi	Chemical structure	Reference
Aflatoxins	*Aspergillus flavus*, *Aspergillus parasiticus*	 Aflatoxin B1 Aflatoxin B2	[8, 9, 34-38]
Citrinin	*Penicillium citrinum*		[39-42]
Cyclopiazonic acid	*Aspergillus* spp., *Penicillium* spp.		[39, 40, 43]
Fumonisins	*Fusarium proliferatum*, *Fusarium verticillioides*, *Gibberella fujikuroi*, *Aspergillus niger*	 Fumonisin B1	[9, 37, 44-48]
Fusarin C	*F. verticillioides*		[10]
Gliotoxin	*Aspergillus fumigatus*		[49]
Moniliformin	*F. proliferatum*		[10]
Ochratoxin A	*Aspergillus* spp., *Penicillium* spp.		[9, 10, 37, 42, 50-52]
Patulin	*Aspergillus* spp., *Penicillium* spp.		[53]
Sterigmatocystin	*Aspergillus versicolor*, *Aspergillus terreus*, other *Aspergillus* spp.		[10, 42, 54]
Trichothecenes	*Fusarium sporotrichioides*, *Fusarium graminearum*, *Trichothecium roseum*	 Deoxynivalenol  T2 toxin	[55, 56]
Zearalenone	*Fusarium* spp.		[57]

Rice, along with other cereal crops, is susceptible to contamination by a wide range of mycotoxin-producing filamentous fungi, which usually infect crops in the field and continue in storage facilities. In addition, rice is a good substrate for mycotoxin biosynthesis by grain fungi such as *P. verrucosum* [32] and *Fusarium subglutinans* [33]. For example, *P. verrucosum* was known to be related with ochratoxin A contamination in rice, which was reported as the most commonly detected mycotoxin with levels above the tolerated limits in Korean rice [9]. Several mycotoxins, including aflatoxins [8, 9, 34-38], citrinin [39-42], cyclopiazonic acid [39, 40, 43], fumonisins [9, 37, 44-48], fusarin C [10], gliotoxin [49], moniliformin [10], ochratoxin A [9, 10, 37, 42, 50-52], patulin [53], sterigmatocystin [10, 42, 54], trichothecenes [55, 56], and zearalenone [57], have been found in rice grains. Rice-associated mycotoxins and their related fungi reported in previous studies are summarized in Table 2.

Aflatoxins. Aflatoxins include 14 different types of fungal secondary metabolites with similar structures. In particular, among the naturally produced aflatoxins are B_1, B_2, G_1, and G_2; the names B and G are derived from the blue and green fluorescent colors, which are produced under UV light on thin layer chromatography plates [58]. Aflatoxins were first discovered after the mass deaths of turkeys due to liver disease in England in 1960, which was named turkey "X" disease since the cause was unknown. It was later revealed that the affected turkeys had been fed on aflatoxin-contaminated feed [59].

The most toxic aflatoxin B_1 is exclusively produced by *A. flavus* and *Aspergillus parasiticus*. This toxin has been shown to have mutagenic, immunosuppressive, and teratogenic properties [60-62]. The relationship between aflatoxin B_1 and liver cancer has been supported by epidemiological studies showing that regions with high aflatoxin dietary intake values had significantly higher incidence of liver cancer [63]. About 4.5 billion people are exposed to uncontrolled amounts of aflatoxins in developing countries, where poor cereal storage conditions promote aflatoxigenic fungi, leading to higher levels of aflatoxins in cereals. Due to concerns about human and animal health, regulatory limitations were issued on the quantity of aflatoxin allowed in food and animal feed in several countries. According to the FAO of the United Nations, the limits vary according to the commodity and the country. For example, in Europe, the limits range between 2~12 μg/kg for B_1 and 4~15 μg/kg for total aflatoxins. In the United States, food safety regulations include a limit of 20 μg/kg for total aflatoxins in all food [64]. For these reasons, elimination of aflatoxin in food is crucial. Several attempts to eliminate or reduce aflatoxin contamination on food have been made; however, the most effective way to control this will be achieved by directly reducing or limiting exposure to the toxin-producing fungal species using traditional methods (e.g., physical separation, heat, or radiation treatments) and commercial fungicides.

CONTROL OF FUNGAL AND MYCOTOXIN CONTAMINATION IN STORED RICE GRAINS

Conventional control of grain fungi. The previous section introduced the predominant rice-storage fungal species and the hazards associated with their contamination of rice. This was analyzed in terms of the direct fungal effect on grain quality, and the impact on the environment and human health (e.g., due to mycotoxins). Several attempts have been made to control such important fungi and minimize the loss of quality and quantity of rice. Actions have included the use of host resistance, traditional practices, and chemical application to reduce the populations of fungal pathogens from field to storage [65]. According to the IRRI fact sheet on the management of storage fungi (available from http://irri.org/), several factors affect the populations of storage fungi on harvested rice grains. These include the temperature and moisture content of the stored grain, the condition of the grains before storage, storage period, and insect or mite activity in the grains. Contaminated grains are often treated by physical and chemical means, such as use of Dithane M-45 and Benlate (benomyl) fungicides. Benlate was reported to inhibit a broad spectrum of fungi, to be relatively non-toxic to plant cells and protoplasts, and to prevent fungal contamination [66]. However, increasing concerns about the risks of chemical control methods, limitations on their use against plant diseases, particularly at postharvest stage (unwanted health and environmental issues and the possible emergence of resistant strains) have sparked a search for safer alternatives to control and manage storage fungi. For example, waste fruit peels were investigated for their ability to inhibit *A. flavus* on rice; they were suggested as a safe method for reducing aflatoxin levels on rice grains for up to four months [67]. These control methods could be used for rice storage fungi; however, there is still a need to develop safe and environmentally sound measures to control rice-storage and aflatoxigenic fungi, possibly by using microbial antagonists as will be discussed in the following section.

Microbe-mediated control of grain fungi producing mycotoxins, including aflatoxins. The antifungal properties of beneficial microorganisms, including bacteria have been known since the 1930s. Extensive efforts have been made to use them for disease control as an alternative to chemical fungicides [68]. The best way for selecting potential biocontrol agents is to screen the diverse communities of non-pathogenic microorganisms that are adapted to the same environment. Such microorganisms are considered to be a large untapped source of protection against plant pathogens [69]. In rice, potential, antagonistic bacteria have been reported on seeds [70], which could be employed as biological control agents against plant pathogens [65]. *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* have been described as effective bacterial agents against *Rhizoctonia solani*, which causes sheath blight [71]. These biocontrol studies

generally focused on the field rice diseases; however, not many studies were conducted against storage fungal pathogens. In fact, research on the biocontrol of rice storage fungi should consider the microbial population colonizing the grains as a source of effective biocontrol agents. Adaptability to and survival on rice grains should be considered for the effectiveness of antagonistic bacteria from sources other than rice grains.

Among the bacteria with biocontrol activity reported to date, the genus *Bacillus*, one of the most studied and common biocontrol agents, is well characterized by its plant growth-promoting traits and its ability to protect against several plant pathogens by production of wide array of compounds, including antifungal peptides [72, 73]. A marine strain of *Bacillus megaterium* was shown to have biocontrol activity against *A. flavus* on peanut kernels [74]. In that study, the bacterial treatment reduced the disease incidence on inoculated kernels, and also inhibited the biosynthesis of aflatoxins in a liquid medium, which was due to the inhibition of aflatoxin biosynthesis-regulatory genes (*aflR* and *aflS*) expression. This result was obtained by quantitative real-time polymerase chain reaction (qRT-PCR) analysis of the relative mRNA abundance of the *A. flavus* genes when co-cultured in the medium with the bacterium. Furthermore, the result was supported by a recent study [75], in which a mechanism of action involving the bacterial interference with aflatoxin biosynthesis was proposed; this will be discussed later. In another study, culture filtrates of biocontrol agents, such as *Trichoderma* spp., different strains of *P. fluorescens*, a strain of *B. subtilis*, and *Rhodococcus erythropolis*, showed considerable biocontrol activities against *A. flavus* and limited the production of aflatoxins. In particular, among the tested microbes, *R. erythropolis* completely inhibited the growth of *A. flavus* and decreased aflatoxin production [76].

On the other hand, for the biocontrol of *Penicillium* spp., a few studies showed biocontrol activity on stored rice grains. Among them, a preliminary study for screening of 460 bacterial strains from Korean stored rice grains for their biological activity against *Aspergillus* and *Penicillium* spp. resulted in selection of several potential biocontrol candidates [77]. In another study, specific formulations of *Pichia anomala* exhibited biocontrol activity against *P. verrucosum*, which was capable of reducing the fungal growth as well as ochratoxin A production on moist grains [78]. In this section, the microbial effect on mycotoxigenic fungi was discussed; studies reporting the direct and microbial degradation of mycotoxins, including aflatoxin, will be discussed in the following section.

Microbial degradation of mycotoxins, including aflatoxins. Microbial degradation represents a promising method for management of mycotoxins contaminating grains and feed. Intensive researches have been conducted to find efficient and safe mycotoxin-detoxifying agents. Previously, rumen microbes could metabolize ochratoxin A, zearalenone, T-2 toxin, and deacetoxyscipenol [79]. Recently, several soil bacteria showed ochratoxin A degradation property; among them, *Acinetobacter calcoaceticus* strain 396.1 and *Acinetobacter* sp. strain neg1 showed consistently the toxin-degradation activity [80]. Similarly, several bacterial strains (belonging to the genera *Anaerofilum*, *Collinsella*, and *Bacillus*) capable of detoxification of deoxynivalenol were isolated and identified using polymerase chain reaction-denaturing gradient gel electrophoresis [81]. Besides, the extracellular extracts of *Acinetobacter* sp. exhibited biodegradation ability of zearalenone and this bacterium was able to grow rapidly on zearalenone as sole carbon source [82].

For aflatoxin detoxification, physical, chemical, and biological methods have been proposed, depending on the nature of the product being treated [83]. Several microbial agents have been shown to effectively degrade aflatoxins under laboratory conditions. The first report showing the possible microbial degradation of aflatoxins came in 1965, paving the way for more sophisticated screenings of microbes with similar properties [84]. Ciegler *et al.* [85] screened 1,000 different yeasts, molds, mold spores, actinomycetes, bacteria, and algae, of which only *Flavobacterium aurantiacum* NRRL B-184 could degrade aflatoxins *in vitro*. Other studies described a similar role for *F. aurantiacum* in irreversibly clearing aflatoxins from different foods (e.g., milk, oil, peanut butter, peanuts, and corn) [85-87], yet the underlying mechanism remained unexplained. Another significant observation by Ciegler and Peterson [88] revealed that aflatoxin B_1 could be converted to the relatively non-toxic aflatoxin B_{2a} by acid-producing molds. It was later found that the conversion was a nonspecific hydration catalyzed by acids and did not require the presence of molds [83]. Since at least part of the aflatoxin was metabolized to water-soluble degradation products, mineralization was proposed as the mechanism of aflatoxin detoxification by *F. aurantiacum* [89]. More recently, crude protein extracts from *F. aurantiacum* have been shown to successfully degrade the aflatoxin, suggesting that the mechanism of detoxification may be enzymatic [90]. In other studies [91], 23 different species of *Bacillus* were screened and *B. licheniformis* was identified as capable of inhibiting the growth of *A. flavus* and *Aspergillus westerdijkiae*, as well as degrading aflatoxin B_1 and ochratoxin A. Recently, *P. aeruginosa* N17-1 was also shown to degrade aflatoxin B_1, possibly through enzymatic degradation [92]. These findings demonstrated that antagonistic bacteria do not only inhibit the growth of deleterious storage fungal species, but can also degrade the mycotoxins present in food. Thus, the bacteria could be utilized as sources of efficient enzymes for mycotoxin degradation in food. The microbial degradation of aflatoxins is not the only way to directly reduce the toxins; other studies have indicated the ability of some bacteria to interfere with the aflatoxin-biosynthetic pathway in the toxin-producing fungi. A historical review [84-86, 88-98] of microbial aflatoxin degradation is summarized in Table 3.

Table 3. Historical reviews on the biological degradation of aflatoxins

Year	Finding	Reference	Comment
1965	First evidence of aflatoxin destruction by microorganisms	[84]	No specific results on microbial aflatoxin biodegradation
1966	First screening for microbial biodegradation of aflatoxin, resulting in the selection of *Flavobacterium aurantiacum* NRRL B-184	[85]	Mechanism of action was not reported
1967	The protozoan *Tetrahymena pyriformis* W exhibited the ability to degrade aflatoxin B_1 but not aflatoxin G_1 in liquid medium	[93]	Aflatoxin G_1 affected growth of the tested organism and the reason for toxicity was not explained
1968	Conversion of aflatoxin B_1 to comparatively non-toxic aflatoxin B_{2a}	[88]	This was later found to occur via a non-specific hydration catalyzed by acids and did not require the presence of molds [74]
1971	Degradation of aflatoxin B_1 by *Rhizopus* spp.	[94]	Culturing of *Rhizopus* spp. with aflatoxin containing medium produced a metabolite derived from aflatoxin G_1 degradation
1972	Partial degradation of aflatoxin B_1 and G_1 in miso after one month fermentation	[95]	Direct evidence of aflatoxin microbial degradation was missing
1988	Reduction of aflatoxin B_1 from peanut milk inoculated with *F. aurantiacum* NRRL B-184	[86]	Results indicated that aflatoxin reduction was more efficient in partially defatted peanut milk
1994~1995	Evidence of the mechanism of *F. aurantiacum* aflatoxin detoxification to be due to mineralization	[89, 96]	Further evidences of enzymatic detoxification were reported later
2000	Crude protein extracts of *F. aurantiacum* could degrade aflatoxin, suggesting that the mechanism of detoxification was enzymatic	[90]	Inhibitory compound was not identified
2006	Reduction of aflatoxin B_1 in chicken feed by *Rhizopus oligosporus*	[98]	Combination with *Saccharomyces cerevisiae* did not improve aflatoxin biodegradation
2007	Screening of 23 different species of *Bacillus*, of which *Bacillus licheniformis* exhibited the ability to inhibit the growth of *Aspergillus flavus* and *A. westerdijkiae* as well as remove aflatoxin B_1 and ochratoxin A	[91]	Mechanism was not explained
2012	Bacterial degradation of aflatoxin in liquid medium and pistachio nuts by 58.66% and 95%, respectively	[97]	Treatment of crude extract with proteinase K resulted in the partial reduction of aflatoxin, which could be due to mechanisms other than enzymatic degradation
2014	*Pseudomonas aeruginosa* N17-1 demonstrated degradation of aflatoxin B_1 by what was proposed to be enzymatic degradation	[92]	Responsible enzyme is not yet identified

Microbial interference in the aflatoxin-biosynthetic pathway. The discovery of the aflatoxin precursor, norsolorinic acid [99], characterization of the biosynthetic and regulatory genes, and the complete sequence of aflatoxin genes in *A. parasiticus* promoted interest in the aflatoxin-biosynthetic pathway [100]. Consequently, the biosynthetic pathway and the roles of different genes governing aflatoxin production are well understood and it is one of the best-characterized fungal metabolites [101]. Such understanding paved the way for further studies on the mechanisms of aflatoxin biosynthesis suppression in *A. flavus* and *A. parasiticus*, by co-cultivation with other microorganisms or physical factors such as water activity and temperature [75, 102].

Since this review focuses the use of microbe-based approaches for aflatoxin suppression, specific details of aflatoxin biosynthesis will not be covered. However, to better understand how microbe-related inhibition of aflatoxin occurs, the aflatoxin-biosynthetic processes and the related genes will be discussed. To this end, there are interesting studies on the possible mechanisms responsible for the down-regulation of the transcription-activator genes *aflS* (formerly *aflJ*) and *aflR* [75, 103] in *Aspergillus* spp. Changes in the *aflR*/*aflS*-expression ratio in response to temperature and water activity have also been reported [102]. The gene *aflR* encodes a sequence-specific DNA-binding transcription activator (AFLR) that regulates aflatoxin biosynthetic genes, while the accessory regulatory gene *aflS* encodes a transcription co-activator (AFLS) [104, 105]. Both genes were reported as essential for aflatoxin production. Meyers *et al.* [106] reported that disrupting *aflS* prevented *A. flavus* from converting intermediates of the pathway to aflatoxins. According to Chang [104], the role of AFLS was to support the binding of AFLR to DNA, thereby activating the

transcription of aflatoxin structural genes, and the lack of this interaction, prevented aflatoxin production. Similarly, over-expression of *aflS* resulted in increased aflatoxin production, suggesting a role in modulating early aflatoxin biosynthetic genes, while the over-expression of *aflR* resulted in higher *aflS*-transcription rates, indicating that *aflS* expression was regulated by *aflR* [107]. Therefore, the exact role of *aflS* remains elusive; however, it could be involved in not only the activation of early and mid aflatoxin pathway genes but also the regulation of structural genes through its association with *aflR*.

In light of this, co-cultivation of *A. flavus* with a strain of *B. megaterium* was proposed as a possible mechanism for the inhibition of aflatoxin biosynthesis through the down-regulation of the regulatory genes [75]. The co-cultivation resulted in the down-regulation of several genes in the aflatoxin-biosynthetic pathway, including *aflS*, as confirmed by qRT-PCR. Down-regulation of *aflS* would alter the *aflR*/*aflS* ratio, inhibiting the regulation of aflatoxin structural genes and blocking the aflatoxin-biosynthetic pathway. Interestingly, a similar effect of down-regulating *aflS* was previously obtained by Yu *et al.* [108] upon increasing the incubation temperature to 37°C. While it remains unknown exactly how the down-regulation of *aflS* leads to the inactivation of structural genes, it is speculated that it is mediated by potential inhibitors in fungal cells. The latter could be similar in size to AFLS and form a dysfunctional AFLS/AFLR activation complex that will bind to the aflatoxin pathway-structural genes, resulting in the inhibition or termination of their transcription (Fig. 1) [75]. In another recent study, *Streptomyces* spp. were used as mutual antagonists against *A. flavus* and *A. parasiticus*, and lowered the aflatoxin levels produced by the *Aspergillus* fungi. In the case of *A. parasiticus*, aflatoxin reduction was

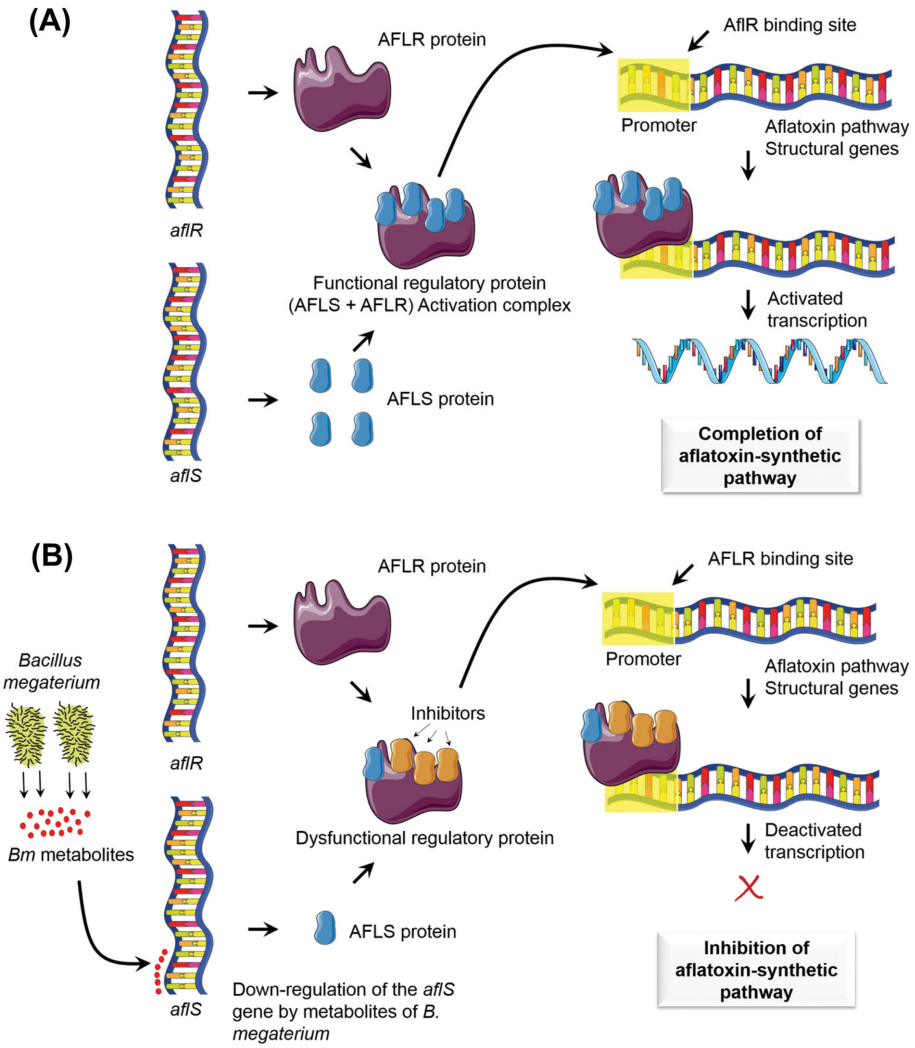


Fig. 1. A hypothetical mechanism illustrating the role of the genes *aflS* and *aflR* in the aflatoxin-biosynthetic pathway in *Aspergillus flavus*: under the normal aflatoxin-producing culture condition (A) and under co-cultivation condition (B) with *Bacillus megaterium*, which inhibits aflatoxin biosynthesis by down-regulating the expression of the gene *aflS*. This figure was re-drawn, with appropriate modification, from Kong et al. [75], with permission of Springer, using Servier Medical Art (www.servier.com).

attributed reduction of fungal growth. No influence on fungal growth was reported in the case of *A. flavus*. Instead, *Streptomyces* spp. appeared to repress *aflM* and *aflS* by altering the *aflR*/*aflS* ratio in a species-specific manner [103]. Microbial interference with aflatoxin-biosynthetic pathway is an important feature of certain beneficial bacteria, which can be applied for prevention of aflatoxin occurrence on stored products, including rice.

CONCLUSIONS

The increased understanding of the impact of rice-grain fungal species on human and animal health, whether in the form of disease-causing spores or dangerous mycotoxins (e.g., aflatoxins), has raised awareness about the control of fungi contaminated food and feed. An effective and safe method for controlling grain fungi during storage may be the microbe-mediated (biological) control measure that, as discussed in this review, can negatively affect grain fungi and detoxify (or biodegrade) mycotoxins or inhibit their biosynthesis. Therefore, further screening and selection of potential biocontrol agents should be encouraged and improved, as these will help finding novel active agents for antifungal compounds or detoxification enzymes and biosynthesis inhibitors of mycotoxins. Based on the reviewed literature, it appears that the selection of antagonistic bacteria that originated on rice grains has been largely neglected. Instead, such antagonistic bacteria from rice could serve as sources of broad-spectrum biocontrol agents against rice grain fungi, as they are more likely to survive and compete against pathogenic rice mycoflora. Taken together, further research should be directed towards the screening of rice-originated bacteria for effective biocontrol agents against mycotoxigenic rice-grain fungal species. In addition, it may be noted that most research on microbe-mediated mycotoxin (including aflatoxin) degradation or production inhibition was made under laboratory conditions. However, it is essential that a safe and effective method for aflatoxin detoxification should be devised on food commodities.

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